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SUMMARY

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TILLAGE EFFECTS ON SOIL ORGANIC CARBON, MICROBIAL BIOMASS CARBON AND BETA-GLUCOSIDASE ENZYME ACTIVITY IN A TYPIC HAPLOXERERT SOIL

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Abstract

Biological indicators allow to define early changes in soil environment due to the impacts in land management over time. This study investigated the effects of long-term (2006-2015) two conventional (CT-1 and CT-2), three reduced (RT-1, RT-2 and RT-3) and a no-till (NT) on soil organic carbon (SOC), microbial biomass carbon (MBC) and beta-glucosidase enzyme activity (BGA) in eastern Mediterranean region, Turkey. Experimental design was randomized complete block with three replications. Results indicated that SOC, MBC and BGA significantly differed among tillage treatments. Non-disturbed soils under NT had nearly 75% higher SOC (8.80 gkg^{-1}), 359% higher BGA (207.66 mg PNP kg^{-1}h^{-1}) and 68% higher MBC (185.9 mg C kg^{-1} soil) compared with highly disturbed soils under CT-1 system. The BGA and MBC concentrations under NT were also significantly higher than the three RT treatments. In contrast to BGA and MBC, the SOC contents under RT systems did not differ from that of NT treatment. Higher concentrations of BGA and MBC under NT and partially under RT compared to CT treatments were most likely related to the increased organic matter contents and non-disturbed environmental conditions.

***Key words**: soil organic carbon, microbial biomass carbon, beta-glucosidase enzyme activity, conventional tillage, conservation tillage.*

INTRODUCTION

Conventional tillage using mouldboard ploughing, subsequent disking and floating for seed bed preparation has been frequently used tillage practice by farmers in many parts of the Turkey. Even in state farms, as in Ceylanpınar that is the largest (163,000 ha) state owned and managed farm in Turkey, conventional practices have been widely used. Farmers prefer this practice for appropriate seed bed preparation by breaking down crop residues along with weeds, incorporating the crushed biomass into the soil, increasing seed-soil contact by breaking larger aggregates or soil clods and providing warmer and drier seedbed for early spring planting (Soane et al., 2012; Dozier et al., 2017).

Despite the short-term benefits of conventional tillage for seed bed preparation, aggressive physical disturbance induces decomposition of soil organic matter (SOM) by creating appropriate soil environment for microorganisms, and subsequently reduces soil fertility and quality (Álvaro-Fuentes et al., 2013; Abdullah, 2014). Alteration in SOM status of soils results in substantial changes in soil microbial biomass and enzyme activities, both are highly correlated with soil organic carbon (SOC) content of soils (Acosta-Martínez et al., 2003; Melero et al., 2008). Therefore, soil microbial properties such as microbial biomass, as a primary source of soil enzymes and pool of SOC and activities of soil enzymes are considered important and sensitive indicators to understand and compare the effects of soil disturbance resulting from tillage practices on soil quality (Álvaro-Fuentes et al., 2013; Kabiri et al., 2016). Conservative tillage systems, reduced or no-till, minimize the soil disturbance and results in improvement of soil quality by increasing SOC with a higher microbial activity, nutrient and water supply and water stable aggregates (Lal, 2015). Minimizing incorporation of crop residue reduces rate of mineralization of organic matter by physically protecting organic matter from microbial decomposition (Tripathi et al., 2014). Experiments on investigating the effects of conservative tillage on microbial properties of soils have been studied elsewhere in many countries of the world, but very few long-term studies were available reporting the effects of tillage practices on microbial properties in Turkey. Thus, this study was conducted to investigate the responses of SOC, microbial biomass C and activity of β-glucosidase enzyme to long-term (nine years) two conventional, three reduced and no-till practices under Mediterranean climate in southern Turkey.

MATERIALS AND METHODS

Study Site and Experimental Details

The experiment was conducted at the Agricultural Research Station of the Cukurova University (37°00′54″ N, 35°21′27″ E; 32 m altitude) with Mediterranean climate in Adana, Turkey. The average annual precipitation is 639 mm, and temperature is 19.2°C. The experimental plots were established in 2006 on a clayey soil classified as smectitic, active, mesic Typic Haploxererts. The initial soil properties in the surface layer (0-30 cm) were 50% clay, 32% silt and 18% sand, pH (saturation paste) is 7.82, electrical conductivity (saturation paste) is 0.15 dS m^{-1}, calcium carbonate is 244 g kg^{-1} (Çelik, 2011).

The plots were 12 m width and 40 m length (480 m^2) with 4 m buffer between each plot. In this study, six tillage systems in rotation of winter wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.) – grain maize (*Zea mays* L.) were applied for nine years.

In all tillage methods, the harvest residues on soil surface were chopped prior to tillage operations except CT-2. The tillage treatments were:

1) Conventional tillage with residue incorporated (CT-1): In CT-1, soil was tilled to 30-33 cm depth using a moldboard plow before winter wheat followed by two passes of disc harrow at 13-15 cm and 2 passes of float. For the second crop, soil was tilled with a heavy tandem disc harrow (HTD) to a depth of 18 to 20 cm, followed by 2 passes of disc harrow to 13-15 cm depth and 2 passes of float.

2) Conventional tillage with residue burned (CT-2); In CT-2, crop residues were burned after each harvest differed from CT-1 and also chisel plow instead of HTD to the depth of 35 to 38 cm was used in second crop.

3) Reduced tillage with heavy tandem disc harrow (RT-1); In RT-1, soil was tilled with a HTD to a depth of 18-20 cm (2 passes) and followed by 2 passes of float before wheat planting. For the second crop, rotary tiller (RoT) was used to 13-15 cm depth and 2 passes of float.

4) Reduced tillage with rotary tiller (RT-2); In RT-2, RoT was used at 13-15 cm depth and followed by 2 passes of float before first and second crop planting.

5) Reduced tillage with heavy tandem disc harrow followed by no tillage for the second crop (RT-3); In RT-3, soil was tilled with a HTD to 18-20 cm depth and followed by 2 passes of float before wheat. A non-selective herbicide (500 g ha^{-1} Glyphosate) was applied for weed management, and NT planter was used for planting of second crop soybean or corn.

6) No-tillage, direct planting (NT); In NT, crop residue on soil surface were chopped as in all other treatments except CT-2, a non-selective herbicide (500 g ha^{-1} Glyphosate) was applied for weed management, and NT planter was used for planting in both the first and the second crop.

Chemical fertilizer application rate was the same regardless of tillage method: 170-180 kg N ha^{-1} and 55-60 kg P_2O_5 ha^{-1} for wheat, 250-265 kg N ha^{-1} and 60-65 kg P_2O_5 ha^{-1} for corn and 120-130 kg N ha^{-1} and 40-45 kg P_2O_5 ha^{-1}

for soybean based on soil analysis. Commercially available corn and soybean cultivars at seeding rates of 8.4 and 23.6 plants per m^2 were planted in the third week of June and harvested in the second week of October.

Soil Samplings and Laboratory Analyses

Disturbed and undisturbed soil samples at the 0-10 cm depth were taken after second crop harvest of corn in 15th of December, 2015.

The activity of β-glucosidase enzyme was determined following the method based on the colorimetric estimation of the p-nitrophenol (Tabatabai, 1982). In this method, 1 g of soil was incubated for 1 h at 37°C with a buffered p-nitrophenyl-β-D-glucopyranoside solution (pH 6.0) and toluene. The p-nitrophenol formed by the hydrolysis of the p-nitro-phenyl-β-D-glucopyranoside at 37°C for 1 h was determined by measuring the yellow filtrate colorimetrically after color development of the soil suspension with 1 mL 0.5 mol L^{-1} $CaCl_2$ and 4 mL of tris (hydroxymethyl) aminomethane buffer (pH=12). The β-glucosidase activity was expressed as micrograms of p-nitrophenol released per gram dry soil per hour.

The amount of soil microbial biomass carbon (MBC, mg C kg^{-1} soil) was determined using the substrate induced respiration (SIR) method (Anderson and Domsch, 1978). In the SIR method, 5 g of moist soil was weighted into small jars, 1 ml glucose was added (0.5% w/w) and waited for 2 hours. After two hours, 2.5 ml of 0.05 M NaOH within small tubes were placed into the jars as an alkali trap. The jars were tightly closed and inserted into the incubator for 4 to 6 hours at 25°C. The same operations were repeated without soil as controls. After the incubation, the NaOH was removed, and 5 ml 0.5 M $BaCl_2$ was added to precipitate the absorbed CO_2 as insoluble carbonate, and the supernatant was titrated with phenolphthalein indicator against 0.05 M HCl to calculate CO_2 released from soil (mg C kg^{-1} soil), against corresponding controls.

Bulk density was determined using soil cores (length 5.1 cm, diameter 5.0 cm) collected from three depths. The soil samples of known volumes were weighed, oven dried at 105ºC for 24 h to a constant weight and weighed to calculate bulk density (Blake and Hartge, 1986).

Soil organic carbon (SOC) was calculated through the dividing soil organic matter content by the Van Bemmelen coefficient of 1.724 organic matter is equal to 58% of carbon (Nelson and Sommers, 1982).

Organic C stock of soils under each of tillage system was calculated on an equal mass basis references to 0-10 cm depth using the organic C concentrations and soil bulk densities of each sampled plot (Lal et al., 1998; Mishra et al., 2010).

$$Cs = OC\ x\ Bd\ x\ D\ x\ A$$

where: Cs is the organic C stock (kg ha^{-1}); OC is the soil organic carbon (g kg^{-1}); Bd is the soil bulk density (Mg m^{-3}); D is the thickness of soil horizon (m); A is the area (ha: 10^4 m^2).

Microbial quotient calculated as the ratio of MBC to SOC (Insam et al. 1989; Anderson and Domsch, 1989).

Statistical Analyses

Kolmogorov-Smirnov test was used to control the distribution of data for normality. The data had normal distribution and no need to use any kind of transformation to normalize the data. The effects of tillage systems and the differences between tillage systems were assessed by analysis of variance (ANOVA) test. Differences among treatments were evaluated by DUNCAN test (P<0.05). The statistical analyses were performed using IBM SPSS statistical package (version 21.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSIONS

Soil Organic Carbon

Tillage effect on soil organic carbon (SOC) was statistically significant (P<0.01) (Figure 1). The SOC increased with the decrease in tillage, and the increase in SOC concentration among tillage systems was in this order: CT-1 > CT-2 > RT-1 > RT-2 > RT-3 > NT. The SOC concentration of soils was ranged from 8.80±0.48 g C kg^{-1} soil (CT-1) to 15.40±0.93 g C kg^{-1} soil (NT) among six tillage treatments. The NT had 75% and 58% higher SOC concentrations than CT-1 and CT-2 treatments.

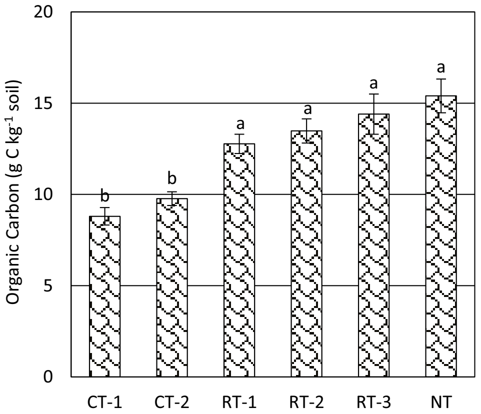

Figure 1. Soil organic carbon concentration (g C kg^{-1} soil) of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at p<0.05

The increase in SOC concentration with minimal or no soil disturbance implies considerable C sequestration potential of soils under RT and NT systems. The final total SOC stock after 9 years under NT treatment (21127 kg C ha^{-1}) was 77 and 61% higher than conventionally tilled CT-1 (11952 kg C ha^{-1}) and CT-2 (13103 kg C ha^{-1}) treatments. The difference in C stock among NT and RT treatments was lower compared to CT, and the differences among NT and RT treatments were not significant (Figure 2). Soil carbon may reach to an equilibrium under NT within 15-20 years or 20-25 years (West and Post, 2002) depending on genetic such as climate, soil type and amount of initial SOC (Schneider, 2007), and dynamic factors i.e., crop rotation and other management options. Thus, our soils can still sequester great amount of C till reaching to equilibrium. Similar to our results on C sequestration, Martinez et al. (2013) stated that after nine years of NT implementation, soil C concentration under NT was 4980 kg C ha^{-1} higher than CT. Karlen et al. (2013) also reported significantly lower SOC concentration under 31 years of conventional tillage using moldboard plough in a corn/soybean rotation and 26 years of conventional tillage in continuous corn cultivation compared to NT. Higher SOC accumulation in surface soils under NT compared to CT systems was ascribed to slow decomposition of crop residue in the compacted surface layer (Martinez et al., 2013), and physical protection of soil organic C within soil aggregates (Souza et al., 2014).

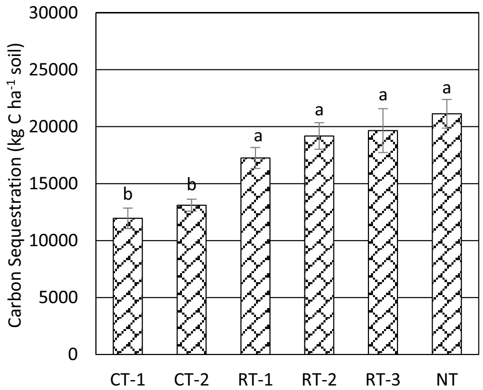

Figure 2. Carbon sequestration (kg C ha^{-1} soil) for nine years under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at p<0.05

Microbial Biomass Carbon

Microbial biomass is defined as the living organic matter fraction of soils and an important source as an energy and nutrient reservoir (Souza et al., 2014). Similar to SOC, the microbial biomass carbon (MBC) concentrations of soils were statistically different (P<0.01) among tillage systems (Figure 3). The MBC concentration of soils presented the same behavior as that observed for SOC which increased with the decrease in soil disturbance. The MBC was the highest under NT (312.3 ± 10.6 mg C kg^{-1} soil) due to better soil conditions for microorganisms, while it was the lowest under CT-2 (178.2 ± 12.5 mg C kg^{-1} soil) where crop residues were burnt after each harvest of crop in rotation.

Higher MBC under NT management implies better soil biological quality under non-disturbed soil environment (Doran and Parkin, 1994). After nine years, the MBC concentrations under RT-1 (247.0 ± 18.4 mg C kg^{-1} soil) and RT-2 (249.9 ± 12.4 mg C kg^{-1} soil) were significantly higher compared to CT-1 and CT-2, however they were significantly lower compared to NT. In other studies, similar increases in MBC with the decrease in soil tillage intensity and frequency have been reported by others. For example, Martin-Lammerding et al. (2015), in a study conducted under the semi-arid region of central Spain, obtained a very high value of MBC under NT system compared to RT and CT systems. In a 6 years old tillage experiment, the MBC concentrations under CT using moldboard and disc plows were found 25 and 43% lower than less disturbed soils using chisel and rotary plows under RT system (Kabiri et al., 2016). The increased MBC under NT and RT in comparison to CT emphasizes the improved environmental conditions due to the minimal disturbance and lower decomposition of organic matter and the importance of conservative tillage practices in Mediterranean climate on enhancing microbial efficiency of soils. In contrast to the reports on significant effects of tillage on MBC, Mbuthia et al. (2015) found no significant influence of long term (31 years) tillage on MBC under continuous cotton at West Tennessee Research and Education Center in Jackson, TN, USA. However, they reported significant alterations in the microbial community structure composition by tillage. This contradictory result was ascribed with a combination influence of soil type (sandy texture) and the low biomass produced under cotton compared to other crops.

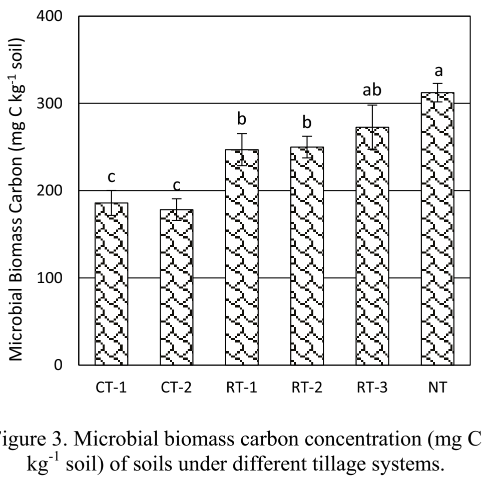

Figure 3. Microbial biomass carbon concentration (mg C kg^{-1} soil) of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at p<0.05

The ratio of MBC to SOC is defined as microbial quotient and widely used for an early indicator of microbial activity due to alteration in soil environment (Sparling, 1992; Martin-Lammerding et al., 2015; Deng et al., 2016). Although both SOC and MBC were significantly different among tillage systems, MBC/SOC ratio slightly different among tillage systems and the difference was not significant (P=0.554). Previous studies reported that MBC represented 2 to 4% of TOC (Moreira and Siqueira, 2006) and 1.13 to 1.43% of TOC (Souza et al., 2014). The MBC concentrations in this experiment were corresponded to 1.82 (CT-2) to 2.11% (CT-1) of SOC obtained.

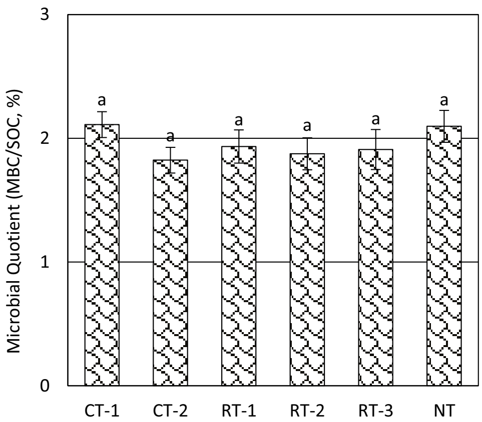

Figure 4. Microbial efficiency rate of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at p<0.05

β-glucosidase Enzyme Activity

β-glucosidase enzyme activity for each tillage practice are represented in Figure 5. The β-glucosidase enzyme activity, one of the important indicators of soil quality (Stott et al., 2010), is sensitive to any change in the management practices that may impact the amount of organic matter (Ekenler and Tabatabai, 2003). Therefore, the decrease in tillage intensity resulted in a significant increase in β-glucosidase enzyme activity being 359 and 365% higher from CT-1 and CT-2 compared to NT, respectively. The activity of β-glucosidase which ranged from 44.86 mg PNP kg^{-1} h^{-1} (CT-2) to 207.66 mg PNP kg^{-1} h^{-1} (NT). The increase in β-glucosidase enzyme activity among tillage systems was in this order: CT-2 > CT-1 > RT-1 > RT-2 > RT-3 > NT (Figure 5). The reduction in tillage intensity resulted in higher MBC which favored the β-glucosidase enzyme activity due to increased availability of substrate and reduction of soil disturbance (Sinsabaugh et al., 2008). Although activities of β-glucosidase were higher under RT (113.03, 121.72 and 134.15 mg PNP kg^{-1} h^{-1} for RT-1, RT-2 and RT-3, respectively) systems compared to CT systems, they were significantly lower than that of NT treatment. Our results are in accordance with those reported by Martin-Lammerding et al. (2015) who indicated that β-glucosidase activity was at the highest level under NT followed by RT, with CT having the lowest level of β-glucosidase activity. Similarly, higher MBC concentration coupled with higher activity of β-glucosidase enzyme under NT in comparison to CT was also reported by Mendes et al. (2003) for a Brazilian Cerrados.

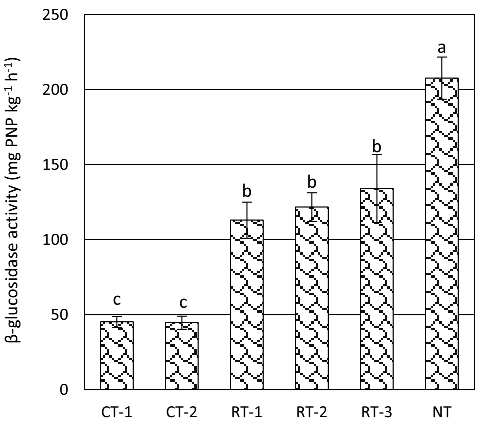

Figure 5. β-glucosidase activity (mg PNP kg^{-1} h^{-1}) of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at $p<0.05$

Pearson correlation was used to evaluate the relationships between SOC, MBC and β-glucosidase activity (Table 1). The significant positive correlations between β-glucosidase activity and SOC (r=0.43) and MBC (r=0.80) revealed that β-glucosidase activity is closely related to SOC and provides information on alterations in SOC concentration of soils. Significant correlations between enzyme activities and SOC and MBC were associated to enhancing the stabilization and activity of microorganisms and protection of extracellular enzymes such as β-glucosidase under conservative tillage systems (Gajda et al., 2013).

Table 1. Pearson's correlation coefficients for soil organic carbon, microbial biomass carbon and β-glucosidase enzyme activity

	SOC	MBC	BGA
SOC	1.00		
MBC	0.37*	1.00	
GEA	0.43**	0.80**	1.00

SOC: Soil organic carbon, MBC: Microbial biomass carbon, BGA: β-glucosidase enzyme activity.
* Correlation is significant at $p<0.05$, ** Correlation is significant at $p<0.01$

CONCLUSIONS

Nine years of conservative tillage practices, reduced and no-till improved soil organic carbon sequestration potential, β-glucosidase activity and resulted in significant SOC accumulations in soil surface compared to conventional tillage practices which have been widely used by the farmers in eastern Mediterranean region of Turkey. The amount of C sequestered was decreased by the increase in tillage intensity. The no-till system led to approximately 10 Mg C ha^{-1} higher soil C sequestration compared to conventional tillage practices.

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THE EFFECTS OF DIFFERENT ZN DOSES AND MYCORRHIZAE APPLICATION ON HORSE BEAN GROWTH AND NUTRIENT UPTAKE UNDER STERILE AND NON STERILE SOIL CONDITIONS

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Abstract

Due to semi-arid Mediterranean climate conditions and soil properties, south east Turkey's soils usually do not contain sufficient amount of plant nutritious elements, especially phosphorous (P) and zinc (Zn). This scarcity conditions is one of the factors limiting agriculture productivity and fertility and consequently, decrease the crop production. Mycorrhiza can be able to increase soil fertility and enhance nutrient uptake and plant growth. The aim of this study was to investigate the effects of mycorrhizal inoculation and different Zn doses on horse bean (Vicia faba L.) growth and nutrient uptake under sterile and non-sterile soils conditions. The experiment was carried out under greenhouse conditions with three replications. Horse bean was used as a test plant and Funneliformis mosseae was used as mycorrhiza species (1000 spore plant^{-1}). Sterile and non-sterile Karaburun soils series from Çukurova region were used. Two doses of Zn (0 and 5 mg kg^{-1}) were used as fertilizer.
In both sterilized, non-sterilized soils mycorrhizal inoculation increased shoot dry matter and nutrient concentration. The results shown that shoot dry matter and nutrient concentration significantly increased of horse bean plants in non-sterilized soils. Maximum value of % P and Zn concentration was determined and in non-sterilized soils mycorrhiza inoculated have 0.28 % P (0 mg kg^{-1}Zn) and in mycorrhiza inoculated sterile soils, 52.1 mg kg^{-1}Zn (5 mg kg^{-1}Zn). The results are encouraging that the mycorrhiza can be used as an organic fertilizer for plant production under large arable conditions.

***Key words**: mycorrhizae, horse bean, zinc, phosphorus.*

INTRODUCTION

Middle East-originated horse bean (*Vicia faba* L.) is among the earliest cultured legumes after chickpea and pea. Horse bean is commonly grown for human nutrition, but it is also used as animal feed (Prolea, 2014). Turkey, especially the Mediterranean Region, has loamy soils with high clay and low organic matter contents because of climate zone and geographical location. Undesired physical, chemical and biological soil characteristics also reduce available plant nutrient concentrations. Soils of Çukurova Region (Eastern Mediterranean Region) with intensive agricultural practices experience nutrient deficiency place to place mostly based on soil parent material and especially phosphorus (P) and zinc (Zn) deficiencies are the common cases in these soils (Ibrikci, 1994).

Mycorrhizal symbiosis between plants and fungi has been one of the most well-known plant-fungus associations since about 450 million years and is of significant importance for growth and persistence of terrestrial plants (Redecker et al., 2000). Obligate symbiotic arbuscular mycorrhizal fungi (AMF) belongs to endomycorrhiza group of *Glomeromycota* family and the fungi is in symbiotic association with about 80% of the plant species (Smith and Read, 2008). Horse bean is also among these plant species which is significantly mycorrhizal dependent (Ortas, 2012a). Turkey has also grown horse bean which can form associations with mycorrhizal fungi (Kaschuk et al., 2010). Plant roots are not the only dominant factors in plant nutrient uptake, but there are some other factors effective in nutrient uptake processes of the plants. It was reported in previous studies that hyphae-producing fungi species, also called as mycorrhiza, which can be seen under microscope and have symbiotic associations with plant roots, may also provide significant supports in plant nutrient uptake from the soils (Ryan et al., 2008). Arbuscular mycorrhiza fungi (AMF) aid in enlargement of root surface area in rhizosphere area and alter rhizosphere pH, thus increase P uptake of plants from the soils (Ortas and Rowell, 2004; Ortas and Akpinar, 2011). In case of an efficient infection between the mycorrhiza and the plants, plants give 5-10% of the carbon to mycorrhiza fungi and in return mycorrhiza facilitates plant nutrient flow (Hodgeand and Storer, 2015) and water uptake (Augé, 2001). It has been found that mycorrhizal inoculation increased plant especially P, Zn and Cu concentrations when effective root infection occurs (Smith and Read, 2008). Besides plant growth, AMF has several positive impacts on soil quality (Celik et al., 2011).

Zn uptake mechanism of mycorrhiza is similar with the phosphorus uptake mechanism and supplies about 60% of the Zn provided to plants through mycorrhizal hyphae from outside the root region (rhizosphere) (Ortas et al., 2011). Arbuscular mycorrhiza not only facilities P uptake of the host plant, but also significantly increase Zn uptake of the plants (Ortas and Akpinar, 2011; Ortas, 2012b). Therefore, existence of mycorrhiza fungi hyphae is a quite significant issue for the uptake of slowly available plant nutrients like Zn. According to Thompson (1990), non-existence of Zn deficiency of the plant species grown in soils with low DTPA-extractable Zn concentrations mostly resulted from Zn supplied from the soils through AM.

Zn is a quite significant microelement for human health. Zn deficiency is experienced about 60% of Turkish soils (Eyüpoğlu et al., 1996), therefore mycorrhiza utilization in agricultural soils is a significant issue to prevent Zn deficiency. Mycorrhiza utilization may have a strategic importance especially in soils of inner semi-arid regions. This study was conducted to investigate the effects of different Zn doses and mycorrhiza inoculations on nutrient uptake and plant growth of horse beans grown in sterilized and unsterilized soils poor in plant nutrients.

MATERIALS AND METHODS

The experiment was conducted on Karaburun soil series (*Typic Xerorthent*) which is nutrient deficient (Table 1). Soil used in the investigation which is silt clay loam, slightly alkaline (pH 7.4), calcareous (23.2%), phosphorus content low (26.0 $kg \cdot ha^{-1}$), and potassium content is sufficient (630 $kg \cdot ha^{-1}$). The half of the soil was sterilized by using an autoclave at 120°C for two hours. Two levels of Zn, 0 and 5 $mg \cdot kg^{-1}$ soil, as zinc sulfate ($ZnSO_4.7H_2O$) were applied.

Plants were inoculated with the AMF *Funneliformis mosseae*. A level of 1000-spore per pot was placed 3 cm below the seeds. The non-inoculated pots received the same amount of mycorrhizal spore-free inoculum.

Horse bean (*Vicia faba* L.) was used as a test plant. In the study, plastic pots with a capacity of 3 kg were used and 5 seeds were planted at the beginning of each pot. After germination, they were reduced to 3 plants. Distilled water was added daily to maintain the moisture at 75% of field capacity.

At the harvest of each pot, total plant biomass (dry weight of root and shoot) were recorded. Plant materials, shoots and roots were washed thoroughly with distilled. Then plants were oven-dried at 65°C for 48 h. The dry material was ground using a Tema mill, and 0.2 g of the ground plant material was ashed at 550°C, then dissolved in 3.3% HCl. The concentration of phosphorus was determined according to Murphy and Riley (1962). An ICP-spectrophotometer (ICP-OES; Thermo ICAP7000) was employed to determine the concentration of K, Zn and Fe in the plant samples. The root staining procedure described by Koske and Gemma (1989). The percentage of AMF colonization was identified by method of Giovanetti and Mosse (1980).

Table 1. Some physical and chemical properties of Karaburun series soils used in experiments

Soil Depth	P_2O_5	K_2O	Fe	Zn	Cu	Mn	pH	$CaCO_3$	Texture
	$kg \cdot ha^{-1}$		$mg \cdot kg^{-1}$					%	
(0-30)	26.0	630.0	3.02	0.38	0.57	3.21	7.41	23.2	SiCL

Mycorrhizal Dependency (MD)
After harvest, the total dry weight of the seedlings was recorded and inoculation effectiveness of the seedlings by AMF was calculated using the following formula by Ortas (2012a):

$$(MD) = \frac{TDW(+M) - TDW(-M)}{TDW(+M)} \times 100$$

TDW= total dry weight; +M, inoculated plant; -M, non-inoculated plant

Statistical Analysis
All statistical analyses (Tukey test and correlation) were performed using the SPSS 22.0 for Windows computer program.

RESULTS AND DISCUSSIONS

Effects of different Zn doses and mycorrhiza inoculation on shoot (SDW) and root dry weight (RDW) production of horse bean were investigated and results are presented in Table 2. Present findings revealed that mycorrhiza inoculations significantly increased both shoot and root dry matter production. While shoot dry matter production was 7.62 g pot^{-1} and 7.44 g pot^{-1} in unsterilized and sterilized control treatment respectively (Table 2). With 5 mg kg^{-1}Zn and mycorrhiza treatments, SDW was 8.36 g pot^{-1} under sterilized and 8.08 g pot^{-1} under the sterilized soil conditions. The greatest shoot dry matter production was observed as 9.18 g pot^{-1} in mycorrhiza-inoculated and non-Zn-treated sterilized control soil. Similar findings were also observed in root dry matter productions. Mycorrhiza had positive impacts on plant growth and development under both sterilized and unsterilized soil conditions. Considering the general averages, it was observed that the greatest shoot dry matter production of horse bean plants in unsterilized soils was 8.21 g pot^{-1}, the greatest root dry matter production was observed as 7.63 g pot^{-1} in sterilized soils. Similarly, Akpinar (2011) also reported that AM inoculations significantly increased dry matter production of horse bean plants. Also Ortas (2012a) showed that during 1997 to 1999 mycorrhizal inoculation significantly increased the horse bean yield under field conditions. Following the soil sterilization, since the other microorganisms, existing in soil and competing with plant roots for nutrients, were removed from the soil, mycorrhiza fungi had much more impacts on plant growth and development. Indigenous mycorrhiza also exists in unsterilized soils to some extend and thus had positive impacts on plant growth and development also in unsterilized soils (Ortas, 2003).

Table 2. Effects of different zinc doses and mycorrhiza inoculation on shoot and root dry matter production of horse bean plants grown in sterilized and unsterilized soils

			Shoot Dry Weight (g pot^{-1})	Root Dry Weight (g pot^{-1})
(-) Sterile	**(–M)**	**(0 mg kg^{-1}Zn)**	7.62 $\pm0.5^{b}$	6.94 $\pm0.0^{ab}$
		(5 mg kg^{-1}Zn)	7.68 $\pm0.1^{ab}$	5.71 $\pm0.6^{b}$
	(+M)	**(0 mg kg^{-1}Zn)**	9.18 $\pm0.9^{a}$	7.61 $\pm0.2^{ab}$
		(5 mg kg^{-1}Zn)	8.36 $\pm0.0^{ab}$	9.56 $\pm1.3^{ab}$
		General Average	8.21^{A}	7.45^{A}
(+) Sterile	**(–M)**	**(0 mg kg^{-1}Zn)**	7.44 $\pm0.2^{b}$	7.36 $\pm0.1^{ab}$
		(5 mg kg^{-1}Zn)	7.81 $\pm0.4^{ab}$	6.27 $\pm0.1^{ab}$
	(+M)	**(0 mg kg^{-1}Zn)**	8.69 $\pm0.0^{ab}$	9.88 $\pm2.5^{ab}$
		(5 mg kg^{-1}Zn)	8.08 $\pm0.1^{ab}$	7.02 $\pm0.1^{a}$
		General Average	8.00^{B}	7.63^{A}

± Standard error, $P<0.05$

The greatest root infection was observed as 65% in 5 mg kg^{-1} Zn-treated and mycorrhiza-inoculated sterilized soils (Table 3). The reason of that high infection rate was considered as absence of other organisms to compete with mycorrhiza. The mycorrhizal inoculum increased the root colonization of horse bean plants compared with the non-inoculated treatments (Ortas, 2012a). Similar with the present findings, previous researchers also reported that Zn fertilization did not influence mycorrhiza infections (Ortas et al., 2002; Subramanian et al., 2009).

Mycorrhizae dependency (MD) was calculated after harvest. As can be seen in the table, MD increased with non-sterile soil. At non sterile soil was 19.31 %, and sterile soil was 13.50 % as calculated (Table 3). This means that mycorrhiza inoculation at the sterile soil was not sufficient. Also, mycorrhizal dependency was found higher in Zn applications as 25.29 %. Ortas (2012a) found that horse bean showed the highest mycorrhizal dependency in all 3 years, especially in P0 plots under field conditions. Tawaraya (2003) reported that mean MD values of 44% for field crops (37 species).

When the present findings were assessed for root infection and mycorrhizal dependence, root infections were observed in control treatments of sterilized soils even at quite low levels because of the existence of indigenous mycorrhiza. Since non-sterile soils have indigenous mycorrhizae which may significantly increase plant growth. As can be seen in Table 2, there is small differences in dry weight in between sterile and non-sterile soil treatments.

Table 3. Effects of different zinc doses and mycorrhiza inoculation on root infection and mycorrhizal dependence of horse bean plants grown in sterilized and unsterilized soils

			Root Infection %	Mycorrhizal Dependency %
		(0 mg kg^{-1}Zn)	10 ±0,0^{c}	
(-) Sterile	**(–M)**	**(5 mg kg^{-1}Zn)**	5 ±7.1^{c}	
		(0 mg kg^{-1}Zn)	15 ±7.1^{c}	13.33
	(+M)	**(5 mg kg^{-1}Zn)**	15 ±7.1^{c}	25.29
		General Average	11.3^{B}	19.31
		(0 mg kg^{-1}Zn)	0 ±0.0^{c}	
(+) Sterile	**(–M)**	**(5 mg kg^{-1}Zn)**	0 ±0.0^{c}	
		(0 mg kg^{-1}Zn)	40 ±0.0^{b}	20.30
	(+M)	**(5 mg kg^{-1}Zn)**	65 ±7.1^{a}	6.71
		General Average	26.3^{A}	13.50

± Standard error, $P<0.05$

When the present findings were assessed for plant nutrients, it was observed that while the lowest P concentration was 0.13% in 5 mg kg^{-1} Zn-treated and mycorrhiza-inoculated sterile soils and the greatest P concentration was 0.28% in mycorrhiza-inoculated unsterilized soils (Table 4). Although the differences between sterilized and unsterilized soils were not found to be significant, P concentrations generally increased with mycorrhiza inoculations as compared to control treatments. It was reported that mycorrhiza fungi significantly increased the uptake of slowly available nutrients, especially the phosphorus under controlled conditions (Grant et al., 2005; Ortas and Akpinar, 2006; Ortas, 2012b). Similar with P concentrations, plant tissue potassium (K) concentrations also increased with mycorrhiza inoculations. With regard to Fe concentrations, the greatest value was observed as 308.4 mg kg^{-1} in 5 mg kg^{-1}Zn-treated and mycorrhiza-inoculated unsterilized soils. The lowest Zn concentration (26.7 mg kg^{-1}Zn) was observed in sterilized and non-mycorrhiza-inoculated control treatments and the greatest Zn concentration (52.1 mg kg^{-1}Zn) was observed in 5 mg kg^{-1}Zn-treated and mycorrhiza-inoculated sterilized soils. Previous researchers also indicated that mycorrhiza treatments increased uptake of immobile nutrients, especially Zn, at acceptable levels (Ortaş and Akpınar, 2006; Smith and Read, 2008). In another study, *Fu. mosseae* inoculations increased nutrient uptake levels of horse bean plants (El-Sayad et al., 2002). Ortas (2012a) found that mycorrhizal inoculation significantly increased the horse bean P and Zn concentrations in three years under field conditions. With regard to general averages, the plants grown in unsterilized soils had higher nutrient concentrations than the plants grown in sterilized soils.

Table 4. Effects of different zinc doses and mycorrhiza inoculation on P, K, Fe and Zn concentration of horse bean plants grown in sterilized and unsterilized soils

			P	K	Fe	Zn
			%		mg kg^{-1}	
(-) Sterile	(–M)	(0 mg kg^{-1}Zn)	0.17 ±0.1^{a}	2.5 ±0.4^{a}	147.3 ±16.5^{bc}	36.0 ±0.0^{cd}
		(5 mg kg^{-1}Zn)	0.22 ±0.0^{a}	3.1 ±0.0^{a}	292.8 ±4.2^{a}	35.7 ±2.2^{cd}
	(+M)	(0 mg kg^{-1}Zn)	0.28 ±0.1^{a}	3.3 ±0.4^{a}	307.8 ±12.7^{a}	46.7 ±3.3^{ab}
		(5 mg kg^{-1}Zn)	0.23 ±0.1^{a}	3.9 ±0.9^{a}	308.4 ±62.6^{a}	41.0 ±3.0^{ac}
		General Average	0.23^{A}	3.20^{A}	264.1^{A}	39.9^{A}
(+) Sterile	(–M)	(0 mg kg^{-1}Zn)	0.15 ±0.0^{a}	2.6 ±0.2^{a}	153.7 ±57.7^{bc}	26.7 ±1.2^{d}
		(5 mg kg^{-1}Zn)	0.13 ±0.0^{a}	3.3 ±0.3^{a}	255.3 ±6.7^{ab}	33.5 ±1.5^{cd}
	(+M)	(0 mg kg^{-1}Zn)	0.19 ±0.0^{a}	3.1 ±0.3^{a}	254.2 ±18.6^{ab}	41.9 ±3.6^{a-c}
		(5 mg kg^{-1}Zn)	0.25 ±0.0^{a}	3.7 ±0.1^{a}	118.9 ±1.6^{c}	52.1 ±4.7^{a}
		General Average	0.18^{B}	3.18^{A}	195.6^{B}	38.6^{A}

± Standard error, P<0.05

The correlation analysis among the traits evaluated in this study is given in Table 5. As seen from the table, a positive relationship was found between shoot dry weight, P and Zn also between root dry weight and mycorrhizal dependency. Previous studies have found similar correlations between certain plant developmental parameters and mycorrhizal dependency (Azcon and Ocampo, 1981; Chalk et al., 2006). In the present study, Zn concentration was positively correlated with shoot dry weight, P, K concentration, root infection and negatively correlated with mycorrhizal dependency (Table 5).

Table 5. Correlation values of parameters evaluated in the study

Parameters	SDW	RDW	P	K	Fe	Zn	Inf.	MD
SDW	1	.430	.516*	.293	.474	.641**	.350	.098
RDW		1	.284	.409	.272	.277	.270	.717*
P			1	.542*	.333	.657**	.379	-.296
K				1	.431	.584*	.340	.074
Fe					1	.076	-.357	.716*
Zn						1	.764**	-.842**
Infection							1	-.650
MD								1

*: P <0.1, **: P < 0.05
SDW: Shoot Dry Weight; RDW: Root Dry Weight; P: Phosphorus; K: Potassium; Fe: Iron, Zn: Zinc; Inf: Root Infection; MD: Mycorrhizal Dependency

CONCLUSION

Present findings revealed that AMF, a beneficial fungi species and already existing in soils, had positive impacts on growth, development and nutrient uptake of horse bean plants. Such findings clearly indicated that mycorrhiza inoculations could play significant roles in growth and development of horse bean and similar plants grown especially in soils poor in plant nutrients. Also the results are shown that indigenous mycorrhizae have significant effect on plat growth and nutrient uptake as well. In this sense, agricultural strategies should be developed for naturally existing mycorrhiza in soils and thus for sustainable agricultural practices. Use of such natural rhizosphere mechanisms in agricultural practices will provide significant contributions to both the ecosystem and the economy of the countries largely dependent on foreign countries for chemical fertilizers. For sustainable agriculture, use of mycorrhiza as a biotical fertilizer will also provide significant contributions in reducing chemical fertilizer-induced environmental problems. Use of mycorrhiza in agricultural practices could also be recommended for healthy nutrition, food safety and quality.

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CHISEL PLOW TILLAGE DEPTH EFFECT ON SOIL CARBON DIOXIDE EMISSION

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Abstract

The aim of this study is to determine the effect of different soil tillage depth using chisel plow on soil CO_2 emission and some soil physical properties. The experiment was carried out using chisel plow at three depths of 15 (A), 25 (B), 35 (C) cm and control (D) treatment and three replications.
According to the obtained results, carbon dioxide emissions are determined for A, B, C and D treatment as: 0.148, 0.172, 0221 and 0.165 g m^{-2} h^{-1} respectively. The highest carbon dioxide emissions were obtained for C treatment and it is statistically significant ($p \leq 0.01$). Soil bulk density for A, B, C and D treatment are 1.33, 1.32, 1.24, and 1.39 g cm^{-3} respectively. The differences between soil bulk density, also porosity between treatments were not significant in statistical considerations ($p \leq 0.01$). Soil penetration resistance for A, B, C and D treatment found as 1.13, 1.12, 1.1, and 1.19 MPa respectively. The soil particle size also increased as the soil tillage depth increased. Soil evaporation for A, B, C, and D treatment found to be 4.51, 5.27, 5.76 and 5.26 g m^{-2} h^{-1} respectively.

***Key words**: chisel plow, soil carbon dioxide emission, tillage depth.*

INTRODUCTION

Soil contributes in global warming by producing main greenhouse gases like (CO_2, CH_4 and N_2O) and emitting these gases to the atmosphere (Batjes, 1996). In term of CO_2 emissions related to land use, soil management such as tillage and fertilization affect carbon build up in soil or the amount of atmospheric CO_2 (Nyakatawa et al., 2012). Soil mixing intensity has an effect on the amount of carbon (C) that disappears from the soil in the form of CO_2. Also, increasing aeration in the soil usually increases soil CO_2 emissions due to decomposition (Dao, 1998). In another hand, zero-tillage system (which considered alternative to conventional or reduced tillage) may enhance soil carbon in shallow soil surface but not in deeper layers (Luo et al., 2010). Furthermore, deep tillage improves soil physical properties and increases crops productivity (Qin et al., 2008; Sornpoon and Jayasuriya, 2013; Cai et al., 2014; Guan et al., 2014), as well, conventional tillage is reducing soil compaction more than no-tillage (Ferreras et al., 2000). Using tillage implements in minimal can reduce soil CO_2 emission by reducing the volume of disturbed soil. Also, reducing tillage depth will reduce influenced vertical soil section. This will reduce microbial potential to generate CO_2 when consuming soil organic matter (Beare et al., 1994), and will not increase exposing of soil surface to exchange more gases with atmosphere.

Soil also emits water vapor, which considered important greenhouse gas. Similar to CO_2, tillage had the ability to mitigate soil H_2O evaporation, by reducing soil compaction, which means more root expansion and utilization of soil water instead of transformation to vapor, by improving soil aggregates to holding water, and by increasing water to penetrate deeper in soil.

Most of researches that were conducted to investigate the effects of soil tillage on CO_2 emissions referred to the fact that the differing in tillage methods, systems or intensively will raise up CO_2 emissions. Researches supports this theory; La Scala et al. (2001) by comparing different tillage systems with non-tilled and Akbolat et al. (2009) by studying the effect of intensive tillage on soil CO_2, all found that the no tillage emit less than any used tillage systems in their experiments. But some other studies like Tóth et al. (2009) reported that ploughing will decrease CO_2 effluxes comparing to non-tilled soil. Also, Akbolat and Kucukalbay, (2014) stated that the direct seeding lead to more CO_2 emission than chiseling.

The amount of soil CO_2 emissions depends on soil moisture and temperature regime, soil type, land usage and production method as well as the amount of soil organic carbon and even crop type (Johnson et al., 2007; Wright et al., 2007; Chianese et al., 2009; Shrestha et al., 2009; Feiziene et al., 2010). Thus, dispute in results for above mentioned studies can be attributed to previous circumstances.

Besides that, soil cover like crop residue affect soil CO_2 emissions. According to Reicosky and Lindstrom (1993) report, more CO_2 emissions occurred when the wastes were mixed with the soil than when left on the soil surface. In addition, Akbolat and Ekinci (2017) had found similar results. Another research done by Silva-Olaya et al. (2013) and reached to that among all used tillage systems the deepest tillage with subsoiler (45 cm in depth) led to more CO_2 emissions in their experiment.

In term of effect of soil tillage depths Reicosky and Archer (2007) reached to that there is a significant difference in CO_2 emission when tillage by moldboard plow at different tillage depths CO_2 emissions were increased with the increasing of tillage depth.

As it can be seen from previous researches, it was not possible to find a study of soil tillage at different tillage depths with chisel plow. Therefore, the aim of this study is to determine the effect of soil tillage depth with the chisel plow on soil carbon dioxide emission.

MATERIALS AND METHODS

This experiment was carried out in Süleyman Demirel University (37°47′N; 30°30′E), Isparta province of Turkey. The soil of study area composed of 33.9% sand, 43.8% silt, 22.3% clay, and organic matter content of C 1.7% at the depth of 0-30 cm with pH 7.87 (Karatepe 2000).

The area was planted with wheat crop and the preparations for experiment implementation began in the day after harvest which was done in 19 July 2017. The average weight of crop residue and length of stubble that covered soil surface was about 6720 kg ha^{-2} and 12 cm, respectively. After the wheat crop harvested, the trial area was irrigated by sprinklers at six hours a day for two consecutive days, then it was left to reach the suitable moisture content for tillage. Based on tillage depths, the experiment had three treatments with three replications for each treatment, thus the total of plots was 12, each plot with dimensions of 3 × 40 m distributed according to randomize block design method.

Tractor with 90 HP was used for power requirement for tilling and chisel plow consists of 5 shanks, distance between front shanks is 50 cm and 25 in rear. The tillage depth was adjusted on the chisel plow shank for each parcel before starting to tillage.

Taking of soil samples, measuring of CO_2 and penetration resistance started immediately after the soil tillage is finished. As for Mean Weight Diameter of soil (MWD), the samples were taken in the following day. Determination of CO_2 emission continued for two months started with five consecutive days. An auger was used soil sample for bulk density and soil moisture.

Soil sample cylinder "Eijkelkamp" with volume of 100 cm^3 were used to collecting the soil samples. From every plot three samples at three different soil depths of 0-10, 10-20 and 20-30 cm taken and weighed by sensitive balance. Samples placed in the oven on 105°C for 24 hrs. after that the samples were cooled off out the oven for 30 min, then, reweighted to determine soil bulk density, porosity and moisture according to (Sims et al., 1994).

Penetrologger "Eijkelkamp" was used to take records till 40 cm soil depth, used cone was 2 cm^2 (base area) with 60° top angle. Data were statistically analyzed to investigate the effect of tillage depth on soil penetration resistance.

Samples equivalent to 2.5 kg from depth 0-30 cm were taken by shovel from each plot and left for 4 weeks to dry in the laboratory then treated by sieves that had diameters of 63, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 mm and weighted by balance. Following equation was used to determine the mean weight diameters (Verhulst et al., 2013):

$$MWD = \sum_{i=1}^{n} X_i W_i \quad (1)$$

Where: MWD = mean weight diameter (mm);

Xi = (previous sieve diameter + diameter of current sieve) / 2 (mm); Wi = weight of sample in current sieve/whole sample weight (%).

PP SYSTEMS (PP Systems, Hitchin, UK) „Soil CO_2 flux system" were used in this experiment to investigate the effect of elected tillage depths on the emitted CO_2 from soil. This device consists of CO_2 CFX-2 flux chamber to measure CO_2 and temperature probe with switch in the device body to change between the soil or air measuring (Akbolat and Ark., 2009). Also, this integrated device measures water (H_2O) evaporation. The measurements were made on days of 0, 1, 2, 3, 4, 6, 9, 14, 20, 27, 32, 37, 42, 47, 56 and 63 after the tillage at the end of which soil CO_2 emission in the plots was near equilibrium. In addition, evaporation and soil temperature were concomitantly measured.

In same CO_2 measuring days, soil samples to the depth of 15 cm from every plot were taken by auger to determining the soil moisture. These samples were weighed then dried by oven on 105°C for 24 hrs. Tukey test with significance level $p \leq 0.01$ was adopted as statistical analyze method for the collected data.

RESULTS AND DISCUSSIONS

The average results obtained at the end of the study to determine the effect of soil tillage depth with chisel plow on soil carbon dioxide emissions are given in table 1.

Results taken directly after tillage referred to that there was significant effect of tillage depths on CO_2 effluxes ($p \leq 0.01$). As shown in the **Error! Reference source not found.**a, the treatment of C emitted more CO_2 than other treatments. This condition completely inverted in the second day. Carbon dioxide emission in all treatments were less than the previous day observations, furthermore, CO_2 emission in the D treatment was higher than the other treatments but the difference was not statistically significant.

Broadly, results for whole days show that the deep tillage (C) emit more CO_2 most of days after tillage followed by the control treatment (D) and even some days non-tilled emit the large quantity of CO_2 (**Error! Reference source not found.**).

These results were compared with weather conditions for the same period of experiment and found that the CO_2 was relatively affected by precipitation. When the soil humidity was decreased the emissions from all tilled soil was decreased too, nevertheless, the non-tilled soil seemed not affected too much comparing to the other treatments (Bowden et al., 1998) say CO_2 can affected by soil moisture and temperature, according to their laboratory study forest soil CO_2 efflux was less in the drier soil. Soil moisture may have affected by atmospheric relative humidity, the researches refer to a complex relationship between humidity, temperature, respiration and even clouds (Reicosky and Archer, 2007). The effect of rainfall was greater than the soil temperature on the CO_2 emissions for all treatments.

Rainfall prompted the soil to emit more CO_2 in all treatment, this effect is especially apparent on the 27^{th} and 37^{th} days after tillage.

Soil CO_2 efflux in D treatment seemed to be almost constant before the day 27^{th} after tillage because of the undisturbed soil or the presence of straw on the soil surface, but it was greatly increased after this date due to the precipitation after the day 20^{th} after the tillage. This condition is similar to (Akbolal and Ekinci, 2017) study that indicates no-till soil surface with straw emit less CO_2 than no-till with bare soil.

Soil carbon dioxide emissions in all treatments reached a minimum level on the 63^{th} day after the tillage. For this reason, data recording has been finished this date.

First day measurement (zero day after tillage) showed that the soil water evaporation behaved like CO_2 emissions when the deep tilth led to more evaporation and non-tilled soil released less H_2O, the different here just the B treatment released more H_2O than the C treatment (Figure 1b).

By illustrating the soil moisture as a chart (considering that the soil moisture is the accountable factor for the water evaporation) and comparing it with this result will be clearly that there is a contradiction between soil moisture and H_2O evaporation regarding to the treatment of C and D. Maybe if the rest of soil properties like available water and field capacity took in account this confusion could be answered since soil moisture was taken up to the depth of 30 cm.

Table 1. The average results obtained at the end of the study

Treatment	Soil CO_2 Emission ($g\ m^{-2}\ h^{-1}$)	Soil H_2O Emission ($g\ m^{-2}\ h^{-1}$)	Soil Temperature (^oC)	Soil Moisture (%)
A	0.148^{b}	4.51^{b}	31.8^{b}	16.5^{ab}
B	0.172^{b}	5.27^{ab}	32.3^{ab}	17.3^{ab}
C	0.221^{a}	5.76^{a}	32.0^{ab}	18.3^{a}
D	0.165^{b}	5.26^{ab}	33.1^{a}	15.5^{b}

Means have the same letter at the same column are not significantly different from each other ($P \leq 0.01$).

In the second day (first day after tillage) the H_2O evaporation for all tilled treatments was decreased comparing with the zero day. The reason of this decreasing can be attributed to the same factors that effected the CO_2 emissions. As shown in Figure 1b, the emitted H_2O from non-tilled soil was orderly increased as a regular raised line till the 5th day of the experiment then sharply went down by 49% this may due to the slight decrease in soil and atmospheric temperatures and moisture as shown in Figures 1c and 1d. The rainfall had affective role on increasing of water evaporation (H_2O emission) for all treatments as shown in Figure 1b. Some rainy days (for example 27th and 37th day after tillage) raised the water evaporation more than the day when the tillage was performed. According to the analyzing of collected data after two months of the experiment regarding to soil water evaporation there was a significant difference between all the treatments at $p \leq 0.01$ (Table 1). The deep tillage which presented by C treatment led to more water evaporation than non-tilled (D) and shallow tillage (A, B).

The relationship between soil H_2O evaporation, temperature and CO_2 shown in Table 2. According to this table there is a positive but weak correlation between H_2O evaporation and soil temperature, also there is a positive correlation between H_2O and CO_2 $p \leq 0.01$.

The result in this experiment regarding to correlation between CO_2 and temperature is not conflict with result from (Qi and Ming Xu, 2001).

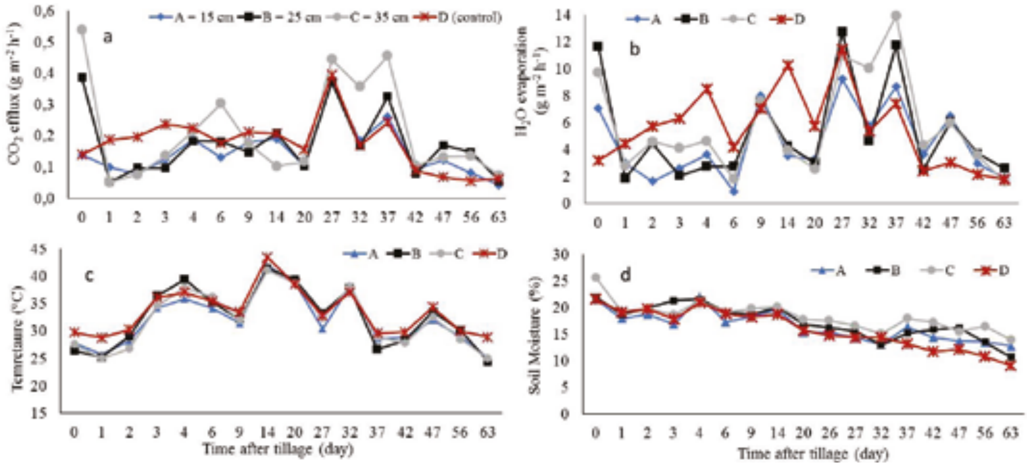

Figure 1. Results of CO_2 (a), H_2O (b), soil temperature (c), and soil moisture (d) depending on time after the tillage

Table 1. Correlation between soil CO_2, temperature and water evaporation

	CO_2	T	H_2O
CO2		0.022	0.481**
H2O	0.481**	0.066**	

** Correlation is significant at the 0.01 level.

The non-tilled soil (table1 and figure1c) was significantly warmer than the tilled soils $p \leq 0.01$.
The results of first day show that the treatment C had more soil moisture than other treatments (Table 1). All tilled soils kept the moisture more than non-tilled plots. the soil nematode values in all subjects declined gradually until the end of the experiment but were not affected by rainfall (Figure 1d.). Treatment of C in terms of soil moisture differed only from D treatment (Table 1).
As average in depth from 0-30 cm, bulk density and porosity had no significant differences between all of the treatments $p\leq 0.01$ (Table 3) Also, there were no differences between the treatments found in the same soil layer. Treatment of C in all soil layers had the lowest bulk density and highest porosity, even so, not considered statistically different $p\leq 0.01$. In the third soil layer (20-30 cm) each the A treatment and the D treatment had higher bulk density than the other treatments. Results in first 10 cm are with agreement with (Logsdon et al., 1999) when tilled soil had less bulk density than (D), but in opposed with it regarding to other depths.
Bulk density had entirely negative correlation with porosity (r= -684), and positive correlation with the depth of soil layer (r=0.684).As the soil depth increases, the bulk density was increased.
The depth of tillage made changes to the MWD (Table 4.) comparing to the non-tilled soil.

Table 3. Mean soil bulk density and porosity

Treatment	Bulk density (g cm^{-3})	Porosity %
A	1.33	49.7
B	1.32	49.8
C	1.24	52.9
D	1.39	47.5

Means have the same letter are not significantly different ($P \leq 0.01$).

As the mentioned table show the deep tillage impact the water stable aggregates by increasing the MWD. Non-tilled and shallow tillage soils were similar in the statistical analyzing estimations $p \leq 0.01$ with respect to the MWD. (Guedes Filho et al., 2013) claimed that the negative impact of chisel will last to 18 months when tilling to the depth of 25 cm, and the enhancement in other soil physical properties will extend to three seasons after tillage (Nunes et al., 2015). The positive effects of increasing in aggregate with large size caused by deep tillage can be come across reducing soil erosion and salting (Tatarko, 2001).
Mean weight diameter in tilled soil profile was increased significantly due to the deep tillage in treatments C and B comparing with D ($P \leq 0.01$), and reduced a bit in shallow tillage (difference is not significant at $p \leq 0.01$).The soil mean weight diameters of treatments is given in table 4.

Table 4. Mean weight diameter of treatments

Treatment	MWD (mm)
C	23.68 ^{a}
B	23.66 ^{a}
D	13.85 ^{b}
A	12.96 ^{b}

Means with same letters are not different ($p \leq 0.01$).

Decreasing soil penetration resistance means increasing of water penetration to deeper soil profile also allows plants roots to expand better in soil. Cone index was decreased with the increasing of tillage depth (Table 5), but these decreasing is not statistically significant at $p \leqslant 0.01$. As said by Zou et al. (2001) relationship between bulk density and soil hardness may vary according to soil roughness. But regardless of soil coarseness, at least in this study, soil bulk density gave more perception than penetration resistance in term of soil compaction. Also, bulk density described the inverse relationship between CO_2 emissions and soil compaction better than con index and this based on studies like (Torbert and Wood, 1992; Novara et al., 2012; Chappell and Johnson, 2015) that touched upon bulk density and soil CO_2 emissions. Procedures to determine bulk density may take more time comparing with cone index process(which was done by Penetrologger "Eijkelkamp" in this study) but it is seems that bulk density or porosity is more accurate than penetration resistance by describing the effects of tillage on soil compaction and CO_2 emissions. Hence, this study is conflict with Tavares et al. (2017) opinion.

Table 5. Mean penetration resistance of treatments

Treatment	Penetration resistance (MPa)
A	1.13
B	1.12
C	1.10
D	1.19

Means with same letters are not different($p \leq 0.01$).

In each treatment, penetration resistance was increased markedly with increasing in soil profile depth (0-40 cm). However, there was no difference between penetration resistance averages between treatments.

CONCLUSIONS

According to this experiment tillage with chiseling more than 35 cm led to more CO_2 emission and causing in more soil water evaporation. Precipitation have positive effect in term of increasing CO_2 emission and H_2O evaporation when tilling at any depth. The increase in tillage depth did not change the soil bulk density and porosity. C and B increased MWD significantly, while A decreased it with no meaningful change comparing with D. The soil penetration resistance did not change with the increase in soil tillage depth. According to the research results, the depth increases in the soil tillage with chisel plow increased soil CO_2 emissions. For this reason, deep tillage should be avoided in seed bed preparation for less greenhouse gas emissions in terms of environmental impact.

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EFFECT OF DIFFERENT MAGNESIUM DOSES ON GROWTH AND YIELD OF PEPPER PLANT IN MYCORRHIZA INOCULATED HARRAN SOIL

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Abstract

The aim of the study was to determine stimulating effect of magnesium on phosphorus uptake. At the field experiment the doses of 0 (Mg0), 20 (Mg2), 40 (Mg4), 60 (Mg6), 80 (Mg8) and 100 (Mg10) kg magnesium ha^{-1} was applied to mycorrhiza inoculated soil. Throughout the experiment fruit yield values determined whereas the fruit nutrient content analysed at the end of the experiment. Magnesium application was considerably effective on pepper yield however dose was critical where the lowest dose (Mg2) is not provided any benefit. Although the highest dose is also provided higher yield compared to control, but the peak value was on Mg8. Mg fertilization increased plant N contents by the increasing application doses, but after Mg8 dose tendency was switched to negative. Phosphorus contents were not strongly influenced from Mg fertilization. Considering all parameters determined it can be said that the dose of Mg8 optimum dose on pepper production.

***Key words**: magnesium, mycorrhiza, pepper, nutrient uptake.*

INTRODUCTION

Magnesium is one of the macronutrient for the plants and it is the main constituent of the chlorophyll. Magnesium also increases plant phosphorus uptake (Rasul et al., 2011) and water use efficiency. Deliboran et al. (2011) concluded that Mg applications increased the P availability in the soil by the effect of water. Increased water improved both magnesium level and yield. Dursun et al. (2017) represent that the Mg application improved mean fruit weight and length as well as dry matter content of cucumber. Xu et al. (2014) reported that the fate of the phosphorus influenced from the initial concentration of phosphate as well as presence of Mg ions. Phosphor availability is rather limited in both low and high pH. At the high pH soil phosphate ions are precipitated by combining Ca ions.

Most proportion of the soils in Turkey has high pH due to climate regime and geographic condition (Dinc et al., 1988). For such pH soil, Mg can be used as an agent that promoting phosphorus uptake. On the other hand Gerendas and Fuhrs (2013) defined Mg as forgotten element and they want to attract the attention to the role of Mg in quality formation. They also point out to importance of Ca/Mg balance for product quality. Proving this suggestion El-Zanaty et al. (2012) recommended soil amendment instead of foliar fertilization due to it realizes soil nutrient balance.

The aim of this research was to evaluate the effects of different Mg doses on the pepper plant growth and nutrient uptake in mycorrhiza inoculated conditions.

MATERIALS AND METHODS

Experiment is carried out on Harran soil series in Kisas province at 2015. The location of implementation field is 37°06′N 38°54′E. The basic soil properties are presented in Table 1.

Table 1. Basic physical and chemical properties of experimental soil

Texture	Saturation (%)	E.C. (dS m^{-1})	pH	$CaCO_3$ (%)
C	82	1.27	7.81	24.8

P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Organic Matter (%)	Mycorrhiza spore number
49	2020	1.4	34

In an 3363 pepper seeds are sown on the viol which filled up by 1:1 peat and perlite mixture as a seedling medium. The medium was sterilized in autoclave at 121°C, two times for 1 hour each. Following the germination, the plants are transferred the holes in the field which all were inoculated by Mikostar BTH-100 mycorrhiza inoculant. Experimental design was randomized complete block with 3 replications. Parcel size was 14.56 m^2. Field was fertilized by 210 kg ha^{-1} nitrogen from ammonium sulphate (Karakus and Anlagan, 1996) and 100 kg ha^{-1} phosphorus from triple super phosphate. Magnesium doses were 0, 20, 40, 60, 80 and 100 kg ha^{-1}. The doses are labelled as Mg0, Mg2, Mg4, Mg6, Mg8 and Mg10, respectively. $MgSO_4.7H_2O$ was used as a Mg source. Phosphorus and magnesium fertilizer applied full dose at the beginning of experiment whereas nitrogen fertilization realized at two different stages of the plant. Throughout the experiment regular cultivation practices are followed. Fruits are harvested when they reach the standard fruit size and reddish colour. At the end of the experiment plant roots were taken, washed with deionized water and sub-sampled to determine mycorrhizal colonization.

Mycorrhizal colonization was done based on the staining procedure by Koske and Gemma (1989). Infection rate was determined by the grid-line intersection method (Giovannetti and Mosse, 1980).

The N concentration of the plant was determined according to Kjeldahl distillation method (Bremner, 1965). P and K analysed by Vanadomolibdophosphoric yellow colour (Kacar and Inal, 2008) and flame photometric methods, respectively. For Ca, Mg and micronutrient analyses the samples were wet digested using nitric-perchloric acid mixture and the nutrient contents of the filtrate determined by ICP-OES (Isaac and Johnson, 1998).

RESULTS AND DISCUSSIONS

The total yield values determined in the experiment are presented in Figure 1. Magnesium application found to be effective on pepper yield however the smallest amount (Mg2) did not provide any benefit and the highest dose (Mg10) provided even lower yield than control (Mg0). Among the doses Mg8 was the optimum application which the highest yield observed. Mg2 seems to be insufficient to provide enough Mg nutrition, probably due to the interactions by other nutrients.

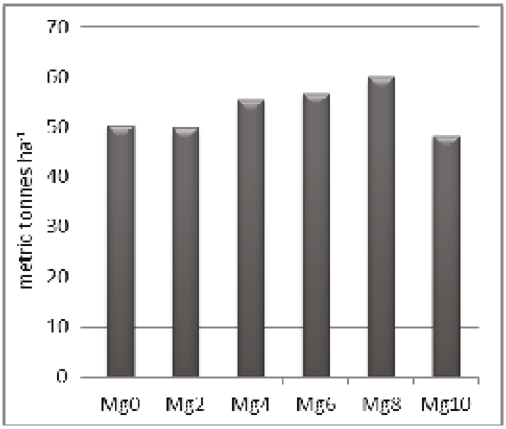

Figure 1. Yield values

Based on the plant height values (Figure 2), slight increases were determined in Mg6 and Mg8 doses, but there was no significant difference within the others. Stem diameter (Figure 2) represent similar values with plant height values; however the thickest diameter obtained from the lowest dose (Mg2). When the yield and stem diameter considered together it can be said that the plant used the photosynthate to build up strong body instead of producing fruit.

This assumption should be further synthesized by prospective studies. Fruit length (Figure 2) showed tendency that the increasing Mg doses increased length of the fruit except Mg10. In general the higher nutrient accumulation expected in case of suppressed plant growth due to the concentration effect, but in this particular case Mg10 decreases both Mg concentration and yield together.

Vitamin C contents of the pepper fruit is presented in Figure 3.

There was no tendency on vitamin C contents depending on Mg doses whereas great fluctuation was observed. The highest vitamin C content determined in Mg8 and the lowest value was in Mg2.

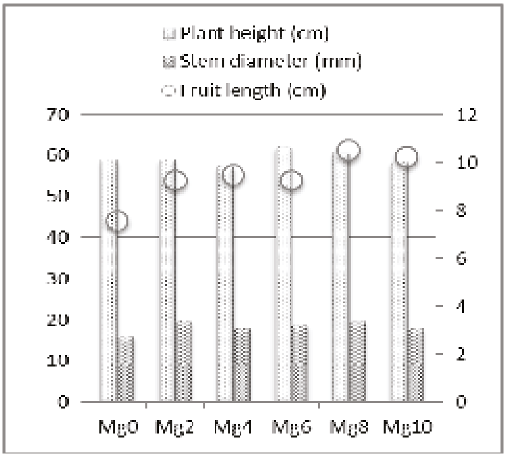

Figure 2. Plant height, stem diameter and fruit length

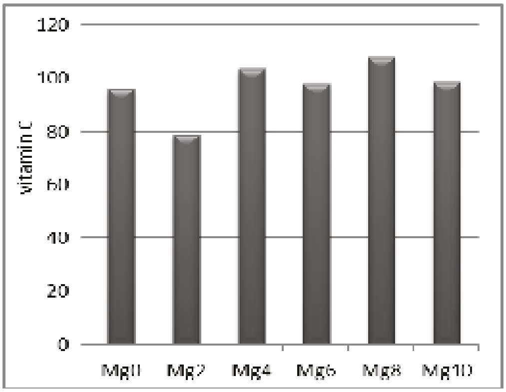

Figure 3. Vitamin C contents

The determined N, P, K, Ca and Mg concentrations are given in Table 2. Fertilization with Mg increased plant N contents by the increasing application doses, but after Mg8 dose tendency was switched to negative. Even though, the higher N contents were determined in Mg applied pots. Phosphorus contents were not strongly influenced from Mg fertilization. In some doses P content increase but for others values were lower than control. This finding is not in accordance with the hypothesis of the manuscript where Mg application is not clearly improved P uptake. However Mg8 dose provide the highest P content. The higher potassium content was also determined in Mg8 and generally all Mg doses except Mg10 stimulated the K uptake evidently. Ca and Mg concentration is not seems to be greatly influenced form Mg application. This was also not expected situation because Mg fertilization should increase plant Mg concentration in normal condition.

Table 2. N, P, K, Ca and Mg concentration (%)

	N	P	K	Ca	Mg
Mg0	3.51	0.243	0.91	4.44	0.96
Mg2	3.70	0.233	1.12	4.49	0.88
Mg4	3.76	0.257	1.77	4.57	1.05
Mg6	3.84	0.237	1.35	4.72	1.01
Mg8	4.24	0.293	1.81	4.88	1.05
Mg10	3.97	0.263	0.97	4.62	0.98

The micronutrient contents as Cu, Fe, Mn and Zn are given in Table 3. Mg8 dose is an exception; none of the Mg dose was effective on Cu content. Fe concentration is greatly influenced form Mg, the highest value was achieved in Mg6 whereas the lowest Fe was in Mg2. Zinc concentration is also influenced from Mg. The highest value determined in Mg8.

Table 3. Cu, Fe, Mn and Zn concentration (mg kg^{-1})

	Cu	Fe	Mn	Zn
Mg0	18	204	201	25
Mg2	18	182	197	21
Mg4	18	247	224	26
Mg6	17	251	216	24
Mg8	20	223	215	33
Mg10	16	232	196	23

Infection rates are presented in Figure 4. No relation was determined between Mg doses and infection rate. None of the application was improved infection over 50%.

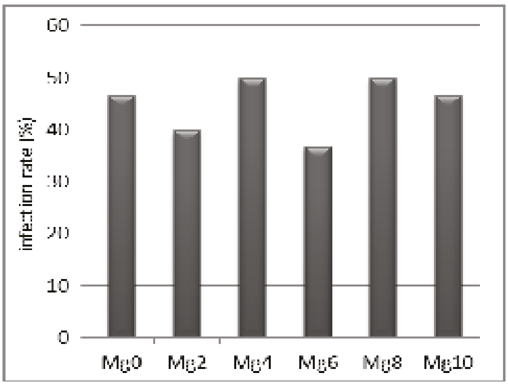

Figure 4. Infection rate

CONCLUSIONS

Based on the overall results magnesium application is effective on most of the determined parameters.

That is clearly indicated that magnesium fertilizer is required for Harran soil. But the dose seems to be critical both lower and the highest doses are not providing benefit, even the highest dose reduced yield which is predominate factor for the farmers. In this research it is evaluated that Mg application is not stimulate P uptake which is contrary to expectation that Rasul et al. (2011) reported. Thus further studies should be studied to clarify the mechanism behind this phenomenon.

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SOIL EROSION RISK MAPPING USING USLE/GIS METHODOLOGY IN ROZE-CHAY CATCHMENT, NORTHWEST IRAN

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Abstract

Soil erosion by water is a serious environmental problem all over the world, particularly in semi-arid regions like Iran, where agricultural water quality and productivity potential of soils have been decreased as a direct consequence of this process. Therefore, the development of a model assessing soil erosion potential and proportional risk maps, would help to protect soil and water resources. Among the empirical models, Universal Soil Loss Equation (USLE) is a widely accepted model developed for soil erosion prediction. The aim of this study was to assess erosion risk by integrating the USLE model and GIS techniques in Roze-Chay catchment, northwest Iran. The map of the USLE factors was produced as follow: rainfall erosivity factor (R) map was interpolated from data point taken from the meteorological stations; Soil erodibility factor (K) map was created by determining the particle size distribution and organic carbon of the 98 soil samples from the study area; Topographic factor (LS) was derived from digital elevation model (DEM); Cropping-management factor (C) was computed from NDVI values derived from Landsat-TM data. Assuming no erosion control practices in the catchment, Conservation practice factor (P) was set to be a unit, in calculations. The results showed that the annual average soil loss for the Roze-Chay catchment was 57 Mg.ha^{-1}.ya^{-1} in 2015. The erosion risk map showed that, 33.6% of the total area has a low erosion risk with annual average soil loss of less than 10 Mg.ha^{-1}.ya^{-1}, but the area of 265 km^2 (≈ 59%) in the catchment showed high to highly severe erosion risk, which should be protect by appropriate conservation practices. The high erosion risk of the catchment can be related to the topography and low levels of the vegetation cover. The developed soil erosion risk map can be used to highlight the erosion risk areas and therefore, assist the farmers and decision makers in implementing suitable conservation program to control soil erosion.

***Key words**: soil loss, Geographical information system, USLE, Roze-chay catchment.*

INTRODUCTION

Soil erosion is considered as one of the most important environmental problems leading to significant reduction of soil fertility and crop yields (Olivares et al., 2011).
In the northwest of Iran, water erosion affects negatively agricultural productivity and reduces water and soil quality (Vaezi and Bahrami, 2014).
Therefore, the assessment of erosion risk at the watershed scale can be very useful to establish conservation measures and soil and water management plans.
Universal soil loss equation (USLE) is the most widely used erosion model throughout the world to estimate average annual soil loss resulting from rill and sheet erosion (Erdoghan et al., 2007; Belasri and Lakhouili, 2016). USLE model can be integrated with geographic information systems (GIS) which allow preparing erosion risk maps in larger areas. In a GIS environment, the USLE can be applied to determine soil erosion risk quantitatively and spatially (Lufafa et al., 2003).
The aim of this study was to assess erosion risk by integrating the USLE model and GIS techniques in Roze-Chay catchment, northwest Iran

MATERIALS AND METHODS

Study area. Roze-Chay catchment is located in the northwest of Iran and approximately 10 km south of Urmia city, west Azarbayjan province. Roze-Chay catchment covers an area of 453 km^2 (Figure 1).
The climate of the region is characterized as semiarid with average annual precipitation of 313 mm.
The minimum and maximum monthly temperature are -2^{o}C and 24^{o}C, respectively.
USLE model. USLE (Wischmeier & Smith, 1978) was used to estimate annual soil loss.

USLE quantitatively estimates soil erosion with the following empirical equation (Eq. 1):

$$A = RK(LS)CP \quad (1)$$

Where: A is the average annual soil loss in (Mg ha^{-1} year^{-1}); R is the rainfall-runoff factor (MJ mm ha^{-1} hr^{-1} yr $^{-1}$); K is the soil erodibility factor (Mg h MJ^{-1} mm^{-1}); LS is the topographic factor, C is the cover-management factor; P is the supporting practice factor.

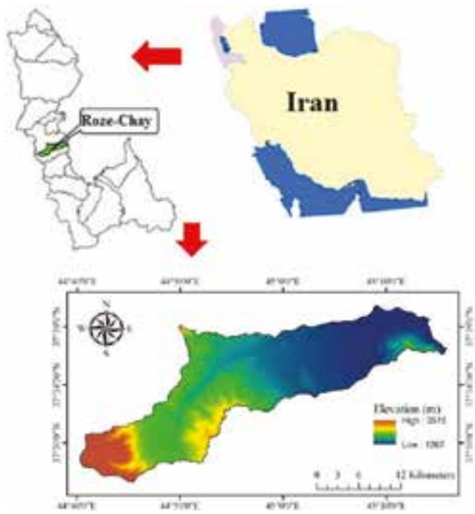

Figure1. Map of Study area

R factor. Rainfall-runoff erosivity factor is derived from the 20 years (1993-2012) precipitation records of 66 meteorological stations in the west Azarbayjan province. The annual R factor was calculated from the following empirical equation (Eq. 2), proposed by Ferro et al. (1999):

$$R = 2.7015\left[\left(\frac{1}{N}\sum_{j=1}^{N}\sum_{i=1}^{12}\frac{p_{i,j}^{2}}{P_j}\right)^{1.41}\right] \quad (2)$$

For the period of N years, $p_{i,j}$ is the rainfall depth in month i (mm) of the year j and P_j is the total rainfall for the same year.
Erosivity map of the West Azarbayjan province was prepared by kriging interpolation method and then the R map of the Roze-Chay catchment was extracted from the province map.

K factor. Total of 98 topsoil (0-15 cm) sample were collected from the catchment by stratified randomized sampling design and erodibility factor was calculated for each sampling point using the following equation (Eq. 3):

$$K_{\left(\frac{Mg\,ha\,h}{ha\,MJ\,mm}\right)} = 2.8\times10^{-7}M^{1.14}(12-a) \quad (3)$$

Where: *M=(% silt+% very fine sand)×(100-% clay)* and a is the percentage of organic matter. Kriging interpolation was used to create a continuous map of K factor from the sample points.
LS factor. Topographic factor was calculated using the digital elevation model and the equation (Eq. 4) (Moore and Burch, 1986):

$$LS = \left[\frac{A_s}{22.13}\right]^{0.4}\left[\frac{\sin\beta}{0.0896}\right]^{1.3} \quad (4)$$

$$A_s = Flow\ accumulation \times cell\ size$$

Where Flow accumulation is the number of cells contributing in a given cell, cell size is the pixel’s side, β is the slope angle in degrees.
C factor. Cover-management factor was calculated from the Landsat 7 satellite image (year 2015) through the Normalized Difference Vegetation Index (NDVI).
The following equation (Eq. 5) proposed by Van der Knijff et al. (1999) was used to prepare a C factor map:

$$C = exp\left[-2\times\frac{NDVI}{1-NDVI}\right] \quad (5)$$

P factor: supporting practice factor set to be 1 for the entire study area, assuming no support practice in the catchment.

RESULTS AND DISCUSSIONS

The maps prepared for R, K, LS, and C factors (Figures 2, 3, 4 and 5) of the USLE model were integrated using Equation (1) within the raster calculator option of the ArcGIS spatial analyst in order to quantify and provide erosion risk map for Roze-Chay catchment.

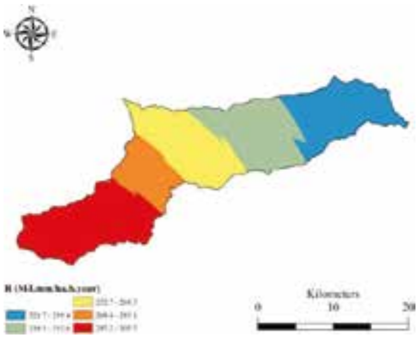

Figure 2. Rainfall-runoff erosivity factor

After the multiplication of the 4 factors, the resulting soil erosion map (Figure 6) was empirically classified into 7 classes: for soil loss < 5 Mg ha^{-1} yr^{-1} the erosion risk characterized "very low", for 5-10 Mg ha^{-1} yr^{-1} "low", for 10-20 Mg ha^{-1} yr^{-1} "moderate", for 20-40 Mg ha^{-1} yr^{-1} "high", for 4-80 Mg ha^{-1} yr^{-1} "very high", for 80-160 Mg ha^{-1} yr^{-1} "severe", and for soil loss >160 Mg ha^{-1} yr^{-1} "highly severe" erosion risk.

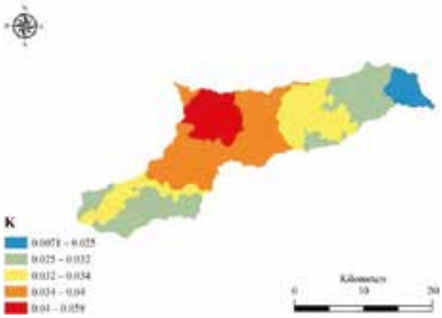

Figure 3. Soil erodibility factor

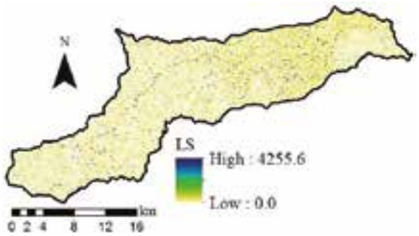

Figure 4. Topographic factor map

Soil erosion risk map showed that the annual average soil loss for the Roze-Chay catchment was 57 Mg ha^{-1} ya^{-1} in 2015.

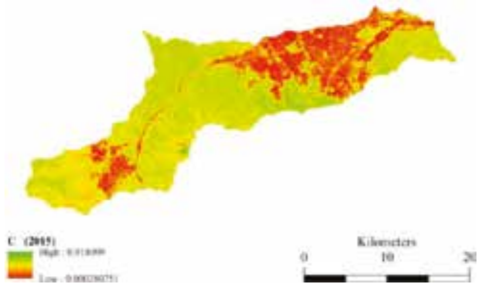

Figure 5. Cover-management factor map

The erosion risk map also showed that, 33.6% of the total area has a very low and low erosion risk with annual average soil loss of less than 10 Mg ha^{-1} ya^{-1}, but the area of 265 km^2 (≈59%) in the catchment showed high to highly severe erosion risk, which should be protected by appropriate conservation practices (Figure 7).

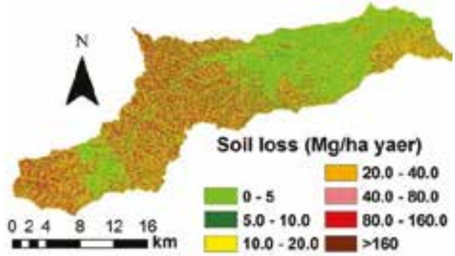

Figure 6. Erosion risk map of the Roze-Chay catchment

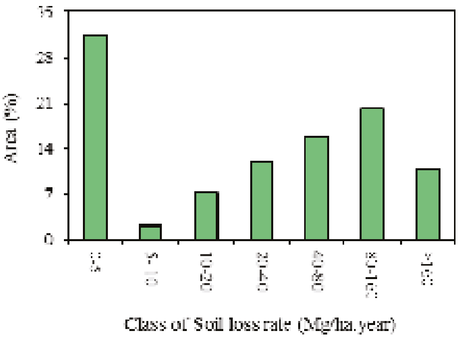

Figure 7. Percentage area of Roze-Chay catchment belonging to each soil loss class

Soil erosion map of the Rze-Chay watershed clearly indicates that the highest soil loss values are spatially correlated with the steepest slopes which shows the high sensitivity of the USLE model to the topographic factor (Moore and Wilson, 1992).

CONCLUSIONS

Erosion risk map of the Roze-Chay watershed, based on USLE model, showed that the most of the catchment has high to highly severe erosion risk, which should be protected by appropriate conservation practices. The high erosion risk of the catchment can be related to the topography and low levels of the vegetation cover. Low erosion risk areas are located at the east parts of the catchment where the topographic factor has low value due to the gentle slopes. Spatial patterns of soil loss shows a clear correlation between topography and soil loss values which may be related to the high sensitivity of USLE model to topographic factor.

Finally, the present study has confirmed that GIS techniques are low cost tools and simple for modeling and mapping soil erosion. The developed soil erosion risk map can be used to highlight the erosion risk areas and therefore, assist the farmers and decision makers in implementing suitable conservation program to control soil erosion in Roze-Chay catchment.

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EVOLUTION OF DARK CHESTNUT STEPPE SOIL UNDER CONDITIONS OF DIFFERENT USE AND CLIMATE CHANGE

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Abstract

The results of studies on the evolution of dark chestnut steppe solonetzic soil under conditions of different uses (virgin lands - from „Askania-Nova" reservation, irrigated and non-irrigated) and climate aridization are presented. It is shown that under the influence of agricultural use, in the arable and long-term irrigated soils with fresh water, the direction of soil formation processes changes. This process affects the properties of soils and the ecosystem services that it provides. The upper part of the soil profile is transformed into a qualitatively new cultivated horizon with altered properties due to soil cultivation and irrigation water action. Irrigation with fresh water promoted the desalinization of the naturally solonetzic virgin soil. The content of Na + K from the sum of the absorbed cations is reduced, from 4.3% to 3.3% (0-25 cm) and from 3.6% to 2.4% (25-50 cm). Due to agriculture use, as well as irrigation by sprinkling, there have been changes in agrophysical indicators of virgin soil. They consist of compaction, structural state deterioration, decrease in the strength of the microstructure and the water resistance of aggregates of dark chestnut steppe soil.

Key words: *agriculture use, dark chestnut soil, evolution, irrigation, soil indicators.*

INTRODUCTION

In recent decades, as a result of increasing the anthropogenic pressures on soils, unbalanced land use, increasing areas of degraded land and climate change, the most urgent and priority area of action, both internationally and nationally, is the sustainable management of soil resources for the purpose protection and balanced use of them, achieving a neutral level of degradation, adapting agriculture to arid conditions for ensuring the country's food security and sustainable development goals.

In recent years, global warming has been observed, the water availability of crops in arid zones has decreased, which necessitates the use of irrigation. Climate, as an environmental factor, affects the development of biological, chemical, physical processes, soil properties.

The introduction of methods and principles for sustainable soil management is also one of the key among the five activities of the Global Soil Partnership of the FAO. To maintain and multiply productive, ecological, biological functions of soils, to ensure the fulfillment of ecosystem services, it is necessary to comply with the scientific and methodological principles of sustainable management (Revised World Soil Charter, 2015).

MATERIALS AND METHODS

To assess the impact of agricultural use, irrigation, climate conditions on the soil formation properties of dark chestnut solonetzic soil, the systematic approach, synthesis methods, comparative analysis were used. Observations were carried out on the stationary sites with various uses - absolutely virgin soil (from Natural biosphere reservation „Askania-Nova"), irrigated (the term irrigation is about 60 years old) and non-irrigated soils. The objects of research are located in the zone of the Steppe Dry Ukraine (Kherson region). For irrigation is used the water from the Kakhovka main canal through a network of inter-farm and on-farm canals. The is represented by a dark chestnut weakly solonetzous, light loamy soil on the loess loam. The content of physical clay is 60-66% in the 0-50 cm soil layer. According to the level of ground water, the soils are characterized by automorphic conditions - more than 10 m from the surface. Irrigated and non-irrigated plots are used in the field crop rotation. The soil indicators were determined be State Standards of Ukraine.

RESULTS AND DISCUSSIONS

As a result of agricultural development of soils and their use in plowed fields, the natural phytocenoses are transformed, and the direction of soil formation processes is changing. The scale of the transformation depends on the degree of human impact on the soil. With extensive use of land, soil processes tend to have the same orientation as under natural conditions, and with the increase in the ameliorative load (under irrigation), the landscape-ecological situation, the direction and speed of elementary soil processes change, the agrogenic transformation of the composition and properties occurs in soils.

The objects of research are located in a zone with a temperate continental climate with hot dry summer (Балюк, 2016). Kherson region is characterized by the lowest values of the Selyaninov hydrothermal coefficient, which varies between 0.71 ... 0.46, thus, the climatic conditions affect the soil productive capacity. In order to increase the productivity of agricultural crops in this zone, it is necessary to develop irrigation.

The results of the studies indicate that in cultivated soils the water, air, nutrient, biological regime changes (Пухова, 2011). It should be noted that the parameters of virgin soil differ from agrozems - anthropogenically transformed soils. Irrigation enhances the spatial heterogeneity of the properties of arable soils. Morphological analysis of soil profiles of the investigated objects indicates that the upper part of the soil profile is transformed into a qualitatively new cultivated horizon with altered parameters and properties due to soil treatment and the operation of good quality irrigation water (Воротынцева, 2017).

For soils of the Dry Steppe, an important index is the composition of the soil-absorbing complex, which determines the physico-chemical, physical properties of the soil, since the dark chestnut soils are solonetzous in nature. It should be noted that as a result of the long-term influence of the factors studied, changes in the content of sodium and potassium solonetizing soils have occurred. As a result of plowing of virgin soil, its introduction into agricultural use, application of ameliorative methods, there have been changes in the orientation of soil processes and regimes, which contributed to decrease in the concentration of sodium and potassium in the 0-25 and 25-50 cm layers of dark chestnut irrigated soil in comparison with the virgin soil: from 4.7% to 4.2% (0-25 cm) and from 2.9% to 2.5% (25-50 cm) (Figure 1).

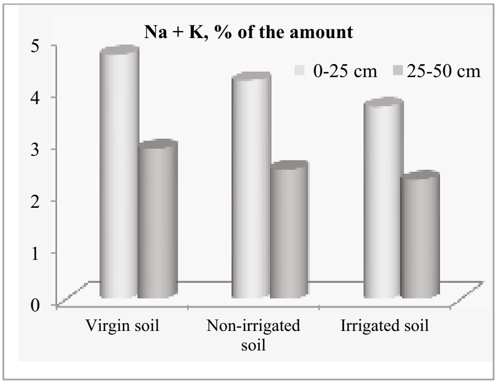

Figure 1. Changes in solonetizing of dark chestnut soil under the influence of agricultural use and irrigation

By the years of research, the dynamics of the absorbed cations content is noted. In irrigated soil under the action of fresh water and the improvement of the water regime, take place the desalinizes of the initial naturally solonetzic soil. According to the results of research conducted in 2016, the content of Na + K from the sum of absorbed cations decreased from 4.7% to 3.7% (0-25 cm soil layer) and from 2.9% to 2.3% (25-50 cm soil layer), which contributed to the improvement of physical and chemical properties of the soil.

In the absorbed cations of the studied soils, the calcium predominates, the content of which in the virgin soil in the 0-50 cm layer is 70-77% of the sum of all cations. As a result of changes in the water, air, and biological conditions of the dark chestnut soil (as a result of agricultural use), quantitative changes occurred in the cation composition of the absorbed cations: in non-irrigated soil, the content of absorbed calcium decreased to 60-70%, and under irrigation conditions - up to 60-66%. But at the same time, the concentration of absorbed magnesium increased from 23-30% in the virgin soil to 28-36% in arable soil.

Therefore, we can assume that the absorbed magnesium influences the solonetzization of the soil, determines the morphological features and agrophysical indices (clumpiness and compaction).

Long-term mechanical tillage of soil is the most significant factor that can cause negative, stable changes in agrophysical indicators and physical condition of soils, which leads to the development of degradation processes. The bulk density indicator of virgin soil differ from agrozemes (Figure 2).

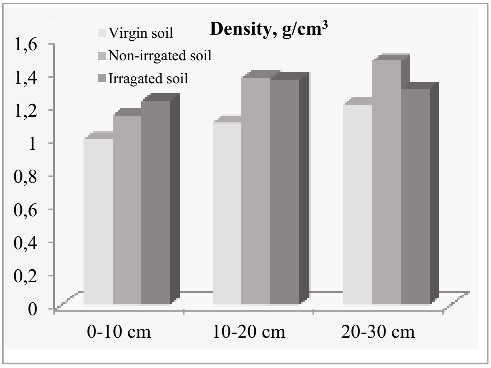

Figure 2. Density of dark chestnut soil for different uses

The results of investigations showed that in virgin dark chestnut soil the equilibrium density in the upper 0-10 cm layer was 1.00 g/cm^3, and with depth increased to 1.22 g/cm^3 (20-30 cm) and 1.37 g/cm^3 (30-40 cm). As a result of prolonged agricultural use of dark chestnut soil in the 0-10-cm layer, this index significantly increases to 1.14 g/cm^3 (non-irrigated soil) and 1.23 g/cm^3 (irrigated soil) - the smallest significant difference (SSD) - 0.08. In arable soils at a depth of 10-30 cm, the plow outsole is formed due to the different depths of tillage, and the density increases significantly to 1.30-1.47 (SSD for 10-20 cm soil layer - 0.08; for 20-30 cm - 0.14).

The bulk density of soils increases with a decrease in the amount of humus. In the upper elluvial layer of virgin soil, the humus content varied within 5.0-5.5% (0-25 cm) and in the upper layer of arable soil it decreased to 2.9-3.2% (0-25 cm), which is associated with a change in the functional structure of the microbial cenosis, type of vegetation, and physical and chemical properties of soil.

The introduction of soils into agricultural use contributed to the deterioration of the structural state of dark chestnut soil - a decrease in the strength of the microstructure and the water resistance of aggregates. Under the influence of irrigation and agricultural use, the structural coefficient decreases. So, in virgin soil, this index in 0-10 cm and 15-25 cm layers was 2.4-2.5, and in irrigated fell to 1.2-1.4, which indicates the deterioration of soil structure as a result of destruction and decreasing the number of agronomically valuable aggregates with a size of 0.25-10 mm and increasing the amount of lumpy fraction (larger than 10 mm) (Воротынцева, 2017).

The results of investigations of the nutrient regime of dark chestnut soils of various uses have shown that there are differences in the content of mobile forms of nitrogen, phosphorus and potassium in virgin soil and arable soils. According to the content of mineral nitrogen, virgin soil is characterized by a high degree of availability, non-irrigated - low, irrigated - medium, according to State Standards of Ukraine DSTU 4362 (Figures 3, 4).

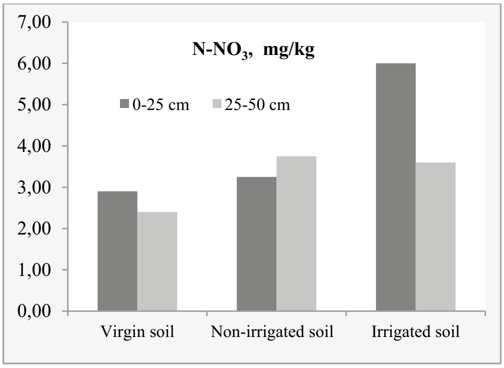

Figure 3. Content of nitrate nitrogen in dark chestnut soil of various uses

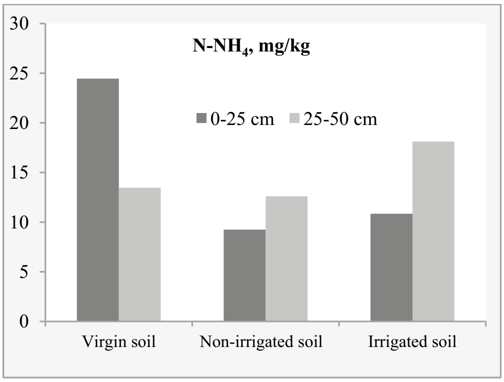

Figure 4. Content of ammonium nitrogen in dark chestnut soil of various uses

According to the availability of mobile phosphorus (Figure 5), dark chestnut soil was characterized by an average (virgin, non-irrigated soil) and its increased content (irrigated soil). An increase in the content of mobile forms of this element can be associated with an increase in the solubility of its compounds as a result of an improved water regime.

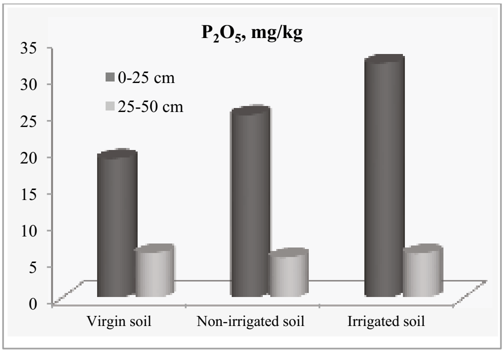

Figure 5. The content of mobile forms of phosphorus in dark chestnut soil of various uses

The content of mobile forms of potassium was characterized by an increased level of content, but somewhat higher in the variant with virgin soil (Figure 6).

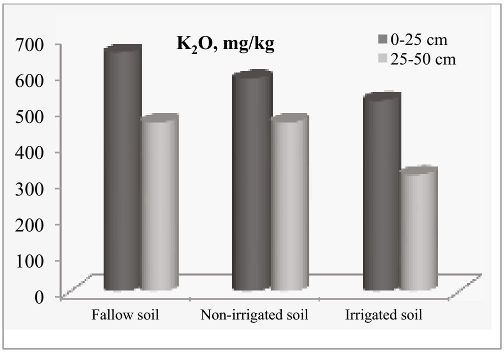

Figure 6. The content of mobile forms of potassium in dark chestnut soil of various uses

Thus, the soil availability of nutrients is influenced by the nature of soil use, the crop culture, the fertilizer application system, and the water regime.

CONCLUSIONS

The introduction of lands into agricultural use leads to a change in the factors and conditions of soil formation, the direction of the evolution of dark chestnut soil. For Dry Steppe conditions the factor that affects the properties and condition of the soil, the performance of its ecosystem services is a climate that is characterized by low values of the hydrothermal coefficient.

The introduction of virgin soil into agricultural use led to a change in the composition of the soil absorbing complex. There is a decrease in the content of absorbed sodium and potassium in non-irrigated soil in comparison with virgin soil from 4.7% to 4.2% (0-25 cm layer) and from 2.9% to 2.5% (25-50 cm layer).

In the irrigated soil under the influence of fresh irrigation water, the process of desalinization was more intense: the content of Na + K from the sum of the absorbed cations decreased from 4.7% to 3.7% (0-25 cm layer) and from 2.9% to 2.3% (25-50 cm), which contributed to the improvement of the physical and chemical properties of the soils.

Long machining and irrigation as anthropogenic factors led to a change in the physical state of the soils: soil compaction, a decrease in the strength of the microstructure and the water resistance of the aggregates. Due to active land use and transformation of the soil microbial coenzyme structure, dehumification processes are developing and changes in nutrient regime.

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INFLUENCE OF SOIL TILLAGE SYSTEMS AND INOCULATION ON SOYBEAN NODULATION AND YIELD

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Abstract

A field study was conducted over two agricultural years, 2015 – 2016, and was aiming to establish the influence of some soil tillage systems and inoculation on soybean nodulations and grain yield. The experimental design of the research, was organized using a split plot method and the following factors were analyzed: Factor A – soil tillage system: Conventional tillage (CT): Ploughing at 20 cm (control, A_{20}); Minimum Tillage (MT): Chisel plow at 20 cm (C_{20}), Chisel plow at 40 cm (C_{40}), Disking at 10 cm (D_{10}), Disking / Ploughing at 20 cm (D/A_{20}) and Disking/ Chisel at 40 cm (D/C_{40}); Factor B – soybean varieties from different maturity groups: Carla, 000, PR92B63, 0; Factor C – soybean seeds inoculation with Nitragin Bac Soya: non - inoculated; inoculated.
Influenced by nitrogen - fixing bacteria and soil tillage systems, the soybean nodulation recorded an increase of 37.9%. After two years of research, the average grain yield for soybean varieties sowed in variants treated with Bradyrhizobium japonicum, ranged from 2177 kg/ha (D) to 2044.6 kg/ha (D/C_{40}). For variants, where soybean seeds weren't inoculated with nitrogen - fixing bacteria, the grain yield ranged in average from 2044.6 kg/ha (D) to 2422.7 kg/ha (D/C_{40}). An increase of soybean grain yield with 5.4 % was brought by seed inoculation with nitrogen-fixing bacteria.

Key words*: soybean, minimum tillage, conventional tillage, nodulation, yield,*

INTRODUCTION

As a basic component of sustainable agriculture, the unconventional soil tillage systems (Marin et al., 2011), reduce the soil erosion (Guş and Rusu, 2011; Marin, 2011; Li et al., 2017), has a favorable influence on soil water conservation (Hatfield and Stewart, 1994; Marin et al. 2007; Busari et al., 2015), involves a lower energy consumption (Rusu et al. 2009; Rusu, 2014) and costs (Höflich et al., 1999; Ignea et al., 2012; Li et al, 2017), and on a long-term supports crops' productivity (Soane et al., 2012; Pittelkow et al., 2015; cited by Li et al, 2017).
Researches carried out in Romania, regarding the influence of unconventional soil tillages systems on soybean grain yield, reported increases between 1.6 % and 7% (Guş and Rusu, 2011; Guș et al., 2013; Căpățână and Ciocan, 2016) compared to the conventional system.
All the physical, chemical and biological properties of the soil are influenced by the tillage systems (Jităreanu and Ailincai, 1999; Feiza and Cesevicius, 2006; Cara et al., 2008; Cociu and Alionte, 2011; Dogan et al., 2011; Sheibani and Ahangar, 2013). Compared to the conventional system, minimum tillage system favors the microbial activity (Höflich et al., 1999), of the arable layer. As an important element of a sustainable agricultural system, the symbiotic nitrogen fixation (SNF) by leguminous plants has a major contribution in influencing cycle of the nitrogen in nature (Ferguson, 2013) and is also an important source of renewable energy (Rotaru, 2009). On long-term, SNF improves, soil fertility (Bohlool et al., 1992; Cass et al., 1994; Tago et al., 2011; Matsumiya et al., 2013; Ferguson, 2013), can successfully replace the synthetic nitrogen fertilizers (Kovačević et al., 2011) and has a positive influence on crops' grain yields (Căpățână et al., 2017).
Soybean *Glycine max* L. (Merrill) is an important commercial crop at a global level (Subramanian and Smith, 2013), primarily cultivated due to its high content of protein and

oil (Hymowitz et al., 1972) and also due to the plants' capacity to fix atmospheric nitrogen (SNF), through roots' nodules resulted from the symbiosis with *Bradyrhizobium japonicum* nitrogen - fixing bacteria (Cheţan et al., 2014).

MATERIALS AND METHODS

This study, conducted during 2015 - 2016, presents results, regarding the influence of conventional tillage (CT) and minimum tillage (MT) on number of root-nodules and grain yield, of soybean crop. The experiment was established on a chromic luvisol with a clay-loam texture, a moderately acid reaction (pH 5.2 - 5.4) and a low humus content that ranged between 2.1% and 2.2%, located at Moara Domneasca Didactic Farm, Ilfov County (Mihalache et al., 2010) based on a split splot set-up with the following factors tested:

Factor A - Tillage system with six graduations:
Conventional system (CS):
a_1 - Ploughing at 20 cm (A_{20} - Control, C);
Minimum tillage (MT):
a_2 - Chisel plow at 20 cm (C_{20}); a_3 - Chisel plow at 40 cm (C_{40}); a_4 - Disking at 10 cm (D_{10}); a_5 - Disking at 10 cm / Ploughing at 20 cm (D/A_{20}); a_6 - Disking at 10 cm / Chisel at 40 cm (D/C_{40}).
Factor B - soybean varieties:
b_1 - Carla, 000 (ISTIS 2015);
b_2 - PR92B63, 0;
Factor C - seeds inoculation with Nitragin Bac Soya before sowing:
c_1 - non-inoculated seeds;
c_2 - inoculated seeds.

For D/A_{20}, and D/C_{40}, the soil tillage were applied alternative, as follows: D for the previous crop and A_{20} and C_{40} for the soybean crop. During the two years of research, the soybean varieties were sown on April 16th 2015 and on April 9th 2016, using an SPC sowing machine at 50 cm between rows. The fertilization was assured by a complex fertilizer $N_{45}P_{60}K_{45}$ (kg/ha a.s), applied at seedbed preparation. Prior to soybean sowing, seeds were treated with Nitragin Bac Soya (pure bacterial culture of *Bradyrhizobium japonicum*) at a dose of 300 g/ha. Weed control was performed, pre-emergent with *S-metolachlor* 960 g/l (Dual Gold, 1.5 l/ha). After plants' emergence, the control of dicotyledonous weeds was assured by using 480 g/l *bentazone* + 150 g/l *wettol* (Basagran, 2 l/ha) and for the monocotyledonous weeds *quizalofop-P-teflil* 40 g/l (Pantera, 1.2 l/ha) was used.
Harvesting date of varieties was different, influenced by the maturity group and by the climatic conditions of the area: a_1 - Carla 000 (ISTIS 2015), September 07th 2015 (144 days after sowing), September 10th 2016 (154 days after sowing); a_2 - PR92B63 0, October 19th 2015 (186 days after sowing), October 21th 2016 (195 days from sowing). The number of nodules per plant for soybean varieties was determined once at every two weeks, from May 31 to June 30 (2015 - 2016). At June 30th, determination of nodules number per plant was conducted when soybean varieties were in different stages of development, Carla - R3 (beginning of pods formation) and PR92B63 - R1 (beginning of blooming).
Climatic conditions of the area significantly influenced soybean plants development. The average amount of annual rainfall was 662.4 mm, higher than the normal values of the area with 106.3 mm and the average temperature was 12.2°C compared to normal of the area 10.5°C (Table 1).

Table 1. Climatic conditions, Moara Domneasca, Ilfov County. Average 2015 - 2016

Month	Average 2015 - 2016			
	Temperature (°C)		Rainfall (mm)	
	Average 2015 - 2016	Normal	Average 2015 - 2016	Normal
October	11.3	11.0	67.1	35.8
November	6.8	5.3	79.9	40.6
December	2.2	0.4	43.2	36.7
January	-2.5	-3.0	48.0	30.0
February	1.9	-0.9	28.5	32.1
March	6.9	4.4	66.4	31.6
April	**13.0**	**11.2**	**33.3**	**48.1**
May	**17.2**	**16.5**	**52.3**	**67.7**
June	**21.7**	**20.2**	**85.8**	**86.3**
July	**24.7**	**22.1**	**4.7**	**63.1**
August	**23.7**	**21.1**	**68.6**	**50.5**
September	**18.9**	**17.5**	**84.6**	**33.6**
Avg. / Sum Oct. - Sept.	12.2	10.5	662.4	556.1
Avg. / Sum Apr. - Sept.	19.9	18.1	329.3	349.3

During the vegetative period (April - September), average temperature was 19.9°C, 1.8°C higher than the normal value of the area (18.1°C). Average rainfall recorded during the vegetative period was 329.3 mm, close to normal values of the area (349.3 mm), (Table

1). The rainfall distribution during the vegetative period had influenced the development of soybean varieties. On average during the two years of research (2015 - 2016), July rainfalls' recorded a critical value of 4.7 mm, which represented 7.4% of the normal values of the area (63.1 mm), having a negative influence on varieties' yield, with them being in the reproductive period, Carla R3 - R5, and PR92B63 R1 - R3.

RESULTS AND DISCUSSIONS

Influence of soil tillage systems on soybean number of nodules

On average in two years of research (2015 - 2016) under the climatic conditions at Moara Domneasca, in variants where the soybean seeds were inoculated before sowing, the soybean number of nodules per plant at 30th of June, recorded differences from -2.3 (D) to 7 (C_{40}) nodules/plant at Carla and between -2.7 (D) and 6.8 (C_{40}) nodules/plant at PR92B63 statistically significant compared to control (A_{20}).

For the Control ($A_{20,}$ C) variant, where the conventional system was applied, soybean varieties recorded on average 33.1 nodules/plant. Nodules number per plant increased with very significant values, statistically assured, for variants in which minimum tillage systems with C_{20}, C_{40}, and D/C_{40}, were applied. The highest number of nodules per plant was recorded by PR92B63 variety, 41.7 nodules/plant for variant C_{40}, with an increase of 19.6 % compared to A_{20}, (Table 2).

Table 2. Soil tillages influence on the number of nodules per plant in variants treated with Nitragin Bac Soya, 30thJune. Average 2015 - 2016

Variants	CARLA				PR92B63				Average - Varities			
	No. Nod./pl.	Diff	%	Signf	No. Nod./pl.	Diff	%	Signf	No. Nod./pl.	Diff	%	Signf
A20	31.3	C	100.0	-	34.8	C	100.0	-	33.1	C	100.0	-
C20	35.8	4.5	114.3	***	39.4	4.6	113.2	***	37.6	4.5	113.7	***
C40	38.3	7.0	122.4	***	41.7	6.8	119.6	***	40.0	6.9	120.9	***
D	29.0	-2.3	92.7	ooo	32.2	-2.7	92.3	ooo	30.6	-2.5	92.5	ooo
D/A20	31.8	0.5	101.7	-	35.1	0.3	100.8	-	33.5	0.4	101.2	-
D/C40	35.2	3.9	112.4	***	38.2	3.4	109.8	***	36.7	3.6	111.0	***

LSD 5% = 1.0; LSD 1% = 1.3; LSD 0.1 % = 1.7
**Note: C – control. ns – not significant. * positive significance. 0 negative significance; *No Nod./pl = Number of. Nodules / plant.*

For the non - inoculated variant (Table 3), the number of nodules for soybean varieties recorded statistically significant increases from 5.2 % to 18.6 %. For both varieties, the number of nodules per plant recorded lower values compared to A_{20} with negative differences between -0.6 (Carla) and -1.2 (PR92B63) for the variants where the minim tillage with D was applied. Under the soil tillage influence at June 30th, the varieties sown in non - inoculated variant recorded between 22.9 and 28.2 nodules/plant, compared to Control (A_{20}) (Table 3).

Table 3. Soil tillages influence on the number of nodules per plant in variants not treated with Nitragin Bac Soya, 30thJune. Average 2015 – 2016

Variants	CARLA				PR92B63				Average - Varities			
	No. Nod./pl.	Diff	%	Signf	No Nod./pl.	Diff	%	Signf	No Nod./pl.	Diff	%	Signf
A20	23.0	C	100.0	-	24.6	C	100.0	-	23.8	C	100.0	-
C20	26.3	3.3	114.3	***	27.6	3.0	112.2	***	27.0	3.1	113.2	***
C40	25.4	2.3	110.1	***	27.2	2.6	110.5	***	26.3	2.5	110.3	***
D	22.4	-0.6	97.3	o	23.4	-1.2	95.3	ooo	22.9	-0.9	96.2	oo
D/A20	24.2	1.2	105.2	***	25.9	1.3	105.4	***	25.1	1.3	105.3	***
D/C40	27.2	4.2	118.3	***	29.2	4.6	118.6	***	28.2	4.4	118.5	***

LSD 5% = 0.6; LSD 1% = 0.8; LSD 0.1% = 1.1
**Note: C – control. ns – not significant. * positive significance. 0 negative significance; *No Nod./pl = Number of. Nodules / plant.*

Influence of Nitragin Bac Soya on soybean number of nodules

Influenced by Nitragin Bac Soya treatment, the number of nodules per plant recorded an very significant increase of 9.7 nodules/plant (37.9 %), very significantly positive, compared to non-inoculated, Control (C). In two years of research (2015 - 2016), the Nitragin Bac Soya inoculation brought an statistically significant increase of nodules number per plant with values between 7.7 and 13.7. For variants were the conventional tillage system (A_{20}) was applied, inoculation with Nitragin Bac Soya, generated an increase of 9.3 nodules/plant compared to the control. The average number of nodules per plant (2015 - 2016) for the variant inoculated with Nitrogen Bac Soya, ranged from 30.6 (D) to 40 (C_{40}) nodules/plant (Figure 1).

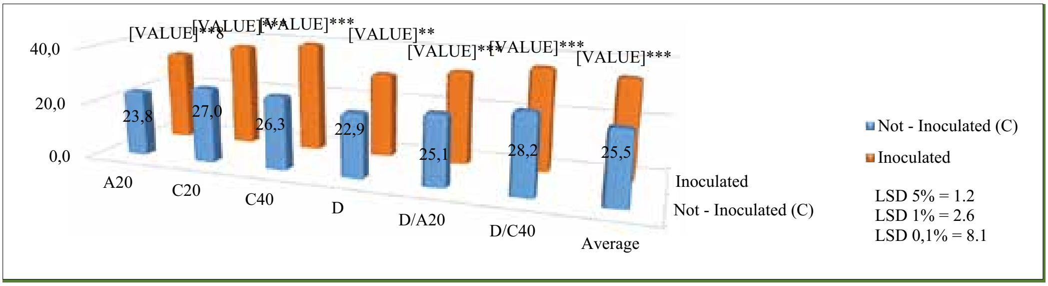

Figure 1. Influence of Nitragin Bac Soya treatment on the nodules number. Average 2015 -2016

Influence of soil tillage systems on soybean grain yield

Under the influence of soil tillage grain yield for the variant treated with Nitragin Bac Soya, in two years of research (2015 - 2016) recorded statistically significant increases ranging from 93.6 kg/ha (PR92B63, D/A_{20}) to 120.1 kg/ha (Carla, D/C_{40}), compared to A_{20} (Table 4). According to data presented in Table 4, varieties' average grain yield compared to control recorded differences from -279.0 kg/ha (very significantly negative, D) to 115.4 kg/ha (distinctly significant positive, D/C_{40}).

Negative differences, statistically assured were compared to compared to A_{20} were recorded for both varieties in variants where the minimum tillage with D and C_{20} were applied.

Influenced by soil tillage and Nitragin Bac Soya inoculation, the highest grain yield was 2639.0 kg/ha (PR92B63, D/C_{40}) and the lowest was 2083.2 kg/ha (Carla, D).

Table 4. Soil tillage influence on soybean grain yield (kg/ha), in variants treated with Nitragin Bac Soya. Average 2015 - 2016

Variants	CARLA				PR92B63				Average - Variety			
	GY kg/ha	Diff kg/ha	%	Signf	GY kg/ha	Diff kg/ha	%	Signf	GY kg/ha	Diff kg/ha	%	Signf
A20	2383.6	C	100.0	-	2528.2	C	100.0	-	2455.9	C	100.0	-
C20	2305.3	-78.3	96.7	o	2449.4	-78.8	96.9	o	2377.4	-78.6	96.8	o
C40	2492.8	109.2	104.6	**	2607.5	79.3	103.1	*	2550.2	94.2	103.8	**
D	2083.2	-300.4	87.4	ooo	2270.7	-257.5	89.8	ooo	2177.0	-279.0	88.6	ooo
D/A20	2421.3	37.6	101.6	-	2621.8	93.6	103.7	**	2521.6	65.6	102.7	-
D/C40	2503.8	120.1	105.0	**	2639.0	110.8	104.4	**	2571.4	115.4	104.7	**

LSD 5% = 68.9 kg/ha; LSD 1% = 92.8 kg/ha; LSD 0.1% = 123.1 kg/ha

**Note: C – control. ns – not significant. * positive significance. 0 negative significance;*

According to Table 5, the highest grain yield for non-inoculated variant was 2492.9 kg/ha (PR92B63, D/A_{20}), with an increase of 2.9 % compared to control variant (A_{20}). Compared to control lower yields were recorded for variants where the minimum tillage with D was applied

(Table 5) by both Carla and PR92B63, with significant negative differences between -297.7 kg/ha (Carla) and -315.2 kg/ha PR92B63. On average, due to the minimum tillage system soybean varieties registered differences in yield statistically assured between -306.5 kg/ha at D and 71.7 kg/ha at D/C_{40} (Table 5).

Table 5. Soil tillages influence on soybean grain yield (kg/ha), in variants not treated with Nitragin Bac Soya. Average 2015 - 2016

Variants	CARLA				PR92B63				Average - Variety			
	GY kg/ha	Diff kg/ha	%	Signf	GY kg/ha	Diff kg/ha	%	Signf	GY kg/ha	Diff kg/ha	%	Signf
A20	2278.9	C	100.0	-	2423.2	C	100.0	-	2351.0	C	100.0	-
C20	2191.1	-87.8	96.1	o	2330.3	-92.9	96.2	oo	2260.7	-90.3	96.2	oo
C40	2358.4	79.5	103.5	*	2467.4	44.2	101.8	-	2412.9	61.9	102.6	-
D	1981.2	-297.7	86.9	ooo	2108.0	-315.2	87.0	ooo	2044.6	-306.5	87.0	ooo
D/A20	2335.6	56.8	102.5	-	2492.9	69.7	102.9	*	2414.3	63.2	102.7	-
D/C40	2366.0	87.1	103.8	*	2479.5	56.3	102.3	-	2422.7	71.7	103.0	*

LSD 5% = 66.6 kg/ha; LSD 1% = 89.5 kg/ha; LSD 0.1% = 118.6 kg/ha

**Note: C – control. ns – not significant. * positive significance. 0 negative significance;*

Influence of Nitragin Bac Soya on soybean grain yield

During 2015 - 2016, inoculation with Nitragin Bac Soya had a favorable influence on soybean yields (Figure 2). Thus, under the influence of Nitragin Bac Soya inoculation influence, the average grain yield of varieties recorded increases between 104.9 kg/ha and 148.6 kg/ha, compared to non-inoculated, statistically assured, Figure 2. The average grain yield increase brought by inoculation was 124.5 kg/ha, higher with 5.4 % compared to the non - inoculated variant. The highest grain yield was recorded at D/C_{40} with a value of 2571.4 kg/ha, by 6.1% higher than control (non-inoculated).

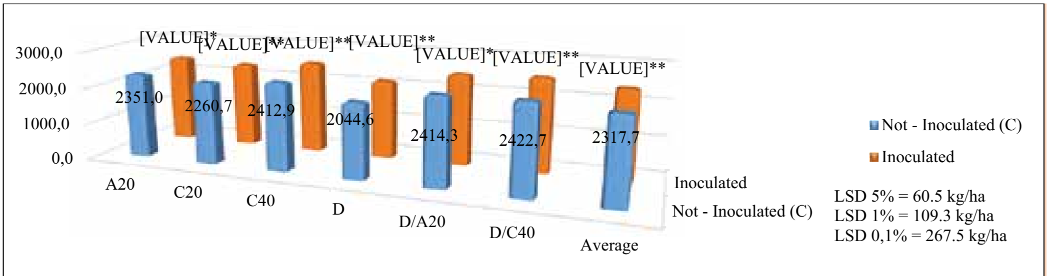

Figure 2. Influence of Nitragin Bac Soya treatment on soyabean grain yield. Average 2015 - 2016

CONCLUSIONS

For the two varieties tested in South - East of Romania, during the agricultural years of 2014/2015 - 2015/2016, the highest number of nodules per plant in variants treated with Nitragin Bac Soya, was 29.2 (PR92B63, D/C40) and the lowest was 22.4 (Carla, D). On average, during the two years of research, under the inoculation with Nitragin Bac Soya (pure bacterial culture of *Bradyrhizobium japonicum*), the number of nodules per plant recorded an increase of 37.9%. The average grain yield of the two varieties (2015-2016), under the influence of soil tillage systems along with inoculation, ranged between 2177.0 kg/ha and 2571.4 kg/ha, and for the non-inoculated variant the average yield varied between 2044.6 kg/ha and 2422.7 kg/ha.

The lowest grain yield was 1981.2 kg/ha recorded by Carla at D, non-inoculated variant, and the highest was 2639.0 kg/ha recorded by PR92B63 at D/C_{40} inoculated variant. For variants inoculated with Nitragin Bac Soya, grain yield recorded increases compared to A_{20} (C) between 2.7 % and 4.7 % for the minimum tillages systems (MT) C_{40}, D/C_{40}, D/ A_{20}. For variants were conventional tillage (CT) was applied the average grain yield of varieties ranged from 2351.0 kg/ha and 2455.9 kg/ha. Regardless of the Nitragin Bac Soya inoculation, the grain yield of varieties recorded for D/C_{40} had on average higher values statistically assured compared to A_{20} with 71.7 kg/ha (non-inoculated variant) and with 115.4 kg/ha (inoculated variant).

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AGROTECHNICAL SYSTEMS TO CONSERVING WATER IN THE SOIL FOR WHEAT CROP

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Abstract

The ecological frame from the Transylvanian Plain is given by the existence of interaction among a great number of factors, of which two are very important for the agroechosystem: i) the first is the thermal background with high time variations and low rainfall, characteristics which impose significant restrictions for crop plants; ii) and the second is the hill orography, with slope fields. The purpose of the research made is to establish the influence of the soil tillage system (conventional and no-tillage), the fertilization system and the phytosanitary treatments applied, on the accumulation and water preservation in the soil, on the degree of weeds in the case of autumn wheat crop and the production. The restoration of water reserve in the soil in the no-tillage system is more reduced than in the conventional system, but also the loss of water in the conventional system is as rapid. This loss of water is due to a more intense water evaporation in the conventional system, without vegetal debris at the surface. The degree of weeds in the case of wheat in the conventional system (with plowing) during the experiment (2012-2016) is more reduced compared to the no-tillage system. The average production of wheat recorded in the no-tillage system during the five experiment years is 6234 kg/ha, compared to the average production obtained in the conventional system, that is 6162 kg/ha, which indicates the suitability of its cultivation in the no-tillage system.

Key words*: climate variability, conservative agrotechnique, no-tillage, wheat.*

INTRODUCTION

Research and agricultural practice have promoted, during the last years, several alternative variants of soil tillage, as solutions of conservative agriculture, with works adapted to concrete conditions regarding especially the type of soil, the climate conditions, the land orography and the available technical equipment. Expert literature cites, most frequently the following soil tillage systems (Dick et al., 1994; Rusu, 2014; Marin et al., 2015; Chetan et al., 2016): conventional system (with plowing), respectively no-tillage (direct sowing in unprocessed land). Between these extremes there are variants like: reduced works (rationalized conventional), minimum tillage (with covering under 30%), mulch tillage (with covering over 30%), ridge tillage, strip till, zone till, direct drill.

The reduction of the number of soil tillage modifies radically the technological approach regarding fighting against weeds, diseases and pests. Special attention in the case of these conservative soil tillage variants is imposed by the phytosanitary protection of crops where the preventive character must prevail. The failure of many attempts of applying conservative soil tillage systems is based on the adequate lack of control of weeds. Special attention must be paid to indirect methods: crop rotation, hidden crops, directed fertilization (Szajdak et al., 2003; Domuta et al., 2012). In the case of moldboard plowing weed seeds are spread in all arable layer, their germination being made in steps, and those buried deep lose their viability. In the case of minimum tillage and no-tillage, seeds are concentrated in the first 10 cm, they germinate explosively in the first year of application, determining excessive weeds, and subsequently the change of weed spectre.

During the last years there are more and more problems with drought, that is why it is necessary to preserve water in the soil, and the choice of the technological management system is the most important thing (Lal, 2007; Dixon et al., 2010; Coyle et al., 2016; Lampurlanes et al., 2016). The limitation of drought effects can also be made by agrophytotechnical measures of accumulating, preserving and efficiently valuing water from rainfall (Halbac-Cotoara-Zamfir et al., 2015; Mu et al., 2015). In the case of conservative soil tillage systems by protecting the soil with vegetal debris (mulch), the loss of water is avoided by evaporation but also the suffocation of grown or upcoming weeds (Wozniak et al., 2014). In the conservative agricultural system, either we apply minimum tillage or no-tillage, the soil mulch (covering) with vegetal debris left after the harvest of the previous crop leads both to the improvement of humus content in the soil and to the water preserve.

The pedo-climate and ecological frame from the Transylvanian Depression is given by the existence of the interaction of a great number of factors, of which two are very important for the agroechosystem (Rusu et al., 2017); the first is the thermal background with high time variations, features which impose significant restrictions for thermophile plants like: corn, soy, sunflower and the second is the hill orography. The land degradation from the Transylvanian Depression and its effects must be regarded from the point of view of the local physical-geographical conditions, over which extreme climate conditions superpose. These conditions create, in general, a frame favorable for the development of morphogenetic processes caused by human activity, besides just like those caused by natural mechanisms, intensifying both the rhythm and their territorial extension. In this regard, we can notice first rainfall, which although under the aspect of the annual amount is lacking, it has a negative influence on the vegetal field by its regime. This is due to the fact that, on one hand, during March-November, when the soil through agro-technical works is always loosened, the quantity of rainfall which causes runoff on slopes is relatively high (40-50% of the total rainfall), and on the other, to torrential rain which has a strong rain aggressiveness. Together with rainfall, relief is also susceptible, by its increased degree of fragmentation and by the slope inclining, especially southern peaks, vegetation by the predominance of cultivated plants and by the advanced stage of land degradation, then lithology by the predominance of friable rocks (sands, bedrocks, freestones etc.). This climate characterization imposes special technological measures of preserving lands.

The interaction of environment factors in relation to the anthropic influence had an impact upon the field state, with a lot of soils degraded by erosion, which impose restrictions regarding the crop structure, the system of machines and tractors which ensure the mechanization of slope works.

The purpose of the research made is the study, under the pedoclimate conditions from the Transylvanian Depression, of the influence of the soil tillage system (conventional and no-tillage) on the accumulation and preserve of water in the soil, on the degree of weeds of wheat crop and of production. The novely of this research lies in the adaptation of an adequate phytosanitary protection system to the soil tillage system applied.

MATERIALS AND METHODS

The research presented in the paper was made at the Agricultural Research and Development Station Turda (ARDS Turda) during 2012-2016 and its purpose was testing two soil tillage systems: the conventional system (with autumn plowing, preparation of land, fertilizing and sowing), respectively no-tillage (direct sowing in the stubble of the pre-emerging crop). The research was made in a 3 years rotation crop (soy-wheat-corn), and for the technological optimization and particularization of the agrotechnical system applied, fertilization and the phytosanitary treatments applied were differentiated/adapted. Arieşan type of wheat was cultivated, which although it is not a very new type, it is productive and adapts easier to different agrotechnical systems (it has a slight genetic polymorphism).

The experiences were made on a vertic phaeosiom type of soil, with the following properties: pH 6.30-7.00, humus 2.21-2.94%, total nitrogen 0.162-0.124%, phosphorus 0.9-5.00 ppm, potassium 126-140 ppm. The experience made is polifactorial, with three repetitions, organized according to the method of subdivized parcels. The surface of an experimental parcel was 48 m^2 (4 m wide x 12 m long).

The experimental factors were:

Factor A - year: a_1 - 2012, a_2 - 2013, a_3 - 2014, a_4 - 15, a_5 - 2016.

Factor B - soil tillage system: b_1 - conventional system (CS), b_2 - no-tillage (NT).

Factor C - phytosanitary treatments: c_1 - without herbicides, c_2 - with herbicides (combinations of treatments presented in Table 1).

Sowing was made with Gaspardo Directa-400 sowing machine directly for the no-tillage variant. The sowing thickness was 550 germinable seeds/m^2, at 18 cm distance among lines and incorporating the seed 5cm deep (the pre-emerging plant is soy). The fertilization of the crop was made in two phases, with $N_{40}P_{40}$ kg active substance/ha in the autumn at the same time with sowing plus an additional fertilization with N_{40} kg active substance/ha in the spring when wheat retakes its vegetation.

The degree of weeds of the crop and the spectre of weeds presented was determined visually and by numbers with the help of the metric frame (0.25 m^2), then gravimetrically by extracting the seeds, separating them according to species, weighting and drying them in the oven. The production of the wheat crop was determined by weighting on the experimental parcels, after taking out the sides and transforming the production according to STAS moisture (14%). The set up of the soil moisture 0-100 cm deep was made according to the gravimetric method (taking soil samples with Theta drill and drying them in the oven). The experimental data was processed according to the variance analysis and by establishing the limit differences (5%, 1%, 0.1%).

Table 1. Products used for the control of vegetation and crop protection during 2012-2016

Variant of treatment	Phenophase of application / dose			
	End of twinning		Skin	
c_1-without herbicides	Polyfeed (foliar fertilizer: 19-19-19+1% Mg+ME)	2.5 kg/ha	Evolus (proquinazid 40 g/l + tebuconazol 160 g/l + procloraz 320 g/l)	1.0 l/ha
	Calypso 480 SC (tiacloprid 480 g/l)	0.1 l/ha	Fastac 10 EC (alfacipermetrin 100 gr/l)	0.1 l/ha
	Falcon 460 EC (167 g/l tebuconazol + 43 g/l triadimenol + 250 g/l spiroxamina)	0.6 l/ha	Trend 90 (900 g/l alcool isodecil etoxilat)	0.3 l/ha
c_2-with herbicides	Fastac 10 EC (alfacipermetrin 100 gr/l)	0.1 l/ha	Polyfeed (foliar fertilizer: 19-19-19+1% Mg+ME)	2.5 kg/ha
	Sekator Progres OD (amidosulfuron 100 g/l+iodosulfuron-metil-Na 25 g/l+mefenpyr dietil 250 g/l) + DMA 6 (600g/l dimetil amina 2.4D)	0.15 l/ha + 0.6 l/ha	Evolus (proquinazid 40 g/l + tebuconazol 160 g/l + procloraz 320 g/l)	1.0 l/ha
	Falcon 460 EC (167 g/l tebuconazol + 43 g/l triadimenol + 250 g/l spiroxamina)	0.6 l/ha	Calypso 480 SC (tiacloprid 480 g/l)	0.1 l/ha
	Polyfeed (foliar fertilizer: 19-19-19+1% Mg+ME)	2.5 kg/ha	Trend 90 (900 g/l alcool isodecil etoxilat)	0.3 l/ha

RESULTS AND DISCUSSIONS

The results obtained must be reported to the evolution of climate factors in order to identify the best agrotechnical measures to adapt to climate changes. In this regard, an analysis of the evolution of the thermal and rainfall regime at ARDS Turda (altitude of 427 m) is presented during the last 60 years, respectively since 1957, date when the station was set up and up to present (Figure 1 and Figure 2). The research area is characterized by a multiannual average temperature of 9.1°C and by multiannual average rainfall of 518.6 mm. But during the last 15 years one can notice a clear tendency of rising temperatures and a fall of the rainfall recorded. The climate changes recorded, as well as the unpredictible ones from the future impose the judicial choice of the biological material which is going to be cultivated and the application of certain agrotechnical systems adequate to the new climate conditions.

Specific for the five years taken into account in the study (2012-2016) was the unequal distribution of rainfall; there were periods of drought, with extended pedologic drought followed by torrential rain.

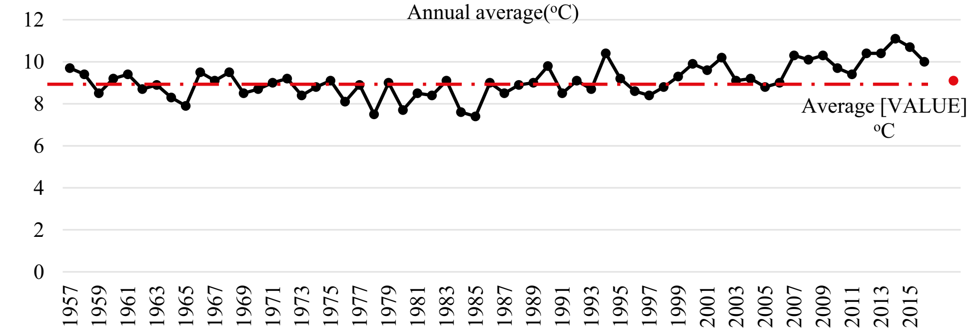


Figure 1. The thermal regime ARDS Turda, 1957-2016

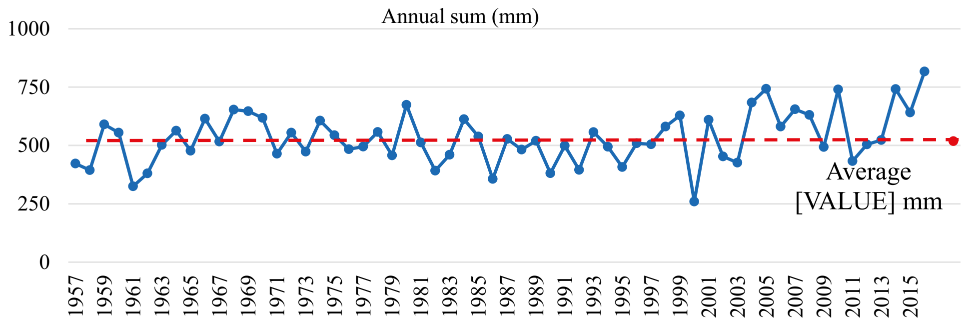


Figure 2. The rainfall regime ARDS Turda, 1957-2016

April and May in 2012 were warm, and during June-August there was a permanent heat which lasted for 21 days in a row; during this period temperatures were over 32°C, and the biological processes of the plant stopped. From the point of view of rainfall all months were very droughty. The rainfall from the two months, April and May somehow restored the water reserve in the soil, which helped plants make good productions.

Specific for 2013 was the alternation of heat waves with waves of cool temperatures, with high differences from one period to another, which resulted in the disruption of biological cycles at certain species of plants. The temperature values deviated from multiannual average by +2.1 up to +2.5°C, generating three warm months, of which April and May, where thermal values exceeded 29°C. During summer, compared to the monthly multiannual average, thermal values exceeded the multiannual average by +1.7 in July and respectively by +2.9°C in August, the months being warm in a whole, the maximum values of temperature exceeded many times the heat threshold. The end of July – beginning of August recorded seven days of continuous heat and which, corroborated with relatively very low moisture (around 20-29%) determined the installation of a stabilized atmosphere drought.The highest value of temperature was +35.8°C on 9.08.2013. In July and August a strong drought installed which caused stress to plants, intensive rain came late, at the end of August, after 25.08.2013.

From a climate point of view, 2014 was a favorable year for agricultural crops, the alternation of months with normal temperatures from a thermal point of view with hot ones was beneficial for the ongoing of vegetative steps. The rainfall from 2014 with the annual amount of 741.5 mm was quantitatively high, especially in the summer, even if the number of rainy days was smaller.

2015 was characterized as a warm and rainy year. The average of the year was 10.6°C, that is 1.5°C higher than the multiannual average in 60 years. The rainfall of this year exceeded by 122.4 l/m^2 the multiannual average in 60 years (518.8 l/m^2). The amount recorded was 641.2 l/m^2.

2016 is characterized as a warm year, with a deviation of +0.9°C compared to the multiannual average, recording an annual average temperature of 10°C. From the point of view of the rainfall regime, 2016 with 816.8 l/m^2 and a deviation of +288 l/m^2 compared to the multiannual average is characterized as an excessively rainy year.

The reduction of the weed degree of the land was made inside the crop rotation by the herbicidation of the wheat stubble with Glifosat (glifosat acid 360 g/l) in dose of 3-4 l/ha in 300 l water, 15-20 days after wheat was harvested. Three weeks later mustard was sowed as green fertilizer in order to keep the soil covered with vegetation until sowing, when mustard was chopped and incorporated in the soil through an artificial soil work. The species of weeds presented in the weed crop before the herbicide treatment were 25, of which 17 annual dicotyledonous (DA): *Xanthium pensylvanicum, Chenopodium album, Polygonum convolvulus, Polygonum aviculare, Polygonum lapathifolium, Erigeron canadensis, Capsella bursa-pastoris, Veronica persica, Sonchus oleraceus, Papaver dubium, Ambrozia artemisiifolia, Galium aparine, Delphinium consolida, Arctium lappa, Viola arvensis, Hibiscus trionum, Amaranthus retroflexus*; 5 perennial dicotyledonous (DP): *Convolvulus arvensis, Rubus caesius, Cirsium arvense, Taraxacum officinale, Lathyrus tuberosus*; 2 annual monocotyledonous (MA): *Setaria glauca, Echinochloa cruss galli* and a perennial monocotyledonous species (MP): *Agropyron repens.*

Inside the experience, in c_2 variant, herbicidation was made at the end of the wheat twinning, when annual dicotyledonous weeds have 2-3 leaves, perennial dicotyledonous weeds like bull thistle (*Cirsium arvense*) are up to 10 cm high and annual monocotyledonous weeds like bristle grass (*Setaria glauca)*, beared grass (*Echinochloa cruss galli*) have 2-4 leaves and are not united. Herbicidation was made using the sistemic products Sekator Progres OD + DMA 6 (0.15 l/ha + 0.6 l/ha) which fight against a large spectre of annual and perennial dicotyledonous weeds and some monocotyledonous.

In the no-tillage system, in the first year of application, the number of weeds was higher compared to the conventional system (Figure 3). After the first year of experimenting, during 2013-2016, one can notice a reduction of weeds in the no-tillage system (from 50 weeds/m^2 in 2012 to 48 weeds/m^2 in 2013, 40 weeds/m^2 in 2014, 31 weeds/m^2 in 2015, 21 weeds/m^2 in 2016). The decrease of the degree of weeds in no-tillage is also due somehow to vegetal debris (mulch) left on the soil surface from the pre-emerging crop (in our case soy) they are incorporated, just like in the case of the conventional system. Also, in no-tillage one can notice a drop of the number of annual dicotyledonous weeds and a rise of the number of perennial dicotyledonous weeds, as well as of the number of perennial monocotyledonous weeds. Among the species of perennial monocotyledonous weeds, *Agropyron repens* was present during all experimental years, fighting against it in the wheat crop cultivated in no-tillage system is harder to achieve even if complex herbicides to fight against weeds were applied.

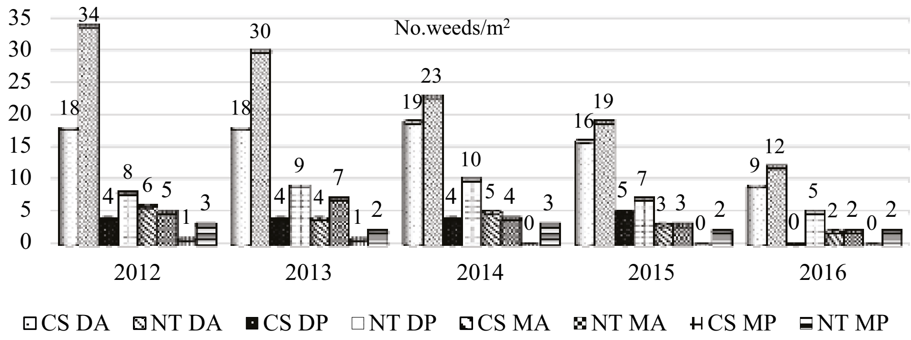


Figure 3. Species of weeds participating at the weed degree of wheat crop, 2012-2016

The degree of weeds of wheat in the conventional system, during all five experimental years, is smaller (compared to no-tillage), the weeds present were 29 weeds/m^2 in 2012, 24 weeds/m^2 in 2013, 28 weeds/m^2 in 2014, 24 weeds/m^2 in 2015 and 11 weeds/m^2 in 2016.

The total mass of weeds determined during 2012-2016 in c_1 variant -without herbicides is 18.5 t/ha in the conventional system and 25.5 t/ha in no-tillage system (Table 2). The highest number percentage in both soil tillage systems was represented by annual dicotyledonous weeds, that is 80 weeds/m^2 and 5.2 t/ha vegetative mass in the conventional system, respectively 118 weeds/m^{2}and 5.6 t/ha in no-tillage system. The highest percentage as vegetative mass was represented by perennial dicotyledonous weeds, with 10.7 t/ha in the conventional system and 14.7 t/ha in no-tillage. Annual monocotyledonous weeds had the lowest degree of participation at the crop weed in both soil tillage systems, of 2.1 t/ha in conventional system and 2.3 t/ha in no-tillage. Perennial monocotyledonous represented 0.5 t/ha in the conventional system and 2.9 t/ha in no-tillage.

Table 2. Weed degree of wheat crop in c_1 variant - without herbicides, 2012-2016

Soil tillage system / year / variant		Annual dicotyledonous			Perennial dicotyledonous			Annual monocotyledonous			Perennial monocotyledonous		
		No/m^2	g/m^2	t/ha	No/m^2	g/m^2	t/ha	No/m^2	g/m^2	t/ha	No/m^2	g/m^2	t/ha
c_1 x conventional system	2012	18	153	1.5	4	368	3.7	6	55	0.6	1	29	0.3
	2013	18	73	0.7	4	249	2.5	4	49	0.5	1	24	0.2
	2014	19	95	1.0	4	222	2.2	5	52	0.5	-	-	-
	2015	16	124	1.2	5	241	2.3	3	29	0.3	-	-	-
	2016	9	83	0.8	-	-		2	22	0.2	-	-	-
Total		**80**	**528**	**5.2**	**17**	**1080**	**10.7**	**20**	**363**	**2.1**	**2**	**53**	**0.5**
c_1 x no-tillage system	2012	34	247	2.5	8	319	3.2	5	63	0.6	3	77	0.8
	2013	30	81	0.8	9	434	4.3	7	58	0.6	2	54	0.5
	2014	23	62	0.6	10	271	2.7	4	61	0.6	3	69	0.7
	2015	19	82	0.8	7	235	2.3	3	31	0.3	2	51	0.5
	2016	12	92	0.9	5	224	2.2	2	20	0.2	2	43	0.4
Total		**118**	**564**	**5.6**	**39**	**1483**	**14.7**	**21**	**233**	**2.3**	**12**	**294**	**2.9**

In c_2 variant- with herbicides, the efficiency of these treatments determined the formation of a vegetative mass of reduced weeds in both soil tillage systems (Table 3). Annual dicotyledonous weeds had a weight of 0.46 t/ha in the conventional system and 0.68 t/ha in no-tillage. Perennial dicotyledonous weeds represented 8.0 t/ha in the conventional system and 12.7 t/ha in no-tillage. The lowest value was recorded at annual monocotyledonous weeds, 0.2 t/ha in the conventional system and 0.3 t/ha in no-tillage and were present only during the first experimental years.

Table 3. Weed degree of wheat crop in c_2 variant - with herbicides, 2012-2016

Soil tillage system / year / variant		Annual dicotyledonous			Perennial dicotyledonous			Annual monocotyledonous			Perennial monocotyledonous		
		No/m^2	g/m^2	t/ha	No/m^2	g/m^2	t/ha	No/m^2	g/m^2	t/ha	No/m^2	g/m^2	t/ha
c_2 x conventional system	2012	2	14	0.1	2	251	2.5	1	11	0.1	1	23	0.2
	2013	1	9	0.09	1	147	1.5	1	13	0.1	1	25	0.3
	2014	1	11	0.1	1	128	1.3	-	-	-	-	-	-
	2015	1	7	0.07	1	143	1.4	-	-	-	1	25	0.3
	2016	1	10	0.1	1	131	1.3	-	-	-	-	-	-
Total		**6**	**51**	**0.46**	**6**	**800**	**8.0**	**2**	**24**	**0.2**	**3**	**73**	**0.7**
c_2 x no-tillage system	2012	2	15	0.2	3	293	2.9	1	12	0.1	2	28	0.3
	2013	2	15	0.2	2	254	2.5	1	11	0.1	1	20	0.2
	2014	1	8	0.08	2	261	2.6	1	13	0.1	1	22	0.2
	2015	1	10	0.1	2	229	2.3	-	-	-	2	27	0.3
	2016	1	11	0.1	2	235	2.4	-	-	-	1	24	0.2
Total		**7**	**59**	**0.68**	**11**	**1272**	**12.7**	**3**	**36**	**0.3**	**7**	**121**	**1.2**

Optimizing the soil tillage system for the autumn wheat crop must ensure the accumulation and preserve in the soil of the entire quantity of water coming from the rainfall during summer and autumn. It is known that during the last years the climate in the Transylvanian Plain has changed, with the increase of the annual average temperature as well as the non-uniformity of rainfall, that is why the agrotechnique applied must be adapted to more oscillating ecological conditions. Following the determinations made during 2012-2016, regarding the moisture existing in the soil in the autumn wheat crop, one can notice that there are certain differences among the soil tillage systems (Figure 4).

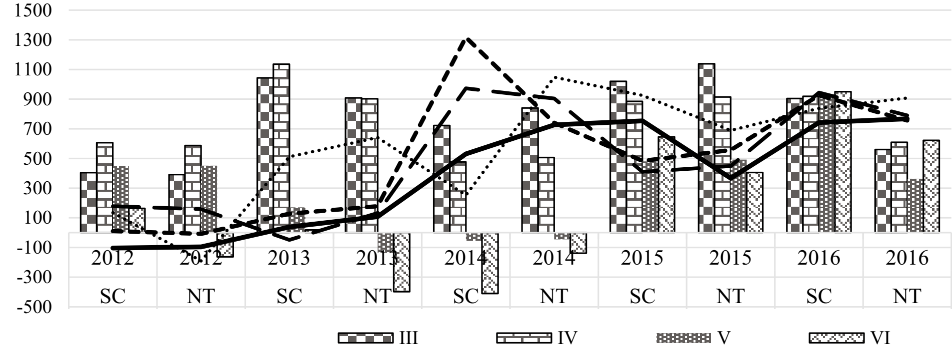


Figure 4. Influence of the tillage system applied to wheat crop on the water reserve of the soil (m^3/ha), 2012-2016

In March-April 2012, although there were before three droughty months and only January was rainy, the accessible moisture reserve kept in normal limits, with a tendency to drop towards minimum values. The results of the lack of rainfall in spring were felt in the following months June-September, with pedologic drought 0-100 cm deep (-104 m^3/ha in the conventional system in September; -163 m^3/ha in June, -8 m^3/ha in July in no-tillage system). In August, excessively droughty, the pedologic drought was accompanied by the atmosphere drought and very high temperatures. The soil moisture renewed in October, which was beneficial for the post-emerging crop from the rotation.

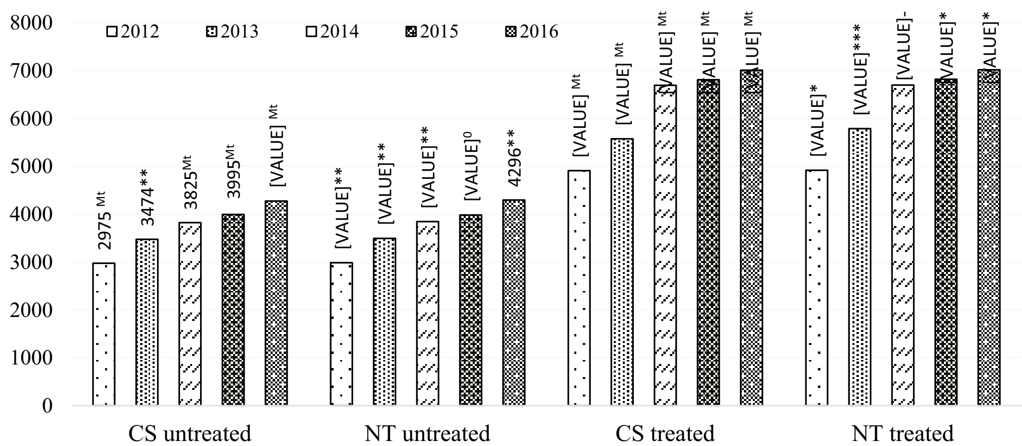
In 2013, wheat followed in the rotation after soy, benefitting from the renewal of the accessible moisture in the soil. The spring months alternating between excessive rain and excessive drought ensured normal moisture reserves to the wheat crop, of 908 m^3/ha. In the autumn of 2013, an autumn in which rainy months alternated with droughty months, the moisture reserve renewed reaching very good values, close to optimal supply (60% of the interval of active moisture).

In the spring of 2014, the values of the accessible reserve maintained to values close to optimal, that is between 506-840 m^3/ha. Only in May and June the values of the accessible reserve dropped under the fading coefficient (-56 m^3/ha and -411 m^3/ha, in the conventional system; -46 m^3/ha and -139 m^3/ha in the no-tillage system).

The renewal of the water reserve during 2015-2016 had to suffer because of short torrential rain when the leakage from slopes was higher than the infiltrations in the soil. In the no-tillage system, the accesible water reserve is kept better in the soil even during drought, the depth water rises through capillaries to the radicular area compensating the lack of water due to drought. The water reserve in the soil in no-tillage is renewed harder than in the conventional system, but here the loss of water is as rapid. The higher degree of weeds in the first years of application in no-tillage leads also to a higher consumption of water, with influence on the wheat production.

The wheat production made during the experimental years 2012-2016 has significant differences, given by the soil tillage system and by the treatments applied, as well as by the climate conditions specific for the years taken into account in the study (Figure 5). Thus, compared to the conventional system without herbicides, where productions ranged between 2975-4275 kg/ha, in the no-tillage system productions ranged between 2987-4296 kg/ha. Except for 2015 where the production made was very close, in both soil tillage systems, with a difference of only 11 kg/ha (3995 kg/ha in the conventional system and 3984 kg/ha in the no-tillage system).

The wheat production achieved in the case of the c_2 variant - treatments including herbicides is significantly higher. In 2013, 5788 kg/ha were achieved in the no-tillage system and 5575 kg/ha in the conventional system, with a difference of 214 kg/ha. In 2012, 2015, 2016 productions ranged between 4919-7016 kg/ha in the no-tillage system. Thus, the no-tillage system influences significantly positive production and is suitable for the wheat crop in the Transylvanian Plain and it mandatory needs at least one treatment against weeds. The conventional soil tillage system recorded productions ranging between 4909-7007 kg/ha. 2014 (a rainy year) determined very close productions between the two soil tillage systems (6693 kg/ha in the conventional system and 6697 kg/ha in no-tillage system).



DL (p 5%) =7.6; DL (p 1%) =11; DL (p 0.1%) = 26

Figure 5. Interaction of factors tillage system x treatments x years on wheat production, 2012-2016

In achieving these wheat productions, a very important role besides the treatments applied was also played by the fractionated application of mineral fertilizers to supplement the reserve of nutritive elements in the soil, crop rotation, correctiveness of execution of all works the soil tillage system involves.

CONCLUSIONS

The no-tillage system determines in the case of wheat crop a higher degree of weeds during the first years of application, but it decreases starting with 2013, determining the progressive growth of wheat production. This decrease of weeds has a positive influence on the water reserve in the soil, and together with mulch influence the rhythm of loss of water in the soil in no-tillage.

The conventional system determines a more reduced total mass of weeds, of 18.5 t/ha in the variant of treatments without herbicides and 9.36 t/ha in the variant of treatments with herbicides. In the no-tillage system, the total mass of weeds was 25.5 t/ha in the variant of treatments without herbicides and 14.88 t/ha in the variant of treatments with herbicides.

The higher values of the accessible water reserve in the soil were recorded, during the first years, in the conventional soil tillage system, but at the same time a more rapid loss is recorded here than in the no-tillage system, where the accumulation of water in the soil is made harder but is lost slower.

The wheat production achieved in the conventional system in the variant without herbicides, during 2012-2016 recorded values ranging between 2975-4275 kg/ha, and in the no-tillage system a higher production was achieved, ranging between 2987-4296 kg/ha. In the no-tillage system where treatments with herbicides were applied, productions ranged between 4919-7016 kg/ha.

The land particularities from the hills in the Transylvanian Plain determined by relief, climate and soil condition impose the use of certain conservative soil tillage alternatives, as it is only such that certain objectives can be achieved, for example: the increase and capitalization of the soil capacity to preserve high water quantities and thus to avoid the surface and depth leaks; the decrease of the number of tillage and the avoidance of water evaporation, structure degradation and the decrease of soil erosion; the ensurance of a favorable crop state to reduce the soil erosion during critical times from the point of view of rain erosion and snow melting; the ensurance of durable growth and development conditions of crop plants.

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HEAVY METALS CONTENT IN ALFALFA CULTIVATED ON VERTISOLS ALONG THE HIGHWAY E75 FROM BELGRADE TO LESKOVAC (SERBIA)

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Abstract

In order to assess the health and safety of animal feed, in ten samples of soil and plant material, collected along the E75 highway from Belgrade to Leskovac, it was examined the content of heavy metals: Cr, Cu, Ni, Pb, Co, As, and their accumulation in alfalfa (Medicago sativa L.) grown on vertisols. Samples of soil and aerial part of the plant material were collected from the both sides of lanes at locations where the studied plant species was cultivated, at 10, 30, 50 and 400 m perpendicular to the direction of the highway. Soil and plant analyses of the metals content were done according to ICP methodology. Analysis of the soil samples showed the following: the content of total forms of Cr and Ni was above the maximum permissible concentration (MPC) in 50% of samples tested; the content of Pb was above the MPC in 30% of samples tested; in the other soil samples the values of the examined parameters were within permissible limits. In five of the ten tested plant samples Cr content was above the toxic concentrations, and in one sample it was above the maximum tolerance levels for animal feed. In one sample of alfalfa it was determined the contents of As and Pb above the toxic levels. In addition, the concentrations of Co and Pb above the normal levels were registered in one sample of plant material, but they were below the maximum tolerance levels for animal feed. The obtained results suggest a caution in the use of alfalfa, grown near the highway route, for animal feed, because of the potential entry of heavy metals into the food chain. The study also revealed that increased concentrations of analyzed elements occurred at all distances from the route lanes.

Key words*: heavy metals, vertisols, animal feed, highway.*

INTRODUCTION

In the part of the E75 motorway, section of Belgrade to Leskovac, in the Republic of Serbia, during the 2010, was studied the impact of the highway on the heavy metal accumulation in the alfalfa plants, grown on soil type vertisols. Samples of soil and plant material were taken at a distance of 10, 30, 50 and 400 m from both sides of the lanes (Pivić et al., 2013). According to Jankievicz et al. (2010), the rapid development of industry, the increase in the number of inhabitants and the intensification of road traffic are among the most important causes of ecosystem pollution in urban areas. Heavy metals are found everywhere in the environment, either as a result of natural or anthropogenic activities, which makes the eco system exposed to the pollution in different ways (Wilson and Pyatt, 2007). Environmental risk assessment of soil contamination is particularly important for agricultural areas, due to the fact that heavy metals potentially harmful to human health exist in the soil and can be transferred in significant quantities to the food chain (Szinkovska et al., 2009). Heavy metals are found in fuels, fuel tanks, motors and other components of vehicles, in catalytic converters, tires and brakes, as well as surface materials on roads (Deska et al., 2011) and as such represent potential pollutants. Adoption of microelements depends on the type and age of plants, and their accumulation varies depending on the plant species and the organ in which it accumulates. Zn, Mn, Ni and B are unequally distributed at the root and in the above-ground part of the plant. Cu, Cd, Co, Mo are more represented at the root than in the tree, while the content of Pb, Sn, Ag, Cr is higher at the root and lower in the tree (Kastori et al., 1997). Microelements are required for plants growth in very small quantities, and if supply is inadequate, there may be disorders in physiological-biochemical processes, reduction in growth and yield, and in the food chain may cause disorders of human and animal feeding (Szinkovska et al., 2009; Pivic et al., 2017).

MATERIALS AND METHODS

Field of study and sampling

Sampling of soil and above-ground part of alfalfa plant material was carried out on the section of E75 from Belgrade to Leskovac during the vegetation period during August and September 2010 (Figure 1). Based on the coordinates of the sampling site registered with the GPS device, using the data from the pedological map of the Republic of Serbia (Institute of Land, Mrvić et al., 2013), the locations of the soil type vertisol (WRB, 2014) were determined, from which a plant species, alfalfa was sampled and studied.

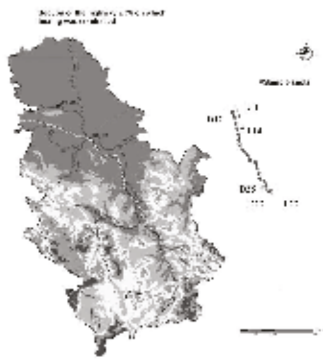

Figure 1. Soil and plant sampling spots in the section of the study with corresponding distances and coordinates

Sample spots (m from route lanes)	Coordinates	
	X	Y
L11(400)	7501072	4931838
D12(30)	7503516	4923959
D12(50)	7503495	4923944
L14(30)	7507509	4909703
L14(50)	7507529	4909705
D26(50)	7544419	4836338
D27(30)	7548003	4830378
D27(50)	7547990	4830558
L27(30)	7548175	4830567
L27(30)	7548193	4830583

Ten soil samples in the disturbed state were sampled to a depth of 30 cm at a different distance from the highway, at 10, 30, 50 and 400 m from the road lanes. The alfalfa plant material, the above-ground part was sampled, with the remark that the year in which it was planted was not recorded. The average sample consisted of 15 to 20 individual samples, where by the cut was carried out by hand cutting at a height of 3-5 cm of the plant.

Preparation and analysis of tested soil samples

In ten composite soil samples prepared in accordance with SRPS ISO 11464: 2004 - Pretreatment of samples for physical-chemical analyzes, sieved through a sieve of 2 mm in diameter, the pH is determined in 1M KCl, potentiometrically (SRPS ISO 10390: 2007 - Determination of pH), calcium carbonate by volumetric method SRPS ISO 10693: 2005- Determination of carbonate content, total contents C, N, S was analyzed on elemental CNS analyzer Vario EL III (Nelson et al, 1996). SOM (soil organic matter) was calculated using the formula:

SOM content (%) = organic C content (%) x factor 1.724 (Džamić et al., 1996)

Available P_2O_5 (determined using spectrophotometry) and K_2O (determined using flame emission photometry) were analyzed by AL-method according to Egner-Riehm (Riehm, 1958). Ca and Mg were extracted by ammonium acetate and determined with an atomic adsorption analyzer SensAA Dual (GBC Scientific Equipment Pty Ltd, Victoria, Australia) (Wright and Stuczynski, 1996). The total contents of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and As in soil samples were determined by inductively coupled plasma-atomic emission spectrometry - THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES, after the digestion of the samples with aqua region (ISO 11466: 1995 Soil quality - Extraction of trace elements soluble in aqua region; ISO 22036: 2008 Soil quality - Determination of trace elements in extracts of soil by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The concentration of trace elements Hg was determined by a flame atomic adsorption spectrophotometer (AAS, GBC, SENSA DUAL HG), method by hydration after the so-called "wet" combustion of samples, i.e. boiled in the mixture of concentrated acids:

HNO_3 and H_2O_2, with filtration and the necessary dilution (AA Hydride system HG 3000, EHG 3000 & MC 3000 Operation & Service manual, 1995).
Reference soils NCS ZC 73005, Soil Certificate of Certified Reference Materials approved by China National Analysis Center Beijing China, and reagent blanks were used as the quality assurance and quality control (QA/QC) samples during the analysis.

Collection, preparation and analyses of the plant material

Medicago sativa L. (Alfalfa) is a perennial leguminous crops, which is regarded as the leading and most important forage crop for the production of high quality feed, and is used in the fresh state and conserved as well as hay, haylage, silage, meal, pellets and pasta (Vučković, 2004; Jakšić et al., 2013). The samples of plant material are air-dried and milled. The sampled plant material were dried at 105^{o}C for a period of 2 hours, using gravimetric method for determination of dry matter contents of plant tissues (Miller, 1998). The contents of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and As in aerial parts were determined in triplicates with THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES after the digestion of the samples with concentrated HNO_3 and redox reaction with H_2O_2 for total forms extraction (Soltanpour et al., 1996). Calibration standards were in the range of 0-10 ppm, except for iron (0-25 ppm).

Processing results

The results of the conducted soil analyze represent the arithmetic means of three replicates of each sampling, their ranges and standard deviations values. The data on microelements and heavy metal concentrations in the studied plant species are presented by figures as bar charts with standard deviation values.

RESULTS AND DISCUSSIONS

Soil type Vertisol WRB (2014) on which was grown tested plant material alfalfa were formed on the substrates containing more than 30% of clay predominantly montmorilonite type.
Vertisols are soils with unfavorable water-air and thermal regime, and some of their properties are the characteristics of hydrogen soils. It swells in a wet state, and in dry cracks with the formation of cracks that can reach one meter in depth. The chemical properties of these soils are considerably more favorable. They are characterized by neutral to low alkaline reaction with high adsorption capacity. Base saturation is up to 90%. Humus, total nitrogen and easily accessible potassium are generally well provided, and in terms of availability of easily accessible phosphorus vertisols are quite poor. These type of soils in terms of benefits for plant production belongs to the third rating class.
Interpretation of the obtained results was carried out on the basis of the Ordinance on the permitted quantities of hazardous and harmful substances in soil and irrigation water and methods of their examination (Official Gazette of RS, 1994), within which the maximum allowed quantities of hazardous and harmful substances have been defined.
The chemical reaction of the tested soil samples ranges from slightly acidic to neutral. The carbonate content is not registered. In relation to the content of easily accessible phosphorus, the examined soils are medium to very high; while the supply of easily accessible potassium is high. The humus content ranges from medium to high supply. In relation to the content of total forms of heavy metal in five soil samples on the positions D16 at 30 and 50 m away from the route lanes, D17 and L17 at 400 m distance from the route lanes and D19 at 30 m away from the highway is determined the content of Cr, and in three samples the Pb content (position D17 and L17 at 400 and D19 at 30 m distance from the route lanes) higher than the maximum allowed concentration (MAC) in the soil sample (Official Gazette of RS, 1994).
The chemical properties of the soil samples tested are shown in Table 1.

Table 1. Properties and composition of Vertisols (means ± standard deviation and intervals)

Property	Value	Property	Value
pH in 1M KCl	5.87±0.44 (5.10-6.60)	Total content of As (mg kg^{-1})	10.71±5.62 (5.07-20.81)
Total content of $CaCO_3$ (%)	below the detection limit	Total content of Cr (mg kg^{-1})	104.13±52.21 (43.09-186.71)
Available P_2O_5 (mg 100 g^{-1})	17.93±12.13 (5.32-40.27)	Total content of Ni (mg kg^{-1})	88.31±55.83 (35.96-192.40)
Available K_2O (mg 100 g^{-1})	32.99±4.32 (27.60-37.30)	Total content of Pb (mg kg^{-1})	64.24±40.57 (26.66-121.20)
Total content of N (%)	0.25±0.08 (0.10-0.35)	Total content of Hg (mg kg^{-1})	0.12±0.09 (0.05-0.31)
Total content of C (%)	2.23±0.81 (0.91-3.57)	Total content of Zn (mg kg^{-1})	67.32±30.69 (40.86-130.11)
Total content of S (%)	0.04±0.02 (0.02-0.08)	Total content of Cd (mg kg^{-1})	1.20±0.55 (0.41-1.95)
SOM (%)	3.84±1.39 (1.57-6.16)	Total content of Cu (mg kg^{-1})	27.29±7.59 (18.29-40.19)

The content of Fe, Mn, Cu, Zn, Ni, Cr, Cd, As, Co, Pb, Hg is determined in the samples of plant material (above-ground biomass sampled for study purposes).

Figures 2-4 show the mean values and standard deviation of the concentration of microelements and heavy metals in the analyzed samples of plant material.

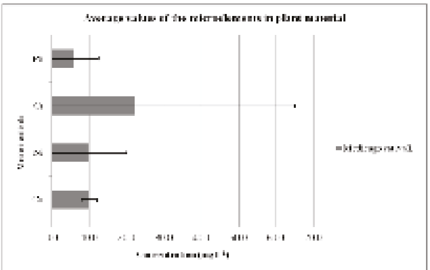

Figure 2. Concentrations of copper (Cu), nickel (Ni), chromium (Cr) and lead (Pb) in the plants aerial parts (mg kg^{-1})

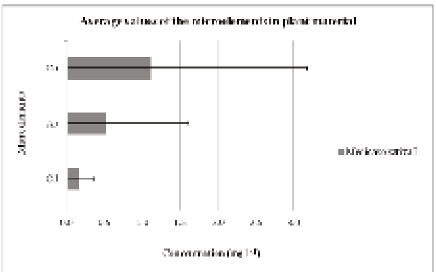

Figure 3. Concentrations of cadmium (Cd), arsenic (As) and cobalt (Co) in the plants aerial parts (mg kg^{-1})

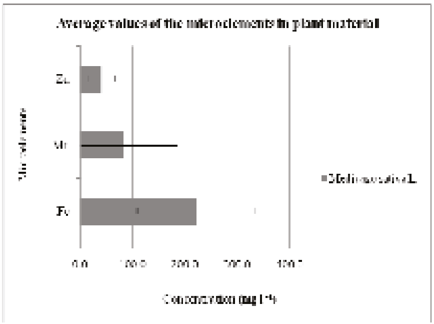

Figure 4. Concentrations of iron (Fe), manganese (Mn) and zinc (Zn) in the plants aerial parts (mg kg^{-1})

The table 2 shows the reference values of the content of microelements in plants relative to normal and toxic concentrations.

Table 2. Reference values for trace elements content in plants according to literature sources

Element	Normal concentrations (mg kg^{-1})	Toxic concentrations (mg kg^{-1})	Maximum tolerant level for fooder (mg kg^{-1})
Cu	3-15[a]	20[b]	12-50[g]
Ni	0.1-5[a]	30[b]	50[g]
Pb	1-5[a]	20[b]	40[g]
Cr	<0.1-1[a]	2[b]	
Cd	<0.1-1[a]	10[b]	1[g]
Mn	15-100[e]	400[b]	
Zn	15-150[a]	200[b]	2000[g]
Co	0.05-0.5[e]	30-40[d]	
Fe	50-250[f]	(>500)[f]	1250[g]
As	10-60[c*]	<2[c]	4[g]

μg kg^{-1}; reference values: [a] *Kloke et al. (1984)*; [b] *Kastori et al. (1997)*; [c]*Kabata-Pendias and Mukherjee (2007)*; [d] *Kabata-Pendias (2011)*; [e]*Misra and Mani (1991)*, [f]*Schulze et al. (2005)*; [g]*NRC* (2005), *Adams (1975)*

Iron (Fe) can be accumulated in plants without any harmful effects (Marić et al., 2013; Simić et al., 2015; Pivić et al., 2017), so it is not uncommon that the contents of this element could be higher than the MPC. The concentrations of Fe adopted by plants in addition to the species and stages of growth, depends on the soil properties. In all tested samples of alfalfa grown on vertisol, the toxic contents of this element were not registered.

The most important role of Mn is reflected in participation in oxidation-reduction processes. Due to rapid transmission through the plant, it is most accumulated in young plant organs and less in the root. In organs of plants, Mn occurs in excess when there are high levels of this element in the soil, low pH and high redox potential (Misra and Mani, 1991; Kastori et al., 1997). No toxicity of this element (>400 mg kg^{-1}) was detected in nine tested alfalfa samples except in sample D33 at a distance of 50 m from the highway route in which the concentration of Mn in the plant material was 277.88 mg kg^{-1} and above normal concentrations.

Copper belongs to the category of micronutrients that have an important role in photosynthesis, respiration, carbohydrate metabolism, nitrogen reduction and fixation, protein metabolism etc. The rate at which the plant adopts the copper largely depends on the type of plant, but also on the origin of the copper present. Young plant organs are particularly exposed to copper deficiency. The surplus of this element usually occurs in acidic soils, and as copper is one of the most vulnerable elements in the acidic environment, its accessibility for plants is growing. Sensitive plant species on copper toxicity are cereals, legumes and spinach. For normal development, copper is required by plants in small amounts of 5-20 mg kg^{-1}, less than 4 mg kg^{-1} is considered a deficit, and more than 20 mg kg^{-1} may cause toxicity (Kloke et al., 1984; Kastori et al., 1997). In the tested alfalfa samples, the toxic value of copper was not registered.

Zinc is an essential nutrient for plant growth and is involved in significant metabolic processes. Soluble forms of zinc are easily accessible for the plant, and the adoption of this element is in linear relationship with the content of this element in the nutrient solution or the soil. The content of zinc in the tested samples of plant material is not registered above toxic values (> 200 mg kg^{-1}).

Adoption of nickel depends on soil properties and the properties of the plant itself. The most important factor is the pH value of the soil. In order to adopt this element, its origin is very important, as studies indicates that anthropogenic deposited nickel is much easier to adopt by plants (Kloke et al., 1984; Kastori et al., 1997). The content of nickel in unpolluted soils varies and depends on ecological and biological factors. Since the nickel is easily mobile in plants, usually all parts of plants show a high concentration of this element. In the tested samples of plant material, nickel content was in five samples above the normal value, but below the toxic level of the above-ground plant mass, for the tested samples from the location D16 (30 and 50 m), L17 (400 m), L23 (30 m) and D33 (50 m) distance from the route lanes. This corresponds to the zones where in the soil has also been determined that the total nickel is above the MPC.

The content of chromium in plants varies and depends largely on the geological substrate. The source of chromium is also a significant factor that affects the solubility and availability of these elements (Adams, 1975; Kloke et al., 1984; Kastori et al., 1997; NRC, 2005). The concentration of chromium is almost always higher in the root than in leaves or trees, while the lowest quantities are recorded in the fruits. The chromium content in the test samples, except for the sample at the location of D33 (at 50 m away from the route lanes) does not exceed the toxicity values of this element for animal feed (50-3000 mg kg^{-1}). In sample D33, the content of chromium in the plant material is 103.35 mg kg^{-1}.

Cadmium is one of the most toxic and harmful elements that adversely affects soil biological activity, plant metabolism and human and animal health. It is easily absorbed through the root system and accumulated in the aboveground plant parts. The pH of the soil solution is cited as the main factor in the adoption of cadmium. The origin of cadmium is also an important factor that affects the solubility and availability of this element. The content of cadmium in the tested samples of plant material ranges in the range of normal values (up to 10 mg kg^{-1}).

Different plant species show a different degree of tolerance in relation to arsenic. Leguminous species are susceptible to arsenic. The most common result of a high content of this element in the soil is a reduced yield of (Kabat Pendias and Mukherjee, 2007; NRC, 2005). In the tested alfalfa samples, the toxic value of the arsenic content was recorded in one sample at the D33 site (50 m from the route lanes).

Measuring the content of cobalt in plant mass has become very important when it was observed that the lack of this element in the soil and consequently in the plant mass causes a disease for sheep, goats and other livestock. In the soil, this element is usually found as a side element of iron, nickel and other heavy metals. In the tested samples of the plant material, a value higher than the normal values was registered, in a sample at D33 (50 m), which was 5.00 mg kg^{-1}, while in other examined alfalfa samples, the value was within normal limits.

Lead is the least mobile element among the microelements of the soil (Kabata-Pendias A., 2011). Lead is poorly adopted and transferred to the above-ground organisms of the plant, except on acidic soils. Plants can accumulate lead either from the soil or absorbed from the air. Most of the lead from the soil is not available to plants.

Non-oganic lead forms become accessible to plants only in acidic soils (Wiklander and Vahtras, 1977). Lead originating from the air is the main source of pollution by this element. According to some studies, about 95% of the total amount of lead in the plant can be originated from the air. In a sample at the D16 site (30 m), the lead concentration was above the toxic values (>20 mg kg^{-1}) and in samples D16 (50 m) and D33 (50 m) above normal values (>5 mg kg^{-1}). Increased content of this element which was in the range of critical concentrations for animal feeding (10-30 mg kg^{-1}) was registered in the sample at the location of D16 (30 m).

Mercury content in all tested samples of alfalfa is below the limit of detection therefore is not presented in the graphic.

CONCLUSIONS

The total content of As, Cd, Zn, Co, and Hg in all tested samples of soil type vertisol were within the limits of maximum permissible concentration (MPC). In the samples from sites D17 (400 m), L17 (400 m) and D19 (30 m) and on the site D16 at a distance of 30 and 50 meters from the route lanes, the total contents of Cr and Ni was above the MPC. The content of lead above MPC was determined in samples D17 and L17 (400 m) and D19 (30 m). In addition, anthropogenic pollution, which is reflected in excessive use of the preparation of pesticides and fertilizers, as well as the air pollution originating from motor vehicles, in some sections of the tests, it is evident geochemical pollution of soil.

In the tested biomass of alfalfa, content of increased concentrations of certain elements with respect to the normal value, is registered in a certain location and at a distance usually 30-50 m from the motorway route. In the sample at the site D33 (50 m) the chromium content exceeds the toxicity values of this element for animal feed (50-3000 mg kg^{-1}). The content of arsenic above toxic concentrations was registered in only one sample, also at D33 (50 m from the route lanes). In a sample at the D16 site (30 m), the lead concentration is above the toxic values (>20 mg kg^{-1}) and in samples D16 (50 m) and D33 (50 m) above the normal values (>5 mg kg^{-1}). It is to be expected that this element is most present in the immediate vicinity of roads as a product of exhaust gases of motor vehicles. The increased content of critical concentrations of lead for animal feed (10-30 mg kg^{-1}) was recorded in the sample at D16 (30 m).

The obtained results indicate caution for cultivation of alfalfa in near vicinity of route lanes for the animal feeding due to the possible entry of heavy metals into the food chain.

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THE EFFECTS OF DIFFERENT SALT DOSES ON YIELD AND NUTRIENT UPTAKE OF TOMATO PLANT

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Abstract

The salinity is a problem in agriculture due to improper use of fertilizer, inadequate irrigation and drainage. This is one of the most important agricultural problems in many parts of the world, especially in arid and semi-arid soil ecosystems. The present study was conducted to investigate the effects of different salt doses on yield and nutrient uptake of tomato plant. The study has been carried out with three replications according to the experimental pattern of randomized plots in the plastic pots with the capacity of 3 kg under the greenhouse conditions. In the study 5 salt doses were applied: 0 dS m^{-1}, 3 dS m^{-1}, 6 dS m^{-1}, 9 dS m^{-1}, 12 dS m^{-1} and NaCl was used as a source of salt. The tomato plants was harvested before flowering and shoot dry weight, macro and micro elements concentrations were determined. The findings have shown that increasing salt doses decreased shoot dry weight of tomato plant. The highest shoot dry weight was determined with 37.33 g pot^{-1} in 0 dS m^{-1} application. Also, N, Ca, Mg, Fe and Cu concentrations were decreased with salt applications. However, the highest P and Zn concentrations were 0.159% P and 30.1 mg kg^{-1} respectively with 12 dS m^{-1} application. Generally, the salt applications didn't affect the yield and macro and micro element concentrations except for P and Zn of tomato plant.

***Key words**: salt, tomato, yield, nutrient uptake.*

INTRODUCTION

Worldwide, more than 800 million ha land area is under the threat of salinity and alkalinity (FAO, 2009). Such problems are experienced over 1.5 million ha land area in Turkey (GDRS, 2011). Salinity is the primary environmental factor limiting and reducing soil fertility and plant yields in various parts of the world, especially in arid and semi-arid regions (Greenway and Munns, 1980). Plants are continuously exposed to various biotic and abiotic stressors and environmental stress factors (Iranbakhsh et al., 2018). The stress factors effecting plants are classified as biotic (plants, microorganisms, animals and anthropogenic impacts) and abiotic (radiation, temperature, water, gases, minerals etc.) stress factors (Larcher, 1995). Salinity is an important abiotic stress limiting plant growth and yields (Zhu, 2016). High soil and water salinity levels significantly restrict agricultural productions in arid and semi-arid regions (Al-Karaki, 2000). It is estimated that salt stress might result in about 50% loss in agricultural productions (Kreps et al., 2002).

Salt stress is generally originated from high soil sodium (Na) and chloride (Cl) concentrations (Ismail et al., 2014). Specific impacts of salt stress on plant metabolism are related to depletion of K and Ca with the accumulation of toxic ions (Na and Cl) (Munns et al., 2002). Soils contaminated with high concentrations of sodium and chloride ions inhibit plant ion uptake and absorption of essential ions (K, Ca, NO_3) through root system (Ashraf and Foolad, 2007). The plants grown in saline ambient have several disadvantages. High salt concentrations of soil solution reduce soil water potential and increase osmotic stress. Increasing sodium (Na) and chloride (Cl) concentrations and Na and Cl accumulation in plant tissue inhibit mineral nutrient uptake of the plants (Marschner, 1995). Salinity is the greatest abiotic stress factor reducing agricultural productivity and influence large area worldwide. Therefore, a need has emerged to grow salt-resistant plants over these lands (Yamaguchi and Blumwald, 2005). Plants have different threshold values against salt stress, some are sensitive (glycosides), some moderately resistant and the rest highly resistant to salinity (halophytes) (Menzel and

Lieth, 2003). Except for halophytes, plant growth and development is negatively influenced by saline conditions (Bewley and Black, 1994). Significant changes are observed in morphologies of the plants grown under salt stress. Effects of salinity on plants generally emerge as smaller leaves, shorter plants, less number of leaves and recessed growth and development. Salinity generally inhibits plant growth and reduce yield levels (Al-Karaki, 2000). Plant sensitivity to saline conditions may vary based on growth stages. Salt has the greatest impact on plant growth and development especially in the germination period (Taiz and Zeiger, 2002). Therefore, salt tolerance of plants should be investigated at different growth stages and threshold values should be determined for each growth stage (Zapata et al., 2004).

Tomato is a significant greenhouse and field crop cultured in semi-arid regions of Mediterranean countries (Sekmen et al., 2005). Tomato (*Solanum lycopersicum* L.) belongs to *Solanaceae* family and has the greatest commercial consumption among the vegetables. Tomato is quite rich in β-carotenenu-cretonne, lycopene, flavonoids, ascorbic acid and other nutrients and all these elements make tomato an effective anti-oxidative and anti-carcinogenic foodstuff (Ahmad et al., 2018). It was proved that 100 gram tomato contained 0.55 mg vitamin B6, 1700 IU vitamin A, 0.10 mg vitamin B1 and 21 mg vitamin C (Sevgican, 1981). With regard to salt tolerance, tomatoes are classified as moderately resistant (Maas, 1986). Knowledge about salt and heat tolerance of the plants to be grown in saline soils will undoubtedly have great contributions to producers both in time and economic aspects (Doğan et al., 2008).

In this study, effects of salt treatments at different doses on yield and nutrient uptake of tomato plants were investigated.

MATERIALS AND METHODS

This study was carried out at greenhouses of Plant and Animal Production Department of Cumhuriyet University Sivas Vocational School. Experiment was conducted in randomized plots design with 3 replications. Experimental soils were taken from 0-20 cm soil profile of experimental fields of the department. Soils were sieved through 2 mm sieve and 3 kg air-dried soils were placed in experimental pots. Soil physical and chemical characteristics are provided in Table 1. Experimental soils were silty-clay-loam in texture, slightly alkaline (pH 7.25), highly loamy (16.2%), unsaline (0.031%), poor in available phosphorus (38.1 kg P_2O_5 ha^{-1}) and sufficient in potassium (942.0 kg K_2O ha^{-1}).

Before sowing, 200 mg N kg^{-1} (in the form of $CaNO_3.4H_2O$), 100 mg P kg^{-1} and 125 mg K kg^{-1} (in the form of KH_2PO_4), 2.5 mg Zn kg^{-1} (in the form of $ZnSO_4.7H_2O$) and 2.5 mg Fe kg^1 (in the form of Fe-EDTA) were applied to each pot as basic fertilizers.

Salt concentrations were arranged as 0 dS m^{-1}, 6 dS m^{-1}, 9 dS m^{-1} and 12 dS m^{-1} by using NaCl. Industrial-type H 2274 tomato cultivar was used as the plant material of the study. Seeds were sown in turf-perlite mixtures (1:1 V/V) in greenhouse, irrigated regularly and seedlings were obtained. Half (1/2) of the salt doses was incorporated into soils during the transplantation of the seedlings into the pots and remaining portion was applied through irrigation water when the seedlings had 7-8 leaves.

Table 1. Physical and chemical properties of experimental soil

Texture	pH	Tuz (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Organic Matter (%)	Lime (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
SiCL	7.25	0.031	38.1	942.0	1.2	16.2	3.25	0.42	2.49	1.21

Plant Analyses

Leaf samples were taken from the tomato plants at the beginning of flowering and harvest was performed then. Vegetative parts of the plants were washed through tap water, rinsed respectively through distilled water, 0.1% N HCl solution and twice though again distilled water. They were placed over coarse filter papers and excess water over them was

removed. Plant parts were then placed in separate paper bags and dried at 65°C until a constant weight. Following the measurement of dry weights, dry samples were ground in a plant mill. About 0.2 g of ground samples were wet digested in H_2O_2-HNO_3 acid mixture in a microwave oven.
Resultant slurry was then completed to 20 ml with distilled water and filtered through blue-band filter paper. Samples were then subjected to P colorimetric measured at 882 nm in a spectrophotometer (Murphy and Riley, 1962), K, Ca, Mg, Zn, Mn, Fe and Cu in an AAS (Atomic Absorption Spectrophotometer) (Shimadzu AA-7000) (Kacar and Inal, 2008). N contents were determined with Kjeldahl distillation method (Bremner, 1965).

Data Assessment

Experimental results were subjected to variance analyses (ANOVA) separately in accordance with randomized plots experimental design. SPSS 22.0 Windows software was used for statistical analyses. Means were compared with Tukey's test at $P<0.05$.
Correlation analysis was performed to assess the relationships between the treatments.

RESULTS AND DISCUSSIONS

Effects of different salt treatments on dry matter production of tomato plants were investigated and results are presented in Figure 1.

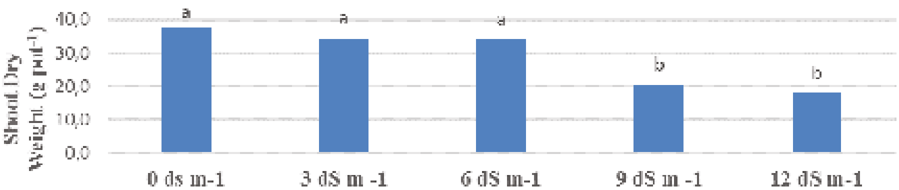

Figure 1. The effects of different salt doses on shoot dry matter production of tomato plant

With regard to shoot dry matter production of tomato plants, the greatest value (37.33 g pot^{-1}) was obtained from the control (0 ds m^{-1}) treatment. Dry matter productions of 3 dS m^{-1} and 6 dS m^{-1} treatments were not significantly different. Decreasing dry matter productions were observed with increasing salt concentrations. Previous researchers also reported decreasing dry matter productions with increasing salt treatments. Al-Karaki (2000) carried out a salt stress study with 3 different tomato cultivars and reported decreasing root dry matter productions with increasing salt concentrations. Cicek and Cakirlar (2002) reported decreasing maize fresh and dry weights with increasing salt stress. Khaled and Fawy (2011) indicated that salt stress negatively influenced maize growth and development and reduced dry matter productions.

Table 2. Effects of different salt doses on N, P and K concentrations of tomato plant (%)

Salt Doses	N	P	K
0 dS m^{-1}	3.10 ±0.02 ^{a}	0.089 ±0.01 ^{b}	4.93 ±1.09 ^{a}
3 dS m^{-1}	2.59 ±0.04 ^{ab}	0.097 ±0.01 ^{b}	4.74 ±0.84 ^{a}
6 dS m^{-1}	2.43 ±0.37 ^{b}	0.102 ±0.00 ^{b}	4.33 ±0.41 ^{ab}
9 dS m^{-1}	2.54 ±0.28 ^{ab}	0.072 ±0.02 ^{b}	4.23 ±0.12 ^{ab}
12 dS m^{-1}	2.88 ±0.57 ^{ab}	0.159 ±0.03 ^{a}	3.34 ±0.84 ^{b}

With regard to N concentrations of tomato plants, the greatest value (3.10%) was obtained from the control (0 dS m^{-1}) treatments as it was in shoot dry matter productions (Table 2). Control treatments was followed by 12 dS m^{-1} treatment with 2.88% N. Yokas et al. (2008) carried out a salt stress study with F1 Target tomato cultivar under greenhouse conditions and reported decreasing N concentrations with increasing salt concentrations. As it was in several studies, Alam (1994) also indicated that

Na intrusion reduced N concentrations. Contrary to N concentrations, P concentrations of tomato plants increased with salt treatments. While P concentration of 0 dS m^{-1} control treatment was 0.089% P, the value was measuredas 0.159% P in 12 dS m^{-1} treatment. The 12 dS m^{-1} treatment was found to be significant with regard to % P concentration as compared to other treatments. With regard to K concentrations of tomato plants, the greatest K concentration (4.93% K) was obtained from 0 dS m^{-1}control treatment. Control treatment was followed by 3 dS m^{-1} treatment with 4.74% K, but the differences between these two treatments were not found to be significant. Kuşvuran et al. (2008) carried a salt stress study with salt-tolerant and sensitive *Cucumis* sp. genotypes and reported significant increases in Na and Cl ions and decreases in K concentrations. Similar findings (decreasing K concentrations) were also reported in various other salt stress studies carried out with tomatoes (Yokas et al., 2008) and soybean (Baran and Doğan, 2014).

Table 3. Effects of different salt doses on Ca and Mg concentrations of tomato plant (%)

Salt Doses	Ca	Mg
0 dS m^{-1}	0.91 ±0.10 ^{a}	0.46 ±0.10 ^{a}
3 dS m^{-1}	0.89 ±0.04 ^{a}	0.46 ±0.07 ^{a}
6 dS m^{-1}	0.81 ±0.03 ^{ab}	0.42 ±0.03 ^{a}
9 dS m^{-1}	0.75 ±0.06 ^{b}	0.36 ±0.03 ^{a}
12 dS m^{-1}	0.76 ±0.04 ^{b}	0.35 ±0.03 ^{a}

With regard to Ca concentrations of tomato plants (Table 3), the greatest values (0.91% and 0.89% Ca) were respectively observed in 0 dS m^{-1} and 3 dS m^{-1} treatments which were placed in the same statistical group. Increasing % Ca concentrations were observed with increasing salt doses. Yakıt and Tuna (2006) applied salt stress to maize plants and reported decreasing Ca concentrations with the intrusion of Na ions. Similar to % Ca concentrations, decreasing Mg concentrations were observed in tomato plants with increasing salt doses. The greatest Mg concentrations were obtained from 0 dS m^{-1} and 3 dS m^{-1} treatments with 0.46% Mg. The changes in plant Mg concentrations under different salt doses were not found to be significant. Similarly, decreasing Mg concentrations were reported for soybean plants with increasing salt doses (Baran and Doğan, 2014).

Table 4. Effects of different salt doses on Fe, Zn, Mn and Cu concentrations of tomato plant (mg kg^{-1})

Salt Doses	Fe	Zn	Mn	Cu
0 dS m^{-1}	126.5 ±15.94 ^{a}	25.3 ±2.72 ^{b}	33.9 ±12.71 ^{ab}	9.4 ±0.23 ^{a}
3 dS m^{-1}	74.3 ±6.22 ^{c}	24.1 ±3.05 ^{b}	31.9 ±3.90 ^{ab}	7.7 ±1.63 ^{b}
6 dS m^{-1}	86.6 ±8.77 ^{bc}	25.6 ±2.59 ^{b}	40.2 ±5.35 ^{a}	8.0 ±0.31 ^{ab}
9 dS m^{-1}	55.9 ±6.47 ^{c}	27.8 ±0.99 ^{ab}	31.4 ±1.17 ^{ab}	8.6 ±0.06 ^{ab}
12 dS m^{-1}	106.4 ±2.97 ^{ab}	30.1 ±0.36 ^{a}	27.2 ±2.15 ^{b}	9.3 ±0.21 ^{a}

With regard to micro element concentrations of tomato plants, Fe concentrations varied between 55.9 mg kg^{-1} Fe and 126.5 mg kg^{-1} Fe with the greatest value (126.5 mg kg^{-1} Fe) in control (0 dS m^{-1}) treatment followed by 12 dS m^{-1} treatment with 106.4 mg kg^{-1} Fe (Table 4). The greatest plant Zn concentration (30.1 mg kg^{-1} Zn) was obtained from 12 dS m^{-1} treatment and the lowest Zn concentration (24.1 mg kg^{-1} Zn) was obtained from 3 dS m^{-1} treatment. With regard to plant Mn concentrations, the greatest value (40.2 mg kg^{-1} Mn) was observed in 6 dS m^{-1} treatment and it was followed by 0 dS m^{-1} control treatment with 33.9 mg kg^{-1} Mn. Different salt doses did not have significant effects on plant Cu concentrations. The greatest value (9.4 mg kg^{-1} Cu) was obtained from 0 dS m^{-1} control treatment and it was followed by 12 dS m^{-1} treatment with 9.3 mg kg^{-1} Cu. Khaled and Fawy (2011) applied different salt doses (0 mM, 20 mM, 60 mM) to maize plants and reported increasing Fe, Cu and Mn concentrations at 20 mM NaCl, but decreasing values at the greatest salt dose of 60 mM.

There was a negative correlation between shoot dry matter production and Zn concentration and there was a positive correlation between N and Fe (Table 5). While there was a positive relationship between Ca and Mg (P<0.01), there was a negative relationship between Ca and Zn (P<0.05). There were also negative correlations between Mg and Zn (P<0.01), positive correlation between Mg and Mn (P<0.05), between Fe and Cu and negative correlations between Zn and Mn and between Zn and Cu (P<0.05).

Table 5. Correlation values of parameters evaluated in the study

Parameters	SDW	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
SDW	1									
N	.009	1								
P	-.375	.237	1							
K	-.240	.197	.080	1						
Ca	.407	.314	-.276	.288	1					
Mg	.440	.091	-.386	.221	.891**	1				
Fe	.200	.648**	.370	.050	.332	.172	1			
Zn	-.669**	.119	.499	.194	-.624*	-.707**	.110	1		
Mn	.273	-.293	-.415	-.340	.421	.632*	-.124	-.552*	1	
Cu	-.312	.532*	.251	.323	-.019	-.088	.580*	.595*	-.218	1

*Significant at P<0.05 **Significant at P<0.01

CONCLUSIONS

This study was carried out to determine the effects of salt treatments at different doses (0 dS m^{-1}, 3 dS m^{-1}, 6 dS m^{-1}, 9 dS m^{-1}, 12 dS m^{-1}) on yield and nutrient uptake of tomato plants. Present findings revealed that salt treatments reduced yields of tomato plants. Salt treatments had significant effects on P, Zn and Mn concentrations and reduced some macro element (N, K, Ca, Mg) and micro element (Fe, Cu) concentrations. Excessive salinity result in yield and quality losses, thus economic loses. Present findings indicated that a special attention should be paid while selecting plant species to be grown over saline lands and salt-resistant tomato cultivars should be selected in this case to grown tomato over saline soils. Further research is recommended to be carried out about the effects of different salt concentrations on different plants for sustainable agriculture over saline lands.

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COMPARING THE EFFECTS OF COMPOST AND VERMICOMPOST ON CORN GROWTH, NUTRIENT CONCENTRATION AND UPTAKE DURING THE DIFFERENT GROWTH PERIODS

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Abstract

In this study, it was aimed to compare the effects of vermicompost (VC) and compost (C) containing same originated organic materials (oil rose processing waste, dairy manure, poultry manure and straw on materials) on the growth, nutrient concentrations and nutrient uptakes of corn plant. Vermicomposts and compost were applied with the rates of 0, 10, and 30 t ha^{-1}to 2 kg soil containing pots. Study was conducted in glasshouse conditions for eight weeks with three replicates. During the growing periods plants were harvested 2 weeks intervals. After each harvest, plants were analysed for mineral nutrient concentrations (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) and nutrient uptakes were calculated using dry weight and nutrient concentrations. No significant differences were observed between C and VC in terms of plant dry weight, but increasing dosage of both C and VC significantly increased plant dry weight. Plant Ca, Mn and Zn concentrations were significantly affected from the individual effects of sources (C and VC) and also Ca, Fe and Zn concentrations were significantly affected from source x week interactions. Plant N and other micronutrient concentrations increased with the dosage. While only plant P, Zn and Cu uptake significantly varied with C and VC and their interactions, all nutrient uptakes significantly increased with the application levels.

Key words: *compost, plant growth, nutrient concentration, nutrient uptake, vermicompost.*

INTRODUCTION

Organic matter (OM) is one of the most effective components on soil fertility. With the direct and indirect roles, organic matter has many roles on preserving and maintaining soil productivity since it has not only a positive effect on soil physical, chemical, and biological properties but also is a source of many nutrients mainly carbon, nitrogen, sulphur and phosphorus (Follett et al., 1981; Barakan et al., 1995; Zink and Allen 1998; Doan et al., 2013; Doan et al., 2014). With these properties soil organic matter, a key component of soils, affects many reactions that occur in these systems. As known, the main sources of soil organic matter are death plant and animal residues at various stages of decomposition, ranging from fresh undecomposed materials through partially decomposed and short-lived products of decomposition to well-decomposed humus. In a fertile soil, it should contain at least 3% OM. But, because of the mineralization process, agricultural practices, natural events, and some other factors, organic matter in the soils decreases continuously. Soil OM content should be preserved for sustainability of soil productivity and diminishing parts of soil should be replaced. There are several ways to protect or increase soil organic matter. Although, the most common way of adding organic matter to the soil is farmyard manure; compost, vermicompost, green manure, and farmyard manure are another sources for OM. Composting is defined as biological aerobic transformation of an organic materials into stabilized products that can be applied to the soil as soil amenders and nutrient suppliers and can be used also as seedling media (Atiyeh et al., 2000; Agegnehu et al., 2015; Showler 2015; Aynacı and Erdal, 2016). Decomposition and stabilization of organic substrates by soil organisms can be defined as vermicomposting. Due to favourable physical properties and available nutrient contents, leading to better growing environment for plants, vermicompost are desirable materials (Edwards, 1988).

The objective of the research is to determine and compare the effects of same-originated compost and vermicompost on the growth, nutrient concentrations, and nutrient uptakes of corn plant.

MATERIALS AND METHODS

Study was conducted under greenhouse conditions as pot experiment during 8 weeks. The experiment was planned according to the randomised parcels design with 3 replications. Compost (*C*) and vermicompost (*VC*) were added to the 2 kg soil containing pots at the rates of 0, 10 and 30 t ha^{-1} and 300 ppm N (as ammonium nitrate), 200 ppm P (as triple super phosphate) and 100 ppm K (as potassium nitrate) were added as basal fertilization. The experiment consisted of two sources (C and VC), 3 doses (0, 10, 30 t. ha^{-1}), 4 growing periods (2, 4, 6 and 8 weeks) and 3 replicates and thus 72 (2x3x4x3) pots were used. Corn plant was used as plant materials. In order to see periodical effects of C and VC on plant growth and nutrient concentrations, plants were harvested two weeks intervals during the growth periods.

In order to determine soil available nutrients, P extracted with $NaHCO_3$ (Olsen et al., 1954), K, Ca, and Mg extracted with NH_4AOC (Jackson, 1967) and Mn, Zn, Fe, and Cu extracted with DTPA (Lindsay and Norvell, 1969). P measurement was done using spectrophotometer; others were measured with Atomic Absorption Spectrophotometer. Soil texture was determined using hydrometer (Bouyoucos, 1954) and $CaCO_3$ content was measured with calcimeter (Allison and Moodie, 1965). pH was determined using pH meter in suspension of soil and water at the rates of 1/2.5. Soil organic matter (OM) was determined based on Walkley and Black (1934).
Some physical and chemical properties of the experimental soil and total nutrient concentrations of C and VC are given in Table 1 and Table 2.
After each harvest, plants were washed with tap water and distilled water. After wards samples were dried, grounded and wet digested with microwave oven. Phosphorus concentrations of samples were determined with a spectrophotometer (Shimadzu UV-1208) at 430 nm according to the vanadomolybdo phosphoric acid method. Potassium, Ca, Mg, Fe, Cu, Zn, and Mn concentrations were determined using atomic absorption spectrophotometer (Varian, 240 FS). Total nitrogen was analysed according to Kjeldahl method. The same procedures were applied to determine total nutrient concentrations of C and VC.
Results were evaluated statistically using MSTAT program for one-way analysis of variance applied to determine any significant difference at 0.05%.

Table 1. Some characteristics of the soil used for the experiment

Texture	pH	$CaCO_3$ (%)	OM (%)	Plant available nutrients (mg kg^{-1})							
				P	K	Ca	Mg	Fe	Cu	Zn	Mn
CL	8.0	15	1.5	14	850	5500	170	2	1.5	0.7	4

Table 2. Total nutrient concentrations of composts and vermicomposts

Nutrients	Sources	
	Compost	Vermicompost
N, %	2.2	2.2
P, %	0.75	0.73
K, %	2.28	2.18
Ca. %	3.87	3.30
Mg, %	1.25	0.93
Fe, ppm	235	238
Zn, ppm	33.7	42.9
Mn, ppm	27	27
Cu, ppm	8.0	8.4

RESULTS AND DISCUSSIONS

Dry weight

Increasing levels of both compost and vermicompost increased plant growth significantly ($P<0.01$). Looking at the interaction effects for each harvest, it can be clearly seen that both sources led to increase in dry weight. Increment rates at the weeks of 2, 4, 6 and 8th week dry weights were 84 %, 337 %, 99 %, 42 % for C and 8 %, 102 %, 94 %, 28 % for VC, respectively. Effects of sources on dry weight showed different effect depending on the harvest time.

Table 3. Effects of C and VC on plant dry weight (g pot^{-1})

Harvest week	Dosage ($t\ ha^{-1}$)	VC	C
2	0	0.13 c*	0.13 c
	10	0.20 b	0.15 bc
	30	0.24 a	0.15 bc
	mean	0.19 A**	0.14 B
4	0	0.40 d	0.40 d
	10	0.68 c	0.45 d
	30	1.75 a	0.97 b
	mean	0.94 A	0.61 B
6	0	1.71 d	1.71 d
	10	2.12 c	2.72 b
	30	3.40 a	3.31 ab
	mean	2.41 A	2.58 A
8	0	3.66 d	3.66 d
	10	4.66 b	4.33 c
	30	5.18 a	4.66 b
	mean	4.50 *A*	4.22 A
General means for VC and C		2.01 A	1.89 A

General means of dosages	
0	1.48 *C****
10	1.91 *B*
30	2.45 *A*

*shows the interaction effect for each harvest week (P< 0.01)
** shows the differences between the sources for each harvest week (P< 0.01)
*** shows the differences depending on the dosages (P< 0.01)

While first two harvests dry weights at 2nd and 4th weeks, significantly affected by VC and C, there were not significant differences between the dry weights obtained at 6th and 8th week. Also no significant variation was observed between the general means of dry weights obtained from VC and C applied pots. Comparing to control, application doses significantly increased general means of dry weights at rates of 29% and 66 % with 10 and 30 t ha^{-1} dosages respectively (Table 3).

Nutrient concentrations

Plant macro element concentrations were given in Table 4. Except for 4th week, plant N concentrations were not affected by dosages and sources and their interaction. Also it was seen that there were not a significant differences in the general means of the values obtained from sources and dosages. But plant K concentrations significantly varied by the effect of dosage x source interactions in every week. Plant K concentrations increased similarly with the dosages of VC and C dosages. There were not significant differences between general means of VC and C applications, but the average means of application levels significantly varied each other and the highest K concentration was reached with 30 t ha^{-1} dosage. Plant Ca concentrations measured from the each harvest period affected significantly by the interaction, but the individual effects of sources had no effect on Ca. Except for first two harvests, plant Mg levels were not affected from the interaction effects. There was no difference between VC and C, both on a weekly basis and in general. Means of the dosages obtained from VC and C showed that plant Mg concentration increased about 21 % and 15 % when compared to control.

Effect of VC and C and their doses on periodical micronutrient concentrations were given on Table 4 As it can be seen from the Table 5, micro element concentrations of plant were affected from the dosage x source interactions generally. Compared to control, plant micronutrient concentrations increased with the dosages at each period with VC and C applications generally. General means showed that increasing dosages of VC and C resulted in increase of Cu, Mn, Fe and Zn, but no significant differences were found between the sources.

Table 4. Effects of VC and C on plant macronutrient concentrations (%)

Harvest week	Dosage (t ha^{-1})	N		K		Ca		Mg	
		VC	C	VC	C	VC	C	VC	C
2	0	2.4	2.4	2.4 c	2.4 c	1.3 c	1.3 c	0.18 b	0.18 b*
	10	2.1	2.1	3.7 b	3.7 b	1.5 b	1.8 a	0.27 a	0.25 a
	30	2.4	2.4	3.9 a	4.0 a	1.6 ab	1.3 c	0.26 a	0.25 a
	mean	2.3	2.3	3.3	3.4	1.5	1.5	0.24	0.23
4	0	1.4 b	1.4 b	3.1 ab	3.1 ab	1.8 a	1.8 a	0.16 d	0.16 d
	10	1.6 ab	1.6 ab	2.9 b	3.4 a	1.7 ab	1.8 a	0.25 b	0.31 a
	30	1.6 ab	1.8 a	3.4 a	3.4 a	1.3 c	1.5 b	0.21 c	0.25 b
	mean	1.5	1.6	3.1	3.2	1.6	1.7	0.21	0.24
6	0	1.0	1.0	2.8 b	2.8 b	1.3 ab	1.3 ab	0.20	0.20
	10	1.1	0.9	3.0 ab	3.0 ab	1.5 a	1.3 ab	0.21	0.19
	30	0.9	1.0	3.4 a	3.3 a	1.2 b	1.1 b	0.21	0.18
	mean	1.0	1.0	3.1	3.0	1.4	1.4	0.21	0.19
8	0	0.7	0.7	2.7 b	2.7 b	1.5 a	1.5 a	0.20	0.20
	10	0.8	0.7	2.9 a	2.9 a	1.5 a	1.1 b	0.21	0.19
	30	0.7	0.8	2.9 a	3.0 a	1.2 b	1.2 b	0.20	0.20
	mean	0.7	0.7	2.9	2.9	1.2	1.3	0.20	0.20
General means for VC and C		1.4	1.5	3.1	3.1	1.5	1.5	0.22	0.22
General means of dosages									
Dosage	0	1.4		2.8 B**		1.5		0.19 B	
	10	1.4		3.1 AB		1.5		0.23 A	
	30	1.5		3.4 A		1.4		0.22 A	

*shows the interaction effect for each harvest week ($P< 0.01$)
** shows the differences depending on the dosages ($P< 0.01$)

Table 5. Effects of C and VC on plant micronutrient concentrations (mg kg^{-1})

Harvest week	Dosage (t ha^{-1})	Cu (mg/kg)		Mn(mg/kg)		Fe(mg/kg)		Zn(mg/kg)	
		VC	C	VC	C	VC	C	VC	C
2	0	8.4 b*	8.4 b	42 d	42 d	102	102	33 bc	33 bc
	10	14.4 ab	14.0 ab	67 a	50 bc	114	106	29 c	40 ab
	30	15.9 a	17.0 a	57 b	47 bcd	112	116	38 b	49 a
	mean	12.9	13.3	55 A	46 B	109	108	33 B	41 A
4	0	8.3 b	8.3 b	70 b	70 b	81 c	81 c	24 ab	24 ab
	10	8.9 b	12.3 a	54 c	71 b	90 bc	99 b	20 b	27 a
	30	10.3 a	10.9 a	75 ab	81 a	106 ab	124 a	24 ab	28 a
	mean	9.2 B**	10.5 A	66 B	74 A	92	101	23	26
6	0	8.1 ab	8.1 ab	92 a	92 a	92 b	92 b	15 b	15 b
	10	6.8 b	9.8 a	92 a	75 b	91 b	86 b	18 ab	18 ab
	30	8.8 a	9.2 a	96 a	97 a	98 ab	122 a	18 ab	21 a
	mean	7.9 B	9.0 A	83	88	94	100	17	18
8	0	6.0 c	6.0 c	96 ab	96 ab	98 c	98 c	17 abc	17 abc
	10	11.1 a	5.7 c	86 b	61 c	109 bc	103 c	19 a	15 c
	30	7.8 b	9.9 ab	99 ab	111 a	112 b	147 a	20 a	16 bc
	mean	8.3 A	7.2 B	94	89	106	116	19	16
General means for VC and C		9.6	10.0	78	74	100	106	23	25
General means of dosages									
Dosage	0	7.7 B***		75 B		93 B		21 B	
	10	10.4 A		70 B		98 B		23 B	
	30	11.2 A		83 A		117 A		27 A	

*shows the interaction effect for each harvest week ($P< 0.01$)
** shows the differences between the sources for each harvest week ($P< 0.01$)
*** shows the differences depending on the dosages ($P< 0.01$)

Plant nutrient uptakes

Nutrients uptakes of corn for each growth period were affected significantly by dosage source interactions.

Looking at the values obtained from both periodically results and general means, the effect of both sources VC and C, had similar effect on plant macronutrient uptakes generally. General means get from both sources showed that macro nutrient uptakes of by plant increased with the dosages (Table 5).

Micronutrient removal of corn for all harvest week significantly varied with source x dosage interactions.

Although VC and C variation had significant effect on plant periodic nutrient removal in some cases, there were not a significant variations at the general means of VC and C.

Comparing the control (0 t ha^{-1}) Increases in VC and C dosages significantly increased plant Cu, Mn, Fe and Zn uptakes by above ground parts of corn (Table 6).

Table 5. Macronutrient uptake of corn by above ground biomass (mg $plant^{-1}$)

Harvest week	Dosage (t ha^{-1})	N		K		Ca		Mg	
		VC	C	VC	C	VC	C	VC	C
2	0	3.1 c*	3.1 c	3.1 d	3.1 d	1.7 c	1.7 c	0.2 b	0.2b
	10	4.2 b	3.2 c	7.4 b	5.6 c	3.0 ab	2.7 b	0.5 a	0.4 ab
	30	5.8 a	3.6 bc	9.4 a	6.0 c	3.8 a	2.0 c	0.6 a	0.4 ab
	mean	4.4	3.3	6.3 A**	4.7 B	2.8	2.1	0.5 A	0.3 B
4	0	5.6 d	5.6 d	12.4 c	12.4 c	7.2 c	7.2 c	0.6 d	0.6 d
	10	10.9 cd	7.2 d	19.7 c	15.3 c	11.6 bc	8.1 c	1.7 c	1.4 cd
	30	28.0 a	17.5 b	59.5 a	33.0 b	22.8 a	14.6 b	3.7 a	2.4 b
	mean	14.8	10.1	29.5 A	20.1 B	15.0 A	10.4 B	2.0	1.5
6	0	17.1 c	17.1 c	47.9 d	47.9 d	22.2 c	22.2 c	3.4 d	3.4 d
	10	23.3 b	24.5 b	63.6 c	81.6 b	31.8 b	35.4 ab	4.5 c	5.2 bc
	30	30.6 a	33.1 a	115.6 a	109.2 a	40.8 a	36.4 ab	7.1 a	6.0 b
	mean	23.7	24.9	73.9	78.3	32.1	31.8	5.0	4.9
8	0	25.6 b	25.6 b	98.8 c	98.8 c	54.9 ab	54.9 ab	7.3 c	7.3 c
	10	37.3 a	30.3 b	135.1 b	125.6 b	69.9 a	47.6 b	9.8 a	8.2 b
	30	36.3 a	37.3 a	150.2 a	139.8 b	62.2 a	55.9 ab	10.4 a	9.3 a
	mean	33.1	31.1	127.5 A	121.0 A	63.0 a	53.5 B	9.2 A	8.3 B
General means for VC and C		27.9	28.3	62.1	59.3	29.1	26.8	4.2	3.7
General means of dosages									
0		20.4 B***		41.4 C		22.1 B		2.80 B	
10		26.7 B		59.2 B		28.6 B		4.39 A	
30		36.8 A		83.3 A		34.3 A		5.39 A	

*shows the interaction effect for each harvest week
** shows the differences between the sources for each harvest week.
*** shows the differences depending on the dosages.

Table 6. Micronutrient uptake of corn by above ground biomass (µg $plant^{-1}$)

Harvest week	Dosage (t ha^{-1})	Cu		Mn		Fe		Zn	
		VC	C	VC	C	VC	C	VC	C
2	0	1.1c*	1.1c	5.5 b	5.5 b	13.3 c	13.3 c	4.3 d	4.3 d
	10	2.9 b	2.1 b	13.4 a	7.5 b	22.8 ab	15.9 bc	5.8 c	6.0 c
	30	3.8 a	2.6 b	13.7 a	7.1 b	26.9 a	17.4 b	9.1 a	7.4 b
	mean	2.5	1.9	10.5 A**	6.4 B	20.7 A	15.1 B	6.3	5.7
4	0	3.3 d	3.3 d	28.0 c	28.0 c	32.4 d	32.4 d	9.6 d	9.6 d
	10	6.1 c	5.5 cd	36.7 c	32.0 c	61.2 c	44.6 d	13.6 c	12.2 c
	30	18.0 a	10.6 b	131.3 a	78.6 b	185.5 a	120.3 b	42.0 a	27.2 b
	mean	8.6	6.4	62.0 A	45.1 B	86.5 A	61.6 B	21.6 A	15.9 B
6	0	13.9 b	13.9 b	157.3 c	157.3 c	157.3 e	157.3 e	25.7 d	25.7 d
	10	14.4 b	26.7 a	195.0 b	204.0 b	192.9 d	233.9 c	38.2 c	49.0 b
	30	29.9 a	30.5 a	326.4 a	321.1 a	333.2 b	403.8 a	61.2 a	69.5 a
	mean	19.0	23.2	200.0 B	227.0 A	226.5 B	258.0 A	41.0	46.4
8	0	22.0 c	22.0 c	351.4 c	351.4 c	358.7 e	358.7 e	62.2 d	62.2 d
	10	51.7 a	24.7 c	400.8 b	264.1 d	507.9 c	446.0 d	88.5 b	65.0 d
	30	40.4 b	46.1 a	512.8 a	517.3 a	580.2 b	685.0 a	103.6 a	74.6 c
	mean	38.0 A	30.9 B	421.6 A	377.6 B	482.3	496.6	84.8 A	67.2 B
General means for VC and C		17.2	15.7	179.1	164.4	205.5	210.0	38.6	34.2
General means of dosages									
0		11 C***		111 B		138 C		31 B	
10		20 B		133 B		187 B		44 B	
30		27 A		203 A		287 A		66 A	

*shows the interaction effect for each harvest week
** shows the differences between the sources for each harvest week.
*** shows the differences depending on the dosages.

If a general evaluation was made looking at the results of plant dry weights, nutrient concentrations and plant nutrient uptakes it was clearly seen that VC and C applications had positive effects on the examined parameters in this research. One of the reasons of the positive roles VC and C can be the organic matter addition to the soil. As indicated previous studies, organic matter has an impressing role on plant growth and mineral nutrition due to its direct and indirect effect (Follett et al., 1981; Barakan et al. 1995; Zink and Allen, 1998). Although some nutrient concentrations in plant were not affected by VC and C applications, uptakes of all nutrients by above plant biomass showed increment with the application and their dosages generally. This could be attributed to dilution of the nutrient concentration in plant tissue with the plant growth (Erdal et al., 2014; Erdal and Ekinci, 2017). In some cases Recently, VC and C are widely used materials for increasing soil fertility and plant nutrition. In a study conducted by Aynaci and Erdal (2016) increasing of plant growth and plant mineral nutrition was attributed to nutrient release from the compost by means of mineralization and some other positive direct and indirect roles of compost. Also it was implied that vermicompost is a good even better source as soil conditioner. As explained previous studies conducted as field or pot experiments, vermicompost can increase soil fertility by means of different ways and thus plant growth and dry matter increase (Nagavallemma et al., 2004; Gutierrez-Miceli et al., 2007; Joshi and Vig, 2010). On these yield increase, slow release of nutrients during the plant growth and decreasing of nutrient loss by means of leakage may have effect as indicated by Cantanazaro et al., (1998). Although Edwards (1998) indicated that vermicompost had higher effect on increasing growth and yield of some vegetables, fruits and flowers when compared to other composts and pot soils, we could not see significant differences between both sources in terms of examined parameters.

CONCLUSIONS

In conclusion, plant growth and mineral nutrition of corn plant positively affected by vermicompost and compost generally. The highest yield and nutrient uptakes were obtained from the 40 t ha^{-1}. Also the effects of VC and C were similar generally.

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EVOLUTION MICROMORPHOLOGY OF ARGILLIC HORIZONS IN SOME ARID SOILS IN THE WEST OF URMIA LAKE IN WESTERN AZERBAIJAN PROVINCE, IRAN

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Abstract

Argillic horizons are subsurface diagnostic horizons and presence and identification of argillic horizons in soils of arid and semiarid regions can be used as an important tool for soil classification, interpretation of soil forming processes and condition of their formation. In this study, argillic horizons were investigated micromorphologically and SEM technique. According to micromorphic studies, accumulations of illuvial clay in these soils are present as coatings on skeletal grains and void faces and their thickness varies from 20 to 30 µm and they comprise about 2-5% of argillic horizons. In some argillic horizons, illuvial clay has filled all of pore spaces and comprises about 10% of horizon. In some soils carbonates have covered clay coatings and in some cases clay coatings have been disappeared. Co-existence of argillic and calcic horizons is indicative of polygenesis. A calcic horizon containing illuvial clay is usually interpreted as indicator of climate change. Thus, the formation of complex argillic-calcic horizon shows that these soils are polygenetic and argillic horizons in these soils have been formed in a more humid past climate.

Key words*: Argillic horizon, clay coating, semiarid, Iran, Western Azerbaijan.*

INTRODUCTION

An argillic horizon is normally a subsurface horizon with a significantly higher percentage of phyllosilicate clay than the overlying soil material. It shows evidence of clay illuviation. The argillic horizon forms below the soil surface, but it may be exposed at the surface later by erosion (Soil survey Staff, 1999, 2014). Because there is little or no evidence of illuvial clay movement in soils on the youngest landscapes, soil scientists have concluded that the formation of an argillic horizon requires at least a few thousand years. On some late Pleistocene landscapes, argillic horizons are more strongly expressed in soils under forest vegetation than in soils under grass. Therefore, the kind of flora and associated fauna is thought to have an influence on the rate of development or degree of expression of the argillic horizon. Climate also is a factor. There are few or no examples of clay films in soils with perudic soil moisture regimes. Textural differentiation in soils with argillic horizons results from one or more processes acting simultaneously or sequentially, affecting surface horizons, subsurface horizons, or both. The degree to which a process or several processes operate varies widely from soil to soil. In some soils clay illuviation is significant, while in others clay illuviation is overshadowed by in situ weathering. Not all of the processes are completely understood. The ones thought to be most important are: a) Clay eluviation and illuviation, b) Clay dissolution in the epipedon, c) Selective erosion, d) *In situ* clay formation, and e) Clay destruction in a subsurface horizon (Ransom et al., 1997; Pal et al., 2003; Soil survey Staff, 1999, 2014).

The argillic horizon represents a time-landscape relationship that is locally and regionally important. Because an argillic horizon forms at a relatively slow rate, its presence indicates that the geomorphic surface has been relatively stable and that the period of stability has been long. If the argillic horizon occurs in an area of an aridic moisture regime and is rarely moist or has free carbonates throughout, it probably indicates an old soil and stable geomorphic surface of such great age that the climate has changed since the formation of the horizon. In the present environment, the precipitation is not sufficient to remove carbonates from the soil or to

translocate clay to the base of the argillic horizon (Pal et al., 2003; Gunal and Ransom, 2006; Soil survey Staff, 1999, 2014).
Regarding above and also importance of argillic horizons in arid and semiarid regions, the argillic horizons in calcareous semiarid soils in the south west of Urmia Lake were studied micromorphologically to demonstrate their genesis and paleoclimatic significance.

MATERIALS AND METHODS

This study was conducted in the south west of Urmia Lake-in the West Azerbaijan province Iran, located about 20 km of Naghadeh and 10 km of Mohammadyar cities, near the south west of Urmia Lake, from 37° 4.74′ to 37° 6.7′ N latitude and 45° 25.97′ to 45° 28.41′ E longitude (Figure 1a). The mean elevation of the study area is 1303.9 m above sea level. A total of 6 soil profiles were dug along a toposequence in order to demonstrate the variation in physiography and topography in the south west of Urmia Lake. The parent material of this area is white grey limestone of Miocene and according to soil moisture and soil temperature regimes map of Iran (Banaei, 1998), the soil moisture and soil temperature regimes of study area are Dry Xeric and Mesic respectively. Profiles 1 and 2 are located in Hills physiographic Unit, profiles 3 and 4 in Plateaux and profiles 5 and 6 in Piedmont Plain (Figure 1b). Soil profiles in each physiographic unit had different slope and elevation. These 6 pedons were described and classified according to Soil Survey Staff (2014) and were sampled using standard techniques. Preparation of soil thin sections was done on undisturbed samples using standard techniques (Benyarku and Stoops, 2005). Micromorphological descriptions and interpretations were done based on criteria and terminologies of Stoops (2003). SEM analyses were performed with a ZEISS DSM 940A Electronic Scanning Microscope.

RESULTS AND DISCUSSIONS

Based on field and micromorphic studies and laboratory data, argillic horizons were present only in soils located in the piedmont plain (profiles 5 and 6, figure 1b), but calcic horizons were present in in soils in the Plateaux (profiles 6 and 4) and Piedmont plain (profiles 5 and 6) physiographic units and profiles 1 and 2 had no argillic nor calcic horizons (Figure 1b). So, profiles 5 and 6 (piedmont plain) had both argillic and calcic horizons.
According to the micromorphic observations, accumulations of illuvial clay in these soils are present as coatings on skeletal grains and void faces and their thickness varies from 20 to 30 μm and they comprise about 2-5% of argillic horizons. In some argillic horizons, illuvial clay has filled all of pore spaces and comprises about 10% of horizon (Figure 2).
SEM observations on some samples containing clay coatings revealed presence of these features on coarse fragments (Figure 3). EDAX analysis showed higher amounts of Si and Al and lower amounts of Ca, Fe and K, which confirm clayey composition of these coatings (Figure 4).
Clay coatings in these soils show weavy extention which indicates well orientation of clays. All pedons with argillic horizons were enriched in pedogenic carbonates and described with pedogenic carbonate features in the B horizons. Although all pedons had >5% pedogenic carbonate accumulations and more than 15% in the B horizons, so, they were classified as having calcic horizons (Figure 2).
Pedogenic carbonate and clay films have been observed at approximately the same depth (Figure 2 c and d). This is an indication of a complex history of carbonate leaching, subsequent illuviation of clay, and redeposition of calcite. Some of calcium carbonate accumulations were superimposed on clay coatings. This suggests that the soils are polygenetic and that clay illuviation preceded carbonate accumulation. Polygenetic soils occur when climate changes are great enough to produce new soil properties without obliterating existing properties (Chadwick et al., 1995). Stoops (2003) described such features as juxtaposed compound pedofeatures. The juxtaposition occurs due to the changing of local or environmental conditions (Stoops, 2003). The clay coatings that were coated with calcans appeared to be undamaged and exhibit birefringence.
Theoretically, the pedogenic processes of clay illuviation and calcium carbonate

accumulations should be contradictory to each other, since large quantities of Ca^{2+} tend to cause clay flocculation and reduce illuviation. However, these pedogenic features may be observed in the same soil at approximately the same depth (Gile and Grossman, 1968; Ransom et al., 1997; Pal et al., 2003; Khormali et al., 2003; Soil survey Staff, 1999, 2014). The presence of both accumulation of calcium carbonate and oriented clay in the same horizon implies a complex history of carbonate leaching, deposition of secondary calcite, and clay illuviation. Gile and Grossman (1968), Ransom et al. (1997), Pal et al. (2003), Khormali et al. (2003), Khademi and Mermut (2003), and Gunal and Ransom (2006) observed obliteration of clay films in some parts of the argillic horizon because of pedogenic carbonate accumulation, especially for those clay coatings that occurred on the surfaces of sand grains and pebbles. Pal et al. (2003) reported that poorly oriented clay platelets are often find associated with $CaCO_3$ grains. However, Ransom and Bidwell (1990) did not observe any evidence of disruption of clay skins in horizons where secondary carbonate was accumulating.

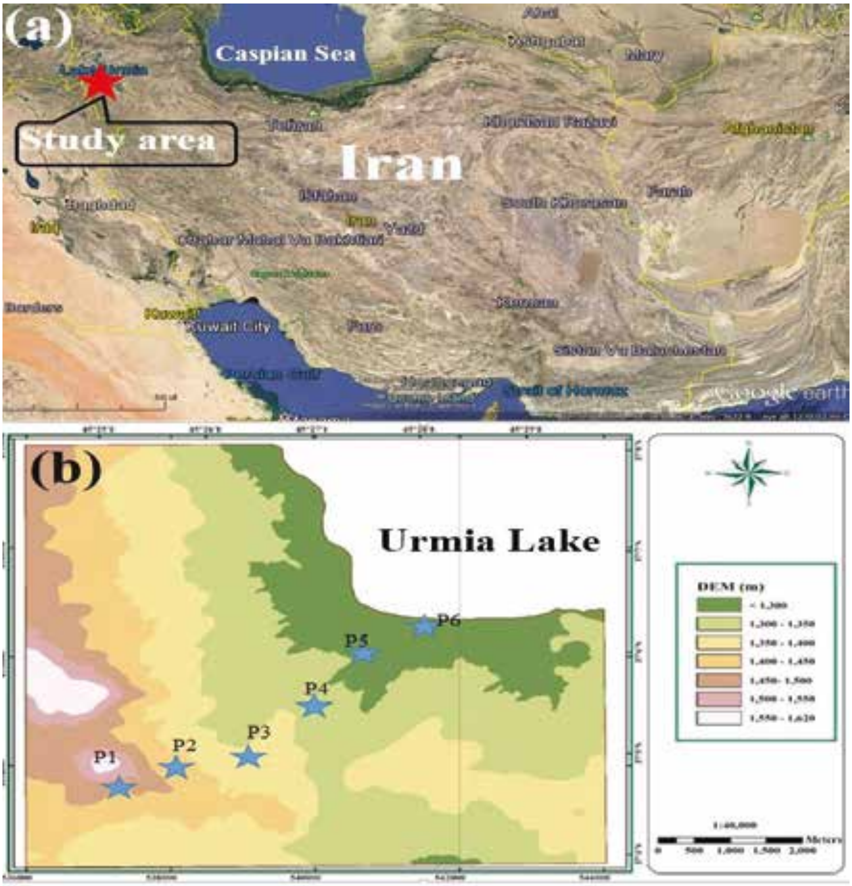


Figure 1. Position of study area in Iran (a), and the location of soil profiles along a toposequence in the south west of Urmia Lake (b)

They reported that the clay coatings were intact.

The coexistence of argillic and calcic horizons in these calcareous semiarid soils is a peculiar combination, suggesting a multistage pedogenesis in this area. Plaeo-argillic horizons were likely developed under a moister environment than today. Sufficient rainfall contributed to the removal of carbonates from the topsoil and subsequent eluviation of clay to form the argillic horizon.

Presence of clay pedofeatures and calcium carbonate is common in soils of arid and semiarid climates.

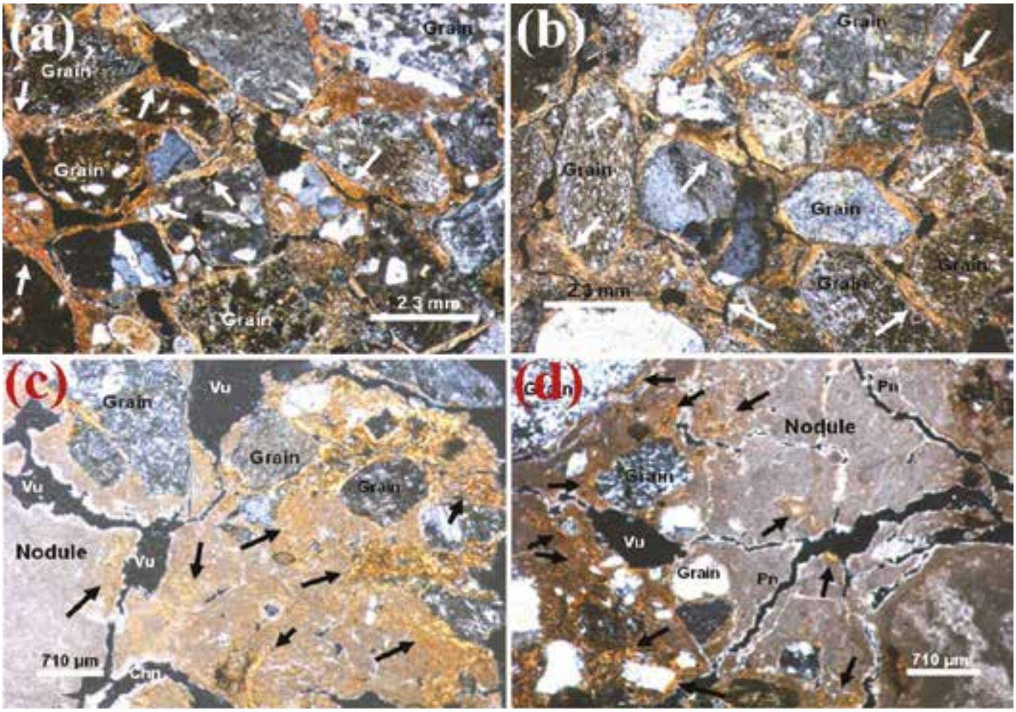


Figure 2. Clay coatings in studied calcareous semiarid soils. Figures a) and b) clay coatings around skeletal grains, and figures c) and d) show clay coatings engulfed by calcium carbonate. Figures have been taken under XPL light

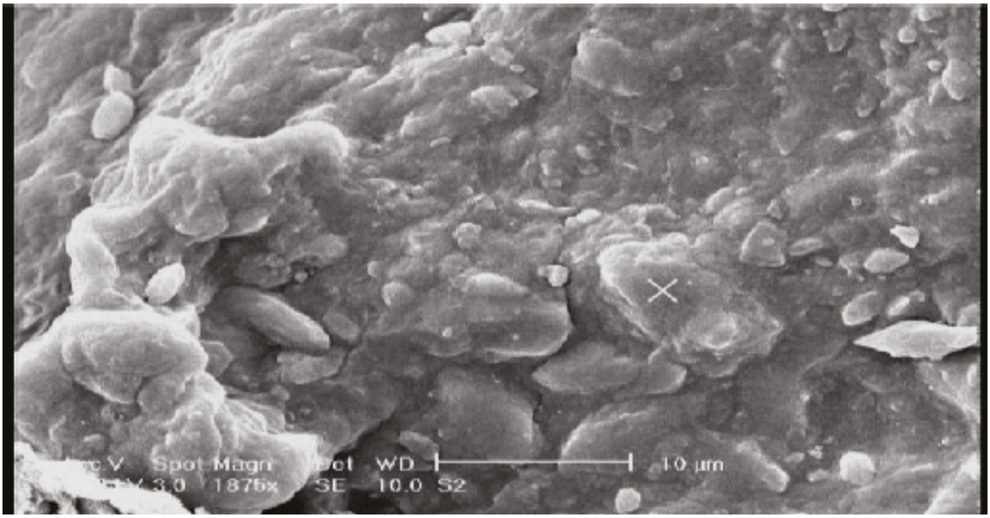
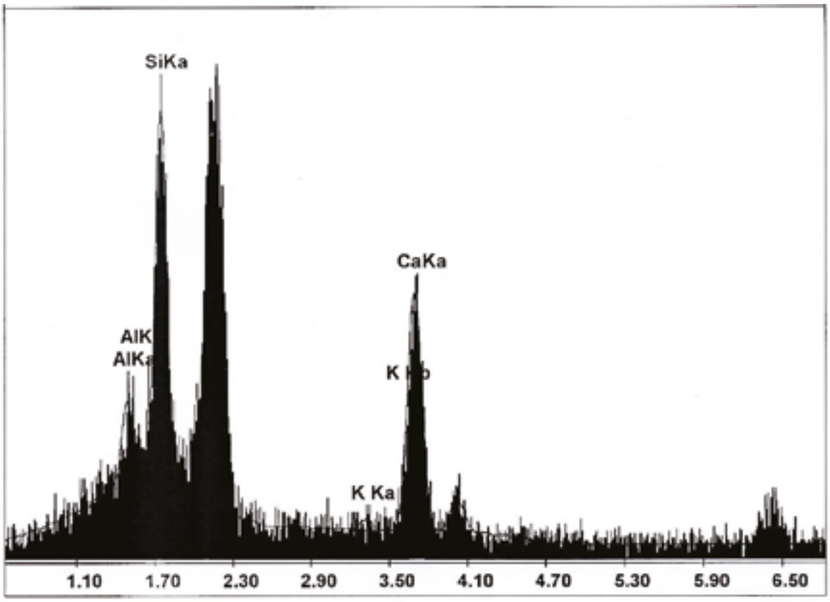


Figure 3. SEM Image and EDAX analysis of clay coatings in studied calcareous semiarid soils.
Figure represents clay coatings on a skeletal grain



EDAX ZAF Quantification (Standardless)
Element Normalized

Element	Wt %	At %
AlK	9.83	11.84
SiK	43.39	50.20
K K	2.07	1.72
CaK	44.70	36.24
Total	100.00	100.00

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
AlK	11.75	12.05	7.69	0.97
SiK	49.18	9.93	2.89	4.95
K K	1.88	5.75	27.15	0.33
CaK	37.09	5.75	3.27	6.45

Figure 4. EDAX analysis of clay coatings in Figure 3

A calcic horizon containing translocated clay is commonly interpreted to represent climate change. Gile et al. (1966) indicated that clay was translocated in a moister climate, but was later engulfed by carbonate when climate become drier, and the clay orientation was also disturbed by accumulation of carbonate. Finally, we conclude that these illuvial features may not be the products of the recent climate, but probably formed in amore humid climate.

CONCLUSIONS

Argillic horizons are subsurface diagnostic horizons and Presence and identification of argillic horizons in soils of arid and semiarid regions can be used as an important tool for soil classification, interpretation of soil forming processes and condition of their formation. According to micromorphic studies, accumulations of illuvial clay in these soils are present as coatings on skeletal grains and void faces and their thickness varies from 20 to 30 µm and they comprise about 2-5% of argillic horizons. In some argillic horizons, illuvial clay has filled all of pore spaces and comprises about 10% of horizon. In some soils carbonates have covered clay coatings and in some cases clay coatings have been disappeared. Co-existence of argillic and calcic horizons is indicative of polygenesis. A calcic horizon containing illuvial clay is usually interpreted as indicator of climate change. Thus, the formation of complex argillic-calcic horizon shows that these soils are polygenetic. In arid and semiarid climates, due to lack of moisture, presence of carbonates and other inhibiting factors, the illuviation of clay does not take place or is very slow, in which, some scientists believe that clay illuviation in arid and semiarid climates is impossible or the amount of illuviated clay is not enough for development of argillic horizons. Regarding that argillic horizons in this study are present in deep horizons and also the climate is arid and semiarid and soils are carbonatic, it seems that dispersion and subsequently illuviation of to the this extent and to these deep depth is impossible. Thus, argillic horizons in these soils have been formed in a more humid past climate.

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PHYSICAL PROPERTIES FEATURES OF ALLUVIAL IRRIGATED SOILS OF DNIESTER AND DNIEPER RIVER BASINS

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Abstract

The features of the physical properties of alluvial-meadow irrigated soils of the Dniester and Dnieper rivers basins have been studied. The irrigated soil of the floodplain of rivers is characterized by the apparent stratification of the profile and the presence of the buried humus horizon. Irrigated soil is more remote from the river bed, their profiles are less stratification, lack of buried horizons; with depth, alleviation of the granulometric composition from sandy loam to cohesive sand. Upper and buried humus horizons of irrigated soils of the Dnieper, after granulometric composition - sandy loam, transition horizon and parent rock - coherent sand. Irrigated alluvial soils in the Dniester meadow are characterized by comparatively homogeneous texture on the profile and fall into the fine clay texture class. The main processes that cause the degradation of fluvial soils in the river meadows are high gleizing and compacting of the 20-35 cm layer.

Key words*: humus horizon, irrigated soil, granulometric composition, physical properties.*

INTRODUCTION

The alluvial process is the accumulation of river alluvium as a result of sedimentation in the floodplain soil surface of solid particles from flood waters on the surface. In the result of the alluvial process on the surface of the floodplain, there is an annual deposition of alluvium immediately involved in soil formation. Therefore, alluvial soils constantly grow upward, obtaining systematically new portions of the soil-forming rock (Варка, 2018). It is important to emphasize that groundwater is an essential factor of alluvial soil formation. In any developed floodplain it is possible to distinguish three essential parts: a pristine elevated part or a crescent tree, the central most levelled part of the floodplain and a terrace lowering.

The soil cover of river floodplains is very variegated, complex, mosaic due to the constant meandering of the river bed and the migration of various parts of the floodplain. Hence the wide distribution of polycyclic, buried soils (Аллювиальные почвы..., 2018).

Ratio in soils of elementary particles of different sizes forms the basic properties of soils - productive and ecological. And the very formation and functioning of the soil as a natural component and object of human economic activity largely depends on the granulometric composition (Medvedev, Laktionova, 2011).

The nature and direction of soil use, agrarian specialization, farming systems, fertilizer and land reclamation techniques are often differentiated depending on the granulometric composition (Baliuk et al., 2015).

Irrigation further complicates the structure of the soil cover, which requires a differentiated approach to both irrigation development and agro-ameliorative measures, which should be based on studying the physical properties of soils. That is why when studying alluvial irrigated soils, they turned their attention primarily to their physical properties.

MATERIALS AND METHODS

The study objects were as follows:
1) Alluvial virgin soils clayey on the marshy deposits, non-irrigated (virgin) and alluvial arable soil, irrigated for 30 years, vegetable crop rotation, from the Lower Dniester meadow (Kopanca commune, Kausheni district, southern area of Moldova).
2) Alluvial soddy soil, used for a long time in organic farming, drip irrigation (20 years), vegetable crop rotation, and non-irrigated alluvial meadow soil used in agricultural production as pasture, Lower Dnieper meadow (Kopani village, Kherson region, southern area of Ukraine).

Field survey of irrigated lands was carried out using the method of analog keys on landfills. In the studied territories, key sites have been laid on irrigated and on non-irrigated soil. Within the sites, the soil profile was laid down to 1-2 m (depth of groundwater) and soil samples were taken from each genetic horizon. Selection, preparation for analysis and preliminary processing of soil samples of the lower reaches of the Dnieper and Dniester, subject to analysis, were carried out according to the normative documents existing in Moldova and Ukraine (Soils quality: 2004; 2007a, 2007b).

RESULTS AND DISCUSSIONS

Alluvial soils in the meadows of large rivers formed by the sedimentation of alluvial deposits, which led to the layered layout of their profile in space and depth, which is very variable regarding physical properties.

A comparative study of the mineralogical state of genetic close, but different in age, alluvial clayey soil of the Dniester tributary floodplain and stagnant chernozem on the Late Pliocene age clayey rock of alluvial origin from one of the watersheds of northern Moldova has been performed. These researches showed the identity of the qualitative composition of primary and clay minerals and the presence in both soils heterogeneity (stratification) of the rock, which in the alluvial soil received a more pronounced manifestation. Alluvial soils are distinguished by a high content of micas, which should be considered as a specific feature in general of heavy rocks of alluvial origin (Алексеев et al., 2016 a; Воробьев, 1974).

A hypothesis has been advanced that the exceptionally high content of total potassium reserve in soils on alluvial deposits, irrespective of age, is their genetic particularity and is due to the plastic form of micaceous minerals that facilitates their migration in the sedimentary material of the catchment basins and accumulation in river floodplains (Алексеев et al., 2016 б).

The investigated soil profiles from the Dniester and Dnieper meadows are less differentiated in the upper horizons and are characterized by buried soils and gleyed horizons in the lower part (Figures 1 and 2).


a b

Figure 1. Alluvial soils: a) non-irrigated and b) irrigated from the Dnieper meadow (Ukraine)

This is due to the fact that in the underground terrace of the meadows the river overflowing were rare, and after the construction of the dykes, they were stopped. The more or less regular genetic horizons are only observed up to a depth of 80 cm. In the depth range of 80-95 cm is situated a humid horizon Abhg, formed in another historical period (Алексеев, 1999, Stegărescu, 2016). This is a common feature for the Dniester and Dnieper soils. Under this horizon there is an extremely pronounced gleic horizon with dark, humid-colored layers. So, the profile of the studied soils consists of the buried, very humid, gleic soil, located more than 80 cm, and the contemporary soil with humeferous developed profile, poorly gleyed in the lower part, situated in the depth range of 0-80 cm.

Alluvial soils from the Dniester meadow.
From the profiling structure the irrigated alluvisoils is divided into three segments:
- contemporary soil (0-80 cm) with homogeny clayey texture;
- humifer horizon of buried marshy alluvisoil (80-115 cm) with fine clayey texture;
- fine-grained clayey horizon (115-200 and deeper) with gray-bluish colour and dark humic thin layers.

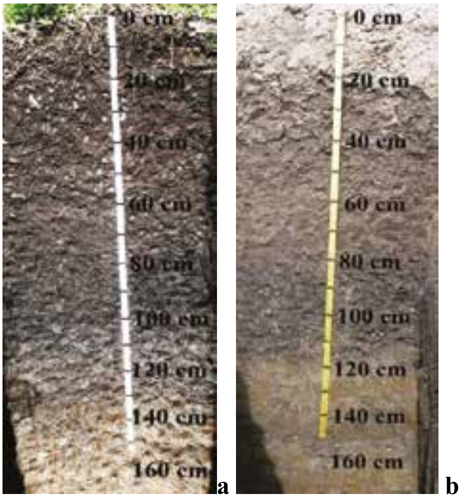

Figure 2. Alluvial soils: a) non-irrigated and b) irrigated from the Dniester meadow (Moldova)

The composition of the alluvial soil profile confirms the cyclicity of the pedogenesis process in the meadow beneath the terraces of the Lower Dniester as a result of the different co-ratio of the manifestation intensity of the following processes: alluvial - accumulation of deposits, subterranean alteration *in situ* and gleizing, pedogenesis (humus formation).
Irrigated alluvisoils are characterized by comparatively homogeneous texture on the profiles. The physical clay content ranges from 82.7% in the arable layer to 88.7% in the highly gleaned horizon, while the fine clay content is at 49.4% to 61.8% (Table 1), indicating a strong argillization in the gleic horizon. Soil falls into the medium and fine clayey texture class. Non-irrigated grounded soil falls into the clayey-loamy texture class.
The accuracy of the average statistical parameters of the physical clay and clay content of irrigated soils is within the limits of 0.8-1.9%.
From the point of view of clayey texture, the studied alluvisoils are a difficult object for irrigation. Being low humid, they are characterized by cloggy structure and low resistance to compaction.
In relation to the decrease of the arable layer thickness from 35 cm to 20 cm, the destruction and decrease of the humus content in the arable layer are the factors leading to the excessive compaction of the soil layer of 20-35 cm of irrigated soils (Table 1).

Table 1. Average statistical parameters (X ± s) on genetic horizons of the granulometric composition of irrigated and non-irrigated alluvisoils from the Dniester meadow

Horizon and Depth, cm	The size of the fractions, mm; content, % g/g					
	0.1-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01
Irrigated Alluvial Soil						
Ahp1 (0-20)	3.3±1.2	13.8±1.2	10.6±1.2	22.9±1.6	49.4±0.9	82.7±1.6
Ahp2 (20-38)	3.7±0.9	13.7±1.4	8.6±1.4	22.3±2.1	51.7±1.4	82.6±1.4
ABh (38-58)	3.9±1.0	12.4±1.2	10.1±1.6	21.7±1.6	52.0±1.2	83.7±0.8
Bhg (58-79)	3.9±0.9	12.8±1.2	8.4±1.5	24.1±1.5	50.8±1.2	83.2±1.1
Abhg (79-95)	2.2±0.8	11.5±1.5	7.2±1.5	22.5±1.9	56.4±1.5	86.1±1.4
Bbhgk (95-115)	2.0±1.0	9.3±1.3	7.6±1.6	19.3±1.9	61.8±1.9	88.7±1.1
Non-irrigated Alluvial Soil						
Ahpț (0-18)	8.2±1.0	23.3±1.1	6.9±1.2	15.5±1.5	45.9±1.0	68.3±1.5
Ah (18-40)	9.3±1.1	23.8±1.1	5.8±1.2	15.7±1.6	45.2±1.2	65.7±1.4
ABh (40-62)	5.2±0.9	26.8±1.2	5.0±1.1	16.4±1.5	46.4±1.3	67.8±1.4
Bhg (62-80)	7.7±0.8	14.7±1.2	6.1±1.2	15.5±1.6	55.8±1.3	77.4±1.4
Abhg (80-100)	9.6±0.8	12.6±1.3	6.8±1.2	16.9±1.7	53.7±1.3	77.4±1.4
Bbhg1 (100-112)	9.1±0.9	13.9±1.3	5.2±1.5	18.2±1.7	53.1±1.3	76.5±1.3
Bbhg2 (112-130)	6.4±0.9	20.9±1.3	7.7±1.5	14.4±1.8	49.3±1.2	71.4±1.2
G1 (130-150)	4.4±0.8	32.2±1.2	5.9±1.4	10.1±1.7	37.2±1.1	53.2±1.1

According to the average statistical parameters of the structural composition (dry sieving) the researched soils (irrigated and non-irrigated) are characterized by a good structure in the layer 0-20 cm (result of the plowing and permanent work between the rows, and grasses of virgin soil) and very unsatisfactory in the compact layer 20-38 cm. Wet sieving data demonstrate that the hydrostability of the aggregates in both the 0-20 cm arable layer and the 20-38 cm layer is very high - the result of the large clay content. The causes of the degradation of the structure of the irrigated soil are the dehumification of the arable layer and its intensive work with heavy machinery.

The hygroscopicity of irrigated soils varies (increases) in profile from 6.6% in the arable layer to 9.1% in the gleic horizon as a result of the increase in the depth of clay content. The coefficient of variation is equal to 5.7% in surface horizons and 19.6% in gleic horizons. The coefficient of hygroscopicity concurrently ranges from 9.6% to 12.0%.

Density in the profile of irrigated aluvisoils ranges from 1.23 g/cm^3 in the arable layer to 1.44 g/cm^3 of the underlying layers. Apparent density on irrigated soils ranges from 2.65 in the arable horizon to 2.75 g/cm^3 in the Bbhgk horizon. The precision of the average indices varies within the range of 1.86-3.03%, the coefficient of density variation in space does not exceed 6.1%. The arable layer is characterized by optimal values of apparent density, but the underlying layer is compacted (Figure 3).

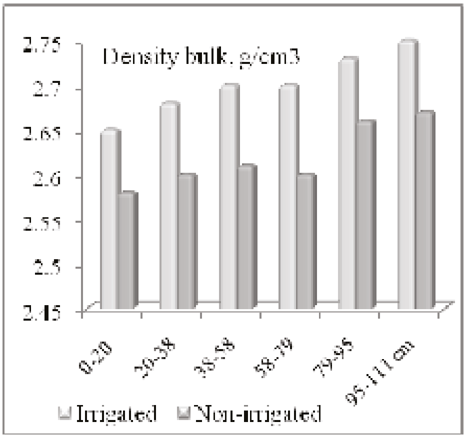

Figure 3. Density bulk in alluvial soils of Dniester floodplain

The total soil porosity values for the studied soil are medium in the recently arable layer (53.7 ± 0.9%) and small in the underlying layers (49.4 ± 0.5%).

According to the size of the compaction, the soil is poorly compressed (3.3 ± 1.3%) in the 0-20 cm layer and moderately compressed (16.0 ± 1.2) in the underlying layers. According to the values of the degree of compaction, the studied soil is characterized by a small degree (low settled soil) of compaction of the recent arable horizon (3.3 ± 1.3%) and a high degree of compaction (moderately settled soil) in the underlying horizons 11.5-16.0 with a deviation of ± 0.8-1.2%.

Generally, the physical properties of arable post swampy alluvisoils are satisfactory for the arable and unsatisfactory for the compacted, post-arable layer (Cerbari, Stegarescu, 2016).

Alluvial soils from the Dnieper meadow.

The profile of the non-irrigated soil in the lower reaches of the Dnieper is characterized by pronounced stratification and is horizontally located layers that are sufficiently contrasting in the granulometric composition.

This significantly worsens the water regime of these soils and contributes to waterlogging during rainy periods and drying out during droughts. There are five genetic horizons in the profile of the non-irrigated soil.

The granulometric composition of the upper horizons (sodden 0-9 cm and humus gleyed 9-25 cm layers) is characterized as sandy-loamy. In the first transition horizon (25-44 cm), the quantity of clay fractions decreases (Figure 4).

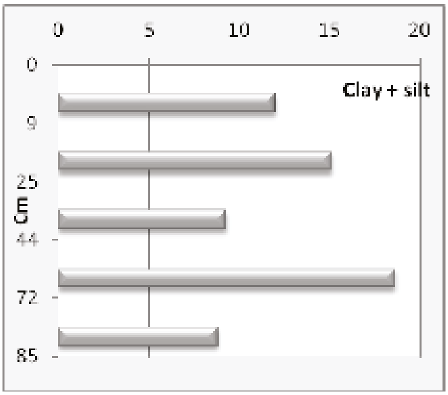

Figure 4. Content and profile distribution of physical clay in non-irrigated soil of the Dnieper floodplain, %

This horizon regarding granulometric composition is a cohesive sand.
In the second horizon (44-72 cm), the sum of fractions less than 0.01 mm decrease in two time, in comparison with the previous one, and it is characterized as sandy loam. It is buried under sandy alluvial deposits the humus horizon. Deeper in the profile, the content of clay fractions decreases - the rock is characterized as cohesive sand.
Alluvial (soddy non-irrigated) soil is characterized by a high degree of structurization and lumpy-granular structure. The humus gleyed horizon (9-25 cm) is poorly structured. The remaining horizons are practically structureless. The first transitional (25-44 cm) is due to a light granulometric composition, deeper due to the high degree of gleying (Figure 4).
When studying the profile of irrigated soil, the lower reaches of the river Dnieper three genetic horizons are distinguished. The irrigated alluvial soil in the 0-20 cm layer (arable horizon) has sandy loam granulometric composition (Figure 5) and is characterized by a high degree of cultivation - contains more than 4 percent of humus, which contributes to its structuring. The lower horizons of this soil, typical of soils formed on sands of alluvial origin, are cohesive sands.

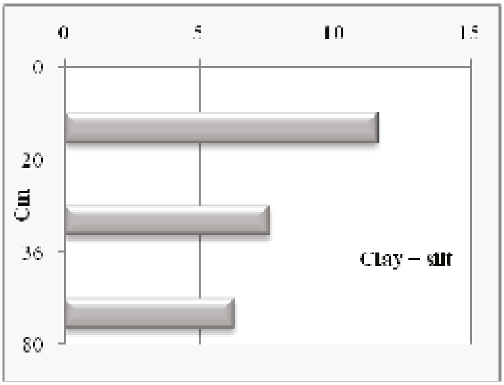

Figure 5. Content and profile distribution of physical clay in irrigated soil of the Dnieper floodplain, %

Sandy and sandy-loamy soils are easy to process without significant effort. However, on light soils there is no mechanism for maintaining the addition parameters created as a result of processing, so they require more frequent loosening (Medvedev, Laktionova, 2011). They are often characterized by lack of structure, caused by a low amount of physical clay, which we observe.
In addition, high water permeability and low water retention capacity create the danger of insufficient supply of plants with moisture and cause the spontaneity of the water regime. These features of the granulometric composition of the studied soils must be taken into account when determining the list of necessary ameliorative effects in irrigated agriculture.
The common characteristic of alluvial soils in the meadow beneath the Dniester and Dnieper terraces is the cyclicity of the pedogenesis processes in them. Alluvial soils are by nature cumulative soils: they grow upward under the influence of the alluvial process. The evolution of the cyclical process of alluvial soil pedogenesis comprises three phases.
The first phase of pedogenesis is the underwater gleic phase, that result in the intense accumulation and gleyzation of the accumulated material in short periods between the overflows of evil, which favors the formation of thin humic layers.
The second phase of aluvisoils pedogenesis was influenced by the radical changes in the course of the river's lateral arms, characterized by long periods without flooding or with very slow overflows, which did not lead to massive accumulations of alluviums and to the process of pedogenesis.
The third phase of pedogenesis is related to the accumulation of a layer (about 80 cm) of homogeneous texture alluviums by massive flooding, which led to the burial of the marshy soil, formed in the previous phase (Calaşnic, 2008).
Under conditions of anthropic degradation of soil cover and global warming, food security can only be ensured by extending the surfaces with irrigated soils. For the development of the irrigated agriculture the most suitable are the lands in the meadows of rivers with non-salinizated and non-solonetzizated soils.

CONCLUSIONS

Irrigated soil of the floodplain of river Dnieper is characterized by visible layering of the profile and the presence of a buried humus horizon. The upper and buried humus horizons in the granulometric composition are sandy-loamy, the transition horizon and the parent rock are cohesive sand. Irrigated soil is more remote from the river bed. Its profile is characterized by lower stratification, absence of buried horizons, gradual, with depth, facilitation of granulometric composition from sandy-loamy to cohesive sand.

Humus horizons of soils are characterized by structurization (varying degrees), deeper horizons of soils are structureless.

Alluvial soil of Dniester meadow have a clayey texture and low content of humus, unsatisfactory physical state of arable layer, it's are hard object to use in agriculture.

Alluvial clayey-loamy soils from Dniester basin and alluvial sandy-loamy soils from Dnieper are suitable for irrigation and use in agriculture for vegetables, potatoes, annual and perennial leguminous (Program, 2004).

The main measure of remediation of the irrigated post-marshy alluvisoils needs to be oriented towards increasing the flow of organic matter into the arable layer and restoring their natural structure.

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IMPACT OF CLIMATE CHANGE ON AGRO-CLIMATIC INDICATORS IN TRANSYLVANIAN PLAIN BETWEEN 2009-2016

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Abstract

Transylvanian Plain (TP), with an area of 395,616 hectares, is considered as an area with a lowest capacity to adapt to existing climate change. In these conditions, for a better sustainable development of agricultural technologies, the deployment of measures to adapt to these changes and climate monitoring are essential.Purpose of this paper is to analyse and monitor the agro-climatic indicators between 2009-2016 and the data obtained will be the basis for the technological development of the recommendations adapted to the current favourable conditions. For the study we used 10 HOBO microstations located in TP that record the temperature of the soil at 10 cm, 30 cm and 50 cm depth, the soil humidity at 10 cm depth, the air temperature at 1 m and rainfall. In TP between 2009-2016, the multiannual average of temperature at 10 cm depth in soil is 11.50°C, respectively 11.47°C at 50 cm depth in soil and the multiannual average of soil moisture is 0.223 m^3/m^3. The multiannual average of rainfall is 514.29 mm. Results highlight evolution of agro-climatic indicators in TP in the period 2009-2016.

***Key words**: soil temperature, soil humidity, precipitations, Transylvanian Plain.*

INTRODUCTION

Extensive research carried out on climate change, highlighted based on observational data and projections made for long periods of time, show that the changes in the evolution of climate, global, regional and local levels are an undeniable fact, and have a negative impact on maintaining the right balance in the relationship between society and environment (Szajdak et al., 2003; Keskitalo et al., 2015).

Climate is represented by dynamics of all meteorological phenomena from the atmosphere, including the soil hydric and thermal regime, from a specific place or region in the world, during a very long period of time (Dumitrescu et al., 2015).

Between 1880-2012, global average temperatures, show an average increasing of 0.85ºC (0.65-1.6ºC) for several series of independent data, with great inter-annual and decadal variability (Haylock et al., 2008; Lereboullet et al., 2013).

Regarding the climate changes, they are caused by the increase of greenhouses gases, which result from simultaneous actionof certain internal or external factors and external anthropogenic factors changing the atmosphere composition (IPCC, 2014a; Perry, 2015; Marin et al., 2016).

Recent studies estimate that at ground level, extreme temperatures will grow faster than global warming, and the probability of temperatures above 48ºC being met every decade as we approach 2100, is 10%, if the global temperature exceeds the level since 1850.

According to three different observational records of global average annual near-surface (land and ocean) temperature, the last decade (2007–2016) was 0.87 to 0.92°C warmer than the pre-industrial average, which makes it the warmest decade on record (EEA, 2017).

The average annual temperature for the European land area for the last decade (2007-2016) was around 1.6°C above the pre-industrial level, which makes it the warmest decade on record. Moreover, 2016 was the second warmest year (after 2014) in Europe since instrumental records began.

Due to the increase of greenhouse gas concentrations, the global average temperature projected climate forecasts an increase by the middle of the century by 0.4-1.6 (Collins et al., 2013).

In terms of rainfall, at European level, since 1950, the annual amounts of precipitation in northern Europe rose by more than 70 mm perdecade and fell in some parts of the South, while changes made to exploiting fieldsmodify the rainfall (Haylock et al., 2008; IPCC, 2014a).

Changes in large-scale circulation patterns play a key role to observe precipitation changes, but it is not clear if the relatively minor land-use changes at European level since the 1950s have influenced observed precipitation trends.

While agriculture is affected by climate change, in turn, agriculture contributes with 13.5% of the total greenhouse gas emissions (GGE). Of GGE emissions at European level, those from agriculture have a share of 2 to 26% with an average of about 14% of the total (FAO, 2009).

Transylvanian Plain (TP) is considered one of the most affected areas with the lowest capacity to adapt to existing climate change.

In the last two centuries, the anthropogenic impact of TP has increased considerably, being now a hilly area with problems of soil sustainability, limited water, rainfall deficit and very low forest cover of only 6.8% (Rusu et al., 2014).

Due to these conditions, it's essential to monitoring the climate and applying adaptation measures to climate changes for the sustainable development (Rusu et al., 2017). The objectives of this paper are to monitor the agroclimatic indicators (soil temperature and humidity, rainfall and air temperature) and climatic conditions for the period 2009-2016 in TP.

MATERIALS AND METHODS

Transylvanian Plain with an area of 395,616 ha is composed of three counties Cluj (CJ), Bistriţa Năsăud (BN) and Mures (MS), and together with Someş Plateau and Târnave Plateau is forming the Transylvanian Depression. TP is located in the central part of the Transylvanian region, being characterized by absolute altitudes ranging between 250-500 m. From an agricultural point of view, the main crops present here are winter wheat, corn, sunflower, soybean and sugar beet, holding the largest share of the total cultivated areas (INS, 2014).

Although the TP relief is hilly, the dominant vegetation is represented by hayfields and forest steppe, due to the annual precipitation deficit, the wood vegetation occupies a low surface area because of deforestation (Coste, 2015).

For the study, we used data of temperature and humidity electronically recorded by 10 microstations type HOBO-MAN-H21-002, during 2009-2016, located in different areas of TP with different elevations (Table 1), and different types of soil (Rusu et al., 2017).

For recording rainfall, stations were provided with rain gauges RG3-M type. Monitoring climatic elements, stations were located in different areas of TP with different pedoclimatic issues.

Soil temperature data were recorded electronically at 10, 30 and 50 cm depth, air temperature and rainfall to a height of 1 m, and soil moisture at 10 cm depth in soil. Data was downloaded from the Micro Stations to the computer using HOBO Ware Pro Software Version 2.3.0.

Table 1. Station location in Transylvanian Plain

Nr. crt.	Station/County	Soil Type and Subtype*	Latitude	Longitude	Elevation (m)
1.	Căianu (CJ)	Cernoziom calcaric	46º79'	23º52'	469
2.	Mociu (CJ)	Eutricambosoil typic	46º47'	24º04'	435
3.	Ţaga (CJ)	Preluvosoil typic	46º97'	24º01'	469
4.	Braniștea (BN)	Eutricambosoil typic	47º17'	23º47'	266
5.	Dipșa (BN)	Phaeoziom typic	46º96'	24º26'	356
6.	Zoreni (BN)	Preluvosoil typic	46º89'	24º16'	445
7.	Silivașu de Câmpie (BN)	Eutricambosoil mollic	46º78'	24º18'	463
8.	Filpișu Mare (MS)	Districambosoil typic	46º74'	24º35'	375
9.	Band (MS)	Phaeoziom argic	46º58'	24º22'	318
10.	Triteni (CJ)	Phaeoziom vertic	46º59'	24º00'	342

CJ = Cluj County; BN = Bistrita-Nasaud County; MS = Mures County;

RESULTS AND DISCUSSIONS

Thermal regime of the soil, is influenced by a number of factors, primarily on the intensity of the solar regime and its time variation, followed by the physical properties of the soil, orientation and tilt of the slopes.

Analysing the thermal regime of the soil, multiannual average temperature at 10 cm depth in soil recorded in TP during 2009-2016 is 11.50°C, the highest temperature was recorded at Filpisu Mare station (13.25°C), respectly 11.47°C at 50 cm depth in soil with the lowest temperature at Triteni station (10.87°C).

The multiannual average of soil moisture recorded in the same period is 0.223m^3/m^3, the values being ranged between 0.188-0.244 m^3/m^3 (Table 2).

Table 2. Multiannual average (years 2009 - 2016) of temperature and water contentat 10 cm soil depth, for 10 stations in TP

Nr. crt.	Station name	T°C at 10 cm in the soil	T°C at 50 cm in the soil	Soil moisture (m^3/m^3) at 10 cm in soil
1	Țaga (CJ)	10.90	11.13	0.212
2	Braniște (BN)	11.77	11.58	0.220
3	Dipșa (BN)	11.53	11.72	0.241
4	Zoreni (BN)	11.37	11.30	0.238
5	Silivașu de Câmpie (BN)	11.12	11.03	0.235
6	Filpişu Mare (MS)	13.25	12.93	0.210
7	Band (MS)	11.40	11.27	0.188
8	Triteni (CJ)	10.67	10.87	0.244
Average TP		**11.50**	**11.47**	**0.223**

CJ = Cluj County; BN = Bistrita-Nasaud County; MS = Mures County

Temperatures recorded at 50 cm in the soil in TP are ranged between 8-15°C.

Regarding the average of the multiannual air temperatures, the lowest value was at Band station with 11.03°C, and the highest one was recorded at Dipsa station with 11.54°C (Figure 1).

In terms of rainfall, multiannual average value recorded between 2009 and 2016 was 514.29 mm, value that falls within the annual average of 500-700 mm/year (Figure 2).

The highest value of annual average rainfall was recorded in 2016, 725 mm and the lowest value was in 2012, 279.40 (Table 3).

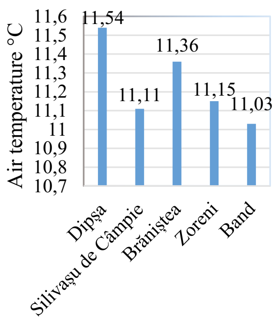

Figure 1. Multiannual averages of temperature (2009-2016), in Transylvanian Plain

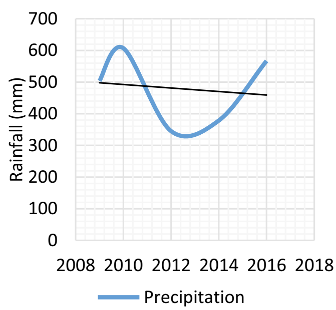

Figure 2. The evolution of the average values of rainfall (mm) in TP, between 2009 - 2016

Table 3. Annual average of rainfall (mm) in TP, between 2009-2016

Station/Year	2009	2010	2011	2012	2013	2014	2015	2016
Brăniştea	653.60	671.40	334.60	279.4	496.00	-	498.40	725.00
Căianu	350.80	662.40	368.60	315.60	343.80	378.60	556.80	514.20
Dipşa	465.40	628.60	396.00	406.00	488.60	-	495.60	-
Silivaşu de Câmpie	581.40	561.60	391.80	315.60	522.80	378.60	556.80	-

The soil moisture at 10 cm depth is directly influenced by the rainfall regime.

As can be observed linear decrease tendency is keeping in 2012 when it was recorded the lowest value (0.202 m^3/m^3) compared to 2010 when it was the highest value (0.260 m^3/m^3), (Figure3).

During period 2009-2016, the analysis of annual mean soil moisture values recorded at 10 cm depth in TP indicates a downward trend.

The highest value of multiannual average was recorded at Triteni station (0.244 m^3/m^3), while the lowest value was recorded at Band station (0.188 m^3/m^3).

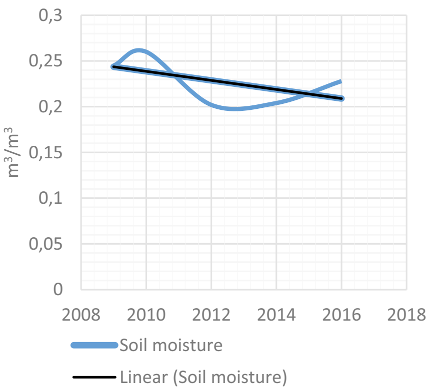

Figure 3. The evolution of the average values of moisture (m^3/m^3) in TP, between 2009 - 2016

CONCLUSIONS

The certainty of climate change has been and is being highlighted by global research, and its effects have a negative impact on all sectors and areas of activity, and can also be observed in the Transylvanian plain.

In Transylvanian Plain, climate changes can be observed both in values of temperature recorded at 10 cm and 50 cm depth, soil moisture and the variation of multiannual averages of air temperature.

The multiannual average of temperature in soil, recorded between 2009-2016 at 10 cm depth is 11.50ºC, respectively 11.47ºC at 50 cm depth. The highest multiannual average of air temperature for the same period was 11.54ºC, recorded at Dipsa station. The multiannual average of soil moisture recorded in the same period is 0.223 m^3/m^3. The multiannual average value of precipitation is 514.29 mm.

In Transylvanian Plain due to the reduced precipitation regime, there is the possibility of increasing the water deficit.

Analyzing the parameters of soil temperature, soil moisture and rainfall, it is recommended to apply measures that help to maintain and increase the water reserves in the soil, thus decreasing the degree of soil erosion.

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HEAVY METALS FROM THE SOIL AND MINERAL FERTILIZATION

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Abstract

The degree of soil loading with heavy metals (Cd, Cu, Mn, Ni, Pb, Zn) has been studied after 39 years of fertilization with nitrogen and phosphorus on a Faeoziom cambic soil in Drăgănești-Vlasca village in Teleorman. Nitrogen from nitrogen nitrate was applied at doses of 0, 50, 100, 150, 200 kgN/ha, and phosphorus from superphosphate at doses of 0, 40, 80, 120, 160 kg P/ha. The soil samples were harvested at a depth of 0-20 cm after harvesting the wheat crop. The heavy metal content did not suffer statistically significant changes following long-term fertilization with mineral fertilizers with nitrogen and phosphorus. No cadmium accumulation process was observed following the application of doses of 40-160 kg/ha $P_2 0_5$ annually for 39 years. The cadmium values in the soil oscillated between 0.9 and 1.2 mg/kg, the loading level being assessed as normal-weak, the copper values were in the low loading range, the manganese is within the range of normal-weak, and copper and manganese in the soil content are within the normal range.

Key words: *long-term experiences, heavy metals, fertilizers.*

INTRODUCTION

Water, air and soil are the most vulnerable environmental resources, but most frequently subject to aggression by pollutants, with direct and serious consequences not only on the quality of the environment but also on the health of humans and other living creatures. The most frequent environmental pollution factors usually come from industry, but lately, agriculture is increasingly being discussed (Dumitru et al., 2003).

One of the important components of sustainable development is environmental protection.

The presence of contaminants can cause immediate or long-term danger to human, animal or plant health, which may lead to limiting or excluding land use (Dumitru et al., 2008).

Heavy metals are found in all types of soil in larger or smaller quantities up to trace levels. Once introduced into the environment as a result of anthropogenic activities, they will be present in the soil in the following forms: dissolved in the soil solution, occupying exchange positions or adsorbed specifically on the inorganic soil constituents associated with organic matter or associated with the structure of primary or secondary minerals.

Although it is a rare element, cadmium is distributed everywhere in the environment.

The cadmium concentration in the earth's crust is estimated to be between 0.08 and 0.5 Cd mg/kg.

Both in the upper horizon of the soil and in the marine sediments the content varies between 0.1-1 mg Cd/kg, in seawater is estimated a concentration of 0.02-0.25 mg/l Cd. The main sources of cadmium are the minerals of zinc (Shulte -Schrepping quoted by Kaarstad, 1991).

The main sources of cadmium in industrialized countries are:

- The natural load of soils in cadmium;

- Atmospheric deposits from the production of non-ferrous metals, iron and steel, burning of fossil fuels;
- Manure from animals with cadmium in their diet;
- From phosphorus fertilizers (Dumitru et al., 2008).

Heavy metals penetrate the depth of the soil through precipitation, through biological accumulation penetrate into plants and through consumption pass to humans and animals (Nicoleta Vrînceanu et al., 2010).

Ingested in high doses, cadmium is toxic to humans, its admissible concentration in foods in European countries is 10 µg/day cadmium, the tolerable weekly intake is 70 µg/day cadmium. Removal of cadmium from the human body occurs much more slowly than other contaminants. Around 20 years are needed to eliminate half the amount (Dumitru et al., 2008).

MATERIALS AND METHODS

The experience was located within SCDA Teleorman. For the field experiments it was used, the method of two-factor sub-division and 3 repetition parcels and consisted in 25 variants.

Fertilizers with nitrogen and phosphorus were applied, nitrogen (N) at doses of 0, 40, 80, 120, 160 N kg/ha as ammonium nitrate, phosphorus (P) at doses of 0, 40, 80, 120, 160 P kg/ha, administered from superphosphate. Soil harvesting was carried out at the end of the wheat culture vegetation period, at a depth of 0-20 cm.

The total concentrations of heavy metals were determined in the soil samples by atomic absorption spectrometry after extraction by the aqua regia - microwave digestion method (SR ISO 11047:1999).

Microwave digestion was performed using 10 mL of aqua regia (7.5 mL HCl and 2.5 mL HNO_3) at 140°C for 30 min.

A certified soil reference material (ERM - CC141) was used to ensure the accuracy of the analytical data.

RESULTS AND DISCUSSIONS

A. Cadmium content in soil

The research carried out within the National Soil Quality Monitoring System 16 x 16 km revealed the following: the cadmium loading level of the soil was normal (values < 1.1 mg/kg) in 88.04% of the cases, weak (1.1-2,0 mg/kg) in 28.45% of cases, medium (2.1-3.0 mg/kg) in 2.55% of cases and strong (3.1-7.0 mg/kg) in 1% of cases (Dumitru et al., 2000).

According to the 2016 statistical yearbook in Romania, in the year of 2014, about 9 kg/ha of phosphorus were applied to agricultural land, far below plant requirements, considering that the supply level of mobile phosphorus was low:

Average phosphorus content in the 0-50 cm layer:
- extremely low < 4 mg/kg 107 sites (11.36%);
- very low 4-8 mg/kg 198 sites (21.02%);
- low 9-18 mg/kg 311 sites (33.01%);
- medium 19-36 mg/kg 195 sites (20.70%);
- high 37-72 mg/kg 89 sites (9.45%);
- very high > 72 mg/kg 42 sites (4.46%).

In order to see the difference of opinion between Romania and the Netherlands regarding phosphorus management in phosphorus mineral fertilizers, we present P-load classes in the Netherlands.

Table 1. Soil content classes in accessible phosphorus of the soil in Netherlands (Anonimous, 2015 a,b)

Phosphorus accessibility classes	Agricultural land (mg P/kg dry soil)
Class I (low accessibility of P)	<120
Class II (target areas for P)	120 - 180
Class III (moderate accessibility of P)	190 - 400
Class IV (high accessibility of P)	>410

The content of heavy metals (cadmium, cooper, manganess, nickel, lead, zinc) have not been modifical statistically significant changes following long-term fertilization with nitrogen and phosphorus.
Cadmium values fluctuated between 0.9 and 1.2 mg/kg. Thus, no cadmium accumulation process mas observed following the application of doses 40-160 kg P/ha annualy for 39 years.
Lupașcu et al. (2017) on a typical chernozem from Valu's Traian found that cadmium values fluctuated between 0.40 and 0.44 mg/kg of copper between 21 and 24 mg/kg of lead was 20 mg/kg and zinc between 83 and 84 mg/kg. The heavy metal content (cadmium, copper, lead and zinc) did not suffer statistically significant changes from long-term fertilization (44 years) with nitrogen and phosphorus.
The European Commission appreciates in the "Proposal for a Regulation of the European Parliament and of the Council on the rules for marketed CE fertilizer products available on the market and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009" Phosphorus fertilizers sold in the EU are contaminated with cadmium, typically somewhere between 32-36 mg/kg of P_2O_5.
It has been argued that 80 mg/kg of P_2O_5 is an appropriate legal cadmium contamination limit since - until recently - it was estimated an average contamination level for "non-accumulation" in the land on European farms . "Non-accumulation" means that the cadmium level in contaminated soil in farm land does not rise above current levels, because all new cadmium additions in soil are either taken up by crops (and ultimately consumed by humans or animals) or are washed from the fertile horizon of agricultural soils.
Forstner (1980) estimated that phosphorus fertilizers have a cadmium content ranging between 2 to 180 ppm.
At moderate levels of cadmium (5-10 ppm) in phosphorus fertilizers, there was no significant correlation between the applied dose and the cadmium concentration in the soil horizon after 20 years of application.
Grant et al. (2010) shows that phosphorus fertilization, especially for flax culture, tended to increase the cadmium concentration and the Cd: Zn ratio and to decrease the zinc concentration in tissues and flaxseed.
Lambert et al. (2007) found that the application of phosphorous fertilizers containing cadmium and zinc increased the cadmium concentrations in the soil solution in both field and laboratory experiments. The increase of the metal contamination level or of the applied dose has increased cadmium concentration in soil extract for field and laboratory experiments. The behavior of zinc is not closely related to cadmium. The lower zinc concentration in the soil solution is, at least in part, due to the addition of phosphorus.
Grant et al. (2013) estimated that through the increase of cadmium reserves in the soil influences the amount of cadmium accessible to crops, but the cadmium concentration in durum wheat and in flax seeds will be strongly affected by soil characteristics and environmental conditions in the season of vegetation. The type of crop and soil characteristics and climatic conditions affecting crop accesibility must be taken into account when assessing the risk of transfer of cadmium into the food chain in phosphorus fertilization.
Gao et al. (2011) estimated an increase of concentration and accumulation of cadmium in durum wheat strains immediately after phosphorus fertilization, primarily, as a result of the reduction of competition between zinc and cadmium for plant absorption, of the improvement of cadmium translocation from the root to the stem and improvement of root development , more than the effect of direct cadmium addition with the phosphorus fertilizer. In the short term, the application of phosphorus fertilizers can increase the cadmium concentration in crops, unrelated to cadmium concentration in fertilizers. An optimal fertilization strategy, such as in combination with zinc application, is of great importance to reduce cadmium concentration and accumulation in crops.
Hong et al. (2010) selected seven phosphorus materials (commercial phosphorus fertilizer - mixture of phosphates, mixture of phosphates and superphosphates and phosphate rock, phosphorus chemicals - $Ca[H_2PO_4]_2 \cdot H_2O$, $[NH_4]_2HPO_4$, KH_2PO_4 and K_2HPO_4) selected

for the incubation test, the mixture of phosphates, Ca $[H_2PO_4]_2 \cdot H_2O$, KH_2PO_4 and K_2HPO_4 significantly decreased the extractable cadmium concentration in NH_4OAc (plant accessible form) with increase of the applied doses . The selected phosphorus sources were mixed with contaminated soil with 0, 200, 400, 800 and 1600 P/kg. In particular, K_2HPO_4 was found to be the most effective, mainly due to the increase of the negative charge produced by soil pH and phosphorus adsorption. Phosphorus induced a relief of cadmium extraction which can be attributed primarily to cadmium immobilization due to the increase in soil pH and negative charge, not the precipitation of cadmium phosphate, and therefore alkaline phosphorus materials such as K_2HPO_4 can be effective in immobilizing cadmium in soil.

Corguinha et al. (2012) show that a major concern in assessing health risk is cadmium consumption, and food intake is an important route of exposure. Although the addition of phosphorus fertilizer can increase cadmium content in soils, its transfer to the plant varies according to the management system. They evaluated the cadmium contents of different potato types fertilized with 560 kg P_2O_5/ha and in soybeans cultivated in different soil management systems with long-term application of different management systems and where they were applied high doses of phosphorus fertilizer. The largest amount of cadmium remains in the potato peel (23-781 μg/kg bw) compared to the tuber (14-43 μg/kg bw), and the values vary according to type and area. For soybeans, the grain content ranged from 10 to 30 μg/kg b.w. in rotational experiments and 23-28 μg/kg b.w. for soils that received different doses of calcium carbonate amendments. All the cadmium content found in the crops studied is in line with the Codex alimentarius guide, so there is no risk to human health.

The Health Risks and Environment Committee (2015) shows that phosphate fertilizers used in the EU had an average content of 36 mg Cd/kg P_2O_5.

Working Group of the Council of the European Commission quoting Smolders and Six (2013) presents on 20-21 September 2016 sources of cadmium in agricultural soils: manure 0.01 g Cd ha^{-1} $year^{-1}$, sludge from municipal wastewater treatment plants 0.05 g Cd $ha^{-1}year^{-1}$, the amendments with calcium carbonate 0.09 g Cd $ha^{-1}year^{-1}$, atmospheric deposits 0.35 g Cd $ha^{-1}year^{-1}$ and phosphorus fertilizers 0.8 g Cd $ha^{-1}year^{-1}$ (where the average dose of phosphorus fertilizer was 100 kg P_2O_5/ha).

The concentration of Cd in phosphate rocks varies greatly with origin, e.g. eruptive phosphate rocks have lower Cd concentrations (0.07-0.25 mg Cd/kg rock) compared to sedimentary rocks (0.01 - 2.60 mg Cd/kg rock). The smallest concentrations of metals are generally found in phosphate rocks from the Scandinavian countries, while the highest values were found in Nauru, Togo and Morocco, where the values varied between 2 and 1500 mg Cd/kg P_2O_5.

Smolders (2013) estimates that an average contamination level of 80 mg/kg P_2O_5 leads to soil growth increase with 3% after 100 years, a contamination level of Cd of 60 mg/kg was estimated to result in a decrease of 7% over the same period of time, a contamination level of 40 mg/kg P_2O_5 leads to a 14% decrease after 100 years, a 20 mg/kg P_2O_5 contamination level leads to a 20% decrease after 100 years.

However, the Working Group of Technical Harmonization proposes in 2016, the following limits for cadmium in phosphorus fertilizers : 60 mg Cd/kg P_2O_5 (initial limit value after application of the Regulation), 40 mg Cd/kg P_2O_5 (three years after the application date of the Regulation) and 20 mg Cd/kg P_2O_5 (12 years after the application date of the Regulation).

The General Secretary Council considered that if the limit of 60 mg Cd/kg P_2O_5 was introduced, even in the absence of an available decadmiation tehnologies, most sources of phosphorus fertilizer usually used in the EC could still be used. When introducing a limit of 40 mg Cd/kg P_2O_5 will require specific efforts from the EU fertilizer industry, namely the introduction of an available decadmiation tehnologies.

Rietra et al. (2017) felt that in the EU only 55% of cadmium taken up by people in the total diet is related to cadmium in the soil. A reduction of

50% of cadmium in soil is estimated to reduce the uptake of cadmium in the diet by 18%. Based on these calculations, cadmium limits for phosphorus fertilizers will have little impact on levels of cadmium in the soil on a time scale of 20-50 years. Taking into account even smaller responses to the crop taking of such limited changes in cadmium levels in the soil, it is estimated that a reduction in cadmium levels in fertilizers will have a marginally lower exposure to cadmium in the diet, in Europe.

Six and Smolders (2014) estimate that the use of phosphorus fertilizers in the EU 27 + 1 has fallen by 40% over the past 15 years.

They studied cadmium leaching in 151 soils covering a wide range of European soils properties, and noticed no tendency in cadmium accumulation time following application of manure, compost, urban sludge and calcium carbonate, all of which on large-scale are small sources of cadmium.

The modeling of future long-term changes in cadmium concentrations in the upper horizon of agricultural soils cultivated with cereals or potatoes will result in a 15% decrease in cadmium concentrations in soil over the next 100 years.

Bolan et al. (2003) showed that the application of KH_2PO_4 increased pH and surface charge, the effect being more pronounced in soils dominated by variable load components.

This process induces the addition of specifically adsorbed anions resulting in increased sequestration of the added cations, thus reducing their accessibility in plants. Addition of phosphates improves cadmium immobilization, as shown by increased adsorption, cadmium redistribution to less accessible fractions, and decreasing accessibility for plants.

In fact, if we consider the 80 mg Cd/kg P_2O_5 content, we would apply 100 kg of P_2O_5/year for 100 years without losing anything in the applied cadmium could increase the cadmium content by 0.267 mg/kg , which does not represent any risk to the environment.

Under these circumstances, I wonder why we need to change the limit of cadmium that can accompany phosphorus from mineral fertilizers?

B. Copper content in soil

The research carried out within the National Soil Quality Monitoring System 16 x 16 km revealed the following: copper loading was normal (< 21 mg/kg) in 57.43% of cases, low (21-40 mg/kg) in 36.67% of cases, medium (41-100 mg/kg) in 5.31% of cases (101-200 mg/kg) in 0.83% and very high (201-400 mg/kg) 0.11% of cases (Dumitru et al., 2000).

Li et al. (2007) showed that after 16 years of fertilization with different fertilizers, the concentrations of Cu, Fe and Mn extractable in DTPA were not significantly altered, while soil-soluble Zn was slightly higher in all treatments compared to unfertilized control. Treatments with NP, NPK, ½ fertilized organic plus half fertilized with N, and organically fertilized, led to increased wheat and corn production and organic matter levels in the soil, which also led to an increase in Zn and Fe extract in DTPA.

Maintaining or increasing organic matter in the soil is very important in providing micronutrients accessible to crops.

Through long-term fertilization with phosphorus, nitrogen or nitrogen and phosphorus has not produced statistically significant changes in the copper content of the faeoziom in Drăgănești Vlașca.

Copper values have been recorded in the low level field. Data in the literature does not show changes in the soil content of copper under the influence of mineral fertilization with nitrogen and phosphorus.

Table 2. Heavy metals content in cambic faeoziom from SCDA Teleorman

Variants	Cd (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Control	1.00	28	884	37	18.7	76
N50	1.06	33	981	40	19.6	85
N100	0.93	30	829	34	17.7	69
N150	0.95	30	846	34	18.0	72
N200	1.02	30	896	36	19.4	77
P50	1.20	32	969	47	19.8	91
P50 N50	0.91	30	855	35	16.0	69
P50 N100	105	30	927	36	17.5	77
P50 N150	1.09	32	937	39	19.0	80
P50 N200	1.10	29	929	38	19.2	80
P100	1.02	31	942	37	19.2	78
P100N50	1.23	32	962	42	20.8	85
P100N100	0.95	28	829	32	15.2	69
P100N150	0.89	28	895	34	17.3	73
P100N200	1.15	33	971	41	21.0	84
P150	0.98	27	888	37	18.7	75
P150N50	1.16	31	1001	40	21.5	83
P150 N100	0.97	27	875	35	16.1	74
P150N150	0.94	27	872	35	17.9	74
P150N200	0.949	28	831	35	16.9	71
P200	0.88	27	829	34	15.1	71
P200N50	1.17	32	963	43	21.0	86
P200 N100	1.00	29	892	37	17.6	78
P200N150	0.97	28	862	34	15.5	78
P200N200	1.07	29	945	38	17.2	80
DL 5%	0.29 mg/kg	6 mg/kg	191 mg/kg	10 mg/kg	6 mg/kg	18 mg/kg
DL 1%	0.39 mg/kg	8 mg/kg	255 mg/kg	14 mg/kg	8 mg/kg	24 mg/kg
DL 0,1%	0.51 mg/kg	11 mg/kg	335 mg/kg	19 mg/kg	11 mg/kg	32 mg/kg

C. Manganese content in the soil

After iron, manganese is the most abundant heavy metal in the earth's crust. Total Mn reserves vary greatly due to the diversity of soil cover from 10-10000 mg/ kg of soil. Excessive potentially toxic manganese content for plants, may occur under the conditions of systematic application of acidifying fertilizers to soils with low buffering capacity. Unlike Cu and Al that accumulate mostly in the roots, manganese is translocated into the plant aerial part. Symptoms of leaf toxicity occur at concentrations above 300 mg/kg (Băjescu et al., 1984).

The research carried out under the National Soil Quality Monitoring System showed the following: the degree of loading with manganese was normal (< 901 mg/kg) in 93.20% of the cases, low (901-1100 mg/ kg) in 3,4% of the cases, average (1101-1500 mg/kg) in 2,23% of cases and strong (1501-2000 mg/kg) in 0.11% of cases (Dumitru et al., 2000).

The data presented in table 2 did not reveal statistically significant changes in total manganese content in the soil under the influence of fertilization with nitrogen, phosphorus or nitrogen and phosphorus. Data from the literature does not provide informations on the influence of mineral fertilization with nitrogen and phosphorus on the total content of manganese in the soil. Manganese values ranged from normal to low loading.

D. Nickel content determination in soil

The research carried out under the National Soil Quality Monitoring System showed the following: the degree of soil nickel loading was normal (< 21 mg/kg) in 21.87% of cases, low (21-30 mg/kg) in 29.82% of cases, mean (31-50 mg/kg) in 37.79% of cases, strong (51-100 mg/kg) in 9.55% of cases and very strong (101-300 mg/kg) in 0.32% of cases.

There were no statistically significant changes in soil nickel content under the influence of mineral fertilization with nitrogen, phosphorus or nitrogen and phosphorus. The soil nickel content was found in the average loading range with this element. In the literature, we have not found data on the influence of mineral fertilization with nitrogen and phosphorus on soil content in nickel.

E. Lead content in soil

Research into the National Soil Quality Monitoring System revealed the following: lead load was normal (< 21 mg/kg) in 22.72% of cases, low (21-40 mg/kg) in 57,32% of cases, medium (41-101 mg/kg) in 18.47% of cases and strong (101-300 mg/kg) in 0.53% of the cases (Dumitru et al., 2000).

The data obtained in long-term experience revealed no statistically growth of lead in the soil under the influence of mineral fertilization with nitrogen and phosphorus. The lead load level was maintained within the normal range.

F. Zinc content in soil

The research carried out within the National Soil Quality Monitoring System revealed the following: The soil load in zinc was normal (< 101 mg/kg) in 78,54% of the weak (101-150 mg/kg) in 9.45% of cases; average (151-300 mg/kg) in 11.04%.

The data presented in table 2 do not reveal statistically significant changes in the zinc concentration in the soil under the influence of mineral fertilization with nitrogen and phosphorus. All values fall within the normal supply range.

IV. CONCLUSIONS

No cadmium accumulation process was observed following the application of doses of 40-160 kg/ha P_2O_5 annually for 39 years.

For a content of 80 mg Cd/kg P_2O_5, the application of 100 kg P_2O_5/year for 100 years without losing any of cadmium applied could increase soil contents of cadmium with 0.267 mg/kg, which is not a risk to the environment.

Although ammonium nitrate has an acidic physiological reaction, the application of doses up to 200 kgN/ha for 39 years has not resulted in statistically assured accumulations of manganese in the soil;

The long-term application of ammonium nitrate and superphosphate does not lead to the accumulation of heavy metals in the soil.

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DYNAMICS OF SOIL PROPERTIES UNDER A POLLUTION GRADIENT IN URBAN AREAS (PLOVDIV, BULGARIA)

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Abstract

Urban ecosystems are comprised of diverse land areas, resulting in different habitats for plants, animals and human within urban landscape. Urban habitat quality results the integration of different abiotic and biotic components, such as air, soil and water quality, microclimate and vegetation. Urban soils differ from the other components by the prolonged retention and accumulation of pollutants. As the traffic is rising as the most serious emitter of harmful substances in the environment, we aimed to assess its effect to some soil properties under a pollution gradient. Soil samples were collected on the 7.5 m, 25 m, and 50 m distance from two of the main boulevards in the city of Plovdiv (Bulgaria), using the transect method. Content of some heavy metals and toxic elements in soils was analyzed using ICP-MS. Data revealed that soil contamination is strongly influenced by the distance from the road, followed by the wind rose and urban gradient. Regarding the microbial soil communities, this study confirms that the anthropogenic pressures (building and road infrastructure, deforestation) are the most important factors affecting the soil quality in the urban areas.

***Key words**: urbanization, pollution, gradient, traffic, microbial communities.*

INTRODUCTION

Urban ecosystems are comprised of diverse land areas, resulting in different habitats for plants, animals and human within urban landscape. Urban habitat quality results from the integration of different abiotic and biotic components, such as air, soil and water quality, microclimate and vegetation. There is a clear relationship between urbanization processes and anthropogenic transformation of the landscapes structure and functioning, revealing to an increment of trace elements content in environment and modifying of their load (Petrova et al., 2014 a, b).

Urban soils are a complex and heterogeneous biogeochemical system with both natural-anthropogenic genesis. Various products from anthropogenic sources fall on the soil surface with dry and wet atmospheric deposition, accumulate into the surface horizons and cause significant changes in their chemical content before to be reintegrated in the natural or technogenic migration cycles.

Urban soils differ from the other components by the prolonged retention and accumulation of pollutants. Some studies have shown that concentrations of technogenic elements in urban soils could reflect the intensity of contamination in the past 20-50 years as the soils are more static and resistant in comparison with the other components such as the air, water, biota etc. (Penin, 1989, 1997, 2003).

Anthropogenic pressure results in pollution of all components of the urban environment, damaging the main soil properties. Microorganisms in soil ecosystems are ubiquitous, abundant, diverse and essential for many soil functions such as carbon and nitrogen cycling and plant productivity. Soil microbiology is a key component in urban ecosystems. Bacterial communities take part in different soil processes as mineralization of the organic matter, humus synthesis, nutrient supply and nitrogen fixation (Beare et al., 1994). They are of primary importance for soil quality and natural productivity.

Many European city environments have a long history of industrialization and urbanization, resulting in elevated concentrations of potentially harmful elements, including heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), vanadium (V) and zinc (Zn), derived from industrial and mineral processing, and the atmospheric depositions from traffic fumes and power generation (Reimann et al., 2005; Wang et al., 2008).

In this context, as the car traffic is rising and is the most important and constant emitter of harmful substances in the urban environment, we aimed to: i) assess its effect on both chemical and biological soil properties; ii) assess the significance of factors “Distance from the road”, “Wind rose”, “Background level” and “General urban activity” for the elements concentrations in soils near road network.

MATERIALS AND METHODS

Research site description

City of Plovdiv (N42°9', E24°45') is one of the biggest and most densely populated settlements in Bulgaria-338,000 inhabitants on 102 km^2.

Soil samples are taken in June 2017 from two of the main boulevards (Hristo Botev Boul. and Vassil Aprilov Boul.) along a pollution gradient using the transect method (Figure 1).

Method of transects

Systematic soil sampling was made along transects, where a sampling line is set up across areas with clear environmental gradients (Buckland et al., 1993). Transects started from the road towards the four directions (N, E, S, W) in order to allow the effect of the wind rose on soil properties be assessed. Twelve samples were collected at the 7.5 m, 25 m, and 50 m distance from the road (Table 1) on the depth of 0-20 cm. Each sample was formed by 5 subsamples (Petrova et al., 2013).

Soil chemical properties

Soil pH was measured using pHotoFlex Set, 2512000, WTW-Germany, and the soil conductivity was measured using Multiset, F340, WTW-Germany.

Content of Cd, Cr, Cu, Mn, Pb and Zn was determined by inductively coupled plasma mass spectrometry (ICP–MS) (Agilent 7700). Data were presented as mean values in mg/kg and residual standard deviation (RSD) in %.

Table 1. Sampling plots description

Boulevard	Sampling plot direction	Distance from the road	Soil samples symbol
Hristo Botev	North	7.5 m	HB-N-7.5
		25 m	HB-N-25
		50 m	HB-N-50
	South	7.5 m	HB-S-7.5
		25 m	HB-S-25
		50 m	HB-S-50
Vassil Aprilov	East	7.5 m	VA-E-7.5
		25 m	VA-E-25
		50 m	VA-E-50
	West	7.5 m	VA-W-7.5
		25 m	VA-W-25
		50 m	VA-W-50

Soil microbiology

Microbiological samples were collected in sterile containers and stored at 4°C in the dark until analysis for no longer than 24 h. Prior to the analysis, the samples were passed through 1 mm stainless steel sieve. A total of 1 g (dry weight equivalent) of each was suspended in 99 ml of sterile saline solution. The suspensions were homogenized by mixing at 200 rpm for 20 min for cells extraction (Goto and Yan, 2011). We used culturing techniques to compare the aerobic heterotrophic bacterial population`s total viable count (TVC) at 22°C and 37°C and fungi in soils (Zuberer, 1994). The presence of *Escherichia coli*, faecal coliforms (FC) and faecal streptococci (FS) was used as a pollution indicator. A volume of 100 ml soil solution was filtered through a membrane filter (Membrane Solutions) with pore size 0.45 µm. The filter was transferred and cultivated on selective growth medium for 24 h (ISO 9308-1:2014; ISO 7899-2 2000).

Multi ANOVA and Student/Fisher test were used for testing the differences of elemental concentrations, both between the soils samples from different road distance and also between the studied sampling sites ($p<0.05$).

Relationships between the element content in soil samples were tested using Pearson correlation ($p<0.05$). Data were also processed with Principal Components Analysis (PCA) in order to find the main factors affecting urban soils quality. All statistical analyses were made with STATISTICA 7.0 statistical package (StatSoft, 2004).

Figure 1. Map of Plovdiv and location of sampling plots

RESULTS AND DISCUSSIONS

According to their genesis, the soils in the Plovdiv area are classified as Fluvisol according to the FAO World Reference Base for Soil Resources (2014). Due to the prolonged human presence on the studied territory (more than 8000 years) and the increasing temps of urbanization in the last decades, soils properties are significantly influenced and now it is more appropriate to discuss them as Technosols.

The pH values of the studied soil samples from the surface layer (0-20 cm) varied between 6.22 and 7.08 (in the neutral zone), and the conductivity was in the range of 102-121 $\mu S/cm^2$. The neutral soil reaction makes difficult the migration of many heavy metals and of other elements into the soil profile so they remain immobilized into the topsoils.

Heavy metals and toxic element such as As, Cd, Cr, Cu, Ni, Pb, V and Zn are derived into the urban environment by many anthropogenic processes and activities, such as mining, smelting, industrial engineering, metal processing and plating, ceramics and electronics, domestic activities, residential heating, incinerators, petrol and diesel vehicles, (Johnson et al., 2011).

Manganese is a natural element in soils and its content generally varies from 0.01% to 0.4% (Kabata-Pendias and Pendias, 2001) both as a result from the rocks layer and the atmospheric deposition. Anthropogenic origin of this element into the air is related with such as mining, smelting, industrial activities (especially heavy engineering, metal processing). In our study, Mn was the most abundant from the studied elements but the content is half less average concentration in Bulgarian and European soils (Table 2). In this study, contrary to the case of other metals, manganese concentrations found at different points did not varied much.

Zinc content in our study varied between 64 and 130 mg/kg with a mean value of 90 mg/kg (Table 2). So, some increment was found in the city of Plovdiv in comparison with Bulgarian and European soils, more pronounced in the soils near the Vasil Aprilov Boul.

Table 2. Content of heavy metals and toxic elements in soil samples [[1]Penin (1989, 2003) and [2]Salminen (2005)]

Soil sample	Mn		Zn		Pb		Cr		Cu		Cd	
	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD %	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD, %
HB-N-7.5	490	2.3	85	4.2	40	1.5	35	3.2	28	1.6	0.41	6.1
HB-N-25	471	1.8	88	3.1	38	2.1	40	2.8	26	2.5	0.38	5.1
HB-N-50	435	2.4	88	1.3	26	1.9	32	2.4	22	5.5	0.26	7.3
HB-S-7.5	453	2.7	98	4.7	37	1.9	32	2.6	34	3.0	0.34	12
HB-S-25	457	2.4	95	1.8	45	1.9	32	2.0	27	2.5	0.47	8.5
HB-S-50	447	2.0	66	3.7	30	2.3	35	3.6	21	4.4	0.32	8.2
VA-E-7.5	528	1.5	108	3.0	63	1.6	39	3.1	40	3.3	0.56	6.1
VA-E-25	511	1.7	64	4.4	23	1.9	18	4.2	26	3.0	0.19	12
VA-E-50	469	2.2	71	4.0	33	1.6	14	5.1	28	2.7	0.32	3.9
VA-W-7.5	335	3.7	130	3.1	67	2.0	26	3.6	38	3.4	0.72	8.0
VA-W-25	451	2.3	94	2.4	41	1.8	37	1.1	29	4.8	0.4	6.7
VA-W-50	457	2.4	98	1.5	117	1.5	48	2.8	34	3.6	0.5	9.1
Average in Plovdiv	458	-	90	-	47	-	32	-	29	-	0.41	-
Soils in Bulgaria[1]	1000	-	75	-	35	-	70	-	30	-	0.32	-
Soils in Europe[2]	810	-	68,1	-	32.6	-	94.8	-	17.3	-	0.28	-

As a whole, lead and cadmium content was also quite elevated in comparison with the average level in Bulgarian and European soils (up to 1/3 higher), with both maximums into soils surrounding the Vasil Aprilov Boul. Copper content was very close to the average of Bulgarian value while the chromium content was half less the Bulgarian soil concentration and 2/3 times lower from the European level (Table 2).

Table 3. Correlation between studied elements, p<0.05

	Mn	Zn	Pb	Cr	Cu	Cd
Mn	1.00					
Zn	-0.54	1.00				
Pb	-0.18	0.56	1.00			
Cr	0.08	0.33	0.57	1.00		
Cu	-0.12	**0.78**	**0.63**	0.18	1.00	
Cd	-0.48	**0.88**	**0.67**	0.33	**0.76**	1.00

Statistical evaluation of the elements relationships showed strong positive correlation between Cd, Zn, Pb and Cu (Table 3). Many authors have shown that there is a strong relationship between many of these contaminants and the use of motor-vehicles – leaded fuels for Pb, tyre wear for Zn and Cd, brake pads for Sb, and catalytic converters and exhaust systems for platinum group elements (Kelly et al., 1996; De Miguel et al., 1997; Zereini et al., 1997; Schafer et al., 1998; Angelone et al., 2002; Cichella et al., 2003).
This relationship was also pointed by the cluster analysis (Figure 2).

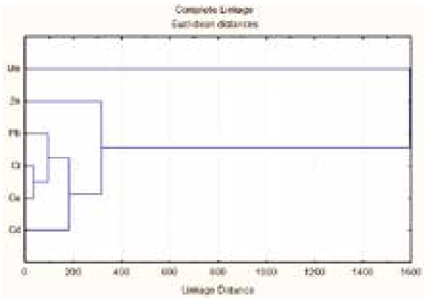

Figure 2. Cluster analysis of studied elements, p<0.05

The strongest similarity was proved between Cu and Cr, and then Pb, Cd and Zn have joined. Mn was found to be quite different from the other elements which fact was not surprising as Mn is naturally more abundant in soils.
Regarding the “Distance from the road” as a factor affecting the soil pollution, our data confirmed the statements of other authors that the concentrations generally decrease away from the main road network and with increasing depth from the ground surface (Johnson et al., 2011).
No significant differences were found between samples at the West direction transect on Vasil Aprilov Boul., but the opposite transect (Vasil Aprilov, South) showed very strong gradient of decreasing pollution from 7.5 m to 25 m and to 50 m samples (VA-E-7.5, VA-E-25 and VA-E-50) (p<0.05). Considering the effect of the wind direction on the dispersion of pollutants

in the environment, this fact is obviously due to the prevailing winds from west to east - 75% of all winds in Plovdiv through the year. That is the reason for the lack of pollution gradient into the windward transect too.

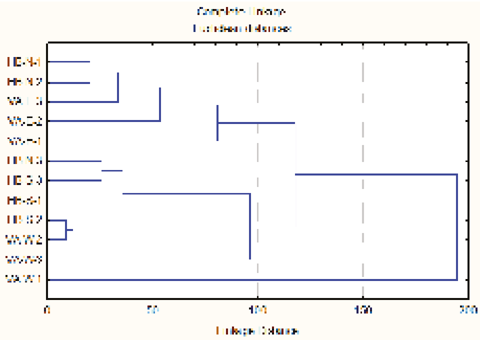

Figure 3. Cluster analysis of studied transects and soil samples, p<0.05

The second boulevard, Hristo Botev Boul., is oriented into the W-E direction (as the wind rose) and the distribution of traffic related elements was quite complex. We could not find strong significant relations in their content with the distance from the road network. Both transects (North and South) showed low correlation gradients, as follows: p=0.13-0.21 for 7.5 m and 50 m samples and p=0.09-0.13 for 25 m and 50 m samples.

Cluster analysis divided the studied soil samples in two main clusters (Figure 3). First one consisted from the first two samples in North direction from the Hristo Botev Boul. and all the three samples in East direction from the Vasil Aprilov Boul. Second cluster included the aggregation of the rest of samples from Hristo Botev Boul. (3 to the South and one to the North) and the three samples in the West direction from the Vasil Aprilov Boul.

Next step of the statistical evaluation of our results was the Principal Component Analysis in order to test the significance of several factors ("Distance from the road", "Background level", "Wind rose" and "General urban activity") for the elements concentrations in soils near road network (Figure 4). Main factor (Factor 1=58.59%), contributing to the element content in soil, was found to be the "Background level" in the area, followed by the factor "Distance from the road" (Factor 2=20.51%) which affected mainly Cd and Zn. "Wind rose" as factor (Factor 3=12.48%) played an important role of the dispersion of Cu, Cd and Zn, while the "General urban activity" contributed to the behaviour of almost all studied elements without Pb (Factor 4= 5.58%).

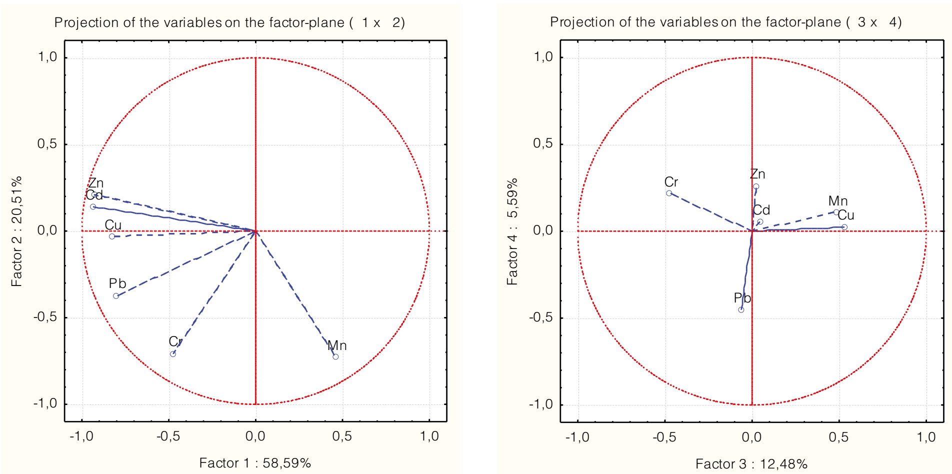

Figure 4. Results from the Principal Component Analysis (PCA), p<0.05

For chromium and lead we could not prove some dependence of the studied factors in PCA. However, Pb showed strong synergism with Cu and Cd, so we can suppose that it is influenced but in lower extent. Cr and V are considered indicative of vehicle emissions (Garty et al., 1996) and partly associated with tire and brake abrasion, as we have shown in our previous studies (Petrova, 2011; Petrova et al., 2013; Petrova et al., 2014 a,b; Petrova et al., 2015). So, this lack of correlation with other traffic-related elements could be a result of some other mechanisms and processes, i.e. higher mobility, phytoextraction etc.

Urban infrastructure and development are some of the most severe threats for microbial communities' structure and well-being (O`Donnell et al., 2001). The results from the microbiological analysis are shown in Table 4. The total numbers of heterotrophic microorganisms cultured at 22°C (TVC 22) and 37°C (TVC 37) were used as an indicator for the soil-forming capacity and anthropogenic pressure respectively. The study showed a strong positive correlation between the parameters (Table 5).

The values for TVC 22 and TVC 37 in the soil samples differed significantly between transects with higher numbers of heterotrophic bacteria near the Hristo Botev Boul. Our data showed a consistency in the distribution of the heterotrophic microorganisms. Their numbers decreased from 7.5 to 50 m (going away from the road) in each transect. This can be explained by the presence of planted trees and shrubs along the road which could have considerable influence on soil microbial communities (Xu et al., 2013), and with the care of the municipality for the seasonal forestry maintenance of these green areas. The observed differences in the bacterial numbers could also be driven by the environmental parameters topology, and site`s geological characteristics.

There is considerable evidences documenting the negative effect of the long-term heavy metal exposure on the soil bacterial community (Sobolev and Begonia 2008; Xie et al., 2016). However, the parameters TVC 22 and TVC 37 showed no significant negative correlation with the tested heavy metals. This is probably due to the relatively low level of contamination compared to the other studies.

The cultivation-based detection and enumeration of microbiological indicators remain the gold standard in the environment protection (Rodgers et al., 2011). We have established clearly defined differences in the distribution of the sanitary state indicators between the two studied areas (Table 4).

The number of *E. coli* correlate positively with the number of FC and FS (Table 5). Studies of fecal coliforms didn`t show statistically significant differences between the soil samples from the Hristo Botev Boul. Fecal streptococci and *E. coli* were unevenly distributed. Their amounts in the northern transect increased in the direction away from the road, with the FC: FS ratio in these samples > 4:1 which indicates the presence of nonpoint source of surface soil contamination. Similar trend was observed for the VA-W transect. In general, the total number of the indicators is not high, and our conclusion, based on their decay speed (Rodgers et al., 2011) is that the pollution in the surveyed areas is significantly low.

Table 4. Average values of the studied microbiological indicators

Soil sample	TVC 22 (cfu.10^5/g)	TVC 37 (cfu.10^5/g)	FC (cfu/g)	*E. coli* (cfu/g)	FS (cfu.g)	Fungi (cfu.10^3/g)
HB-N-7.5	417±15	69±6	18905±8200	115±9	78±7	20±3
HB-N-25	256±15	46±3	8360±3000	467±23	108±10	96±6
HB-N-50	143±10	18±2	7384±1460	952±61	330±26	107±3
HB-S-7.5	521±14	79±2	10860±3040	1100±111	823±225	42±2
HB-S-25	337±11	79±2	11930±4040	300±10	287±23	45±8
HB-S-50	184±18	45±9	7485±1854	4±3	0	204±5
VA-E-7.5	86±8	8±0.1	480±120	0	8±0	4±0.2
VA-E-25	37±2	8±0.2	650±320	0	18±0	9±0.1
VA-E-50	15±2	2±1	540±200	0	8±1	10±0.4
VA-W-7.5	159±11	41±0.9	5000±160	900±114	1080±44	46±2
VA-W-25	94±5	41±5	18100±2100	1320±98	1340±84	56±2
VA-W-50	31±6	16±2	18300±3400	3240±249	2100±200	34±1

Table 5. Correlation between element concentrations and the studied microbiological indicators, $p<0.05$

Variable	Mn	Zn	Pb	Cr	Cu	Cd	TVC22	TVC37	FC	FS	*E. coli*	Fungi
Mn	1.00											
Zn	-0.54	1.00										
Pb	-0.18	0.56	1.00									
Cr	0.08	0.33	0.57	1.00								
Cu	-0.12	0.78	0.63	0.18	1.00							
Cd	-0.48	0.88	0.67	0.33	0.76	1.00						
TVC22	-0.06	0.15	-0.25	0.17	-0.03	0.01	1.00					
TVC37	-0.22	0.16	0.07	0.25	-0.04	0.15	0.86	1.00				
FC	-0.10	0.14	0.33	0.62	-0.07	0.11	0.36	0.42	1.00			
FS	-0.46	0.50	0.65	0.50	0.31	0.30	-0.06	0.08	0.58	1.00		
E. coli	-0.28	0.37	0.83	0.54	0.36	0.34	-0.21	0.06	0.58	0.91	1.00	
Fungi	-0.14	-0.30	-0.20	0.30	-0.48	-0.09	0.32	0.39	0.29	-0.20	-0.17	1.00

Fungi fulfill a range of important ecological functions particularly those associated with nutrient and carbon cycling processes in soil. The bacteria-to-fungi ratio tends to be lower in acidic, low nutrient soils and high C-to-N ratio, whereas bacteria are increasingly prominent in high N+P, saline, and alkaline soils. Previous studies demonstrated the higher resistance of the fungi to heavy metals (Hiroki, 1992; Ashraf and Ali, 2007). Despite that our research show low numbers of fungi indicating that soil-forming capacity of the studied areas is low and strongly influenced by the urbanization of the territory.

CONCLUSIONS

Our study revealed that the contamination of soils along the road network with Cd, Pb, Cu and Zn is in direct relationships both with the distance from the road and the location of the boulevard itself according to the wind rose. This situation reflected also on the soil microbial community structure, leading not only to the damages in the physico-chemical properties of soils but to the decrease into their quality and functions. As a consequence of the data obtained, we could make some recommendations to use some plant species with high bioaccumulation capacity for green casting the road surrounding.
Finally, it can be added that the data provided are part of a project covering the whole territory of the city of Plovdiv and all the results obtained by us will show a more complete and detailed picture of the anthropogenic impact in the urbanized area.

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THE USE OF CHEMICAL AND ORGANIC FERTILIZERS FOR SUNFLOWER CULTURE ON A STERILE DUMP

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Abstract

Agricultural recultivation of sterile dumps involves, besides landscaping works, the mandatory use of mineral and organic fertilization. The paper presents the experimental results obtained for the sunflower crops on a sterile dump in the Mehedinti County, following the application of mineral fertilization with nitrogen and phosphorus and of organic fertilizers such as taurine compost. On a constant background of P_{60}, progressive doses of N_{40} and N_{80} were administered, and the compost was applied in doses of 10 t/ha, 20 t/ha, 30 t/ha every 3rd year, 2nd year and annually. Determinations of sunflower crops were targeted at plant height, calatidian diameter, production, MMB and MH, which were analyzed in correlation with the applied mineral fertilization, compost fertilization and compost application time.

The findings have shown that on sterile dumps that are poor in organic matter and nutrients, plant cultivation can be achieved with satisfactory results if mineral fertilizers are applied annually, and organic fertilizers are applied annually or at least every 2 years.

Key words*: compost, fertilizers, sunflower, steril dumps.*

INTRODUCTION

Lignite exploitation technology does not allow for the excavation and selective deposition of sterile on geological bedding, so that the resulting dump composition is a heterogeneous mixture of rocks that are unsuitable for agricultural crops. Thus, a complete and sustainable transformation of the initial configuration of the territory and of its economic use takes place.

Studies have determined that mixed overburden materials are capable of supporting various types of vegetation and have demonstrated higher productivity potential compared to adjacent undisturbed land when used as a topsoil substitute (Angel, 1973 quoted by Angel, 2017; DeLong et al., 2012; Ng, 2012; Toups, 1986).

The physical and chemical properties of sterile dumps limit the recultivation process to a high degree, therefore plants with reduced calcium requirements and high tolerance to acidity and high concentrations of iron, manganese, aluminum and sulfur (Angel, 1973) must be used.

Naturally (without nitrogen fertilization) it will take many years for plant species to grow on the dump and contribute significantly to the formation of organic matter in the substrate to stimulate the ammonation and nitrification processes that are dramatically reduced in dumps; the use of high doses of nitrogen presents the risk of leaching, which depends on the amount of precipitation, the applied fertilizer dose and the nature of the crop (Lixandru et al., 1990).

The rapid fixing of slopes with the help of vegetation and maintenance of a viable vegetal cover that reduces the movement of water and oxygen in the dump is an important step for erosion control, dump stabilization and final recultivation (Daniels et al., 1996; Daniels, 2005).

The humidity conditions of the dump, as decisive factors in the recultivation process, are better compared to the soil ones. In dumps, the water storage capacity increases and internal drainage decreases (Angel, 1973).

Using appropriate recultivation leads to the recovery of mines to their previous use or to new uses.

The research and experiments carried out on the sterile dumps in the country after the "every day" mining focused on different situations in order to obtain the elements necessary for the development of a recultivation technology.

The weak alkaline reaction of the subsoil requires higher doses of phosphorus in a slightly assimilable form because some of it is stablely fixed to the soil and cannot be used by plants. In the case of a soil poor in mobile phosphates (the same as the materials in the dumps), the initial rate of accumulation of mobile phosphates is low due to the high soil absorption capacity for phosphate ions (Borlan, Hera, 1984).

In sterile dumps, organic fertilizers should be applied along with mineral ones to ensure both nutrient and organic matter, soil biological activity and structural improvement. However, the lasts for 2-3 years (from the start, the plants do not have sufficient nutrients in their accessible form). Thus, 35%, 20%, 15% of organic nitrogen is durably retained in soil in humic substances (Borlan et al., 1994) in the first year, in the 2nd year and in the 3rd year, respectively. On poor soils such as the podzolic and anthropogenic ones, compost, an organic fertilizer with a valuable chemical composition, can be used, which provides the soil structural fertility enhancers, being sufficient in doses below 20 t/ha so that the desired effect is boosted.

Research regarding the application of different compost doses and with different application times, supplemented with mineral fertilizers, has highlighted that the application of chemical fertilizers increases the effectiveness of compost. In terms of the application time of the compost, in the case of an every 2-year application, the decrease in production was insignificant, compared to the annual application, and in the case of the every 3-year application, the decrease is significant (Ionescu et al., 1985).

It has been shown that the ameliorative effect of organic fertilizers is due to the contribution of organic matter, which is made up of easily and hardly degradable compounds. The more stable organic matter, consisting mainly of lignin, persists for a long time in the soil, determining the long-lasting effect of organic fertilizers on soil improvement, including the humus regime (Davidescu, Davidescu, 1991; Lăcătuşu, 2006).

Sterile dumps can be turned into areas used for the cultivation of plants which are important melliferous sources for the development of apiculture, such as sunflower, rapeseed, facet, vetch, acacia, lime, hazelnut, alder, sea buckthorn, oleaster, used mostly for increased productivity, but also for fixing the slopes (Nastea et al., 1991).

MATERIALS AND METHODS

The experimental device was placed on an uncovered sterile dump with fertile soil in the Husnicioara quarry, Mehedinti County, on a typical four-layer psyllium entiantrosol. In order to consolidate and fix the slopes resulted from the leveling works of sterile dumps, lucerne was cultivated. In a stationary three-year rotation, there were grown, in three iterations, sunflower, corn, and chickpeas crops, which are suitable for the agricultural recultivation of sterile dumps, in accordance with the specific crop profile of the area.

The factors related to the sunflower crops were the following:

Factor A - The applied compost dose (organic fertilizer) with 4 graduations: a_1 - unfertilized with compost; a_2 - 10 t/ha taurine compost; a_3 - 20 t/ha taurine compost; a_4- 30 t/ha taurine compost.

Factor B - The application time interval of the three-graduations compost: b_1 - applied annually; b_2 - applied every 2 years; b_3 - applied every 3 years.

Factor C - The application of mineral fertilizers with 3 graduations and different values of nitrogen on a background of P_{60}: c_1 - N_0P_0; c_2 - $N_{40}P_{60}$; c_3 - $N_{80}P_{60}$.

Phosphorus fertilizers were applied each autumn (in the form of simple superphosphate with 20% P_2O_5), and the compost in the established doses, then, it immediately showed at a depth of 20-22 cm with the plow in aggregate with a stellate harrow, which provided the incorporation of applied fertilizers and vegetal rests through the work.

Considering the chemical composition of the wet compost with 1.054% total N, 0.447% P_2O_5, 0.095% K_2O, 0.993% CaO and 0.051% Mg (Table 1), it results that, in relation to the established doses, the following nutrients were annually introduced in the dump (kg/ha):

- for the compost dose of 10 t/ha = 105.4 kg N; 44.7 kg P_2O_5; 9.5 kg K_2O; 18.6 kg CaO and 5.1 kg Mg;
- for the compost dose of 20 t/ha = 210.8 kg N; 89.4 kg P_2O_5; 19 kg K_2O; 18.6 kg CaO and 10.2 kg Mg;
- for the compost dose of 30 t/ha = 316.2 kg N; 134.1 kg P_2O_5; 28.5 kg K_2O; 27.9 kg CaO and 15.3 kg Mg.

On the variants for which the application of compost every 2 and 3 years was experienced, the nutrient intake was different compared to its annual application.

Nitrogen fertilizers (ammonium nitrate with 33.5% N) in the established doses were applied in a fractional manner, namely: 1/2 from the dose for seeding and 1/2 for the second hand hoeing.

No herbicides have been applied, not knowing their effects on such soils.

Various determinations and measurements of the calatidian diameter (cm), plant height (cm) and production (kg/ha) were made in the field. The measurements and determinations, for each year of experimentation, were performed on 10 plants from each experimental variant, in 3 iterations in order to obtain the most realistic data and to allow to be statistically processed.

Obtaining the biological fertilizer

The compost was completed within 6 months, using manure from cattle, straw and special cultures of bacteria, under conditons of controlled fermentation, humidity and temperature.

In order to accurately determine the composition of the compost and an an average was established (Table 1).

Table 1. Composition of the used compost

Characteristics and composition		Sample 1	Sample 2	Sample 3	Average
Humidity %		40.0	40.2	39.0	39.7
pH		7.58	7.69	7.62	7.63
Total N %	Humid	1.125	1.035	1.002	1.054
	Dry	1.875	1.730	1.642	1.749
P_2O_5 %	Humid	0.469	0.432	0.440	0.447
	Dry	0.782	0.720	0.721	0.734
K_2O %	Humid	0.086	0.096	0.103	0.095
	Dry	0.143	0,160	0.168	0.157
CaO %	Humid	0.088	0.093	0.097	0.093
	Dry	0.146	0.155	0.159	0.153
Mg %	Humid	0.048	0.054	0.051	0.051
	Dry	0.080	0.090	0.083	0.084

The results were compared with the average composition of natural fermented cattle manure (according to Davidescu, Davidescu, Table 2).

Table 2. Average composition of naturally fermented cattle manure (after Davidescu, Davidescu)

Characteristics	Humidity %	Nt %	P_2O_5 %	K_2O %	CaO %
Values	77.5	0.34	0.16	0.40	0.31

K_2O and CaO content is higher in naturally fermented cattle manure in comparison with the biological matter produced by directed composting. By comparing the values obtained for the main compost components with those of the naturally fermented cattle manure, it is obvious that these values were higher.

The results of the analyzes show that the compost prepared as an entirely valuable organic material, easier to transport and apply than ordinary manure with low water content, having a lower volume and a high fertilizing effect (much higher on the same product unit).

Also, the materials used for preparing it and the way of preparation are accessible to those who raise animals (taurines), contribute to a better exploitation of the internal resources, resulting in suitable fertilizers that can successfully replace the intensive mineral fertilizers and contribute to the reduction of the sources of pollution in the nature.

The obtained agricultural products are ecological, the quantity of the used chemical fertilizers being reduced and the expenditures incurred are substantially diminished.

RESULTS AND DISCUSSIONS

On the sterile dump, the choice of a crop structure from which sunflower was a part, was based on the climate and soil conditions of the area, the requirements of the area's population, the agrofitotechnical requirements of the cultivated species and the efficiency of the improvement of sterile dumps from a pedological and economic point of view.

Under the influence of the above mentioned factors, a series of determinations were made of the height of the sunflower plants (Figure 1), the diameter of the calatidium (Figure 2), production rate (Figure 3), the mass of 1000 grains (Figure 4) and the hectolitric mass (Figure 5). Mineral fertilization, composting (organic) fertilization and the compost application interval led to a series of assessments regarding the cultivation of sunflower on sterile dumps, based on the results obtained for the analyzed parameters.

Height of the plants

The determinations of the size of the sunflower plants showed the positive effect of organic matter and applied mineral fertilizers on the height of the plants on the Huşnicioara sterile dumps.

The use of compost in different doses positively influenced the crop size, which increased at the same time with the increase of the amount of organic matter, from 107 cm for unfertilized to 115 cm for the dose of 30 t compost/ha (Table 3).

The average calculation of the experimental years showed a constant height for the witness (a_1) and the lowest value of 107 cm, an increase at 108 cm for the following variant a_2, 110 cm when fertilized with 20 t/ha compost (a_3) and 115 cm for the variant with the maximum dose of organic matter (a_4). The obtained results showed high values and statistically ensured within the limit of 1-0.1% only for the variant that received 30 t/ha of compost, the others showing annual variations.

Analyzing the influence of the composting interval on this biometric feature of plants, we observe a constant sunflower size at the annual application of compost and different values for the other variants.

When the compost was applied annually, the height of the plants showed values ranging from 113 to 115 cm (b_1). The application every 2 years (b_2) positively influenced plant growth in the year of application and after, as a permanent effect (b_2). With reference to a 3-year application (b_3), the growth was of 107 cm in the first year, 107 cm in the 2nd and of 113 cm 3rd year.

The recorded negative differences were generally low, mostly within the error margins, except for the first year of experimentation.

The 3-year average for the sunflower plant height showed higher values for the annually fertilization variant with compost (b_1) - 114 cm, lower to that at which organic matter was applied every 2 years (b_2) - 110 cm and the lowest for the fertilized variant every 3 years (b_3) - 109 cm (Table 4).

The value differences on the average plant size range within experimental error margins, due to the large variation over the years, with no significance.

The mineral fertilization with nitrogen and phosphorus in increasing doses positively influenced the plant height (Table 5).

The average plant height was 107 cm for the witness (c_1), 111 cm for $N_{40}P_{60}$ (c_2) and 114 cm for $N_{80}P_{60}$ (c_3) doses. The sunflower size differences in the unfertilizedvariant of 4 cm and 7 cm statistically observed the limit of 0.1%.

The diameter of the sunflower calatidium

The diameter of sunflower inflorescence (calatidium) was influenced by the biological fertilizer doses, their range of application and mineral fertilization.

The use of organic matter in the form of compost in certain doses compared to the non-fertilized witness showed an increase in the calatidium size, simultaneously with increased doses, from 14.5 cm for unfertilized crops to 19.5 cm for the dose of 30 t compost/ha (Table 3). The value differences between the witness and the experimental variants statistically observed the limit of 0.1%.

Following the influence of the composting interval on the calatidium diameter size, we can observe that the highest calatidians (with an average of 19.8 cm) are obtained in the case of annual use followed by the every 2-year application (17.3 cm) and the every 3-year application (16 cm) with variations b_2 (14 - 19.2 cm) and then b_3 (14.4 - 19.0 cm) (Table 4). The negative differences regarding the witness are highly significant for all variants, and the calculation of the average of the analyzed interval showed small differences which are within the error margins (from - 2.5 to - 3.8 cm).

The application of chemical fertilizers in progressive doses led to the increase of the calatidium diameter in all years of experimentation.

The values obtained by calculating the average of the analyzed interval showed the same tendency of the variants: without chemical fertilizers the resulting diameter was of 16.4 cm, at the dose of $N_{40}P_{60}$ it reached 17.6 cm and at the maximum nitrogen dose 19.3 cm (Table 5).

The differences of the calatidium diameter between the witness and the first dose of nitrogen statistically ranged within the error margin (5%) in the first year and were highly significant (1%) in the average case, and for the $N_{80}P_{60}$ dose they were significant (0.1%) in all cases.

The production of sunflower

Sunflower yields were quantitatively significant in the sterile dump conditions. The applied fertilization (organic and mineral) played an essential role in the success of this crop, the results obtained being even higher than those on the areas cultivated in the adjacent area.

The use of compost in different doses led to a higher production of 109-212% as compared to the unfertilized variant, due to the high capacity of the radicular system to extract the necessary nutrients, including the ones from in depth. The crop gains obtained at the application of 20 and 30 t/ha compost statistically ranged within the limit of 0.1%.

The average production for the 3 years was the same in terms of quantity: 619 kg/ha for the witness, 708 kg/ha for a_2, 1058 kg/ha for the a_3 and 1167 kg/ha for the last variant - a_4 (Table 3).

The data presented indicate that the biological matter used as an organic fertilizer had a direct effect on the sunflower production, which had been applied in autumn and incorporated to the basic work, the plants having the necessary nutrients at the start of the vegetation.

The influence of the application interval of the compost on the sunflower yields revealed that, in the conditions of the sterile dump, the annual application has a direct effect but also a remanent effect for the doses used every 2 years. The negative yield differences were significant in the years when no compost was applied compared to the variant that was organically fertilized annually (year I for b_2 and b_3, year II for b_3 and year III for b_2).

The average of the analyzed interval was of 1085 kg/ha for the annually fertilized variant, 815 kg/ha for the 2-year interval and 764 kg/ha when applied every 3 years (Table 4).

The effect of mineral fertilization with different doses of nitrogen on the background of P_{60} on sunflower yield was positive, increasing simultaneously with dose increase. The yield increase was highly significant in the first and second year for the $N_{80}P_{60}$ fertilized variant, the others being within the error limit.

The calculation of the 3-year average showed close production between the three variants, respectively, 792 kg/ha for the witness, for the $N_{40}P_{60}$ fertilization - 867 kg/ha and for the last variant - 979 kg/ha, statistically ensured within the 5-1% limit (Table 5).

Sunflower yields through mineral fertilization highlighted the fact that plants use less fertilizer than other crop plants, and moderate nitrogen doses are recommended in this case. The situation is also confirmed by research conducted by Hera et al., 1984; which showed that the yield gains from nitrogen fertilizers are modest, and high doses of nitrogen are ineffective.

The sunflower mass of 1000 grains (MMB)

MMB values were directly influenced by applied compost doses.

The average of the analyzed period for the experimental variants recorded values for MMB ranging from 61.3 g for the witness and 63 g for the application of 30 t/ha compost (Table 3).

The value differences between variants in the 3 years generally ranged within the limits of the years except for the 30 t/ha compost variant, which was distinctly significant in 2 years (a_4 - 1%). For the average, the differences were distinct and highly significant.

Concerning the application time interval of the compost, the determinations made showed very close values for the mass of 1000 grains during the experimentation period, when it was organically fertilized annually. The negative differences in MMB ranged from distinctly significant to very significant.

The calculation of the average for that period indicated MMB values that decreased from b_1 to 63.3 g, b_2 to 61.7 to b_3 to 61.6 g (Table 4).

The negative differences were within the limit of experimental error margins of 5% determined by large differences between the annual values for variants c_2 ($N_{40}P_{60}$) and c_3 ($N_{80}P_{60}$).

Mineral fertilization, through the applied doses, determined different values of the mass of 1000 grains per variants, but also during the years of experimentation.

The value increase for the first dose of chemical fertilizer compared to the witness was distinct and highly significant, while for the second dose are highly significant.

The average of variants in the three years showed values of the mass of 1000 grains that rose from the witness (60.3 g) to the $N_{40}P_{60}$ dose (62.5 g) and the maximum $N_{80}P_{60}$ (63.8 g), the increases being highly significant (Table 5).

The hectolitric mass of sunflower seeds

The hectolitric mass (MH) was influenced by fertilizers applied differently in relation to their nature and application times.

The use of compost in different doses determined the increase of the hectolitric weight from the unfertilized variant to the fertilized one with the maximum dose in all three years of experimentation.

The differences between the unfertilized and fertilized compost variants were highly significant in the first year, in the second year from significant to very significant, and in the last year from distinctly significant to very significant.

The average hectolitre mass of sunflower seeds increased at the same time with the applied compost dose, namely: 39.5 kg at a_1, 40.1 at a_2, 40.5 at a_3 and 41.2 kg at a_4, the statistical values ranging from 5% to 0.1% (Table 3).

The composting interval also showed that annual application had a positive effect compared to the one every two or three years.

The average variants and years of hectolitric mass indicated values that were reduced from the witness (b_1 - annual application) of 41.1 kg to 40.0 kg (b_2 application every 2 years) and 39.9 kg (b_3 - application every 3 years) - Table 4. The differences are not significant (the values range within the error margin) due to the large variations in years for b_2 and b_3.

Mineral fertilization positively influenced the amount of hectolitreal mass for the sunflower seeds. The enhanced rates of hectolitric mass increase depending on the doses of applied chemical fertilizers statistically ranged from 5 to 0.1% (for $N_{40}P_{60}$) and 0.1% (for $N_{80}P_{60}$).

The average hectolitric mass values were 39.4 kg for the unfertilized variant, 40.2 kg when the $N_{40}P_{60}$ dose was applied and 41.4 kg ehrn the $N_{80}P_{60}$ dose was applied, the first dose increase being distinctly significant, and for the 2nd highly significant (Table 5).

Table 3. The influence of compost fertilization on the analyzed parameters

Compost fertilization	Height (cm)	Calatidium diameter (cm)	Yield (kg/ha)	MMB (g)	MH (kg)
Unfertilized	107	14.5	619	61.3	39.5
10 t/ha	108	18.2	708	62.2	40.1
20 t/ha	110	18.9	1058	62.7	40.5
30 t/ha	115	19.5	1167	63.0	41.2

Table 4. The influence of the application interval of the compost on the analyzed parameters

Composting interval	Height (cm)	Calatidium diameter (cm)	Yield (kg/ha)	MMB (g)	MH (kg)
Annually	114	19.8	1085	63.3	41.1
Every 2 years	110	17.3	815	61.7	40.0
Every 3 years	109	16.0	764	61.6	39.9

Table 5. The influence of mineral fertilization on the analyzed parameters

Mineral fertilization	Height (cm)	Calatidium diameter (cm)	Yield (kg/ha)	MMB (g)	MH (kg)
N_0P_0	107	16.4	792	60.3	39.4
$N_{40}P_{60}$	111	17.6	867	62.5	40.2
$N_{80}P_{60}$	114	19.3	978	63.8	41.1

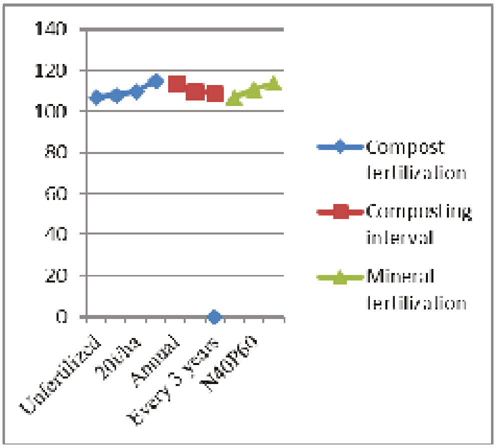

Figure 1. The influence of compost fertilization, application interval and mineral fertilization on plant height (cm)

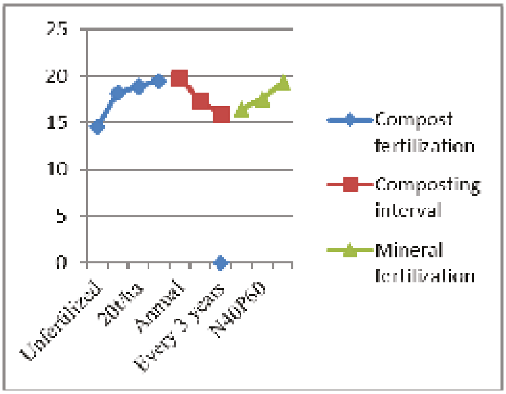

Figure 2. The influence of compost fertilization, application interval and mineral fertilization on the calatidium diameter (cm)

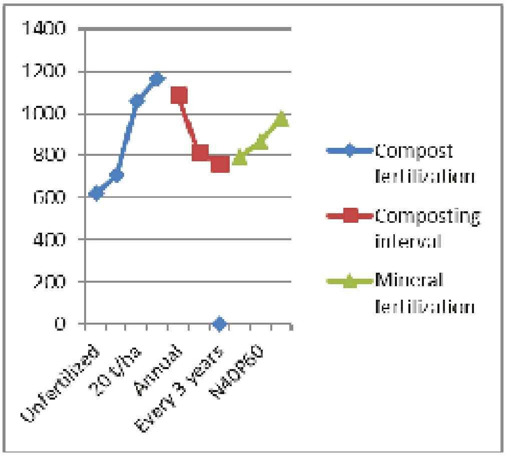

Figure 3. The influence of compost fertilization, application interval and mineral fertilization on yield (kg/ha)

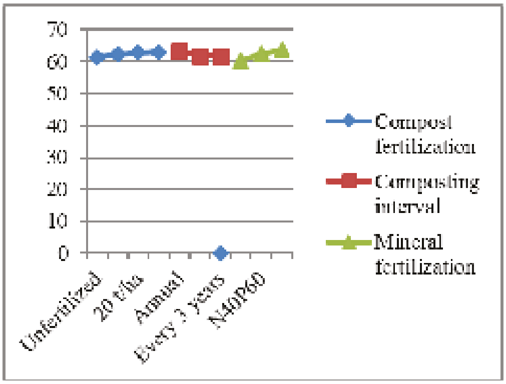

Figure 4. The influence of compost fertilization, application interval and mineral fertilization on MMB (g)

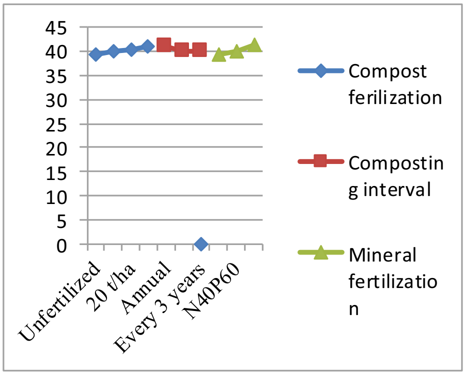

Figure 5. The influence of compost fertilization, application interval and mineral fertilization on MH (kg)

CONCLUSIONS

The use of organic fertilizers of the compost type and nitrogen and phosphorus-based mineral fertilizers on sterile dumps is the main objective of biological recultivation and contributes to increasing the content of organic matter and nutrients to provide the necessary nutrition to the plants that will be cultivated subsequently.

In the case of sunflower crops on the sterile dump, the influence of experimental factors was strong on all the performed determinations (plant height, calatidium diameter, yield, MMB and MH value) with higher values for the biological fertilizer used, regardless of the dose applied or the time of application.

Applying N_{40} and N_{80} doses on a constant P_{60} basis at the same time with compost-type organic fertilizers had a positive effect on all analyzed parameters, the sunflower making full use of the two types of the applied fertilizers.

The influence of the compost application interval highlighted the effect of its annual use, supported by mineral fertilization.

The ability of the sunflower crop to deliver good results and respond to the conditions created by the fertilization variants applied recommends this plant to be successfully used in the biological recultivation of sterile dumps.

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INFLUENCE OF SYSTEM TILLAGE UPON WEEDING LEVEL IN THE MAIZE CROP GROWN AT MOARA DOMNEASCĂ – ILFOV

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Abstract

Soil tillage consists of technological elements that control weeds by cutting, soil incorporation, plant organs fragmentation and their bringing to soil surface where they freeze in winter and dehydrate in summer. Also, the weed seeds from soil surface are ploughed into the depth where there are no conditions foremergence.
In agriculture, weed-produced damage is huge (from 10-20% up to total crop loss) and is reflected by increased production costs, reduced crop yields, and product quality depreciation. The maize crop is sensitive to weeds and the crop loss can reach 70%. The research results presented in this paper were obtained from the experimental field of the Moara Domnească Didactic Farm, Ilfov County, belonging to USAMV Bucharest. The experimental variantson the tillage system were the following: plough 20 cm (control), 20 cm chisel, 40 cm chisel, 10 cm disc, the disk alternation/plough 20 cm disc, 40 cm disc/chisel. The grown hybrid of maize was P0216 (FAO 450). The maximum values for the production of fresh weed biomass and dry substance, depending on soil cultivation, were recorded in the variant worked with the disc (1,942 f.m. kg/ha and 419 d.m. kg/ha) compared to the control variant (plough 20 cm) which resulted in 1,100 f.m. kg/ha and 229 d.m. kg/ha, respectively.There is no direct link between the weed amount and the crop. The maximum yield was recorded in the classic version, 6754.6 kg/ha, while the minimum was recorded in the disc version, 5139.5 kg/ha with a production of 1525.6 kg difference/ha compared to the control.

Key words*: soil tillage, weed biomass, Zea mays* L., *yield.*

INTRODUCTION

Weeds compete with crop plants for the growth factors, have a negative influence on the quality of tillage, and reduce the effect of the measures applied in crop technology by the consumption of the water from the useful edaphic soil layer, crop and soil shading etc. Tillage plays a clear role to weed species as part of the weed control strategy (Marin, 1999).
The effect of tillage upon weed control depends on the tillage type, time period of application, quality, grown crop etc.
Maize production can be adversely affected by the presence of weeds, which results in its decrease by 30-70% (Şarpe, 1975; Budoi, Penescu, 1996; Oancea, 1998; Bîlteanu, 2001; Berca, 2004; Guş, Rusu, 2008; Rusu, Guş, 2008).
Research shows that weed-caused damage depends on a series of factors such as the level of infestation, crop species and phenological phase, the climatic conditions and the time when weeds are controlled (Bosnic, Swanton, 1997; Clay, 1998; Perron, Legere, 2000; Bogdan, 2001; Chirilă, 2001; Fukao et al., 2003; Rusu et al., 2009). Previous studies showed that, in time, soil conservation works can lead to an increase in the density of perennial weeds.
Out of the total harvested gain achieved by various methods of maize plant cultivation, 26% is weed control, 20% fertilizers and 10% density (Bîlteanu, Bârnaure, 1989). Weed control should be integrated, primarily by using agro-technical methods (crop rotation, tillage, mechanical maintenance works), supplemented by the use of herbicides. The choice of herbicides should be based on the dominant weed species, the rotation crop, the soil humus content (Roman et al., 2011).

MATERIALS AND METHODS

Research was conducted on the reddish preluvosoil of the experimental field of the Moara Domnească didactic farm, Ilfov County. The experimental variants were as follows: a_1-

plough at a depth of 20 cm (control), a_2-chisel 20 cm, a_3-chisel 40 cm, a_4-disk 10 cm, a_5-disc/plough 20 cm, a_6-disk/chisel 40 cm, disc tillage in the sequence of disc/chisel and disc/ploughing was performed in the previous winter wheat. Basic soil tillages was performed in the last decade of September.

Sowing was done in the second decade of April. The biological material was the hybrid maize P0216 (FAO 450). Fertilization was carried out with the dose $N_{120}P_{60}K_{60}$ kg/ha of 15:15:15 NPK complex fertilizer and urea.

For weed control we applied herbicide (Dual Gold dose of 1.4 l/ha (*s-metolachlor* 960 g/ l) - preemergence and Dicopur Top 1.0 l/ha (344 g/l *acid 2.4 D* + 120 g/l *dicamba*) + Titus 25DF, 50 g/ha (25% *rimsulfuron methyl*) – post-emergence). Also, we performed mechanical hoeing.

During the experiment we made observations and measurements including: seedling emergence date, height growth rate, leaf formation rate, fruition rate, biomass acumulation rate, numer of grains per cob, TGW, HM, weed level in maize crop.

The influence of soil tillage on weed level was emphasized by the dynamic determination using the gravimetric method and analytical work on each variant. The data are also shown in terms of participation according to biological classification (annual monocotyledonous, annual dicotyledonous, perennial dicotyledonous plants).

To characterize the climate conditions we used the data recorded by the Găneasa Meteorological Station.

From the climatic viewpoint, the years 2015-2016 were characterized by average annual temperatures that exceeded the normal standards for the area (Figure 1). For the maize vegetation period (April-August), the average temperature was 20.1ºC, with 1.9ºC above the normal standards in 2015 and 19.9ºC, with 1.7ºC above the normal standards in 2016.

In 2015, the amount of rainfall recorded 153.0 mm, (Figure 2) with 76.4 mm below the average annual values (very low recorded in months: April, May and July). In terms of rainfall, 2016 was different from 2015, as it accumulated an amount of 343.4 mm, with 114.0 mm over the normal standard during the growing season.

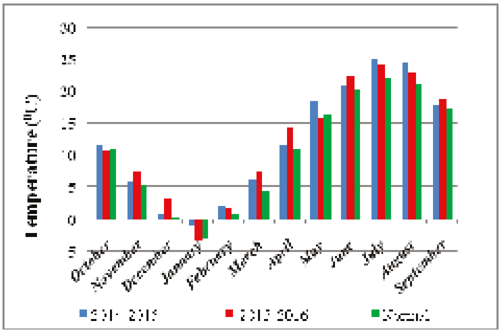

Figure 1. Temperature (ºC), Moara Domnească, Ilfov County (2014-2016 and Normal)

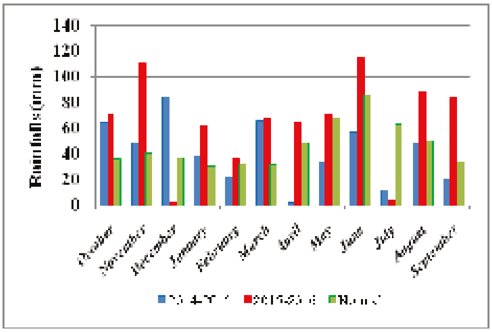

Figure 2. Monthly rainfalls (mm), Moara Domnească, Ilfov County (2014-2016 and Normal)

RESULTS AND DISCUSSIONS

The influence of basic tillage on the weed biomass in the maize crop.

Tables 1 and 2 show the results of the research in 2015-2016 regarding the influence of soil tillage on fresh and dried weed biomass at the end of the vegetation period in the maize crop.

The lowest weed level was recorded in the disc/plough 20 cm variant, i.e. 977 f.m. (fresh matter) kg./ha or 212 d.m. (dry matter) kg/ha representing 89% of the control, 20 cm plough, which resulted in 1100 f.m. kg/ha (229 d.m. kg./ha). In the unconventional system, the highest levels of weed biomass was recorded in the disc-worked variant (1942 f.m. kg/ha and 419 d.m. kg/ha, respectively).

Regarding the amount of fresh and dried biomass obtained in a nonconventional system, the value closest to the control was recorded in 20 cm plough, alternately worked with disc/chisel 40 cm.

The data analysis of the soil influence on the biological weed groups, the annual monocotyledonous plants recorded the highest rate (shown in participation percentage). Their biomass ranged between 803 f.m. kg./ha alternative in the traditional work, disc/plough 20 cm, and 1493 kg f.m. kg/ha in the disk version, followed by annual dicotyledonous plants.

Table 1. Influence of basic tillage on fresh weed biomass in maize, Moara Domnească- Ilfov, average August 2015-2016

Tillage variant	Total weed biomass			Of which						% species participation						
				Annual monocotyledonous		Annual dicotyledonous		Perennial dicotyledonous		*Echinochla crus-galli*	*Setaria viridis*	*Digitaria sanguinalis*	*Chenopodium album*	*Portulaca oleracea*	*Polygonum aviculare*	*Convolvulus arvensis*
	kg f.m./ ha	%	Diff.	kg f.m./ ha	%	kg f.m./ ha	%	kg f.m./ ha	%							
A20	1100	100	C	939	100	100	100	61	100	45	20	20	2	7	0	6
C20	1731	157	631	1375	146	253	253	103	169	33	29	17	5	5	5	6
C40	1608	146	508	1308	139	212	212	88	144	34	33	16	9	3	0	5
Disc	1941	176	841	1493	159	302	302	146	239	41	22	16	8	5	2	6
D/A20	977	89	-123	803	86	40	40	134	219	30	31	21	0	5	0	13
D/C40	1294	118	194	1010	107	198	198	86	141	38	26	16	11	2	1	6

$LSD_{5\%}$ = 112.4 kg f.m./ha ; $LSD_{1\%}$ = 159.8 kg f.m. /ha ; $LSD_{0.1\%}$ = 231.4 kg f.m./ha

The annual dicotyledonous biomass ranged from 40 f.m. kg/ha (disc/plough 20 cm) to 302 f.m. kg/ha (disc) and from 7 d.m. kg/ha (alternative disc/plough 20 cm) to 46 d.m. kg/ha (disc). The weed species commonly found in maize fall with in the annual monocots class. Among these, the predominant species was *Echinochloa crus-galli* with one green biomass total participation between 30 and 45% and 27-50% on the total dry biomass, according to tillage. From the annual dicotyledonous, the *Chenopodium album* species was predominant, with a level of participation between 2 and 11% of the total weed biomass.

Table 2. Influence of basic tillage on dry weed biomass in maize, Moara Domnească- Ilfov, average August 2015 - 2016

Tillage variant	Total weed biomass			Of which						% species participation						
				Annual monocotyledonous		Annual dicotyledonous		Perennial dicotyledonous		*Echinochloa crus-galli*	*Setaria viridis*	*Digitaria sanguinalis*	*Chenopodium album*	*Portulaca oleracea*	*Polygonum aviculare*	*Convolvulus arvensis*
	kg d.m. /ha	%	Diff	kg d.m./ ha	%	kg d.m./ ha	%	kg d.m./ ha	%							
A20	229	100	C	209	100	13	100	7	100	50	21	20	2	4	0	3
C20	367	160	138	312	149	42	323	13	186	36	34	13	5	3	3	6
C40	304	133	75	254	121	39	300	11	157	27	39	17	11	2	0	4
Disc	419	183	190	356	170	46	354	17	243	42	26	16	8	3	1	4
D/A20	212	92	-17	188	90	7	54	17	243	33	35	20	0	4	0	8
D/C40	269	118	40	231	110	28	215	10	143	43	28	15	8	2	1	3

$LSD_{5\%}$ = 16.5kg d.m./ha ; $LSD_{1\%}$ = 23.5kg d.m./ha ; $LSD_{0.1\%}$ =34.1 kg d.m./ha

Influence of tillage system on weed number.

Table 3 shows the results of basic tillage influence on the number of weeds/m^2. Before harvesting the average weeding data obtained from the red preluvosoil of Moara Domnească revealed a number of 17-19 weeds/m² in the conventional variants, and 24-35 weeds/m² in the unconventional variants.

The analysis of weeds/m^2 in the conventional and unconventional system shows the dominance of the annual monocotyledonous species, at a rate of between 66-79% of the total weed number, followed by the annual dicotyledonous species.

Compared with the plough 20 cm variant, a reduced number of weeds/m^2 was recorded in the alternative disc/plough 20 cm (17 plants/m^2). The unconventional system recorded the maximum number of weeds/m^2 in the disc variant, of which 66% represent annual monocots species.

Table 3.Influence of tillage system on the number of weeds/m², average August 2015-2016

Tillage variant	Total weeds/m²	%	Annual monocotyledonous		Annual dicotyledonous		Perennial dicotyledonous	
			No. weeds/m²	%	No. weeds/m²	%	No. weeds/m²	%
A20	19	100	15	79	3	16	1	5
C20	32	100	21	66	6	19	5	15
C40	28	100	21	75	4	14	3	11
Disc	35	100	23	66	7	20	5	14
D/A20	17	100	13	76	3	18	1	6
D/C40	24	100	16	66	4	17	4	17

The influence of soil tillage on maize production.

The tillage system influenced the maize yields, along with the weed level. The highest production (Table 4) was recorded in the classic alternative disc/plough 20 cm (6754.6 kg/ha), with a difference of 89.5 kg/ha compared to the control (ploughing 20 cm), which resulted in 6665.1 kg seeds/ha.

In the nonconventional system, production represented 77.1-90.6% compared to the control, with negative differences of 624.9-1525.6 kg grains/ha. In the application of minimum tillage system, the productions that were the closest to the control were obtained in the chisel 40 cm worked variants, i.e. 5917.4 and alternatively 6040.2 kg grains/ha, respectively.

Table 4. Influence of basic tillage on maize yield, Moara Domnească-Ilfov, average 2015-2016

Tillage variant	Yield kg/ha	%	Diff. kg/ha
A20	6665.1	100	C
C20	5418.9	81.3	-1246.2
C40	5917.4	88.7	-747.7
Disc	5139.5	77.1	-1525.6
D/A20	6754.6	101.3	89.5
D/C40	6040.2	90.6	-624.9

$LSD_{5\%}$ = 208.7 kg./ha ; $LSD_{1\%}$ = 296.7 kg /ha ; $LSD_{0.1\%}$ = 429.6 kg/ha

CONCLUSIONS

The research performed during 2015-2016 showed that the lowest weed level was recorded in the classic version, i.e. 20 cm.

The fresh and dry biomass obtained in the nonconventional system recorded values that were the closest to the control (20 cm) in the alternate variant disc/chisel 40 cm.

In both conventional and nonconventional systems, in terms of weed number/m², annual themonocotyledonous species were dominant, with 13 to 23 plants/m², representing 66-79% of the total number of weeds, followed by dicotyledonous species from the annual group.

In the application of minimum tillage system, the closest quantitative yields were obtained in the chisel 40 cm variants, i.e. 5917.4 kg/ha, and in the disc/chisel 40 cm sequence, i.e. 6040.2kg/ha.

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MYCORRHIZAS AS A TOOL IN MAPPING AGRICULTURAL SOILS

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Abstract

Current agronomic practices are defined by two components: technology and biological dynamics. The response of agroecosystems to applied technologies is visible in the structure and interrelations of the biological component, which defines the actual fertility of soils. In soil-plant-soil biota system, both soil and plant have a rich history in terms of analysis and dynamics. Soil biota is a relatively new component studied, but without an integrated mapping. The response of soil micro-flora and -fauna to technological pressure is found in fertility level, resilience potential, yield stability and conservative status of soil. Mycorrhizas are co-evolutionary symbiotic partners of most cultivated plants. The complex of fungi-roots is defining overall nutrients absorbtion, plant nutrition and productivity. In this context, development of a mycorrhizal mapping system has a high value represented by an improvement of agronomic technologies. The design of mycorrhizal maps can serve to create a management adapted to the specific conditions of agricultural holdings and to increase productivity.

***Key words**: symbiotic status, biological fertility, agroecosystems, technological improvements.*

INTRODUCTION

Agriculture in the 21st century underwent major changes due to the steady increase in feed requirements and the need to increase productivity at the surface unit (Ewert et al., 2005; Long et al., 2014; Quintero-Angel, González-Acevedo, 2018). Current agricultural systems are defined by an integrative management of soil-technological inputs-biological components (Jones et al., 2017). In this type of management has increased the importance of microorganisms for their efficiency in nutrient utilization and transfer processes to plants (Hodbod et al., 2016; Li et al., 2017). The presence of microflora in biogeochemical cycles makes them perfect candidates for plant growth promoting with a rhizospheric and trans-rhizospheric activity (Olivares et al., 2017; Mishra et al., 2018; Techenand Helming, 2017). In addition, the microbial community reduces the pressure of technological inputs and stimulates ecosystem resilience (Müller et al., 2016; Preece, Peñuelas, 2016; van Lenteren et al., 2018).

An intimate contact and the most performant mechanisms are found in symbiotic interactions, with plant benefiting from a broad-based interface and the highest level of microbial activity (Saleemi et al., 2017; Schloter et al., 2018). Rhizospheric symbiosis permanently changes the size of populations under the combined influence of cultivated plants and technology (Andreote et al., 2017; Denison et al., 2011; Vimal et al., 2017). The specificity of management induces changes to existing microbial resources in soil, both oriented to reorganize specific feeding niches and to restructure local diversity (Compant et al., 2016; Terrazas et al., 2016).

The efficiency of using native soil resources and technologically applied (i.e., different types of fertilizers) is evolutionarily conditioned in most plants by the presence of mycorrhizal fungi in the rhizosphere (Ehrmann and Ritz, 2014). Intimate contact with plant roots promotes this type of symbionts as evolutionary nutrition regulators, connecting the rhizosphere to a large number of species and maintaining the stability of agroecosystems (Bonfanteand Genre, 2010; Kraigher et al., 2013; Zemunik et al., 2015). Current applications focus on either boosting native communities in the soil or supplementing them withdifferent microbial consortia (French et al., 2017; Martinez et al., 2010; Munyanziza et al., 1997; Sruthilaxmi et al., 2017; St-Denis et al., 2017). In the first case, long-term studies with a high number of variables are needed: fertilizers, pests, diseases and weeds management combined with different soil management systems. In the second case, it is necessary to follow a large number of inoculation variants to ensure the installation of the most effective symbiotic mechanisms. Both variants require long test periods, high financial resources in order to identify a recipe adapted to each agroecosystem.

The context has created the possibility of developing an effective alternative for stimulating mycorrhizal activity by identifying areas with high symbiotic potential and transferring active rhizosphere into similar agroecosystems. The proposed concept aims to create maps of the efficiency and dependence on mycorrhizal mechanism in agroecosystems, integrating soil type, regional eco-climatic factors, assemblage of native vegetation and microbial communities.

MYCORRHIZAS - MANDATORY MICROBIAL RESOURCES IN AGROECOSYSTEMS

Mycorrhizal symbiotic fungi have evolved to be the regulators of the host plants they interact with. Current estimates have established a 1:5 ratio of mycorrhizal fungi against host plants (Heijden et al., 2015), with a predominance of vesicular-arbuscular fungi (VAM) as symbionts. This ratio is due to the plant's need for complex soil nutritional solutions but which are limited as solubility in parent material (von Tucher et al., 2018; Zhang et al., 2016; Zhu et al., 2018). The potential for the development of extensive hyphal systems in search for nutrients has progressively promoted them as agents to facilitate exchanges within plant communities (Angel et al., 2016; Garcia et al., 2016; Wang et al., 2017). Mycorrhizal hyphae have small diameters, the fungi being able to access the spaces too small for the roots of the plants (Smithand Read, 2008). Plants with thick roots are much more susceptible to association with mycorrhizae than those with thin roots. VAM diversity in agricultural soils depends on the development of the host plant interface, soil type and eco-climatic conditions (Giovannetti, Avio, 2002; Rúa et al., 2016). The reduced specificity of the host plants, together with the adaptive capacity, makes them potential partners in agricultural crops (Karandashov, Bucher, 2005). However, at local level the specificity of the plants for a small number of species prevails over the high diversity of VAM fungi (Goss et al., 2017; Veresoglou et al., 2012). In agroecosystems, fungi act as regulators of biogeochemical circuits, a service that is visible in the phenomena of plant growth promotion (Barea et al., 2002; Jeffries et al., 2003; Nadeem et al., 2014). Between nutrients, phosphorus transferred from fungi to plants can reach high quantities, and nitrogen can be absorbed in high amounts in the mycelium during this process (Basu et al., 2018). Mobile forms of phosphorus have a high rate of immobilization in soil solution which affects normal plant growth. The phenomenon is countered by mycorrhizas that provide an alternative to transporting phosphorus through the hyphal networks in root of host plants (Cavagnaro et al., 2015).

Translocation of carbon has generally been assessed in forest ecosystems, but new agronomic concepts for returning secondary production to soil will reveal the role of VAM in this biogeochemical circuit. Through their extra-radicular mycelial extension, mycorrhizas contribute to a reduction of nutrient losses, either organic or mineral, while maintaining ecosystem sustainability at a high level (Thornley, Parsons, 2014; Wu et al., 2013).

SOIL - SUBSTRATE FOR MYCORRHIZAL NETWORK DEVELOPMENT

In soil, mycorrhizas stimulate the settlement of new seedlings, release of nutrients from organic matter simultaneous with stabilization of formation and aggregation processes of the particles (Lehmann et al., 2017). Mycorrhizal plants possess an increased resistance to water stress, heavy metal accumulation and soil borne pathogen attack (Latef et al., 2016). Current agronomic practices are reevaluated for taking into account the pressure on soil biological communities and, in particular, the link between mycorrhizal fungi and plant (Buczko et al., 2018). VAM dynamics in soil tends to produce complex mycelia, creating extensive interconnected networks (Cabral et al., 2016; Hussain et al., 2016). Networks serve as a point of insertion of host plants into the nutrient flow and favor interspecific metabolites exchanges. The regulation of flows to and towards plants is dependent on the number of individuals/surface unit, the transferable carbon capacity to fungal symbionts and the transfer strategy of mycorrhizas (Gu et al., 2016; Sciacca et al., 2013).

Agronomic pressure on soil in food-based ecosystems acts both on the extension of mycorrhizal networks and on potential host plants (Islas et al., 2016). In general, conventional soil cultivation systems severely fragment VAM by reducing the localization of symbiosis only in the host plant's rhizosphere in the first phase of growth (Kabir, 2005). Soil plowing or the frequent movement of soil particles blocks the formation of trans-rhizospheric networks, leading to a nutritional imbalance of cultivated plants (Brito et al., 2012). Shallowor no-tillage systems maintain VAM networks and stimulate the installation of a high-functionality symbiotic transfer interface (Verhulst et al., 2010). Thus, the plants grown in successive vegetation cycles always benefit from the extra-radicular systems of the precursor. At the same time, reduced soil tillage maintains propagule function of mycorrhizal mycelium and stimulates the increase in the number of spores present in the upper layer of the soil (Isobe et al., 2008). Soil processing also induces changes in VAM species that colonize crop plants by selecting species adapted to each type of management (Martin et al., 2017; Oehl et al., 2004). The phenomenon of selection is visible in the quality of the symbiotic interface, each species having its own colonization pattern based on spores or mycelial fragments. To this is added the change of host plant from one cycle to the next growing season. In monoculture conditions, the mechanism of selection of species adapted to the rhizosphere type is activated by a certain host but not always the most prolific symbiotic partners (Dharand Pagano, 2016). The cultivation of plants in complex rotations balances the representation of each mycorrhizal species in soil, the symbiotic association being much more efficient (Bakhshandeh et al., 2017). Species that are not associated with the main crop survive as symbionts in weeds rhizosphere and plants located outside the parcels (Asmelash et al., 2016; Hodge, Fitter, 2013). Selection induced by the principal crop serves as the main source of variation in mycorrhizal community, allowing the restoration of the population of each species over different time periods.

MYCORRHIZAL MAPS - AN AGRONOMIC SCENARIO

Sustainable agronomy requires efficient, high-performance technologies and interdisciplinary integrative approaches (Wang et al., 2009). Soil systems have developed extremely strongly in last decades by adapting existing technologies or innovation (Hagemann, Potthast, 2015, Poppy et al., 2014). But the development of these systems has generally become independent of soil biology, at this time there is a low database of biota dynamics under different management conditions (Baez-Rogelio et al., 2017; Kumar, Gopal, 2015). Reverse site analysis methodology where different soil work systems have been applied can be a base for monitoring and extrapolation of results.

Generally, independent of the pressure of technologies, soil type or native vegetation from agroecosystems, techniques of biological anlysis of fauna and flora have been developed (Jones, Bradford, 2001; Lemanceau et al., 2015; Parker, 2010; Zalibekov et al., 214; Wall et al., 2001; Wachira et al., 2014). The efficiency of a particular type of farming system is segmented analyzed, without taking into account the whole range of factors (Culman et al., 2010). Mycorrhizas are symbionts present in any type of agro ecosystem with a high number of host plants. The dynamics of mycorrhizal interaction with plants go from symbiosis to parasitism, in close dependence with ensuring of optimal eco-pedological factors. This point to the need for analyzing the current state of mycorrhizal mechanisms in agroecosystems, and to integrate the results into complex databases. The ability to collect mycorrhizal inoculum and transfer to similar agroecosystems can increase the efficiency of soil nutrient cycles and increase long-term production and sustainability.

The mapping system we propose takes into account biotic and abiotic components with high integrative capacity (Figure 1). The general foundations of the system relate to the monitoring of the current state of agroecosystems with the possibility of completing the historical data on each site.

The type of soil is required to establish potential ecotrophic indices, water dynamics and biogeochemical cycles. The soil demarcation of agricultural crops defines the range of soils that can be cultivated simultaneously with the same plant. Soil technological management induces changes in natural vegetation present in each area, defining the conservation status of the symbiotic rhizosphere. The assessment of native vegetation allows identification of the rhizospheric similarity between geographically distant agroecosystems. Main crops serve as a base for active rhizosphere sampling and targeted transfer to adapted areas. The active rhizosphere also serves as the base for laboratory testing in order to identify the most effective partnerships with host plants and site-specific bioproduct synthesis. The functionality of mycorrhizal mechanisms, the extension of extra-radicular hyphal networks and the efficiency of symbiotic association define the potential of connecting crop plants to the biological nutrient networks in the soil. Identifying and transferring the best performing mycorrhizal partners increases the economic and biological efficiency of a crop and the agroecosystem stability.

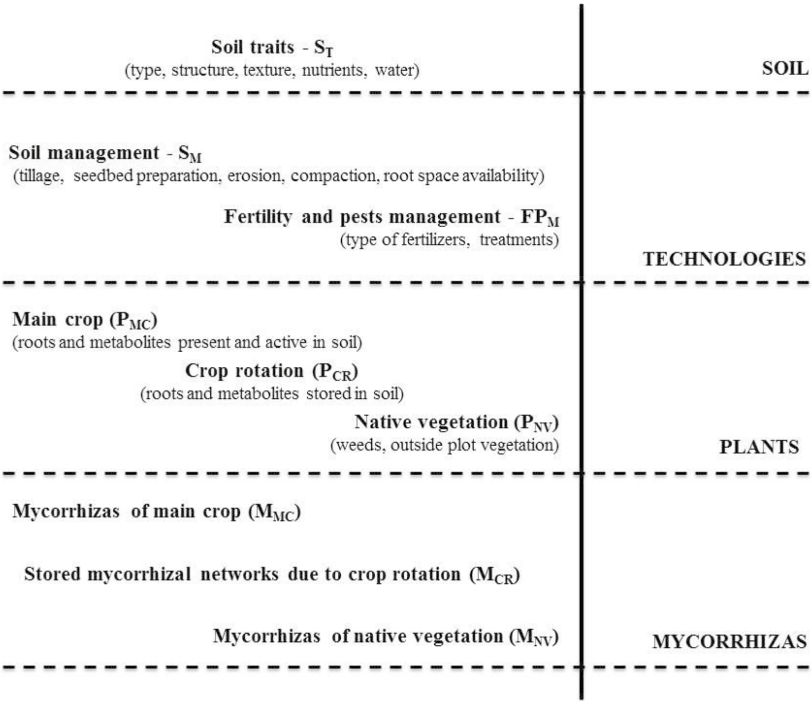

Figure 1. Methodological framework for mycorrhizal maps

The agronomic utility of complex mycorrhizal maps comes from the constant expansion of databases, facilitating access to the best symbiotic resources. The geographic assemblage of similar agroecosystems on complex maps streamlines the targeted transfer processes of mycorrhizal rhizosphere into areas requiring improvements for these mechanisms. Identifying as many as possible of similar characteristics leads to the obtaining of rhizospheric grafts with high adaptive potential under transfer conditions.

Soil analysis (S_T), technologies (S_M, FP_M) and present and past vegetation (P_{MC}, P_{CR}, P_{NV})

have a historical character, as a basis for assembling inter-plot transfer scenarios. The mycorrhizal potential (M_{MC}, M_{CR}, M_{NV}) indicates the efficiency of the current symbiotic mechanism related to the state of the crop and the associated vegetation. Mycorrhizal testing is done in real time to identify the optimum time for collecting the rhizosphere when the number of propagules is the maximum.

CONCLUSIONS

Future agriculture tends to resize and adapt technologies to soil-plant-microflora complexes. The transfer of active rhizosphere will stimulate the sustainable development of agroecosystems. Mycorrhizal maps serve to streamline plant nutrition processes by identifying the most successful symbiotic partners.

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MANAGEMENT OF FUSARIUM WILT (*Fusarium oxysporum* f. sp. *lycopersici)* OF TOMATO WITH ORGANIC AMENDMENTS

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Abstract

Tomato is an important fruit vegetable in Nigeria. Its production is affected by many diseases and Fusarium wilt is one of the most important. The disease is controlled by chemical application especially methyl bromide which is highly hazardous. Alternative methods of control that could be safe, easy and affordable are therefore desirable. Experiment was conducted to evaluate the effects of green manure (cabbage, onion and garlic) in the management of Fusarium wilt of tomato. Results shows significant reduction in the incidence and severity of the disease on plants grown on organic amended soil compared to the control. Similarly, growth parameters and yield of tomato increased significantly with organic amendment compared to the control. Organic amendment could be used in an integrated disease management program for Fusarium wilt of tomato.

Key words*: Fusarium wilt, green manure, incidence, severity, tomato.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the major vegetable crops widely cultivated in Nigeria where it has been an important component of the daily diets.
The crop is rich in vitamins, minerals and antioxidant compound lycopene which play a significant role in human health due to its anti-cancer effect (Miller et al., 2002).
Nigeria ranked as the 14th world producer and second leading producer of tomato in Africa with average production of 1.7 metric tonnes (FAO, 2010).
The production of the crop is affected by many pests and diseases. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the major fungal diseases of tomato. It is a soil-inhabiting fungus that is highly destructive and difficult to control once established (Seremi, Burgess, 2000; Hadian et al*.,* 2011).
The difficulty in the management of the disease was due to the emergence of new pathogenic races (type 2 and 3), indiscriminate use of pesticides leading to the development of resistance and death of antagonistic biocontrol agents (Dewaard et al., 1993; Juliano, Bettiol., 2005). Management of *Fusarium* wilt of tomato under large scale production depends largely on the use of methyl bromide which has been found to have severe environmental hazards and destruction of beneficial microorganisms (Fuchs et al., 1999; Santos et al., 2006). Apart from human and environmental concerns continuous use of chemicals may lead to the development of multi-resistant strains (Njue et al., 2012). This necessitates the search for eco-friendly ways that are effective and affordable in managing the disease.
The objective of this study therefore, was to evaluate the use of organic amendments in the management of *Fusarium* wilt of tomato under field conditions.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the teaching and research farm of Federal College of Horticulture, Dadin Kowa, Gombe state, Nigeria. Gombe State is located on latitude 10° 18^1 N and longitude 11° 31^1 E in the Sudan Savannah ecological zone of Nigeria. The area is characterized with a single peak of rainy season starting from May to October and a dry season spanning from November to April.

Isolation and Purification of *Fusarium oxysporum* f sp. *lycopersici*

Fusarium oxysporum f sp. *lycopersici* was isolated from diseased tomato plants that had shown symptoms of wilting and brown discolouration of vascular vessels. The stems of the infected plants were washed in distilled water to remove any foreign materials.

Five-millimetre pieces were cut, dipped in 70% alcohol and then sterilized in 0.5 % sodium hypochlorite solution for 3 minutes. The samples were dried by blotting. Chloramphenicol at 0.5 mg per litre of molten potato dextrose agar (PDA) was added to suppress bacterial growth. The dried sterile infected plant samples were placed in the center of Petri dishes containing amended PDA and incubated at 38°C for seven days.

Colonies showing morphology of *Fusarium* were further sub-cultured for another 7 days on potato dextrose agar (PDA) to obtain pure cultures as described by Booth (1977). Spore suspension of *Fusarium* pathogen was prepared and adjusted to 10^7 spores/ml using haemocytometer.

Experimental set up and procedures

The experimental plot was cleared of remains of crop residues from the previous season, ploughed and harrowed to give a fine tilt. The plot was marked out in to plots measuring $10m^2$. The plots were inoculated with conidial suspension (10^7 conidia/ml) of Fusarium wilt according to the procedures of Misrak et al., 2014. Twenty kilogrammes of cabbage and 5 kg each of garlic and onion bulbs were sliced into 2–3 cm pieces and incorporated separately into the soil in each plot. The amended soil was moistened with tap water to near field capacity, covered with polythene material and left for 14 days for decomposition to take place. An un-amended soil and a synthetic fungicide CAMAZEB® (60% mancozeb and 40% carbendazim WP) were included as control and check respectively.

The treatments were laid in a randomized complete block design with three replications. Three weeks old tomato seedlings were transplanted in each of the treatments 15 days after organic amendment.

Data on number of leaves, branches and plant height was recorded bi-weekly. Disease incidence (%) was recorded at weekly intervals by simply dividing the number of plants showing symptoms by the total number of plants assessed and the product multiplied by 100%. Mathematically, incidence = (number of infected plants/number of plants assessed) x 100%.

Disease severity was recorded weekly using the scale of Sibounnavong et al. (2012): where 1 = no symptoms, 2 = plant showing yellow leaves and wilting (1 - 20%), 3 = plant showing yellow leaves and wilting (21 - 40%), 4 = plant showing yellow leaves and wilting (41 - 60%), 5 = plant showing yellow leaves and wilting (61 - 80%), 6 = plant showing yellow leaves and wilting (81 - 100%) or death.

Disease severity (S) was calculated using the formula: $S = (\sum n) / (N \times 6)$. Where $\sum n$ = summation of individual ratings, 6 = highest score of severity and N = total number of plant assessed. Tomato yield was recorded in $kgplot^{-1}$ at final harvest.

Data was subjected to analysis of variance using GenStat 17th Edition, Release 17.1 (2014). Treatment means were separated using LSD at 1% level of significance.

RESULTS AND DISCUSSIONS

The results show significant reduction in incidence of *Fusarium* wilt on tomato plants grown on soil amended with organic materials than the control (Table 1).

Table 1. Effect of Bio-fumigant crops on incidence of *Fusarium* wilt of tomato

Bio-fumigant crop	Weeks after transplanting						
	7	8	9	10	11	12	13
Cabbage	7.8	36.9	43.0	41.8	40.2	39.9	39.7
Garlic	8.4	37.5	44.4	43.3	41.6	41.0	40.0
Onion	32.6	39.3	45.7	43.9	41.8	41.8	40.8
[a]Camazeb	15.6	40.1	46.8	45.4	45.0	44.9	44.8
[b]Control	33.6	41.7	45.0	45.4	45.7	46.8	47.8
LSD P≤0.001	0.33	0.52	0.31	0.20	0.67	0.21	0.29

[a]CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP) as check; [b]Un-amended as control.

Incorporating cabbage, garlic and onion leaves in to the soil infected with *Fusarium* wilt significantly lowered the incidence of the disease on tomato plants than the un-amended soil.

Cabbage leaves significantly reduced number of infected tomato plants than the use of garlic and onion leaves respectively.

The reduction in the incidence of *Fusarium* wilt on tomato grown on amended soils could be attributed to the biocidal volatiles released by the green manure.

Cabbage and garlic are known to contain biologically active compounds that inhibit a wide range of soil borne fungal pathogens (Avato et al., 2000) and some of these compounds are released in form of gases during decomposition that acts as fungi toxic to many soil inhabiting pathogens. This observation agrees with previous findings by Adel et al. (2011), Brown et al. (1999) and Ramsey et al. (2007) who reported reduction in soil borne pathogens on crops grown on soil amended with cabbage and garlic.

Tomato plants grown on soil amended with bio-fumigant crops had lower disease severity than the control (Table 2).

Table 2. Effect of Bio-fumigant crops on severity of *Fusarium* wilt of tomato

Bio-fumigant crop	Weeks after transplanting						
	7	8	9	10	11	12	13
Cabbage	1.1	1.2	1.4	1.7	1.8	2.6	2.7
Garlic	1.2	1.5	1.9	2.2	2.2	2.9	3.0
Onion	1.3	1.7	1.9	2.3	2.3	3.2	3.4
[a]Camazeb	1.2	1.4	1.6	1.7	1.7	3.2	3.5
[b]Control	1.5	2.2	2.3	2.7	2.7	3.6	4.1
LSD (P≤0.001)	0.14	0.36	0.36	0.31	0.36	0.20	0.28

[a]CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP) as check; [b]Un-amended as control.

The effectiveness of cabbage leaves in reducing the severity of the disease was as effective as the synthetic fungicide Camazeb.

Cruciferous plants are known to contain methyl-isothiocynate which is release during decomposition and affects a wide range of soil borne fungi. Research findings have shown reduction in disease severity when crucifers are added to soil due to antagonism, antibiosis and competition (Brown, Morra, 1997; Buskov et al., 2002; Zasada, Ferris, 2003).

Soil amendment with bio-fumigant crops significantly (P≤0.001) increased plant height than the control (Table 3).

Taller tomato plants were obtained when cabbage and garlic leaves were incorporated in the soil than the onion. The use of cabbage was as effective as the synthetic fungicide.

Table 3. Effect of bio-fumigant crops on plant height (cm) of tomato

Bio-fumigant crop	Weeks after transplanting			
	7	9	11	13
Cabbage	19.5	27.2	43.8	55.2
Garlic	18.7	26.3	42.9	51.4
Onion	18.0	25.1	41.7	48.9
[a]Camazeb	18.1	24.4	41.0	49.3
[b]Control	16.7	24.0	40.5	46.7
LSD(P≤0.001)	0.26	0.27	0.33	0.50

[a]CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP) as check; [b]Un-amended as control.

Similarly, tomato leaf production was enhanced by the addition of bio-fumigant crops. Incorporating cabbage, garlic and onion in to soil before transplanting has increased number of tomato leaves than the control (Table 4).

Table 4. Effect of bio-fumigation on number of leaves of tomato

Bio-fumigant crop	Weeks after transplanting			
	7	9	11	13
Cabbage	27.4	45.2	63.9	105.8
Garlic	24.9	43.0	60.2	101.9
Onion	22.3	38.9	54.8	96.5
[a]Camazeb	19.8	32.8	54.8	96.8
[b]Control	20.8	36.3	47.2	88.8
LSD(P≤0.001)	1.41	1.23	1.43	0.99

[a]CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP) as check; [b]Un-amended as control.

Addition of cabbage enhanced tomato leaf production more than garlic which in turn increased leaf production than the onion. Soil amended with cabbage produced tomatoes with the highest yield (16.5 kg plot^{-1}) followed by tomatoes grown on soil amended with garlic (16.2 kg plot^{-1}) and onion bulbs (16.0kg plot^{-1}). Lowest yield was obtained on soil not treated with bio-fumigant crop (13.7 kg plot^{-1}) as presented in Table 5.

Table 5. Effect of bio-fumigant crops on yield of tomato

Bio-fumigant crop	Yield (kg plot^{-1})
Cabbage	16.5
Garlic	16.2
Onion	16.0
Camazeb	15.7
Control	13.7
LSD (P≤0.001)	0.1

Decomposition of green materials enhanced the activities of soil micro-organisms which had adverse effect on *Fusarium* wilt pathogen and improve tomato growth and yield. It also increase nutrients availability by supplying both macro- and micro-nutrients needed for healthy growth of tomato. Combination of these attributes has contributed in increased growth parameters and consequently in the final yield of tomato. This conforms with the findings of Smolinska (2000) and Pakeerathan et al. (2009) who reported dual effects of combined use of animal manure and plant materials for improvement of diseases control and plant growth.

CONCLUSIONS

Incorporating cabbage and garlic in to soil before transplanting tomato is effective in controlling *Fusarium* wilt and increased tomato yield. These green materials could therefore be used as an alternative to synthetic fungicides in integrated management programme for *Fusarium* wilt during tomato production.

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EFFECT OF RHIZOBIUM BACTERIA ON NITROGEN FERTILIZER REQUEST OF COMMON VETCH (*Vicia sativa* L.)

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Abstract

Common vetch (Vicia sativa L.) is a single-year legume feed plant, grown in plant-animal production systems for grass and grain purposes in many parts of the world, and is widely used in the feeding of farm animals. Turkey is the genetic center of common vetch and 59 of the existing species are naturally present in the Turkish vegetation.
The aim of this study was to determine the effect of nitrogenous fertilizer on plant and the appropriate dose of nitrogenous fertilizer and to investigate the effect of plant growth, yield, amount of N uptake by the plant, which is Rhizobium bacterial inoculation. Experiment was carried in controlled greenhouse conditions and used 'Tamkoc 2000' variety of common vetch seeds and applied Rhizobium leguminosarum L. bacterial and 3 doses of nitrogen (0-3-6 kg/da nitrogen) fertilizer.
According to the results of the research it was observed that only the difference in the content of N in grain among the examined parameters. This value is 3.89% N in bacteria-overgrown plants and 3.39 % N in bacteria-free plants.

***Key words**: Common vetch, nitrogen fertilizer, Rhizobium leguminosarum* L.

INTRODUCTION

Common vetch (*Vicia sativa* L.) is an annual forage legume which is cultivated in numerous parts of the world in plant and animal production systems and commonly used in the feeding of the livestock (Cabellero et al., 2001; Ramos et al., 2000; Açıkgöz, 2001; Chowdhurry et al., 2001; Han, 2010). Roughage obtained from legumes is an important source of fodder and is richer than the other roughage in terms of minerals, vitamins and most importantly proteins (Ensminger et al., 1990; Karabulut et al., 2007). The chemical composition of the legume forages varies depending on the species, geographical regions and agricultural practices and their protein content can range between 20-45% (Dixon, Hosking, 1992; Abreu, Bruno-Soares, 1998; Filya et al., 2002). In areas where the production of oilseed residues is low or costly due to their high crude protein content, legume forages are used as an alternative source of protein. The amount of cultivated land in Turkey, including fallow lands, is 20.5 million hectares, and within this cultivated land, the area for the cultivation of fodder crops has reached to 1.8 million hectares due to the governmental incentives (TUIK, 2013). Again, with the governmental incentives, among all the forage cultivation lands, common vetch cultivation land has ranked the second with an area of 499000 hectares, following alfalfa cultivation land (629000 hectares).

Seed of the common vetch plant is green and has a good fodder. Common vetch is also a good alternation and green manure crop (Avcıoglu, Soya, 1977). Common vetch has 150 species (Tosun, 1974) and 59 of these species grow naturally in Turkish vegetation (Elci, Acıkgoz, 1993). In particular, *Vicia cracca* are found as natural plantations in certain regions of Anatolia, and as well as being a good source of nectar, these plantations are used as quality crude fodder after being reaped and dried during the fruit-set period (Tamkoc, 1999).

Use of legumes in the fallow lands of Europe and USA as a source of biological nitrogen fixation has been a revived focus of attention. The amount of nitrogen fixed by the legumes in fallow lands (BNF) can be affected and change due to a wide spectrum comprising factors such as the soil characteristics, environmental conditions and land use management (Ledgard, Steele, 1992). Common vetch, which is one of these forage legumes, is the most frequently cultivated vetch species (Anonymous, 2011). Fodder of common vetch is very tasty and nutritious due to its high crude protein content. Extensive studies on the effect of *Rhizobium* inoculation on nitrogen use are inadequate for common vetch plant.

The aim of this study is to identify the effect of appropriate *Rhizobium* inoculation for common vetch on the vetch's requirement for nitrogenous fertilizer, to determine the appropriate fertilizer dose, and to investigate its effects on plant development, yield and the amount of nitrogen bound by the plant.

MATERIALS AND METHODS

This study was performed in the greenhouse of Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition under controlled conditions. In the study, common vetch seed of 'Tamkoc 2000' cultivar was used. Trial were established based on the pattern randomized block design and replicated 4 times. Suitable bacteria *(Rhizobium leguminosarum* L.) for common vetch were taken from Ankara Soil, Fertilizer and Water Resources Central Research Institute. Bacterial inoculation was carried out at a ratio of 1 gram of bacteria per 100 grams of seed. In order to ensure better adherence of the inoculation material to the seeds, 4% sugar solution prepared at a dimly lit location which does not receive sunlight and was added on the seeds. Than the seeds were dried out and planted and irrigation was performed with pure water. The trial plan is as follows:

- Inoculated with bacteria;
- Uninoculated;
- 3 nitrogen doses (0-30-60 kg ha^{-1} nitrogen) (using 46% urea) application.

The experiment was established on April 2016. The soil samples used in the experiment were taken from a depth of 0-30 cm at Selcuk University of Faculty of Agriculture Sarıcalar Experimental area in Konya. The collected soil sample was passed through a 4 mm sieve. Than the experiment soil was sterilized for 2 hours at 121^0C. 0.8 kg soil has been put in each pot. The test plants were irrigated by using pure water and maintain field capacity during plant growth process. The experiment was terminated when the capsule formation was complete at the end of a development period of 4 months.

Measurements and analyzes performed on plants

Plant height (4 measurements were made at 10-days intervals after the first plant exit), bean length, number of grains per bean, leaf chlorophyll amount (with SPAD 502 chlorophyll meter), the grain yield values in the pot were observed. At the end of the trial, the plant was cut from the soil level and shoots and roots were weighed separately and then washed. After being dried at 65°C and being subjected to the required pretreatments.

They were solved by wet method using sulfuric acid and hydrogen peroxide (Bayraklı, 1987). The phosphorus in the plant extracts obtained after wet decomposition was read at the spectrophotometer based on the yellow color method and the potassium was read using atomic absorption (Kacar, Inal, 2010), and the % nitrogen content was calculated using Kjeldahl procedure. The obtained nitrogen values were multiplied by 6.25 to calculate crude protein ratios.

The amount of Nitrogen fixed from the atmosphere by the plants

The plant weight and nitrogen fixation capacity were determined during the development period of the plants.

The amount of nitrogen fixed from the atmosphere was calculated using the following formula (Yaman, Cinsoy, 1996; Öğütücü, 2000).

mg N / pot = [(root dry weight x root nitrogen content %)] + [SB (plant stem biomass dry weight or weight of aboveground parts of plant) x stem nitrogen content %] + (amount of dry weight of grain x grain nitrogen content %) / 100) x 1000

The amount nitrogen fixed nitrogen (kg N/da)

It was calculated using the equation: *Total nitrogen amount of the inoculated plant – total nitrogen amount of the uninoculated control* (Yaman, Cinsoy, 1996; Öğütücü, 2000).

SE (symbiotic efficiency) (%)

It was calculated using the equation: *Amount of nitrogen of the plant grain to which the isolate was inoculated / Amount of nitrogen in grain of nitrogen control × 100* (Beck et al., 1993; Materon et al., 1995).

Efficiency level

Equation: *Mean dry matter weight of test plant/ mean dry matter weight of nitrogen control plant) x 100*

The following limits have been taken into consideration when grouping the isolates based on their efficiency levels:

- 100: Highly efficient;
- between 100-75: Efficient;
- between 75-50: Moderately efficient;
- between 50-25: Slightly efficient;
- <25: Inefficient.

Soil analysis

Some of the physical and chemical properties of the test soil before the trial are given in Table 1. The soil used in the study is clay loam, mild alkaline (pH 7.75), limy (12.94%), less salty, little organic matter and its useful phosphorus concentration is very little. It was found that iron and zinc content is sufficient, and the copper and manganese content is high.

Table 1. Physical and chemical properties of the soil used in experiment

Parameters	Value	Parameters	Value
Texture class	Clay loam	P (mg kg^{-1})	5.15 (very little)
$CaCO_3$ (%)	12.94 (limy)	Fe (mg kg^{-1})	3.69 (enough)
pH (1/2.5 H_2O)	7.75 (mild alkaline)	Cu (mg kg^{-1})	1.85 (high)
EC (mS/cm)	249.0 (less salty)	Mn (mg kg^{-1})	14.73 (high)
Organic Matter (%)	1.98 (little)	Zn (mg kg^{-1})	0.74 (enough)
Volume weight(g/cm^3)	1.25	Field capacity (%)	27.34

Statistical analysis

The data were subjected to analysis of variance using the MINITAB 16 statistical package and means were separated according to the least significant differences (LSD) test.

RESULTS AND DISCUSSIONS

The effect of applying different doses of nitrogen fertilizer to the common vetch plant and the effect of *Rhizobium* bacteria inoculation on developmental parameters such as root weight, stem biomass, grain weight, bean length, number of seeds in the bean were found to be statistically insignificant (Tables 2 and 3). The highest root weight and stem biomass were observed in N30B + application. It was previously reported that inoculation with *Rhizobium leguminosarum* of common vetch (*Vicia sativa* L.) compared to non-inoculated cultivars were increased seed yield, biological yield, straw yield, pod length, number of seed per pod, number of pods, main stem length and thousand seed weight of common vetch cultivars (Albayrak et al., 2006).

Table 2. The effect of nitrogen fertilizer doses and bacterial inoculation on the mean values of various plant developmental parameters

N Doses (kg/ha)	Root weight (g/pot)	Stem Biomass (g/pot)	Grain weight (g/pot)	Bean length (cm)	Number of bean seeds (piece)
N0	1.04	14.16	1.48	2.84	2.01
N30	1.05	13.75	0.95	2.49	1.67
N60	0.70	13.05	1.05	2.52	1.85
Bacteria inoculation	Root weight (g/pot)	Stem Biomass (g/pot)	Grain weight (g/pot)	Bean length (cm)	Number of bean seeds (piece)
B+	0.89	13.65	1.06	2.62	1.72
B-	0.97	13.66	1.26	2.61	1.97

Table 3. The effect of nitrogen fertilizer doses and bacterial inoculation on plant developmental parameters

N Doses (kg/ha)	Bacteria inoculation	Root weight (g/pot)	Stem Biomass (g/pot)	Grain weight (g/pot)	Bean length (cm)	Number of bean seeds (piece)
N0	B+	1.25	14.39	1.46	2.89	1.83
N0	B-	0.84	13.93	1.50	2.78	2.19
N30	B+	0.91	13.93	0.74	2.42	1.62
N30	B-	1.20	13.58	1.17	2.57	1.71
N60	B+	0.52	12.64	0.99	2.55	1.99
N60	B-	0.88	13.46	1.10	2.49	1.99

The effect of nitrogenous manure application on and bacterial inoculation to the common vetch plant on the plant height values measured at 10 day-intervals was found to be statistically insignificant; the simultaneous effect of nitrogen application and bacterial inoculation was found to be significant only in the second measurement period ($P<0.05$). At the last measurement, the plant height varied between 32.8-37.4 cm (Table 4). The highest plant heights were measured at a field experiment in Gümüşhane, Turkey, as 41.20 cm, 38.90 cm, 38.70 cm and 38.60 cm (Yolcu, 2011).

It was found that nitrogen application and bacterial inoculation on the leaf chlorophyll content have a significant effect. The highest chlorophyll content (39.0) was observed upon N0B + application ($P<0.05$) (Table 4).

Table 4. The effect of nitrogen doses and bacterial inoculation on plant height and leaf chlorophyll (SPAD)

N doses (kg/ha)	Plant height (cm)				Chlorophyll
	1.	2.	3.	4.	
N0	27.4	32.3	32.4	33.9	36.54
N30	30.7	33.7	33.5	35.1	34.64
N60	30.5	33.7	35.8	35.1	35.35
Bacterial	1.	2.	3.	4.	Chlorophyll
B+	30.7	33.6	33.8	35.6	34.44
B-	28.4	32.8	34.0	33.8	36.58
N doses and Bacterial	1.	2.	3.	4.	Chlorophyll
N0 B+	29.3	31.8	32.4	34.4	39.0
N0 B-	25.5	32.8	32.4	33.5	34.0
N30 B+	33.3	38.5	36.1	37.4	31.8
N30 B-	28.0	29.0	30.9	32.8	37.5
N60 B+	29.4	30.7	32.8	34.9	32.5
N60 B-	31.5	36.7	38.8	35.2	38.2
LSD Value P<0.05	-	*	-	-	*

Statistically significant difference was observed in the K content of the grain and root of common vetch plant upon both bacterial inoculation and the interaction of nitrogen dose and bacterial inoculation. The nutrient concentration values obtained from the leaf, grain and root of plants are given in Table 5. There was no significant difference between the applications in terms of leaf, grain and root mean nitrogen contents. Bacterial inoculation increased the content of crude protein in grain ($P<0.01$). When the potassium contents of grain and root have been examined, it has been detected that nitrogen dose, bacteria inoculation, interaction of nitrogen dose and bacteria inoculation have statistically significant. The P contents of the grain and root were affected from nitrogen doses ($P<0.01$). The highest grain K content, root K and P contents were observed at the application of N30 dose (30 kg N/ha).

When the effect of nitrogenous fertilizer application and bacterial inoculation on the crude protein content of the grain was analyzed, it was found that bacterial inoculation increased crude protein ($P<0.05$), with the highest crude protein content being 24.75% upon N60B+ application (Figure 1). Phosphorus content of the grain was the highest with 0.62%, upon N30B+ application (Figure 2). Crude protein content was reported to be between of 15.15-20.69% under the ecological conditions of Iğdır (Temel et al., 2015). Crude protein content of 21 different vetch genotypes grown in Kahramanmaras was reported to vary between 23.47% and 18.80% (Yılmaz, Erol, 2012), and in studies carried out under different ecological conditions it was found that the crude protein contents of vetch varieties ranged between 9.08-22.30% (Yücel et al., 2012; Kaplan, 2013; Yücel et al., 2013; Yılmaz, Erol, 2015).

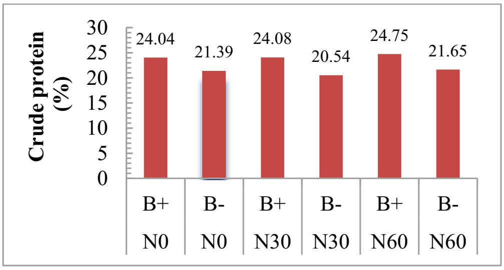

Figure 1. The effect of nitrogen doses and bacterial inoculation on the crude protein content of the grain (%)

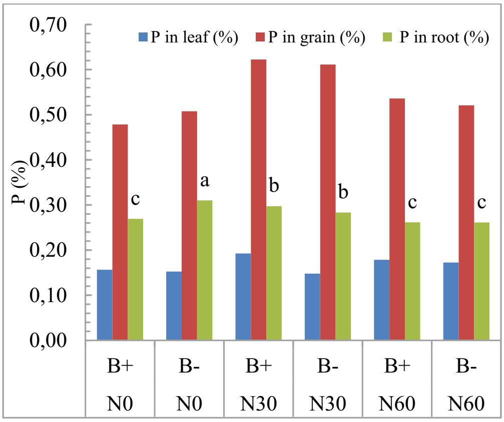

Figure 2. The effect of nitrogen doses and bacterial inoculation on the phosphorus content of leaf, grain and root (%)

Table 5. Effect of nitrogen doses and bacterial inoculation on N, P, and K concentrations of common vetch plant

N doses (kg/ha)	Leaf N	Grain crude protein	Root N	Leaf K	Grain K	Root K	Leaf P	Grain P	Root P
	(%)								
N0	1.26	22.71	2.14	2.50	1.25 b	1.79 a	0.15	0.49 b	0.29 a
N30	1.40	22.31	2.30	2.24	1.53 a	2.04 a	0.17	0.62 a	0.29 a
N60	1.45	23.2	2.59	2.34	1.29 b	1.17 b	0.18	0.53 ab	0.26 b
LSD Value P<0.01	-	-	-	-	0.174	0.305	-	0.09	0.009
Bacterial	Leaf N	Grain crude protein	Root N	Leaf K	Grain K	Root K	Leaf P	Grain P	Root P
	(%)								
B+	1.42	24.29	2.45	2.48	1.31	1.85 a	0.18	0.54	0.28 b
B-	1.32	21.2	2.24	2.24	1.40	1.49 b	0.16	0.54	0.28 a
LSD Value P<0.01	-	*	-	-	-	**	-	-	**

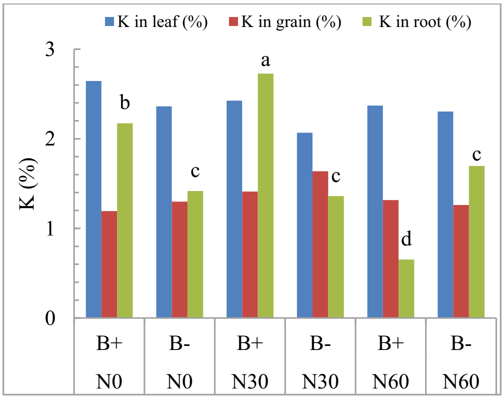

Figure 3. The effect of nitrogen doses and bacterial inoculation on the potassium content of leaf, grain and root (%)

The amount of nitrogen fixation at the application of N30 dose in the plant inoculated with *Rhizobium* bacteria was 34.42 mg N/pot, which was the highest (Table 6). The amount of nitrogen fixation varied between 6.99 and 10.74 kg N/da. In this case, it can be said that the amount of nitrogen fixed from the atmosphere by the vetch plant is significantly increased by bacterial inoculation. This increase is more pronounced when 30 kg N/ha nitrogenous fertilizer is applied. Symbiotic efficiency in the plant inoculated with the bacterial isolate varies between 102.9-108.3. This value is higher when 30 kg N/ha is applied, compared to the other values (Table 6). The isolate belongs to the "highly efficient" group in terms of its efficiency level (Holding, Kong, 1963).

Table 6. The effect of bacterial inoculation on the amount of nitrogen bound by the plant, symbiotic efficiency and the level of efficiency

N doses (kg/ha)	Nitrogen bounds by the plant (mg N/pot)	Nitrogen bounds by the plant (kg N/da)	Symbiotic efficiency	Level of efficiency
N0	24.91±2.60	7.81±0.77	107.60±4.81	118.58±4.89
N30	34.42±6.76	10.74±2.12	108.35±4.65	113.18±27.96
N60	22.46±7.99	6.99±2.52	102.90±1.01	99.95±18.88
LSD value P<0.05	-	-	-	-

CONCLUSIONS

The results of the study show that the common vetch plant should be inoculated with the appropriate *Rhizobium* bacteria prior to seeding and the seeding must be performed only afterwards, and 30 kg N/ha nitrogenous fertilizer application would be sufficient. In today's economic conditions, fertilizer prices are gradually increasing. Excessive use of nitrogenous fertilizer causes the problem of nitrate pollution in the soil and the water. Therefore, bacterial inoculation on vetch before being grown is an important practice. We must be performed these application for economic reasons and in order to eliminate the problem of environmental pollution.

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EFFECT OF WHEATGRASS (*Triticum aestivum* L.) JUICE ON SEEDLING GROWTH AND *Rhizoctonia solani* ON CORN

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Abstract

In this study, possibilities of using wheatgrass juice, as an encouraging or activator of seedling growth on corn, were investigated. In order to obtain grass juice, wheat seeds (bread wheat) were sown in plastic boxes and were harvested on the 10th day. Wheatgrass juice was obtained by using an electronic juicer. Pots with 10-15 cm diameter were filled with perlite+peat mixture and three corn seeds were sown into each pot. Fresh wheatgrass juice was used to irrigate the corn seeds and tap water was used for control pots. Seedlings were removed and washed at the end of 30th day. Stem and root lengths and weights of the seedlings were determined.
In order to determine the effect of wheatgrass juice on Rhizoctonia solani, the pathogen isolate was grown on Petri dishes with Potato Carrot Agar medium and transferred onto pots with perlite. Fifteen corn seeds were sown in each pot. Seeds were then irrigated with distilled water (control group: Rhizoctonia solani+distile water) or wheatgrass juice (Rhizoctonia solani+wheatgrass juice). Disease severity was determined two weeks after sowing.
As a result of the study, wheatgrass juice was significantly reduced both lengths and weigths of the corn seedlings (root+stem). It was also found that wheatgrass juice increased the severity of disease symptoms caused by Rhizoctonia solani.

***Key words**: wheatgrass juice, root-stem length and weight, Rhizoctonia solani.*

INTRODUCTION

Germination/sprouting causes extensive changes on the seeds. During this stage, the synthesis of useful compounds such as vitamins and phenolics occurs. Wheat seedling germinated and grown over a period of 6-10 days is generally called wheatgrass. During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts, and they reach the maximum antioxidant potential (Kulkarni et al., 2006a). It is reported that wheatgrass contains high amounts of vitamins, antioxidants and minerals in bioavailable form. Additionally, the concentrations of vitamins C and E, β-carotene, ferulic acid and vanillic acid increase during the germination period of wheatgrass (Hanninen et al., 1999). The inhibitory effect of wheatgrass on oxidative DNA damage and the high antioxidant content of wheat sprouts were shown (Falcioni et al., 2002). Antioxidants are capable of neutralizing the deleterious effects of free radicals.

In another study, it is shown that wheatgrass powder contained protein, carbohydrate, dietary fiber, chlorophyll, fat, calcium, potassium, vitamins (A, B_{12}, C, E, B_{17}, B_9), and aminoacids (histidine, isoleucine, lysine, leucine, threonine, tryptophan, valine, methionine, trozine, alanine, proline, serine, phenylalanine) (Pant et al., 2013).

Plant activators are natural or synthetic chemicals that help the plant to get the nutrients better from the soil, increase the yield and quality of the product especially under stress conditions by improving the soil structure and natural defense system of the plants. In different studies, it was reported plant extracts obtained from different plant parts contained more antioxidant compounds and their use reduced oxidative stress and encouraged development (Liu, 2004; Joseph et al., 2007; Pan et al., 2009; Pant et al., 2013).

In recent years, it was reported that there were various forms of wheatgrass as a healthy food, such as a ready-made juice, tablet or powder and that 100 g of wheatgrass powder is equal to 23 kg of fresh vegetables. Wheatgrass packs a nutritional punch, including (per 3.5 grams) 860 mg protein, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C and an abundance of micronutrients, such as B complex vitamins and amino acids. Also wheatgrass juice is high in vitamin K (Rana et al., 2011).

Rhizoctonia solani is a soil-borne pathogen causing pre- or post-emergence damping off disease resulting in seedling losses. If the pathogen attacks seeds before germination or just before the emergence of young seedling, seed or seedling root occurs and it is called pre-emergence damping off. If the fungus attacks stem of young seedling after emergence which then falls over the soil and dies, this symptom is called post-emergence damping off. Sometimes the pathogen causes late infections which don't kill the plant, but decreases yield.

The pathogen also causes reddish brown lesions on the roots which prevents nutrient uptake and causes nutrient deficiency symptoms (Agrios, 2005; Aşkın and Katırcıoğlu, 2008).

While there are many researches on the effects of wheatgrass juice on human health, there is no study showing its effects on plants and their diseases. In this study, the possibility of using wheatgrass juice, which is rich in proteins and amino acids, as a bio-fertilizer and to determine its effects on the disease symptoms caused by *Rhizoctonia solani* on corn plants.

MATERIALS AND METHODS

Two different experiments were carried out to determine the possibility of using wheatgrass juice as a bio-fertilizer and its effect on *Rhizoctonia solani,* on corn plants. The experiments were carried out as a factorial in base of completely randomized design with 4 replicates and conducted in the laboratory of the Department of Field Crops, Faculty of Agriculture, Süleyman Demirel University. In order to obtain grass juice, bread wheat seeds (cv. Gün 91) were sown in 35x50 cm plastic boxes and were harvested on the 10th day, by reaping with scissors. Then wheatgrass juice was obtained by using an electronic fruit juicer.

Determination of the effect of wheatgrass juice on plant growth

In the experiment carried out for the determination of the effect of wheatgrass as a bio-fertilizer, 10-15 cm diameter pots were filled with perlite+peat mixture and three corn seeds were sown into each pot. Initially, 100 ml of fresh wheatgrass juice was used to irrigate the corn seeds and same amount of tap water was used for control pots. Every two days pots were controlled and irrigated with 100 ml of wheatgrass juice or tap water for controls, as needed. Seedlings were removed and washed at the end of 30th day after emergence. Roots and stems were cut with a razor blade at their juncture and their lengths were measured with a millimeter ruler. The average root/stem length was determined as cm/plant by taking the average of the replicates per application. Separated stems and roots of the seedlings were placed on blotter paper and dried and they were weighed with mg precision analytical scale. Thus fresh weights of roots and stems of the corn seedlings were found. Samples were weighed again after they were dried at 70°C for 48 hours and dry weights of roots and stems were determined. Average fresh/dry weights were calculated as mg/plant. Nitrogen contents of the aboveground parts of the seedlings were determined by using Kjeldahl method. Crude protein ratios were estimated by multiplying with 6.25 factor (Kacar, İnal, 2010).

Determination of the effect of wheatgrass juice on *R. solani* caused disease

In order to determine the effect of wheatgrass juice on disease symptoms caused by *Rhizoctonia solani*, the pathogen isolate, obtained from the Department of Plant Protection, Faculty of Agriculture, Süleyman Demirel University, was grown on Petri dishes with Potato Carrot Agar medium at 25°C, in the dark for 5 days. Mycelia of the pathogen with agar were cut into small pieces with a scalpel and this inoculum was transferred onto pots with perlite, under aseptic conditions (Figure 1). Fifteen corn seeds were sown in each pot and four replicate pots were used. Seeds were then irrigated with wheatgrass juice or distilled water for control group. Plants not inoculated with the pathogen, irrigated with wheatgrass juice and irrigated only with distilled water were evaluated as negative controls. At the end of two weeks growth period, plants were examined for root and crown rot symptoms and evaluated by using a 1-4 scale to determine the disease severity rates, where 1 represented healthy plant, 2 slight browning on lateral roots, 3 severe browning on roots and crown and 4 totally wilted or dead plant (Türkkan, Karaca, 2006). Disease severity was calculated by using Townsend and Heuberger's formula:

Disease severity (%) = $\Sigma(nv)$ / NV X 100,

where: n was the degree of infection according to the scale, v was the number of seedlings per category, V was the total number of seedlings screened and N was the highest degree in the scale (Townsend, Heuberger, 1943).

All data were statistically analyzed by using SAS program and differences between the applications were compared with LSD test.

Figure 1. Application of *R. solani* inoculum

RESULTS AND DISCUSSIONS

Effect of wheatgrass juice on the growth of corn seedlings

As a result of the experiment carried out in order to determine the effects of wheatgrass juice on seedling growth, there were statistically significant difference between the root ($P<0.01$) and shoot ($P<0.05$) length values obtained from seedlings irrigated with wheatgrass juice and controls. It was found that wheatgrass juice decreased both root and shoot growth of corn plants (Figure 2). Mean root length of the corn seedlings irrigated with wheatgrass juice was 4.63 cm, while it was 27.28 cm in the control group. Similarly, shoot length of the plants irrigated with wheatgrass juice was 14.54, while it was 23.68 in the control group (Figure 4).

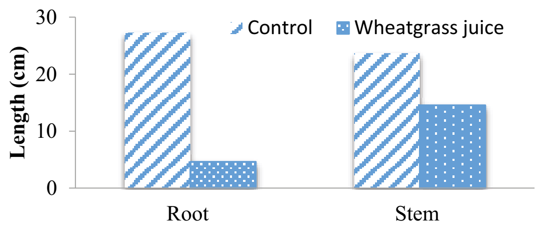

Figure 2. Effect of wheatgrass juice on root and shoot lengths (cm) of corn seedlings

Correspondingly, the effect of wheatgrass juice on root and shoot fresh and dry weights of corn seedlings were statistically significant ($P<0.01$). It was found that wheatgrass juice decreased both fresh and dry weights of roots and shoots (Figure 3). Mean fresh and dry weights of the roots of corn seedlings irrigated with wheatgrass juice were 250 mg/plant and 50 mg/plant, while those were 790 mg/plant and 120 mg/plant in the control group, respectively. Mean fresh and dry weights of the shoots of the corn seedlings irrigated with wheatgrass juice were 250 mg/plant and 70 mg/plant, whereas the means of the control plants were 890 mg/plant and 110 mg/plant.

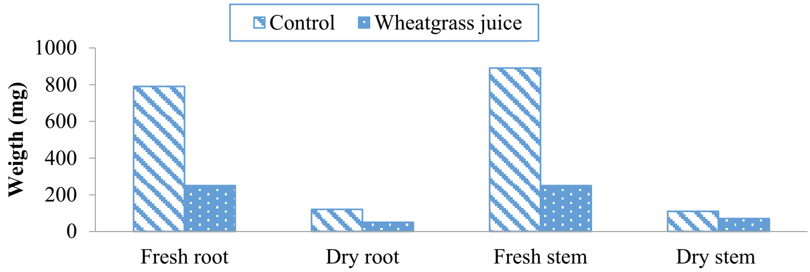

Figure 3. Effect of wheatgrass juice on the fresh and dry weigths of corn seedlings

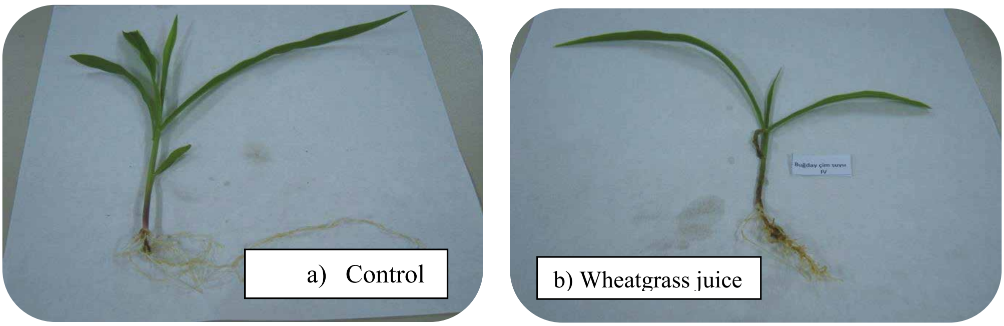

Figure 4. Difference between the root and shoot growth corn seedlings irrigated with distilled water (a. control) and wheatgrass juice (b)

As a result of protein analyses, it was found that the crude protein contents of the corn seedlings increased with the wheatgrass juice application. Crude protein content of the plants irrgated with wheatgrass juice was 19.17% while it was 11.28% in control plants (Figure 5). This difference between the applications were found statistically significant ($P<0.01$). Although wheatgrass juice slowed down the seedling growth, it caused an increase in protein contents of the plants depending on its high nutritive content.

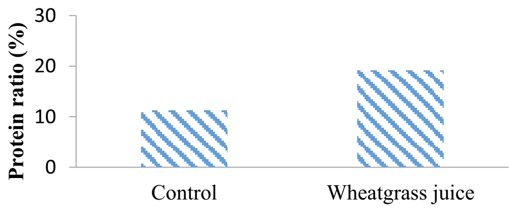

Figure 5. Protein contents of the corn seedlings irrigated with wheatgrass juice and distilled water (control)

Effect of wheatgrass juice on disease symptoms caused by *Rhizoctonia solani* on corn seedlings

As a result of the experiment conducted to determine the effect of wheatgrass juice on disease severity caused by *R. solani* on corn plants, it was found that the mean disease severity of the plants irrigated with wheatgrass juice was higher than the plants irrigated with distilled water. Mean disease severity of the plants irrigated with wheatgrass juice was 79.60%, while that of those irrigated with distilled water was 64.18%. Most of the plants irrigated with wheatgrass juice had less root mass and roots with severe discoloration (Figure 6). Plants without pathogen inoculation showed no disease symptoms.

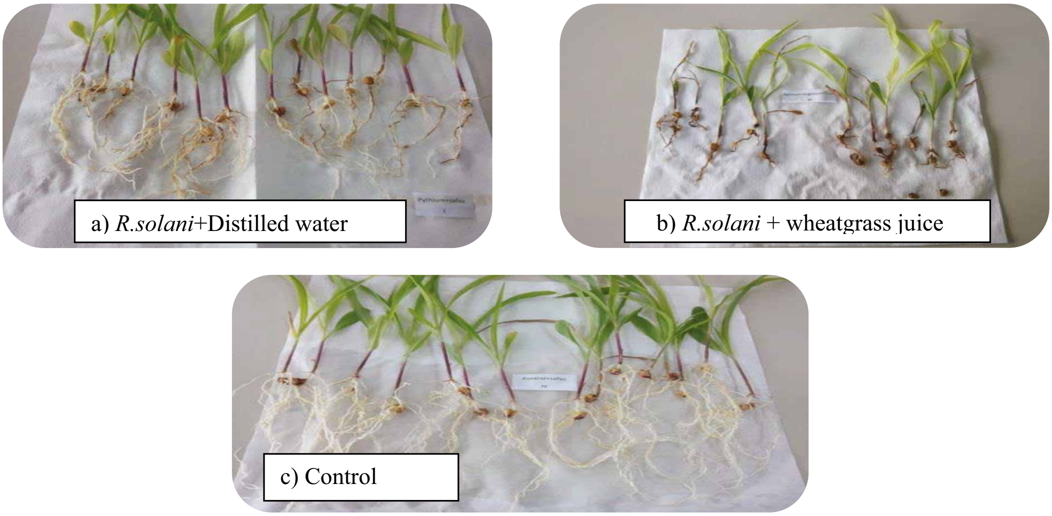

Figure 6. Effect of wheatgrass juice on disease symptoms caused by *R.solani* on corn seedlings: a) *R. solani* inoculated corn plants irrigated with distilled water; b) *R. solani* inoculated plants irrigated with wheatgrass juice; c) corn plants irrigated with distilled water (control)

It was found in this study that wheatgrass juice significantly reduced seedling growth on corn plants. On the other hand, corn seedlings irrigated with wheatgrass juice had higher protein contents. This indicates that wheatgrass juice contained macro and micro nutrients. As a matter of fact, different researchers reported that the extracts of wheatgrass were rich in macro and micro nutrients, amino acids and vitamins (Raheja et al., 2010; Rana et al., 2011; Pant et al., 2013). However, wheatgrass extract also contains important phenolic materials including flavonoids (Falcioni et al., 2002; Kulkarni et al., 2006b; Raheja et al., 2010). Ashok (2011) reported that wheatgrass juice contained alkaloids, saponins, gums, musilages, proteins and amino acids. These materials, which are found in wheatgrass juice, are thought to have an allelopathic effect on germination and development of corn plant. Allelochemicals can affect plant development by acting on metabolic pathways such as photosynthesis, respiration and ion uptake mechanisms. This effect may vary depending on the type and concentration of the allelochemical substance (Jose and Gillespie, 1998; Terzi, 2003).

In the study, wheatgrass juice was determined to increase the severity of *Rhizoctonia solani* caused disease. This is thought to be related to the decreasing effect of seedling and root development of wheatgrass juice. In addition, the nutrient content of wheatgrass juice may provide a suitable environment for fungus growth.

CONCLUSIONS

As a result, it was determined that direct use of wheatgrass juice as a plant activator or biofertilizer was not sufficient, hence wheatgrass juice had a negative effect on seedling growth on corn plants.

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DETERMINATION OF YELLOW RUST DISEASE (*Puccinia striiformis* f. sp. *tritici*) RESISTANT OF THE WHEAT LANDRACE COLLECTED FROM ISPARTA AND BURDUR PROVINCES

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Abstract

In this study, we aimed to determine the genetic diversity of wheat genotypes grown in Isparta and Burdur provinces, to protect valuable genotypes as a germplasm source and to take them into breeding programs. In the study, about 104 villages were visited and 72 wheat seed samples were taken from 45 different locations. The resistance of the sampled wheat varieties/populations to yellow rust disease was examined and the following results were obtained; the rate of resistant genotypes (R) was 6% (disease severity 1-2%), the rate of moderate resistant genotypes (MR) was 33% (disease severity 6-20%), the LM rate was 33% (21-40%), the rate of those in the M group was 19% (disease severity 41-60%), and the rate of HM, which has a little less tolerance, was 8% (disease severity 61-80%). Very few of the collected material was found to be resistant (6%) and most of the material was tolerant (85%).
As a result, it can be said that a large part of wheat genotypes grown in Isparta and Burdur provinces are tolerant to the yellow rust disease and a small part is resistant. In particular, we report that a large part of the population defined by local names (88%) is tolerant, and very few of them are resistant (2%).

***Key words**: Landrace, stripe rust, wheat.*

INTRODUCTION

Turkey is one of the gene pools of wheat as other many plant species. The richness of wheat varieties determined by the evaluation of genetic materials obtained from sampling trips at different times confirms this (Anonmys, 2012). The cultivated wheat consisted of local varieties with high heterogeneity until the late 1800s (Feldman et al., 1995). However, as a result of breeding studies in order to increase productivity and quality in recent years, the genetic diversity of culture forms has decreased; susceptibility to pests, environmental stress, and different diseases have increased, and generally, the genetic basis of all culture wheat has remained narrow (Feldman, Sears, 1981; Reif et al., 2005; Yeşbek, 2007).
Stripe (yellow) rust disease (*Puccinia striiformis* Westend f. sp. *tritici)* appears in leaf stalk and head of plants, although it is seen as intense at leaves. On the upper surface of leaves, yellow color pustules appear in the form of machine stitch, so it can be called "stripe rust" (Şahin, 2009). Stripe rust is generally observed in wheat in almost all regions in our country and differs according to the sensitivity of varieties, environmental conditions, etiological source, years and regions (Zeybek, Yiğit, 2004). Stripe rust disease seen in high altitude areas, cold climates (2-15^{0}C), northern latitudes reduces the green portion where photosynthesis takes place in plants, thus reducing yield and grain quality and causing loss in rates ranging from 10% to 70% (Temel, 2006), as well as decreasing the quality value since it allows grains to be wrinkled and weak (Furan, Yüce, 2009).
As in the entire country, genetic erosion in wheat is increasing in our region. Local wheat species/varieties that have adapted to the region where it grows in a long time period are of great importance as rich gene sources. Therefore, determining, collecting and protecting material that may be important gene source is important in securing our future.

This study aims to determine the sensitivity of wheat genotypes in Isparta and Burdur provinces, against stripe rust disease. In these regions where a genetic diversity has been determined before, protection of germplasm resources which can be used for different purposes, evaluation them in breeding programs, and investigation of the resistance of sampled materials to yellow rust disease have been targeted.

MATERIALS AND METHODS

The wheat seeds used in this research were obtained from registered varieties and landrace populations cultivated in the regions of Isparta and Burdur provinces and their districts and villages. Seed samples are named by number plate system (Table 2). According to this system, each material is numbered first with the number plate of the province where it was taken, and then the first letter of the name of the district, and then the sample number. In this study, 104 villages were visited to collect wheat samples, then 72 wheat seed samples were taken from 45 different locations [Eğirdir (5), Aksu (9), Keçiborlu (4), Yalvaç (6), Gelendost (6), Yenişarbademli (1), Sütçüler (9), Şarkikaraağaç (6), Burdur Central Villages (7), Bucak (3), Çavdır (1), Çeltikçi (1), Kemer (2), Gönen (2), Altınyayla (4), Gölhisar (5) and Ağlasun (1)].

The seeds of 72 wheat genotypes sampled from different locations were planted in separate plots with sowing machine in autumn 2008. Each plot area was 4.8 m^2 and consisted of 6 rows. Seedling rates were 500 seeds/m^2. The basic pre-sowing fertilization rates for all plots were 30 kg $N \cdot ha^{-1}$ and 40 kg $P \cdot ha^{-1}$, the rest of 30 kg $N \cdot ha^{-1}$ was applied at the early spring (stem-elongation stage). The collected wheat genotypes were evaluated in terms of yellow rust disease. 10 random plants in each trial plot were picked up and a total of 2 leaves of each plant including the flag leaf and the leaf below were used to estimate the severity of disease (Zadoks, Schein, 1979).

Disease severity was calculated according to the following Tawsend-Heuberger formula by taking the average of 2 leaves from 10 plants (Karman, 1971).

Table 1. Defining the type of infection of yellow rust disease in wheat genotypes

Signs and Symptoms for Infection Types	Codes	Disease index or Infection type	Disease severity (%)
No visible signs or symptoms	0	0	0
Necrotic and/or chlootic flecks; no sporulation	VR	1	< 1
Necrotic and/or chlorotic blotches or stripes; no sporulation	R	2	1-5
Necrotic and/or chlorotic blotches or stripes; trace sporulation	MR	3	6-20
Necrotic and/or chlorotic blotches or stripes; light sporulation	LM	4	21-40
Necrotic and/or chlorotic blotches or stripes; intermediate sporulation	M	5	41-60
Necrotic and/or chlorotic blotches or stripes; moderate sporulation	HM	6	61-80
Necrotic and/or chlorotic blotches or stripes; abundant sporulation	MS	7	81-95
Chlorosis behind sporulating area; abundant sporulation	S	8	96-99
No necrosis or chlorosis; abundant sporulation	VS	9	99

H=High, L=Light, M=Moderate, R=Resistant, S=Susceptible, V=Very

Disease severity (%) = [(Total scale value/Total number of plants) x (Scale value excluding "0") x 100]

According to the disease severity results, 0=0, 1=<1%, 2=1-5%, 3=6-20%, 4=21-40%, 5=41-60%, 6=61-80%, 7=81-95%, 8=96-99%, 9=99% scales were used (Stubbs et al., 1986). Infection type was determined according to the 0-9 scale in disease evaluation (Table 1).

The total rainfall was 514.4 mm during the plant growth period of 2008-2009, and the average temperature was 11.5°C (Table 3).

Table 3. Important climate data for the experiment year and many years of Isparta

Climate Factor	Year/ Month	Sept	Oct	Nov	Dec	Jan	Feb	March	Apr	May	June	July	Mean/Total
Average Temp. °C	2008-2009	18.0	12.8	9.0	3.7	3.4	4.0	5.5	10.9	14.9	21.0	23.6	**11.5**
	Means for many years (1930-2000)	18.4	12.9	7.5	3.5	0.0	2.7	5.6	10.6	15.4	19.7	23.1	**10.9**
Total Precipitatio (mm)	2008-2009	26.2	32.0	60.0	5.4	124.0	68.2	53.6	39.0	61.2	26.8	18.0	**514.4**
	Means for many years (1930-2000)	15.1	36.7	44.7	91.2	79.8	70.9	61.4	52.4	55.1	33.6	13.4.	**554.3**

*Isparta Meteorology Regional Directorate records

RESULTS AND DISCUSSIONS

Collected wheat genotypes were identified with local names and 18 with variety names (Table 2). Stripe rust has been widely seen in the trial area in 2008-2009. Therefore, the evaluation of the sampled material in terms of stripe rust was done. The results are given in Table 2. As for resistance of wheat varieties/populations to stripe rust; resistant genotypes (R) ratio was 6% (with necrotic/chlorotic lines and without spore), moderately resistant (MR) ratio was 33% (disease severity is between 6% and 20%, spore traces are seen besides necrotic/chlorotic lines), LM ratio was 33% (necrotic/chlorotic lines and light sporulation), M ratio was 19% (necrotic/chlorotic lines, a greater proportion of sporulation, disease severity 41-60%), and the ratio HM, slightly less tolerant, was 8% (necrotic/chlorotic lines as well as moderate sporulation, disease severity was 61-80%) (Figure 1). A small part of the sampled material was found to be resistant (6%) and the majority of the material was tolerant (85%).

A genotype named as Hatay-85 were thought to be the Atay-85 variety and it showed the characteristics of Atay-85 in terms of resistance to stripe rust. Again, the material collected under the name of Cumhuriyet was compared with Cumhuriyet-75 type and Cumhuriyet-75 type was described through variety registration as sensitive. The type of Cumhuriyet used in this trial was determined to be tolerant. Other wheat genotypes sampled with variety names showed similarity as defined in the variety registration.

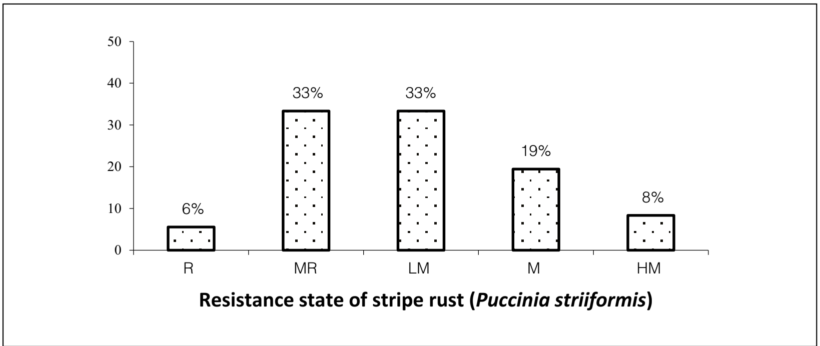

Figure 1. Resistance state of stripe rust (*Puccinia striiformis*) in wheat genotypes

2% of the population identified with local names was found to be in the resistant group (R), 31% in the moderately resistant (MR) group, 35% in the LM group, 22% in the M group and 9% in the less tolerant group (HM). 17% of the samples possessing a variety name were found to be in the resistant group (R), 39% in the moderately resistant (MR) group, 28% in the LM group, 11% in the M group and 6% in the tolerant (HM) group (Figure 2).

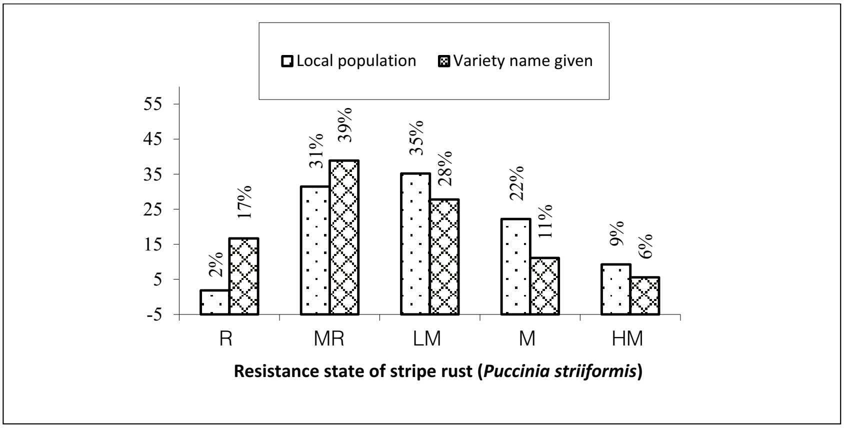

Figure 2. Resistance state of the stripe rust (*Puccinia striiformis*) in collected wheat landrace population and varieties

In the study, disease severity varied for the samples from different places with the same names. Although a local population, defined as Kocabuğday, was generally moderately resistant to yellow rust disease, the severity of the disease varied slightly according to the regions and was coded as LM, HM and M similar situation was detected in other genotypes. This indicates that there may be resistance to different disease strains. Indeed, in a study conducted to determine yellow rust breeds in the USA, 20 wheat samples collected from 20 different states were analyzed, including Clement (Yr9, YrCle), Compair (Yr8, Yr19) and Yr8 and Yr9 isogenic lines. In addition to the previously identified 21 strains, 21 new ones have been identified (Chen et al., 2002). In our country, the most comprehensive study on the determination of yellow rust strains of seedlings was carried out by Ateş and Biçici (2007) in the climate room and cropland conditions. In the study, reaction studies were carried out under natural inoculation conditions, consisting of 137 wheat varieties and lines. According to the results of seedling inoculation of domestic commercial wheat varieties, while the Panda was very resistant to the stripe rust *P. striiformis*; Segatario, white wheat were resistant, and Series 82 was susceptible. In addition, yellow rust assessment was made in natural inoculation conditions of domestic varieties; so, Balatilla, Chils, Amanos, Doğankent, and Yüreğir varieties were found to be resistant (R); Ceyhan 99, Gerek, Dariel varieties (M) were found nearest to susceptible. Wheat varieties which are known to be resistant to rust disease can be susceptible later. In addition, due to long-term cultivation and due to break the resistance by a pathogen, the varieties can be sensitive depending on the climate conditions, environment, and rust strain. Likewise, Mert et al. (2016) detected unexpected stripe rust infections in winter wheat cultivars grown in the Northwest region of our country in 2014 season. Some species known to be resistant (Enola, İzgi 2001) have been reported to be fully susceptible to certain stripe rust races.

In general, a large part of the collected wheat genotypes are tolerant to the stripe rust disease and a small part is resistant. In the study, no sensitive genotypes for stripe rust were determined. Kurt (2013) reported that the Golia, Zenit, Bezostaja and Gerek 79 cultivars were sensitive, while BasriBey 95 and Tahirova 2000 were resistant. Although this literature reported that Gerek 79 and Bezostoja varieties were susceptible, in the present study conducted under Isparta climate conditions, Gerek 79 variety was included in the MR and LM group, and Bezostaja variety in the R, MR and HM group. Bezostaja was registered as resistant and Gerek 79 as tolerant.

Stripe rust is generally observed in wheat in almost all regions in our country and differs according to the sensitivity of varieties to disease, environmental conditions, etiological source, year and region (Zeybek, Yiğit, 2004).

Stripe rust was seen in high altitude areas, cold climates (2-15^{o}C), northern latitudes reduces the green portion where photosynthesis takes place in plants, thus reducing yield and grain quality and causing loss in rates ranging from 10% to 70% (Temel, 2006), as well as decreasing the quality value (Temel, 2006) since it allows grains to be wrinkled and weak (Furan, Yüce, 2009).

Bicici et al. (2000) reported that the number of hot and rainy days during the season may be important for the formation of yellow rust epidemic.

In different climatic conditions of our country, similar studies have been carried out before, investigating on the sensitivity and resistance of wheat varieties. Alp and Sağır (2009) tested 50 hard wheat samples from 11 different local strains for stripe rust. They displayed that 15 of the 50 wheat samples were the stripe rust-resistant, 19 was medium-resistant and 14 were medium-sensitive. In addition, high rust density was found in a group of Aşure local strain (61.68%) and Ruto local strain (0%) was in the resistant group.

Reaction studies of 126 bread wheat varieties in natural inoculation conditions for rust diseases were carried out by Ay (2013) in Adana city. According to the results, it was determined that 49 bread wheat varieties were stripe rust-resistant, 6 bread wheat varieties were medium-resistant to the stripe rust and 2 bread wheat varieties were mild-sensitive. Among these, Ziyabey 98 was found to be stripe rust-resistant, Kaşifbey and Genç 88 varieties were mild-sensitive.

CONCLUSIONS

As a result, in the areas where wheat cultivation is performed, yield loss occurs due to rust diseases when chemical control cannot be done. Therefore, the most important control method of rust diseases is the development and use of resistant varieties. However, due to the continuous emergence of new varieties of rust disease, studies for resistance improvement require continuity. Our country has rich genetic resources of wheat. In this way, the evaluation of gene sources, by scanning, especially for use in the determination and breeding studies of novel varieties will be important for developing and using resistant varieties against rust diseases. We report that most of the local populations (88%) in the present study are resistant, and very few of them are resistant (2%).

Table 2. Evaluation of yellow rust disease (*Puccinia striiformis*) of wheat genotypes

Label No	**The name of collected wheat variety**	**Resistance state of stripe rust (*Puccinia striiformis*)**	**Label No**	**The name of collected wheat variety**	**Resistance state of stripe rust (*Puccinia striiformis*)**	**Label No**	**The name of collected wheat variety**	**Resistance state of stripe rust (*Puccinia striiformis*)**	**Label No**	**The name of collected wheat variety**	**Resistance state of stripe rust (*Puccinia striiformis*)**	**Label No**	**The name of collected wheat variety**	**Resistance state of stripe rust (*Puccinia striiformis*)**
32E01	Kırmızı buğday	MR	32K17	Gerek-79	MR	32YB34	Hatay-85	MR	32Ş75	Bezostaja	MR	15G80	Sarıkılçık	MR
32E02	Çavdar + arı	R	32K18	Kocabuğday	M	32S36	Name unknown	MR	32Ş76	Gerek 79	LM	15M63	Çakmak-79	LM
32E03	Cumhuriyet	R	32K19	Rumeli	MR	32S37	Hatay-85	MR	15M46	Kocabuğday	M	15M67	Kocabuğday	MR
32E04	Ziraat Buğdayı	LM	32Y20	Yerli kırmızı buğday	LM	32S38	Çavdar+arı buğdayı	M	15B47	Aydın	LM	15M68	Kocabuğday	LM
32E05	Ziraat Buğdayı	MR	32Y21	Yerli kırmızı buğday	LM	32S39	Ziraat buğdayı	MR	15B48	Sarı misli (gökala)	LM	15M69	Kocabuğday	M
32A06	Cumhuriyet	LM	32Y22	Ç-1252	LM	32S40	Arıbuğday	MR	15B49	Kocabuğday	MR	15M69-1	Çakmak-79	MR
32A07	Kocabuğday	HM	32Y23	Kırmızı buğday	LM	32S42	Buğday	M	15Ç52	Kaymakam	M	15M70	Kocabuğday	LM
32A08	Kocabuğday	HM	32Y24	Gün-91	MR	32S43	Kızılevi	LM	15ÇE53	Akça	LM	15A71	Morkılçık	MR
32A09	Kocabuğday	HM	32Y27	Gün-91	R	32S44	Çavdar+arı	LM	15K54	Kocabuğday	LM	15A72	Karakılçık	LM
32A10	Kocabuğday	M	32G28	Kızıltan	MR	32S45	Çavdar+arı	M	15K55	Kaymakam	LM	15A73	Hatay-85	MR
32A12	Kocabuğday	M	32G29	Kırmızı buğday	HM	32GÖ56	Kızıltan	M	15A58	Çakmak	LM	15AĞ81	Sarı Buğday	MR
32A13	Kocabuğday	LM	32G30	Ç-1252	M	32Ş64	Hatay-85	MR	15G59	Karakılçık	LM			
32A14	Kocabuğday	HM	32G31	Kırmızı buğday	M	32Ş65	Ankara	MR	15G60	Kocabuğday	LM			
32A15	Kocabuğday	LM	32G32	Düz buğday	M	32Ş66	Konya	MR	15G61	Karakaşık	MR			
32K16	Bezostaja	R	32G33	Name unknown	LM	32Ş74	Hatay-85	MR	15G62	Bezostaja	HM			

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USING DIFFERENT METHODS OF ADDING HERBICIDES IN CONTROLLING WATER HYACINTH (*Eichhornia crassipes*) AND REDUCE IN WATER ENVIRONMENT POLLUTION

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Abstract

The experiment was conducted during the growing season 2017 at Baghdad (Iraq). The aim of study is controlling Water hyacinth and decrease pollution in water by using two herbicides, glyphosate and 2.4-D in a different concentration. The experiment application was set out as factorial experiment in randomization complete block design with three replicates and three factors:1-the types of herbicides with different doses and different methods application, 2-the replication of controlling and 3-the duration time, to get the readings after controlling. The results showed that Glyphosate herbicide it more effective than 2.4-D in reducing fresh weight plant and in percentage of disreducing regrowth up to 17.59% in all durations after controlling. The addition of the wiping gave results similar to the spraying method during different time periods and gave same result with spraying in different period specially when use Glyphosate in rate (1:10) and (1:5) (herbicide: water) concentrations and decrease pollution. It was observed that the period between two applications of 15-30 days is more favorable for controlling water hyacinth plant in which it gives 92.13% control when using glyphosate at dose of 450 g/acre when applied as spray or wiping.

***Key words**: water hyacinth, Glyphosate, 2.4-D, wiping, pollution.*

INTRODUCTION

Water hyacinth plant is the one of an aquatic weed perennial, floating plants on the surface of the water, scientific name is *Eichhornia crassipes* (Mart) Solms-Laubach, belongs to the family *Pontederiaceae*, native to the Amazon River Basin in Brazil, he having the ability to live in different types of water (Center et al., 2005).

Currently spreads in more than 50 countries between latitudes 39 North latitude and 39 in the South (Martinez, Gomez, 2007).

It's one of the most dangerous invasive aquatic plants, and is one of the 100 most dangerous plants in the world ranked 20 among them (Crooks, 2002; Villamagna, 2009) it grows in the form of a thick, broad vegetative mat on the surface of the water and a large root mass that spreads under the surface of the water.

It is be a large biomass of in a short period of time it's a height (50-100 cm) and increases in height with plant density (Tellez et al., 2008; Williamns et al., 2005). This plant has two ways of reproduction the first vegetative method, the asexual reproduction by daughter plant, and the second reproduction is sexual reproduction by seeds (Julien et al., 1999) with the temperature suitable for him of 28-30°C (Center, Dray, 2010).

It is fast responsive and adaptive to environmental changes and severely affects the aquatic ecosystem as a result of blocking light access to other aquatic plants, it absorbs large amounts of dissolved oxygen, which negatively affects aquatic life (Khalil et al., 2009).

Water accounts for 93-96% of plant weight, increasing the rate of transpiration by 2.67-3.2times (Supmaneenan, 2003).

It is the host of many insects that affect human health, such as mosquitoes that transmit malaria and cholera and have a negative impact on water quality (Jones, 2009).

This plant entered Iraq in the mid-eighties and became the presence of this plant in a few years a growing problem day by day, characterized by its ability to reproduce and rapid spread under the conditions of the Iraqi environment from the South to the North and one of the most successful methods used in combating it.

The most important herbicides used are Glyphosate and 2.4-D which have proved very successful in reducing the growth and spread of this plant (Smith et al., 2004; Yirefu et al., 2007).

Chemical control was caused water pollution. The aim of the study was to use the chemical control of this weed with both the glyphosate herbicide and 2.4-D with different combinations and using the wipe machine.

MATERIALS AND METHODS

Basin with 1×1.5 m with a depth of 35-40 cm were used in the soil and used the agricultural plastic to covered this basin and it cut to separate between experimental units 1-1.5 m and the distance between the repeater and the other repeater is 2 m in order to avoid the transfer of the herbicide (Table1) from one experimental unit to another.

There are 9 variants (Table 2).

To the end of the experiment and cultivated with water hyacinth plants homogeneous in age and density and height of 25-35 cm, and it was identified area affected by this plant in the Tigris River as a second site to apply the experiment.

Table 1. The trade name, the common, the ratio of the effective material and the rate of use of the herbicides of the experiment

Trade name	The common name	Active ingredient	Rate of use: cm^3 effective material / ha for weed perennial
Touchdown S4®	Glyphosate	36%	1,440-1,800 (age 1-2 years) and 2,160-2,880 (2 years and above)
Difor Amine 72 SL	2.4-D	72%	1,080-1,800

Table 2. Transactions combating with different ways of reducing environmental pollution with repeated controlling

Experiment Factors	Variants
1. Control coefficients 2. Repeated addition(once and twice)	T1 Comparison without herbicide (water spray only)
	T2 (glyphosate spray) Surface spraying with recommended concentration (450 g active ingredient / acre)
	T3 (herbicide 2.4-D) Surface spraying with recommended concentration (360 g active ingredient / acre)
	T4 (glyphosate herbicide added by a 5: 1 herbicide to water)
	T5 (glyphosate herbicide added by a 10: 1 herbicide to water)
	T6 (glyphosate herbicide added by a 15: 1 herbicide to water)
	T7 (herbicide 2.4-D added by a 5: 1 herbicide to water method)
	T8 (herbicide 2.4-D added in a 10: 1 concentration of herbicide to water)
	T9 (herbicide 2.4-D was added in a survey method with a concentration of 15: 1 herbicide to water)

The process of adding or spraying of the herbicide solution when applying the treatment. The water hyacinth plants in the experimental units were wiping in opposite directions using the wiping machine manufactured as shown in Figure 1 where the water hyacinth plants are cleared for wetness as a result of contact with the cotton tissue, concentration (5:1), (10:1) and (15:1) herbicide to water (volume/volume) and opposite directions to pass on the leaves of the plant without contact with water. In spraying method, it was used the dorsal spray. Components of the manual wiping machine: 1- The herbicide solution tank is a PVC plastic pipe length 150 cm diameter 2 inch; 2- contact part (survey group) which represents the work width or the wiping interface 150 cm length; 3- holder for machine is a tube light weight of iron at length 180 cm; 4- lumbar fabric.

Figure 1. Method of using manual wiping machine to add chemical herbicides to control water hyacinth

The data obtained from the water hyacinth plants.

a. Dry weight of water hyacinth plants for area of one m^2. The dry weight was calculated after 15 and 50 days of control.

b. Measuring the percentage of water pollution caused by the control, after one day for control Water samples were taken from each experimental unit to measure the percentage of water pollution caused by the use of two methods of addition to spray and wiping and by knowing the wavelength of each herbicide where the samples are read by the spectrophotometer and recording the readings of the device (Absorbance) per sample. The obtained data analysis from the field experiment according to experiments method randomized complete block designed (RCBD), using a computer according to a program (SAS) and using test Duncan multi-Range was used to compare the experimental averages, obtained in field for comparing the averages before and after flowering of water hyacinth plants, the differentiate averages that are different from each other in letter different in the level 0.05 according to (Al-Rawi, Abdul-Aziz, 1980).

RESULTS AND DISCUSSIONS

Effect of control coefficients and repeats and time periods after control in dry weight (g/m^2) for water hyacinth plant. Table 3 shows that the glyphosate gave a significant difference in decrease the dry weight of the water hyacinth plant compared to the surface spray of herbicide for 2.4-D and the difference rate of 78.3%. When comparing surface spraying with the wiping of glyphosate method, there were no significant differences between T2 (surface spray of glyphosate herbicide), T5_wiping method (1:10 herbicide: water) and_showing don't significant differences between T4 (1:5 herbicide: water), T6 (1:15 herbicide: water). In both cases (spraying and wiping) of the glyphosate gave desirable results compared to the comparison treatment. In general, the superiority of the glyphosate in the wiping method was observed on all survey coefficients of herbicide 2.4-D and the relationship between surface spraying and the wiping of herbicide 2.4-D. Table 3 indicated that the wiping method was superior in T7 (1:5 herbicide: water) on the rest of the transactions indicating that the use of high concentration in wiping is better than spraying the herbicide. This result is consistent with the findings of the (Fryman, 2009).

Table 3. Effect of control treatment, number of addition times and time periods after control and interference between these factors in dry weight (g/m^2) of water hyacinth

Effect of time periods	Effect of addition times	Effect of interference between number of times and treatment	Number of days after control: 50 days	Number of days after control: 15 days		Number of times addition	
			1975.5 a	1323.3 c	once		
			1051.3 d	1678.2 b	twice		
	1649.37a	3713.3 a	4141.3a	3285.3 b	T1	once	Treatment x The number of times
		645.3 h-i	325.3 m-o	965.3 h-m	T2		
		2088b c	2373.3d-c	1802.7 d-g	T3		
		621.3 h-i	816 j-o	426.7 m-o	T4		
		810.7 g-h	880 i-o	741.3 k-o	T5		
		1272f g	1861.3 d-f	682.7 k-o	T6		
		1594.3 d -f	2042.7 d-e	1056 g-m	T7		
		2013.3 b-d	2432 c-d	1594.7 e-i	T8		
		2131 b	2907.3 b-c	1354.7 e-k	T9		
	1364.74b	3712 a	2880 b-c	4544 a	T1	twice	
		197.3 i	165.3 o	229.3 n-o	T2		
		1796.7 b-e	1722.7 d-h	1850.7 d-f	T3		
		837.3 g-h	357.3 m-o	1317.3 e-k	T4		
		232 i	266.7 n-o	197.3 n-o	T5		
		773.3 g-h	592 i-o	954.7 h-n	T6		
		1336 e-f	1088 f-m	1584 f-h	T7		
		1818.7 b-e	1205.3 f-k	2432 c-d	T8		
		1589.3 c-f	1184 f-k	1994.7 d-e	T9		

	T9	T8	T7	T6	T5	T4	T3	T2	T1	Number of days
1500.54a	1674.7 b-d	2013.3 b	1320 c-e	818.7 f-g	469.3 g-h	872 e-g	1826.7 b-c	597.3 g-h	3914.7 a	15 days
1513.37a	2045.7 b	1818.7 b-c	1565.3 c-d	1226.7 d-f	573.3 g-h	586.7 g-h	2048 b	245.3 h	3510.7 a	50 days
	1860.2 b	1916 b	1442.7 c	1022.7 d	521.3 e	729.3 d	1937.3 b	421.3 e	3712.7 a	Effect of treatment

The value with the same letters is not significantly different from each of the study factors and at each interference at a probability of 5%.

The addition of the herbicide in two batches is better than adding the herbicide once and may be due to increased lethal dose when adding twice the dose of one-time killer, there were no significant differences between the average number of days after control (15 and 50) days, which indicates that the effectiveness of the herbicide is continuous and that the plants are under the influence of the herbicide even after 50 days of control. This result is good because the target is not only the temporary killing but the sustainability of the herbicide effect. The results showed significant differences in the interference between recurrence of the addition in the number of days after the control and gave the treatment of the addition twice after 50 days of control best results in reducing the dry weight of the plant compared with 15 days after, but reverse when it addition one time, it was showed with an increase in dry weight after 50 days compared to 15 days after control. There was also a significant differences between the addition repetition and the control treatment. It was observed that the best treatment to achieve the dry weight reduction of the water hyacinth plant was for the glyphosate when addition repetition, the treatment was T2 (surface spraying glyphosate) and T5 (1:10 herbicide: water) was 197.3 and 232 g/m^2 respectively, while the treatment of T9 (1:15 herbicide: water) was 2.4-D highest dry weight when added once or twice and amounted to 2131 and 1589.3 g/m^2, respectively. In general, herbicide 2.4-D showed less effective results in reducing dry weight, either by spraying or wiping compared to the glyphosate. It is also showing from the wiping treatments that the glyphosate treatment of T5 (1: 10 herbicide: water) was distinguish by high efficiency in reducing the dry weight of the water hyacinth plant taking into account the volume, age and density of the plant in the area unit. The results showed significant differences in the number of days after control and control factors. It is clear that the results of the glyphosate are better than the herbicide 2.4-D and that the efficacy of the glyphosate herbicide lasts longer than the herbicide 2.4-D where the ratio between spray of glyphosate and 2.4-D compared to comparison treatment after 50 days of addition to 93%, 41.7%. There was no significant difference between treated T2 added by spraying method, T4 (1:5 herbicide: water) and T5 (1:10 herbicide: water) added by wipe after 50 days of control can be replaced by spray method to wipe and this is an important indicator of the success of one of the objectives.

Research to reduce environmental pollution caused by herbicide spraying. The results showed a significant difference between the repetition of addition and the control treatment in this adjective it was showed a herbicide surface spray glyphosate is superior in addition twice on the one-time addition where it gave a control ratio of 94.38 and 81.37%, respectively, the method of wiping was superiority in the same herbicide in concentration of (1:5 herbicide: water) for one time added and the wipe method in concentration (1:10 herbicide: water) for two time addition, and these ratios are good in control operations, especially those whose proportions are more than 90%. The herbicide 2.4-D showed a lower efficiency in the killing than in the glyphosate herbicide. However, the best treatment for this herbicide was observed when spraying the herbicide twice and the wipe at a concentration of T7 (1:5 herbicide: water). When comparing between the two herbicides in spraying method, the difference between them 36.05% for the addition of one time and 44.4% for the repeat control while the difference between the best treatment of the glyphosate herbicide was T5 (1:10 herbicide: water) and the wipe method 2.4-D T7 (1:5 herbicide: water) when the wiping repetition to 29.08%. The results in Table (3) showed a significant interference between the number of days after the control and the control treatment. The best control treatments at 15 or 50 days were in the treatment of spraying of T2 (glyphosate surface spray) and wiping in concentration T5 (1:10) and in general, there are no significant differences in the herbicide, either by spraying or wiping method because its efficiency lasts for 50 days, for the herbicide 2.4-D, the treatment in concentration of T7 (1:5 herbicide: water) is better than the spray treatment after 15 or 50 days after the control. The difference between the glyphosate herbicide and the herbicide 2.4-D surface spray after 50 days gave a difference of 49.4%, while the wiping for the same period and at the concentration of (1:10) of the herbicide, reached 35.3%. Indicating that the efficiency of the glyphosate herbicide, either by spraying or by wiping, is better than that of herbicide 2.4-D in the control ratio. Table 3 shows a significant difference the triple interference between the experimental treatments. The results showed that the treatment of the glyphosate spray surface and wiping T5 (1:10 herbicide: water) was treated twice after 15 and 50 days after the control, in addition to the treatment of the glyphosate spray surface for once after 50 days of control that the lowest proportion of control was observed in the following treatments. Treatment of 2.4-D surface spraying and once after 15 days of control, herbicide 2.4-D added on concentration T8 (1:10 herbicide: water), twice addition 15 days after control and 2.4-D in T8 treatment (1:10 herbicide: water) one time after 50 days and surface spraying of the same herbicide after addition to twice in 50 days after control. The results show that the glyphosate herbicide is better than the herbicide 2.4-D in the killing and repetition the spraying of the glyphosate herbicide or treated with a concentration of T5 (1:10 herbicide: water) gives sustainability longer and kill more and preferably the wiping on spraying. It is also not preferable to increase concentration in the wiping method if a herbicide 2.4-D is used add in wiping better manner than spraying, especially at the concentration T7 (1:5 herbicide: water).

Effect of different addition methods for the herbicides used in the control in percentage of pollution in water. Figure 2 indicate that the stagnant water contamination levels when using the wiping method for addition of the glyphosate herbicide with a concentration of 1:5 herbicide: water (T4) is the lowest compared to other concentrations, although there are no significant differences in wiping method. While the treatment of the addition of this herbicide in a spray method with a concentration of 450 g (effective substance)/ acre it gave a pollution of 0.314 mg/l. indicating that spraying method to plants caused high levels of pollution both in the first and second readings.

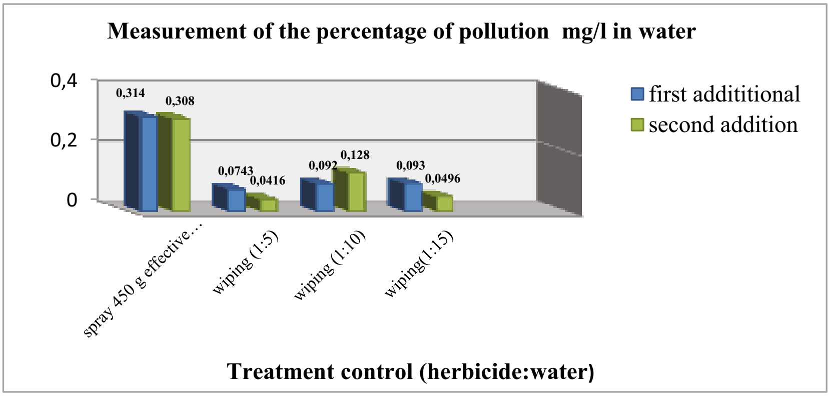

Figure 2. Measuring the percentage of contamination (mg/l) in water resulting from the addition of the glyphosate herbicide using the conventional spraying method and the wiping method in the control of the water hyacinth plant in 2017

We conclude that the wiping method reduced pollution by up to 76% for the first reading after one day of the control and 86.05% after one day of repeated the control, taking into consideration that the process of adding the wipe was done by using the paint roll in opposite directions compared to the use of special equipment designed for this purpose, it will also reduce the percentage of pollution better. The allowed pollution rate of the glyphosate herbicide is 37 mg/l (Ashwini et al., 2007). And for the herbicide 2.4-D it was observed that the percentage of pollution, whether by spraying or wiping, is higher than in the glyphosate herbicide (Figure 3), in general, the rate of surface spray contamination, especially after one day of the first control, reached 77.2% compared to the wipe at the concentration of T8 (1:10 herbicide: water) it 92.8% in the second reading. The lethal dose (LD_{50}) of this herbicide is 639 mg/kg on rats (US EPA, 2005).

We conclude from this that the method of wiping is better than the spraying method for both herbicides, taking into account the use of appropriate concentrations to lead to the process of killing the water hyacinth plant at a high rate and achieve the lowest percentage of pollution in stagnant water, noting that this percentage of pollution depends on the rate of plant density of water hyacinth, which covers the water areas and the height of the plant on the surface of water and the total number of vegetative and depends on the rate of wax covering the leaves, which causes the drop of spray droplets from the surface treatment, which may increase the percentage of pollution and the speed of control in addition to the experience of the control and the efficiency of the machine used. It is important to note the type of herbicide used since there is a percentage of non-effective substances added to the herbicide at manufacturing, including wetness factor or adhesion to the surface of the leaves is of great importance in the aquatic weeds control and reduce the proportion of pollution to the extent that does not affect the aquatic environment. *Comparison of the efficiency of addition wiping with spraying method in reducing pollution*. Figures 2 and 3 showed that the efficiency of the wiping method was significantly higher in the reduction of the pollution resulting from the arrival of the herbicide to the water after the day of the addition of the first and second compared to the method of spraying and both the herbicides. Indicates the effectiveness of this method in achieving control on this dangerous weeds.

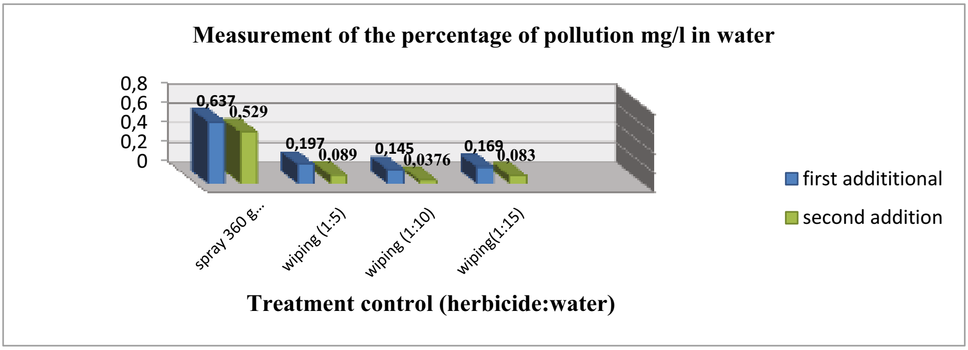

Figure 3. Measurement of the percentage of contamination of water (mg/l) resulting from the addition of herbicide 2.4-D using the spraying method and the wiping method in the control of the water hyacinth plant in 2017

CONCLUSIONS

The Glyphosate herbicide is better than the herbicide 2.4-D in the killing and repetition the spraying of the glyphosate herbicide or treated with a concentration of T5 (1:10 herbicide: water) gives sustainability longer and kill more and preferably the wiping on spraying. It is also not preferable to increase concentration in the wiping method if a herbicide 2.4-D is used and add in wiping method it better than spraying, especially at the concentration T7 (1:5 herbicide: water). The efficiency of the wiping method was significantly higher in the reduction of the pollution resulting from the arrival of the herbicide to the water compared to the method of spraying.

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ANTIFUNGAL ACTIVITY OF NANO CALCIUM POLYSULFIDE AGAINST PATHOGENIC FUNGI ON TOMATO

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Abstract

Many organic and inorganic compounds are known to exhibit widespread anti-fungal activity with successful usage in agricultural applications. The action mechanism of sulfur agents is that sulfur interacts with organic substances and forms sulfides and pentatonic acid having anti-microbial and anti-parasitic activities. Nowadays elemental sulfur is widely used in plant growing as a fungicide and acaricide. In recent years great considerable attention has been paid to the synthesis methods of antifungal properties of nanoscale sulfur due to the development of nanotechnology. Micronized sulfur and sulfur nanoparticles have antifungal and inbition effects of sporulation against pathogenic fungi. In this study, the antifungal activities in vitro of nano calcium polysulfide were investigated against two pathogenic fungi caused disease on tomato (Fusarium oxyporium lycopersici, Botrytis cinerea). Different concentrations of nano calcium polysulfide (0,0.5, 1, 2%) were tested for growth inhibition of Fusarium oxyporium lycopersici, Botrytis cinerea. Growth inhibitions of tested pathogens were measured in vitro. Nano calcium polysulfide showed low inhibiton against Fusarium oxyporium lycopersici, Botrytis cinerea. Mycelial growth inhibition was observed at a maximum of 2% nano calcium polysulfide application for Botrytis cinerea (34,4%) and 0.5% application for Fusarium oxyporium lycopersici (30.72). Nano calcium polysulfide inhibited only spore germination. Mycelial growth in both fungi was very weak increased in nano calcium polysulfide compared to the control.

Key words*: calcium sulfide, tomato, Fusarium oxyporium lycopersici, Botrytis cinerea.*

INTRODUCTION

Diseases are a major source of crop and plant damage that can be caused by a number of plant pathogenic (disease-causing) organisms. Fungi are the number one cause of crop loss worldwide. The effective control of plant diseases have been used chemical fungicides. Fungicides play a key role in disease control in resistance management strategies, but equally important are new fungicides with established modes of action with enhanced characteristics such as systemicity, curativity, and longevity of disease control. According to recent studies a new crop protection product takes around 10 years and approximately 260 million USD from discovery to first sales (McDougall, 2010). Many of the pesticides have been associated with health and environmental issues. Residues of pesticides can be found in a great variety of everyday foods and beverages, including for instance cooked meals, water, wine, fruit juices, refreshments, and animal feeds. Taking into consideration the health and environmental effects of chemical pesticides, it is clear that the need for a new concept in agriculture is urgent. This new concept must be based on a drastic reduction in the application of chemical pesticides, and can result in health, environmental, and economic benefits.

One of the promising directions of development of environmentally safe and efficient fertilizers, means of protection and stimulation of plant growth are nanotechnologies.

The use of nanoformulatioms leads to increased resistance to adverse weather conditions and the increase the harvest of almost all products (Fedorenko et al., 2006). Various nanoparticles are employed in plant disease management. Among them are involved micronized sulfur and sulfur nanoparticles which have antifungal and inbition effects of sporulation against pathogenic fungi (Deshpande et al., 2008; Massalimov et al., 2012; Ahmed, Lee, 2015). In this study, Antifungal Activity of Nano Calcium Polysulfide against Pathogenic Fungi on Tomato were investigated. Lime sulfur production usually include calcium sulfite, calcium sulfate, and metal sulfides.

MATERIALS AND METHODS

In this study, the antifungal activities of nano calcium polysulfide were investigated against two pathogenic fungi caused disease on tomato (*Fusarium oxyporium lycopersici*, *Botrytis cinerea*) *in vivo and in vitro.*

Dual culture. Different concentrations of nano calcium polysulfide (0,0.5, 1, 2%) were added into PDA media. After that a 0.6 cm agar plug with *Fusarium oxyporium lycopersici*, *Botrytis cinerea* were placed in the middle of the agar plate one by one. The dual culture plates were incubated at room temperature. The radial growth was measured each day, until the dishes were completely colonized by the fastest mycelium. Radial growth reduction of *B. cinerea*, in presence of nano calcium polysulfide was calculated in relation to the growth of the pathogen, by using the following formula that measures the percentage of the inhibition of the radial mycelial growth:

Inhibition of the mycelial radial growth (%)= C-T/C × 100,

where:

C is the radial growth measurement of the pathogen in control and

T is the radial growth of the pathogen in the presence of nano calcium polysulfide.

All the experiments in laboratory were replicated 2 times.

In vivo experiment. Five pots were allocated for each group. The nano calcium polysulfide were applied against *Fusarium oxyporium lycopersici* and grey mould caused by *Botrytis cinerea* (Table 1). Two control (untreated) groups for each fstudy were used during this study. The nano calcium polysulfide applications were prepared in 1 litre of water and applied at different dosages (0, 0.5, 1, 2%) Tomato plants were dipped in different doses for 2 minute and planted out. Applications were made from soil, soil+foliar and foliar. After 48 hours the spore suspension of *Fusarium oxyporium lycopersici* were adjusted at 10^6 spore/ml and twenty ml were applied to soil. *Botrytis cinerea* was applied on the leaf surface of tomate by counting 10^6spore/ml. After 30 days, the plant symptoms were rated according the following evaluation scale:

For *Fusarium oxyporium lycopersici:*

0 = symptomless plants; 1 = 50% of leaves chlorotic or wilted; 2 = >50% of leaves wilted but plants not dead; and 3 = dead plants.

For *Botrytis cinerea*

0 = no symptoms, healthy plants; 1 = less 10% of infected stems and leaves with lesions for no more 10% of shoot length; 2 = less 20% of infected stems and leaves with lesions for no more 20% of shoot length; 3 = less 40% of infected stems and leaves with lesions for no more 50% of shoot length; 4 = less 80% of infected stems and leaves with lesions for no more 80% of shoot length; 5 = infected areas covering whole the stems and leaves causing wilting and death of.

Table 1. Combination table of applications against *Fusarium oxyporium lycopersici*, *Botrytis cinerea*

Treatment (%)	Concentration of application (%)	Number of plant
Soil application	0.5	4
	1	4
	2	4
Soil+foliar application	0.5	4
	1	4
	2	4
Foliar application	0.5	4
	1	4
	2	4
Control for *Fusarium oxyporium lycopersici*		
Contol for *Botrytis cinerea*		

The disease severity was evaluated using Townsend Heuberger's formula (Towsend, Heuberger, 1943). The percentage effect of the applications was calculated using the Abbott formula (Abbott, 1925).

Statistics. All experiments were carried out independently at least twice. Data were analyzed by analysis of varience (ANNOVA) to detect differences between treatments. Mean comparisons were made using TUKEY's tests; all statistical tests were conducted at a probability level of $P \leq 0.05$. All analyses were performed using the SPSS 21 software.

RESULTS AND DISCUSSIONS

Dual culture. Growth inhibitions of tested pathogens were measured *in vitro*. Nano calcium polysulfide showed low inhibiton against *Fusarium oxyporium lycopersici*, and *Botrytis cinerea.* Mycelial growth inhibition was observed at a maximum of 2% nano calcium polysulfide application for *Botrytis cinerea* (34.4%) and 0.5% application for *Fusarium oxyporium lycopersici* (30.72%). Nano calcium polysulfide inhibited only spore germination (Figure 1). Mycelial growth in both fungi were very weak increased in nano calcium polysulfide compared to the control (Table 2). Ismail et al. (2016) investigated the effect of different chemicals characters (sulfur and silver nanoparticles; micronized particles of tetramethylthiuramdisulfide, tebuconazole, carbendazim and inorganic peroxides - CaO, SrO, BaO; water solvent of miramistin and 22 flucytosine) against some fungal pathogens (*Alternaria alternate, Aspergillus niger, Candida albicans, Fusarium graminearum, Penicillium notatum*). It was reported that two types of elementary inorganic substances nanoparticles (sulfur and silver) had been antifungal effect to *Botrytis cinerea* and *Fusarium oxyporium lycopersici*.

Nano calcium polysulfide provided heavy disease pressure. Foliar application of nano calcium polysulfide (1-2%) reduced *Botrytis cinerea* on tomato plants and severtiy (leaf and stem infected area) wtih 82.46-82.60% effectiveness (Table 3).

Figure 1. Mycelial growth inhibition of *B. cinerea* in presence of nano calcium polysulfide

Table 2. Inhibition of *Fusarium oxyporium lycopersici* and *B. cinerea* growth in presence of nano calcium polysulfide

Doses of nano calcium polysulfide (%)	Mycelial growth inhibition (%)	
	Fusarium oxyporium lycopersici	*Botrytis cinerea*
0.5	20.18±4.8	8±0.0
1	24.12±2.7	22.22±2.2
2	30.72±3.4	34.42±1.1

Table 3. Efficiency of the nano calcium polysulfide used in the experiment against *B. cinerea*

Treatment	Disease severity (%)	Effect (%)
0.5K	28	67.3 f
1K	25	71.83 ef
2K	23	73.53 de
0.5K+Y	20	76.35 cde
1K+Y	16	81.33 abc
2K+Y	16	81.4 ab
0.5Y	19	77.13 bcd
1Y	16	82.46 a
2Y	14	82.60 a
Control	86	

The fungicidal activity of the nano calcium polysulfide were tested against *Fusarium oxyporium lycopersici*. The results presented in Table 4. The nano calcium polysulfide was supressed with concentration to 1-2%. As shown in tthe present study, the soil application of nano calcium polysulfide was very effective for *Fusarium oxyporium lycopersici* control.

Table 4. Efficiency of the nano calcium polysulfide used in the experiment against *Fusarium oxyporium lycopersici*

Treatment (Fus)	Disease severity (%)	Effect (%)
0.5K	18	77.39 b
1K	10	82.56 a
2K	10	83.3 a
0.5K+Y	19	77.35 b
1K+Y	11	81.86 a
2K+Y	8	82.48 a
0.5Y	58	11.28 d
1Y	56	12.93 d
2Y	50	22.4 c
Control	65	

Jamar et al. (2017) investigated new fungicide formulations available for organic pear farming. The study shows that protective applications, at 300 degree-hours (DH) before inoculation, of copper hydroxide (0.1%), wettable sulphur (1%), lime sulphur (2%) and potassium bicarbonate (1%) significantly reduced pear scab severity with more than 96% effectiveness. Biological properties of sulfur nanoparticles such as antifungal and antibacterial activities have been studied, with physical and chemical properties. Antifungal effects of micronized sulfur and sulfur nanoparticles against two types of pathogenic fungi *Aspergillus niger* and *Fusarium* on sporulation, ultrastructural modifications and phospholipid contents of fungal strains have been studied and obtained results revealed to perspectives of using sulfur nanoparticles (Choudhury et al., 2011). Antibacterial activity of sulfur nanoparticles (5.7 nm) was very high against Gram-positive *Staphylococcus aureus*, while this type of nanoparticles has not antibacterial activity against Gram- negative bacteria (Suleiman et al., 2015)

Nanotechnology is an interdisciplinary research field. In recently it has been investigated to improve agricultural yield in nanotechnology. The green revolution resulted in blind usage of pesticides and chemical fertilizers which caused loss of soil biodiversity and developed resistance against pathogens and pests as well. Nanoparticle-mediated material delivery to plants and advanced biosensors are possible only with nanoparticles or nanochips (Prasad et al., 2017; Kim et al., 2017). Nano-encapsulated conventional, pesticides fertilizers and herbicides that help in the slow and sustained release of nutrients and agrochemicals, resulting in precise dosing of the plant.

The use of sulfur-based products has resulted in the suppression of tested pathogenic organisms, particularly in the form of nanoparticles of nano-calcium polysulfide.

The global consumption of pesticides is about two million tons per year. Careless and arbitrary use of pesticides increases resistance to pathogens and pests, reduces soil biodiversity, kills useful soil microbes. Environmentally friendly nano calcium polysulfide may be used to reduction of the presence of pathogens in the soils and on the plants.

CONCLUSIONS

Preliminary results indicated that nano calcium polysulfide supressed *Fusarium oxyporium lycopersici* and *B. cinerea* on tomato plant. Causes organic enlargement of pesticides, pollinators decreases and destroys the natural habitat of farm. The potential uses and benefits of nanotechnology are enormous. We recommend the nano calcium polysulfide as alternative complex fungicide may be useful to reduce the pesticide load on the environment.

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EVALUATION OF CYTOTOXICITY OF THE HERBICIDE GALIGAN 240 EC TO PLANTS

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Abstract

Excessive use of pesticides in agriculture in the context of increased ecosystems pollution justifies assessing the cytotoxicity of these chemicals. The purpose of the research was to evaluate the cytotoxicity of the herbicide Galigan 240 EC to plants, using as a test plant the species Allium cepa (onion), which is commonly used in the cytotoxicity tests. Exposure to various herbicide concentrations (0.5, 1.0 and 1.5%) for 3, 8 and 24 hours had different effects on the mitotic index. Thus, a decrease in the percentage of the mitotic index was found, in direct correlation with the exposure time and herbicide concentration. The study also reveals a direct correlation between herbicide concentration, exposure time and chromosomal aberrations/nuclear alterations identified in meristematic cells of onion. The most common chromosomal aberrations identified were stickyness and laggards, while the nuclear alterations were represented by binucleated cells and cells with nuclear erosion.
These results suggest caution when applying the herbicide Galigan 240 EC to agricultural crops.

***Key words**: herbicide, onion, mitodepresive, chromosomal aberrations.*

INTRODUCTION

In the modern agriculture, a large number of herbicides are being used to control a wide variety of weeds.
Herbicides are chemical means of plant protection against weeds, obtained by formulating and conditioning some biologically active ingredients of a toxic nature, which requires their use with great care. Herbicides used in modern farming practices can have dangerous effects. They can be transformed into mutagens by the crop plants that absorb polluted nutrients and act as toxic vectors for humans and animals.
Herbicides pollute soil and air, but also the aquatic environment. From this point of view, the herbicides are introduced into the aquatic environment both inadvertently through runoff events and intentionally through the use of those registered for use in waterways (Freeman, Rayburn, 2006). Also, intensive use of herbicides without adequate knowledge on its effects on soil enzymes may have adverse impact on soil biochemical processes and cycling of nutrients (Sireesha et al., 2012).
Soil pollution/contamination may affect or inhibit plant growth or may introduce toxic elements into the nutrient chain by absorbing them from plants and their accumulation in organic tissue. The agricultural plants are the raw material for many food products, and the pollution of the vegetable raw material has a direct influence on the quality and safety of food products (Bonea, 2013).
Galigan 240 EC is an herbicide of the diphenyl-ether group used for selective weed control in a wide range of fruit trees, vegetables, field crops, and non-crop areas. Galigan 240 EC contains oxyfluorfen (240 g/liter), an active substance in the diphenyl ether group, with long-lasting herbicidal contact and residual contact. Oxifluorfen is classified as potentially carcinogenic ingredient (Dragoeva et al., 2012).
The superior plants may serve as genetic tests for the screening and monitoring of various environmental pollutants, such as pesticides or heavy metals (Sărac et al., 2015; Petrescu et al., 2015). Two of the species best suited to citotoxicity testing of pesticides are *Allium cepa* (onion) and *Allium sativum* (garlic).
The effects of chemical substances on chromosomes are especially studied on meristematic tissues from root tips because they are easily obtained, experiences can be performed throughout the year and are less expensive.

MATERIALS AND METHODS

The biological material used was represented by dried and healthy onion bulbs, without any signs of disease or pest attack. First, the onion bulbs were immersed in glasses of water for 72 hours, when the meristematic roots reached the length of 15-20 mm, followed by immersed in dilutions of various concentrations of the herbicide Galigan 240 EC (0.5, 1.0 and 1.5%) for 3, 8 and 24 hours at room temperature. A number of 10 onion bulbs were used for each treatment variant as well as an untreated control that was immersed in tap water.

After expiration of the exposure time, the roots were harvested using a scalpel and processed according to the protocol of fixation, hydrolysis and staining to highlight the cytological activity and eventual presence of chromosomal aberrations.

In order to highlight chromosomes and chromosomal aberrations was used the Feulgen-Rossenbeck method. The microscopic (temporary) preparations were performed according to the squash method, on the principle of tissue tightening between the microscopic blade and the microscopic lamella.

The experiments took place in the Genetics Laboratory of the Faculty of Agronomy Craiova.

RESULTS AND DISCUSSIONS

The exposure to various herbicide concentrations for 3, 8 and 24 hours had different effects on the mitotic index.

The analysis of the results demonstrates a decrease in the percentage of the mitotic index, in direct correlation with the exposure time and herbicide concentration (Figure 1).

Thus, at a concentration of 0.5%, the mitotic index recorded values from 36.8% (at 3 h exposure time), 33.7 (at 8 h exposure time) and 24.7% (at 24 h exposure time).

Also, at the 1.0% herbicide concentration, the mitotic index decreased to 36.6% (3 h), 31.1% (8 h) and 23.0% (24 h), respectively. But the mitotic index had the lowest values at the 1.5% herbicide concentration: 34% (3 h), 30.8% (8 h) and 20.1% at 24 h exposure time.

The mitotic index is a parameter that allows estimation of the frequency of cell division, and inhibition of mitotic activity is often an indicator of the effect of plant cytotoxicity (Marcano et al., 1998).

The decrease in mitotic index level shows the mitodepresive effect of the herbicide Galigan on cytological activity to onion, or the disturbance to a certain extent of the mitotic division. Also, the higher herbicide concentration, correlated with the higher exposure time, resulted in an increase in the frequency of prophases and, at the same time, a decrease in telophase frequency (Figure 2). Thus, at a concentration of 1.5% herbicide, prophase recorded a values from 66.1% (3 h) to 69.3% (8 h) and 71.1% (24 h). In the same vein, telophases values were between 17.5% (3 h), 13.0% (8 h) and 11.6% (24 h).

In terms of chromosomal aberrations, have been observed changes in organization and morphology of the chromosomes in the meristematic cells of onion exposed to treatment with the herbicide Galigan 240 EC. Thus, two categories of mitotic abnormalities have been identified: stickiness chromosomes and laggards chromosomes. In terms of nuclear anomalies, these were binucleated cells and cells with nuclear erosion.

The frequency of chromosomal aberrations and the frequency of nuclear abnormalities increased with the increase of the herbicide concentration and exposure time (Table 1). Thus, at a 1.5% herbicide concentration, the frequency of stickiness chromosomes was 2.7% (3 h), 4.1% (8 h) and 7.5% (24 h), while at the same concentration (1.5%), the frequency of laggards chromosomes was 3.1% (3 h), 3.9% (8 h) and 5.3% (24 h).

Nuclear abnormalities have been identified especially at the herbicide concentration of 1.5% and 24 hours (11% binucleated cells and 5% cells with nuclear erosion).

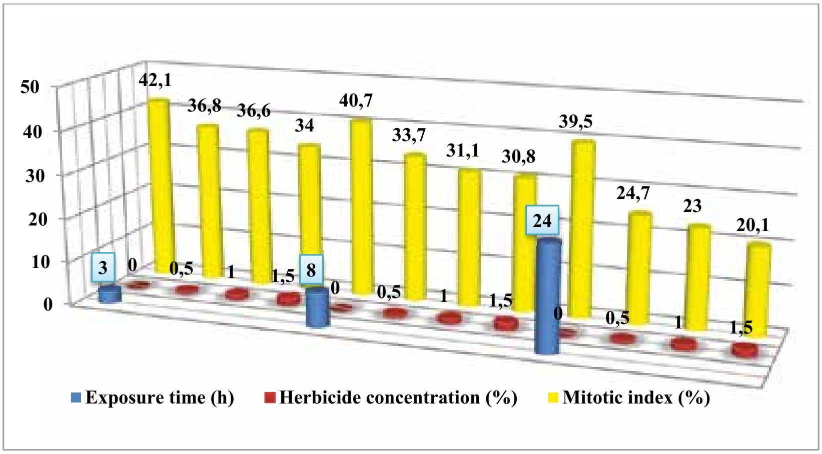

Figure 1. The decrease of mitotic index directly proportional to increase of herbicide concentration and increase of exposure time

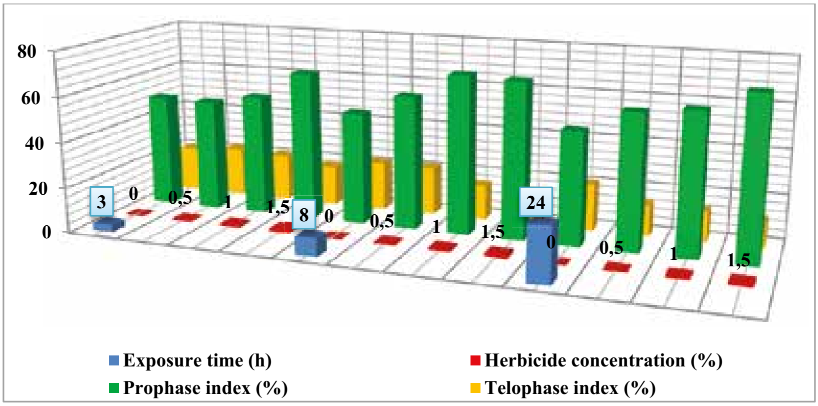

Figure 2. Graphical representation of increase of prophases frequency and decrease of telophases frequency with increasing concentration of herbicide Galigan 240 EC and increasing exposure time

Table 1. Quantification of chromosomal aberrations and nuclear abnormalities identified in meristematic cells of onion treated with the herbicide Galigan 240 EC

Exposure time (h)	Herbicide conc. (%)	Chromosomal aberrations		Nuclear abnormalities	
		Stickiness (%)	Laggards (%)	Binucleated cells (%)	Cells with nuclear erosion (%)
3	0 (Ct)	0	0	0	0
	0.5	1	0	0	0
	1.0	0	1	0	0
	1.5	2.7	3.1	2	1
8	0 (Ct)	0	0	0	0
	0.5	0	1.9	1	0
	1.0	0	2.0	1	1
	1.5	4.1	3.9	3	2
24	0 (Ct)	0	0	0	0
	0.5	0	2.3	3	2
	1.0	0	4.1	8	2
	1.5	7.5	5.3	11	5

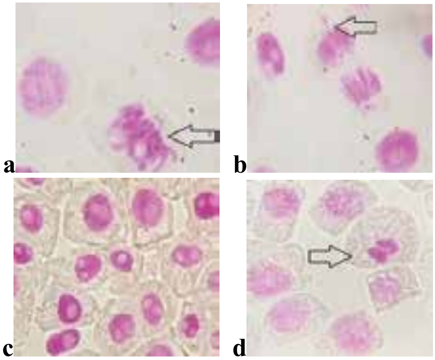

Figure 3. The chromosomal aberrations and nuclear abnormalities identified in meristematic cells of the onion exposed to treatment with herbicide Galigan 240 EC: stickiness chromosomes in methaphase (a); laggards chromosomes in anaphase (b); binucleated cells (c); cell with nuclear erosion (d)

CONCLUSIONS

The study reveals the mitodepresive effect of treatment with the Galigan 240 EC herbicide to onion, and shows the direct correlation between herbicide concentration, exposure time and cytological mutagenic effects identified in meristematic cells. The results suggest caution when applying the herbicide Galigan 240 EC to agricultural crops.

Repeated and without discernment use of herbicides invariably leads to the accumulation of phytotoxic substances in plants and soil and contaminates the environment.

The optimal solution for avoiding cytotoxic effects can be the application of minimum concentrations of herbicides and the combination of these chemical methods of weed control with biological methods, in the context of sustainable agriculture, for the protection of the environment and the increase of the quality of life.

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GROWTH AND GRAIN YIELD PARAMETERS OF SINGLE-PLANTED AND IN-CANOPY GROWN WHEAT (*Triticum aestivum* L.)

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Abstract

Growing high-yielding wheat (Triticum aestivum L.) genotypes in dense populations under a competitive environment of field conditions is the main goal of the modern cropping systems. However, genotypic potential of yield related traits of single-planted wheat may differ with the plants grown under canopy pressure. A field experiment was conducted during two consecutive years to assess the relations between grain yield parameters of single-planted and field-grown wheat plants. The experiment was installed in a randomized complete block design with 3 replications and 8 bread wheat cultivars were used. Plant height, biomass, spike number, grain number, thousand grain weight and grain yield were determined under both conditions. Average grain yield decreased from 2.90 ton/ha in 2012 to 1.71 ton/ha 2013. Although grain weights of single-planted wheat also decreased in the second year (2.83 g/plant) comparison to first year of the experiment (4.93 g/plant), varied response were found among genotypes under single-planted and with in-canopy conditions.

Key words: *wheat, single-planted, in-canopy, grain yield.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops among the world with 750 million tons of production (FAO, 2016). Yield and yield related traits are important selection criteria for wheat farmers and growing high-yielding wheat genotypes in dense populations under a competitive environment of field conditions is the main goal of the modern cropping systems. Yield performance of a wheat cultivar is affected by various factors such as genetic background, environmental effects and farming practices. It is well known that plant density plays a crucial role in the formation of the yield. Numerous studies have been previously reported the effects of plant density on yield (Joseph et al., 1985), radiation use efficiency and green fraction (Whaley et al., 2000), grain weight and grain number per spike (Kazan and Doğan, 2005). Moreover, it has been also reported that the optimum plant density differs with the change in sowing dates (Spink et al., 2000). Wheat plants can compensate the reduction in plant density with their tillering capacity which depends on environmental conditions and genetic background of the plant (Jin et al., 2017). But, decrease in plant density may cause increase in weed population such as jointed goatgrass (*Aegilops cylindrica*) in wheat growing areas. Wilson and Swanson (1961) also reported a progressive decrease in grain yield as plant density dropped below 20 plants per square foot. Puckridge (1982) investigated the influence of size and distance of neighbouring plants on the development of individual plants in wheat and barley populations and at the end of the statistical analyses it has been found that there was not any direct effect of space available or the location of near neighbours on the growth of individual plants. To achieve the optimum yield of a given wheat cultivar we should know its genotypic potential as a single plant, and its performance under canopy pressure. However, relative differences between the performances of single planted and in-canopy grown wheat plants have not been clearly explained yet. The aims of this study were i) to investigate the yield and yield related traits of 8 bread wheat cultivars under two growing conditions as single-planted and in-canopy, ii) to understand the responses of measured traits to different environmental pressure conditions and iii) to identify relative performances of the cultivars used in the experiment in terms of measured traits.

MATERIALS AND METHODS

This study was conducted in 2011/2012 and 2012/2013 growing seasons at the experimental site of Ege University, Faculty of Agriculture, Department of Field Crops, Izmir-Turkey (38°27'6", 27°13'32"E). The soil structure of experimental field was clay loam, mild alkaline and moderate calcic.

The experiment was established during two consecutive years for evaluating the relation between grain yield of single-planted and in-canopy wheat plants. The dimensions of in-canopy plots were 1 m x 0.6 m and it was conducted as three replicates in randomized complete block design. The single-planted plots were designed as four replications that sowed the single plants on corner of square which side length was 40 cm. Eight different bread wheat cultivars were used as plant materials. These were Menemen (MEN), Alibey (ALI), Basribey (BAS), Kaşifbey (KAS), Cut (CUT), Cumhuriyet (CUM), Meta (MET) and Sagittario (SAG) cultivars which are adapted to Mediterranean climate conditions.

Initially, 6 kg N and 6 kg P_2O_5 fertilizer were applied as ammonium sulphate (21%) and triple super phosphate (45%), respectively. And also 6 kg N were applied as ammonium nitrate (33%) in jointing stage of wheat.

After removing border lines, all of the plots were harvested. Plant height, biomass, spike number, grain number, thousand grain weight, and grain yield of plants were determined separately for in-canopy as well as single-planted plots.

RESULTS AND DISCUSSIONS

Yield and yield related trait values of 8 bread wheat cultivars in two growing season under two growing conditions are shown in Table 1. Bread wheat cultivar (cv.). SAG create relatively more dry weight as a single plant than other genotypes in both years (120.4 and 69.8 g). But cv. SAG was not at the first place in terms of total dry weight under canopy pressure (Table 1). Cv. BAS had the highest grain yield in first year (3509 ton/ha) while cv. MEN showed highest grain yield in second year (2465 ton/ha). For thousand grain weight, cv. CUT had the highest values for both as a single-plant (45.8 g in 2012; 28.0 g in 2013) and in-canopy (52.0 g in 2012; 33.5 g in 2013) for two growing seasons.

Table 1. Some agronomic traits of single-planted (snPL) and in-canopy-grown (inCP) 8 wheat cultivars during 2012 and 2013 growing seasons

Cultivars	Treatments	Total dry weight (g/plant)		Grain Yield (g/plant)		Grain Yield (ton/ha)		Thousand grain weight (g)		Grain number (num./spike)		Spike number (num./plant)		Plant height (cm)	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
MEN	*inCP*	3.0	2.9	1.1	1.0	2787	2465	34.2	27.0	27.1	30.2	1.2	1.3	66.5	69.8
	snPL	55.0	68.4	12.9	16.8	-	-	27.7	19.8	26.5	56.8	16.8	14.5	54.4	54.8
ALI	*inCP*	3.5	2.4	1.4	0.5	3489	1217	35.8	20.3	29.5	18.4	1.4	1.4	64.5	73.0
	snPL	87.7	37.5	32.4	9.9	-	-	30.5	19.1	44.1	44.8	24.3	11.8	60.9	57.8
BAS	*inCP*	3.3	2.1	1.4	0.6	3509	1473	41.4	25.0	28.3	19.7	1.2	1.5	67.0	68.2
	snPL	61.4	29.9	12.8	7.8	-	-	22.2	18.8	25.3	55.7	22.5	7.5	58.3	57.4
KAS	*inCP*	3.2	1.5	1.3	0.4	3156	893	34.5	24.9	32.7	17.1	1.1	0.8	81.9	74.2
	snPL	49.0	48.0	12.0	10.4	-	-	26.9	22.6	23.4	52.2	19.3	9.3	52.7	60.5
CUT	*inCP*	2.8	2.3	1.0	0.7	2605	1651	52.0	33.5	20.7	19.7	1.0	1.0	78.1	85.8
	snPL	93.4	67.5	26.4	12.2	-	-	45.8	28.0	28.7	24.2	20.5	18.3	69.7	70.8
CUM	*inCP*	3.2	3.0	1.2	0.8	2890	1990	35.8	35.6	31.2	19.4	1.1	1.2	84.5	84.4
	snPL	114.0	61.2	26.7	10.2	-	-	41.2	24.4	21.5	26.7	30.3	15.5	71.2	70.2
MET	*inCP*	3.4	3.0	1.3	0.9	3226	2316	38.2	24.8	25.7	28.3	1.3	1.3	73.5	80.9
	snPL	96.7	36.8	32.4	8.8	-	-	27.1	27.4	21.8	33.0	33.5	9.8	62.7	63.8
SAG	*inCP*	2.6	2.6	0.7	0.7	1779	1690	32.5	24.8	23.6	21.8	0.9	1.3	55.6	64.6
	snPL	120.4	69.8	41.0	20.1	-	-	33.3	23.6	37.4	51.9	33.0	17.0	51.0	50.5

Cv. KAS had the highest grain number per spike in-canopy conditions in first year (32.7). But as a single-plant cv. ALI had the largest grain number per spike value in first year (44.1). In second year cv. MEN had the largest values for both as single-plant (56.8) and in-canopy (30.2) in terms of grain number per spike (Table 1). The spike number values of the genotypes were close to each other in-canopy conditions for both years (Table 1). However, the spike number values of the single-plants differed among the genotypes. The largest spike number values were observed in cv. MET (33.5) in first year. Cv. CUM had the longest plant height in-canopy and as single-plant in the first year and cv. CUT was the longest cultivar in second year (Table 1).

The mean values of measured traits under two growing conditions (in-canopy and single-planted) for both seasons were presented in Figure 1. Notable differences between the growing types and the growing seasons for all measured traits can be seen from Figure 1. Spink et al. (2000) found significant decrease in yield with the reduction in plant density. Also it has been showed that total crop dry matter increases with an increase in plant density to an optimum level (Holliday, 1960; Donald, 1963; Spink et al., 2000). In our study the yield related traits such as total dry weight, grain yield and spike number per plant decreased dramatically in canopy conditions compare to single planted plants. On the other hand the some traits such as thousand grain weight and plant height presented less variation among the growing conditions. Mosanaei et al. (2017) evaluated two different plant densities as 350 and 420 plants per m^2 for wheat and they did not find significant differences between two plant densities in terms of plant height and thousand grain weight. However, Yonggui et al. (2015) determined an increase in plant height with the increase in plant density from 250 to 500 plants per m^2. In the present study, the mean values were slightly higher in-canopy plants than those of single-planted ones in terms of thousand grain weight (Figure 1). The higher competitive conditions to reach photosynthetic radiation in canopy led wheat cultivars to become taller comparison to single planted conditions (Figure 1). For grain number per spike, single-planted wheat individuals had higher values in both years. But the gap between two growing types in terms of grain number per spike was much bigger in the second year.

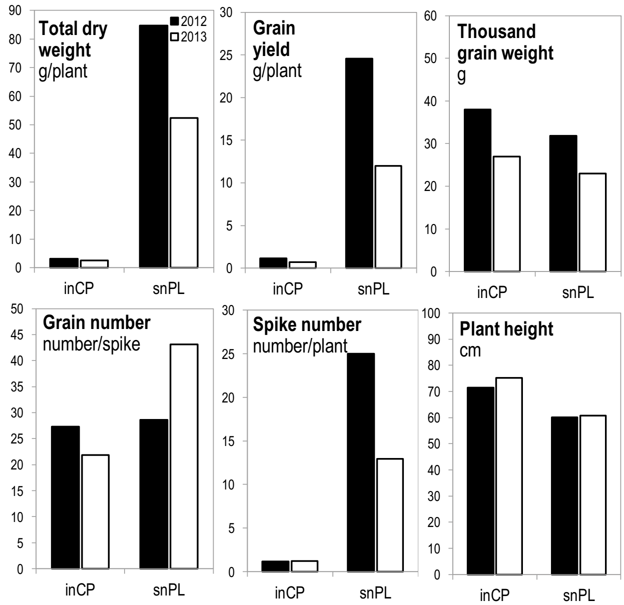

Figure 1. Some agronomic traits of single-planted (snPL) and in-canopy-grown (inCP) 8 wheat cultivars during 2012 and 2013 growing seasons

This result may be attributed to differences between the environmental conditions of two growing seasons. Mosanaei et al. (2017) found significant difference between two plant densities in first year of their research with respect to grain number per spike. When two growing season were evaluated, it can be said that there are obvious differences between two growing seasons in terms of measured traits like Mossanaei et al. (2017) found in their study. The mean values of first year measurements were relatively higher than the second year for total dry weight, grain yield per plant and thousand grain weights for both growing types in-canopy and single-planted (Figure 1). But the mean values were slightly higher in the second year for both growing types in terms of plant height (Figure 1).

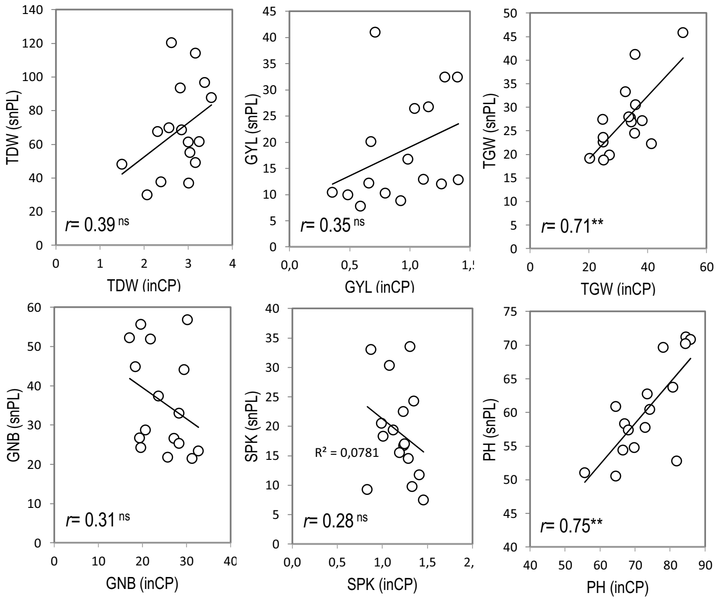

Figure 2. Correlations between some agronomic traits of single-planted (snPL) and in-canopy-grown (inCP) 8 wheat cultivars during 2012 and 2013 growing seasons. [**TDW**= Total dry weight (g/plant), **GYL**= Grain yield (g/plant), **TGW**= Thousand grain weight (g), **GNB**= Grain number (number/spike), **SPK**= Spike number (number/plant), **PH**= Plant height (cm)]

Figure 2 represents the correlations between in-canopy and single-planted values for each trait measured. Statistically significant relations were found between in-canopy and single-planted wheat plants in terms of TGW (*r*=0.71) and PH (*r*=0.75). Hence it can be said that there were close relationships between the values obtained from the single-planted and in-canopy plants in terms of plant height and grain weight. Also, these two traits were not affected by canopy density. However no significant correlation coefficient found between in-canopy and single-plant applications with respect to total dry weight per plant, grain yield per plant, grain number per spike and spike number per plant. These results indicate that the measurements obtained from single-planted individuals did not represent the measurements in-canopy with respect to these four traits.

CONCLUSIONS

The wheat cultivars did not represent their whole genotypic potential while they were in a competitive environment. The performances of single-planted wheat plants in terms of yield and yield related traits are quite different from the wheat plants grown in-canopy. Besides, the relative performances of evaluated cultivars were changed in different growing conditions. As a conclusion, although it is possible to reach the genotypic potential of a given genotype under minimum environmental pressure, this data would be not fully informative about the relative performance of the cultivar growing in-canopy.

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QUALITATIVE MODIFICATIONS PRODUCED IN FEED OF *Festuca rubra* L. AND *Agrostis capillaries* L. UNDER INFLUENCE OF UAN LIQUID FERTILIZER

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Abstract

The paper aimed to present the analysis of the qualitative changes in feed obtained as a result of fertilization with UAN liquid fertilizer of Festuca rubra - Agrostis capillaries. The experiment was established in 2014 in the Baisoara Mountain village (Cluj county), at the altitude of 1240 m about sea level. The experience includes four variants in three repetitions, which are: first variant (V1) – control variant (unfertilized); the second variant (V2) – fertilizer with 50 kg UAN/ha^{-1}; third variant (V3) – fertilizer with 75 kg UAN/ha^{-1}; fourth variant (V4) – fertilizer with 100 kg UAN/ha^{-1}. The grass was cut once in 2014 and in 2015, botanical composition were determined and also feed quality. The natural meadow of Festuca rubra L. and Agrostis capillaris L. responded very well to the application of the liquid fertilizer. The use of liquid mineral fertilizer (UAN) can be taken into account in mountain conditions at moderate doses of up to 75 kg/ha of active substance applied at deworming. The floral composition evolving over the dominance of Agrostis capillaris L. with Festuca rubra L., which produce good quality hay.

***Key words**: Festuca rubra L., Agrostis capillaris L., liquid fertilizer, natural grassland, productivity, feed quality.*

INTRODUCTION

Semi-natural grasslands are particularly important in mountainous areas of Romania, and for many farms, they are the only source of forage. An important part of efficient livestock production is ensuring the sufficient grass for hay and pasture. However, low soil nutrient levels often limit forage production. In Romania, grasslands are an important forage resource, but irrational management systems during the last period have led to their present state of degradation (Vîntu et al., 2011). For fifty years, animal production in most European countries has grown considerably, and the economic efficiency of milk and meat production has improved. Demand for more digestible feed resulted in mowing earlier in the spring and more frequent pastures, but nitrogen fertilization had to be increased.

Intensive pasture management has brought with nitrogen fertilization an increase in economic efficiency and has led to a reduction in the number of plant and animal species. Modern farming methods have been criticized for their impact on the environment and the landscape. Grazing management faces a choice between the opportunities offered by modern technologies and the demand for high biodiversity and attractive landscapes. There is a European-wide move towards extensive management systems as a result of agricultural reform, politics and the desire to promote biodiversity and preserve rural communities. This is particularly true in the regions where the landscape with semi-natural meadows dominates, and where it has the potential to sustain or increase biodiversity (Nösberger, 1998).

The floristic composition and the potential of grassland productivity is an essential, ecological and demographic phenomenon, representing the net result of a complex set of physiological, ecological, and evolutionary interactions in demographic and physical processes. In most cases, an increase in plant productivity due to fertilization leads to a decrease in the number of plant species coexisting in a given area. As a result of the application of fertilizers, the semi-natural grasslands have gradually been transformed into intensely managed meadows (Nösberger 1998).

MATERIALS AND METHODS

The experiment was established in 2014 in the eastern part of Apuseni Mountains at Baisoara Mountain village (Cluj county), at the altitude of 1240 m. The experience was placed after experimental technique method. The surface of experimental plots is 20 m^2. The experience includes four variants in three repetitions, which are: first variant (V1) – control variant, (unfertilized); the second variant (V2) – fertilizer with 50 kg UAN/ha^{-1}; third variant V3 – fertilizer with 75 kg UAN/ha^{-1}; fourth variant (V4) – fertilizer with 100 kg UAN/ha^{-1}. Experience has been placed on grassland *Festuca rubra* - *Agrostis capillaries* type of that is specific of nemoral floor, beech forest the sublevel and mixed beech resinous (Ţucra et al., 1987) determined after Braun-Blanquet (1932). Natural grasslands of *Festuca rubra* with *Agrostis capillaris* responded particularly well to mineral fertilization with liquid fertilizer UAN which is a mixture of ammonium nitrate and urea Chemical formula: NH4NO3 NH2-CO-NH2. Average annual temperature was 6.6°C (the year 2015). Average annual precipitation: 1069.2 mm / m^2 (the year 2015). The soil type is *Litosol Skeletal.*

RESULTS AND DISCUSSIONS

After applying treatments in the second year of experiment changes in phytocoenosis are noted. Graphical representation in space 2-dimensional (2D) allowsus an explanation of floristic changes in proportion of 94.7%. The most important is axis 1 (87.7%) andonly a small proportion of explanation can be attributed to Axis 2, respectively 7.1% (Table 1).

Table 1. Importance of axis and recommended ordinarion space in 2015

Axis	Axis importance (*r*)	Cumulative	Recommended solution
1	87.7	87.7	2D
2	7.1	94.7	

r-the determination coefficient for the correlations between the ordinal distances and the original distances in dimensional space.

The effect of applying liquid fertilizers is replicated on axis 1. The application of 75 kg UAN/ha^{-1} (correlation -0.416) and 100 kg UAN/ha^{-1} (correlation -0.600) is found in the negative space of axis 1.

In the positive space are shown the effects of treatments with lowe ramounts of fertilizers (50 kg UAN/ha^{-1}) and variants without fertilizers. The application of 50 kg UAN/ha^{-1}presents only a tendency to explain the floristic composition on axis 2, the effect be ingstatistically not statistically assured: p≥0.05 (Table 2) .

Table 2. The significance of ordination axes in 2015

Experimental factors	Axis 1		Axis 2	
	r	Significance	r	Significance
50 UAN/ha^{-1}	0.181	not statistically assured	-0.216	not statistically assured
75 UAN/ha^{-1}	-0.416	*	0.171	not statistically assured
100 UAN/ha^{-1}	-0.600	**	0.009	not statistically assured

The changes found this year are minor and fall within the type of meadows, a phenomenon supported by small floristic distances between the separate groups following the ordering (T 0.9562 without statistical assurance and T -2.793 with significant differences, p≤0.05). (Păcurar, Rotar, 2014). When comparing the control variant with variants V3 and V4, but also variants V2 with V4 (Table 3).

Table 3. Comparison of the floristic composition of experimental variants in 2015 (MRPP)

Treatments	T	A	p-value	Significance
V1 vs. V2	-1.24568390	0.06604944	0.09699409	not statistically assured
V1 vs. V3	-2.58826401	0.27203282	0.02373384	*
V1 vs. V4	-2.79310580	0.34235917	0.02289233	*
V2 vs. V3	-1.04145652	0.06186578	0.13965715	not statistically assured
V2 vs. V4	-1.98965091	0.15360126	0.03832053	*
V3 vs. V4	0.95622641	-0.06452769	0.83048504	not statistically assured

In 2015, the dry matter yield is directly proportional to the large quantities of applied fertilizers (75-100 kg UAN/ha^{-1}, p≤0.001). Harvest growth is mostly attributable to plants in the *Poaceae* family which are directly proportional to harvest and high fertilizer quantities (p≤0.001). Plants in the *Cyperaceae* and *Juncaceae* families prefer treatments with small amounts of fertilizers (p≥0.05). Plants of the *Fabaceae* family have a greater weight in the phytocoenosis of the control variant and

after application of the fertilizers they will be reduced ($p \geq 0.05$). Plants from other botanical families respond well to either the control variant or the 50 kg UAN/ha^{-1} fertilization. Floral diversity is reduced due to the application of the treatments, the greatest diversity is recorded in the control phytocoenosis ($p \leq 0.001$), respectively ($p \geq 0.05$).
Most species prefer the control variant (without applied treatments) or the application of small amounts of fertilizers such as: *Briza media* L., *Luzula multiflora*, *Trifolium panonnicum* L., *Campanula abietina* Griseb., *Genista tictoria*,. *Crepis biennis* L., *Galium verum* L., *Genista sagittalis*, *Hieracium aurantiacum* L. etc.
Phytocenosis of the control variant (V1–unfertilized) is represented by the type of grass *Festuca rubra* L. With *Agrostis capillaris* L., but for the other treatments (75-100 UAN / ha^{-1}), the species *Agrostis capillaris* L. will be dominated (Figure 1).

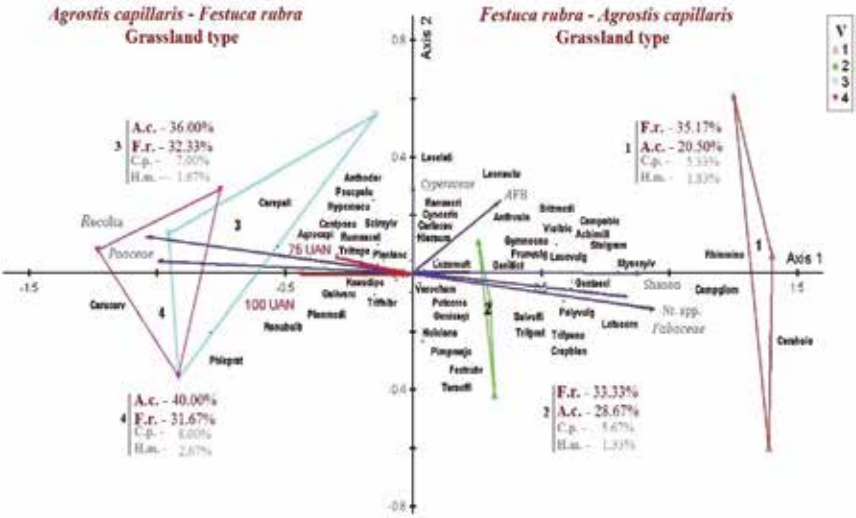

Figure 1. Ordering of floristic composition by applied treatments in 2015:
V1 – control variant (unfertilized); V2 - 50 kg UAN/ha^{-1}; V3 - 75 kg UAN/ha^{-1}; V4 - 100 kg UAN/ha^{-1}; F.r.- *Festuca rubra* L., A.c. - *Agrostis capillaris* L., C.p .- *Centaurea pseudophrygia* C.A. Mey., H.m. – *Hypericum maculatum* Crantz

Concerning the chemical analysis of the feed produced in 2015, it revealed that UAN fertilization results in a decrease of the protein content from 8.38%, in the control variant to 6.03% in fertilization with 75 kg UAN/ha^{-1}. The results indicate a very close relationship between the protein and the weight percentage of the plants from *Fabaceae* family participation of the cannopy.
The nitrogen content is the same as that of the protein, according to the values (Figure 2). Crude fat content reduced from 4.44% to 3.41% in the control variant selected fertilization with 75 kg UAN/ha^{-1}. Crude ash presents a slight decrease this case selected tome asure thea mount of fertilizer to increase, varying between hard 5.42% (control) to 4.80% (V3-75 kg UAN/ha^{-1}) (Figure 2).
The results presented for crude cellulose show a slight increase from 35.10% in the case of the control to 38.52% when applying 75 kg/ha of UAN.
The results on the NDF feed content of 2015, show a significant increase from 50.86% in the control variant to 68.62% when applying 100 kg/ha of UAN.
The results obtained in our experience it shows that the average NDF content, which is the total cellwalls, is roughly double to the gross fiber content.
The content of ADF increases this year from 43.24% in the control variant to 46.35% for the variant fertilized with 100 kg/ha of UAN (Figure 3).

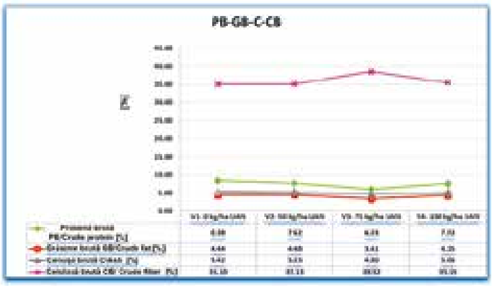

Figure 2. Influence of fertilization with UAN on some quality indexes

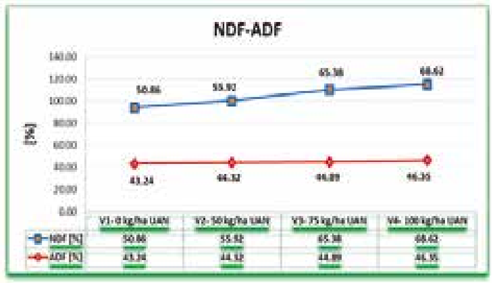

Figure 3. Influence of fertilization with UAN on the content of NDF and ADF

CONCLUSIONS

The use of liquid mineral fertilizer (UAN) can be taken into account in mountain conditions at moderate doses of up to 75 kg / ha of active substance applied at deworming. This yields crop yields of up to 5.64 t / ha SU. The floral composition evolving over the dominance of *Agrostis capillaris* L. with *Festuca rubra* L., which produce good quality hay.

Large doses of liquid fertilizers in liquid form (100 kg/ha of active substance), we do not recommend on mountain meadows because they cause degradation of the cannopy by the appearance of some species, *Centaurea pseudophrygia* C.A. May up to 17.50% and *Hypericum maculatum* Crantz. up to 9.50%, contributing to a degradation of the feed quality by reducing the protein content from 8.38% to 6.03%.

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ALLELOPATHY AND ALLELOCHEMICAL INTERACTIONS AMONG PLANTS

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Abstract

Allelopathy determines the dynamics of plant species in different environments. Plants can release chemicals into the environment that suppress the growth and establishment of other plants in their vicinity, a process known as 'allelopathy'. Chemicals with allelopathic functions have other ecological roles, such as plant defence, nutrient chelation and regulation of soil biota in ways that affect decomposition and soil fertility. In this review we explore allelopathy in the context of environmental characteristics that control the production and release of allelochemicals in both natural and agricultural systems. This study highlights the current understanding of how evolution might affect the intensity and importance of allelopathic interactions. Understanding this biological phenomenon could help to understand the environmental changes caused by allelochemicals and summarizes the knowledge about the mechanisms of action of these compounds.

***Key words**: allelochemicals, allelopathy, evolution, plant secondary metabolites.*

INTRODUCTION

Plants can release chemicals into the environment that suppress the growth and establishment of other plants in their vicinity, a process known as '*allelopathy*'.

Allelopathy is a biological phenomenon where a plant inhibits the growth of another plant. By releasing the allelochemical substances, some plants can greatly affect the development of other plants, either in good or bad way through leaching, decaying etc. Essentially, plant allele-pathy is used as a means of survival in nature, reducing competition from nearby plants.

Allelopathy can be considered as a form of communication between plants. Chemicals with allelopathic functions have other ecological roles, such as plant defence, nutrient chelation and regulation of soil biota in ways that affect decomposition and soil fertility (Bais et al., 2004; Weir et al., 2004; Yoneya, Takabayashi, 2014).

Plants synthesize a multitude of compounds through secondary metabolism.

The production of these compounds depends on the existence of precursor molecules and the activation of specialized genes. Activation of genes required for allelochemical biosynthesis is often dependent on environmental stimulants (Croteau et al., 2000).

Allelopathy was first defined by Hans Molisch in 1937 as its reciprocity or the mutuality of the sufferings or the effects of a plant on the other, and about sixty years later a much more detailed and complete definition has been established by the International Society of Allegiance, according to which allelopathy means any process involving secondary metabolites produced by plants, algae, bacteria and mushrooms that influence the growth and development of agricultural and biological systems (Cheng, Cheng, 2015).

In this review we explore allelopathy in the context of environmental characteristics that control the production and release of allelochemicals in both natural and agricultural systems. This study highlights the current understanding of how evolution might affect the intensity and importance of allelopathic interactions. Understanding this biological phenomenon could help to understand the environmental changes caused by allelochemicals and summarizes the knowledge about the mechanisms of action of these compounds.

PLANT DEFENCE AND THE ROLE OF ALLELOCHEMICALS

Plant cannot move away from the potential threats or towards beneficial entities. During the course of evolution, plants have developed both physical and chemical mechanisms of defence from pests and pathogens (Bernards, 2010). Traditionally resource competition has been considered as the single most important factor that influences the patterning of plant communities (Niklas, Hammond, 2013).

However recent research has described allelopathy as an important aspect of plant defence that impacts plant community diversity (Fernandez et al., 2013). In this process plants release secondary metabolites that are considered to interact with the surrounding environment by inhibiting the germination or growth of neighboring plants (Ben, Jordan, Osborn, 2006; Fernandez, 2016).

The majority of the allelochemicals in the plant kingdom are found in vascular plants, but also in ancient terrestrial nonvascular plants such as mosses or liverworts, has increased over the years.

Therefore, allelochemicals can play an important role in plant succession through their release by pioneer plants (Bryophytes) which contribute substantially to the accumulation of above ground biomass, particularly in cold temperate biomes including boreal forests and peatlands (Chiapusio et al. 2013; Michel et al., 2011).

Allelochemicals are non-nutritive substances produced as plant secondary metabolites or decomposition products of microbes. Examples of allelochemicals that predominate in plants are alkaloids, phenols, terpenoids, glycosides but also acid cinnamic, benzoic acid, flavonoids and others.

Allelochemicals consist of various chemical families and are classified into the following 14 categories based on chemical similarity (Rice, 1974): water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; simple unsaturated lactones; long-chain fatty acids and polyacetylenes; benzoquinone, anthraquinone and complex quinones; simple phenols, benzoic acid and its derivatives; cinnamic acid and its derivatives; coumarin; flavonoids; tannins; terpenoids and steroids; amino acids and peptides; alkaloids and cyanohydrins; sulphide and glucosinolates; and purines and nucleosides. Plant growth regulators, including salicylic acid, gibberellic acid and ethylene, are also considered to be allelochemicals (Cheng, Cheng, 2015).

ALLELOCHEMICAL MODE OF ACTION

Allelochemicals actively released by plants or passively produced during the decomposition process of both above and below-ground plant residues affect abiotic and biotic processes in the ecosystem and thereby influence the invasion process (Inderjit, 1996; Uddin et al., 2012; Uddin et al., 2014b).

Most of these substances are initially found to be inactive. Subsequent transformations (hydrolysis, oxidoreduction, methylation and demethylation) generate new products with distinct allelopathic properties.

Different parts of plants can have these allelopathic properties, from foliage and flowers to roots, shell, soil and mulch. Most of the allelopathic plants retain their protective chemicals in their leaves, especially during autumn. As the leaves fall and decompose, these toxins can affect the nearby plants. Some plants also release toxins through their roots, which are then absorbed by other plants and trees (Figure 1).

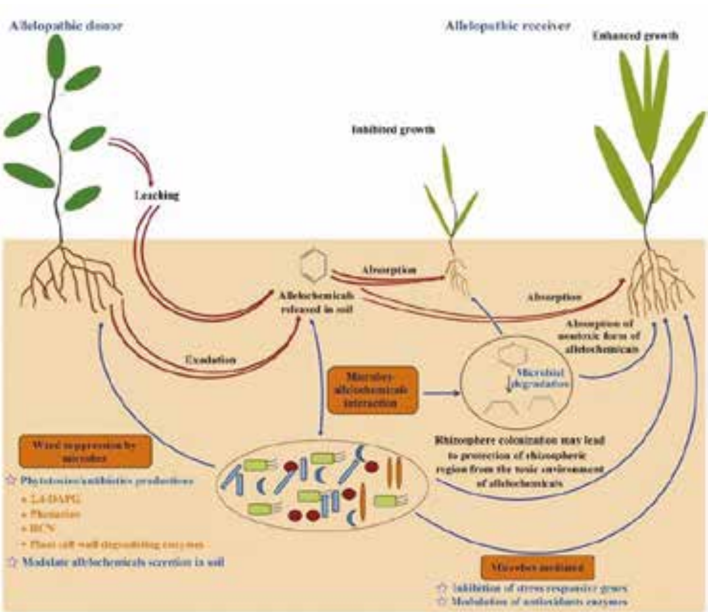

Figure 1. A schematic diagram showing the toxins released through their roots, the interaction of allelopathic donor-receiver species (Barazani, Friedman, 1999; Bais et al., 2006; Mishra et al., 2013; Cheng, Cheng, 2015)

Common plants with allelopathic properties include: *Prunus laurocerasus*, *Arctostaphylos uva-ursi*, *Rhus* spp., *Rhododendron* spp., *Sambucus* spp., *Forsythia* spp., *Solidago* spp., some species of fern, and some species of *Secale* spp., *Festuca arundinacea, Poa pratensis, Centaurea maculosa, Alliaria petiolate, Casuarina* spp. *and Allocasuarina* spp.

In the allelopathic woody species, trees are excellent examples of allelopathy in plants. For example, many trees use allelopathy to protect their space by using roots to draw more water out of the soil so that other plants cannot thrive. Some use their allelochemical substances to inhibit germination or prevent the development of nearby plant life. Most allelopathic trees release these chemicals through their leaves, which are toxic once absorbed by other plants.

Juglans nigra is a prime example of this. In addition to its leaves, the walnuts store allelopathic properties in buds, endocarp and roots. The chemical responsible for its toxicity, called juglone, remains in the soil around the tree. Other trees that are known to exhibit allelopathic tendencies are maple, pine and eucalyptus. Root-root and root-microbe communication can either be positive (epiphytes, mycorrhizal, fungi, nitrogen-fixing bacteria) or negative to the plant (parasitic plants, parasitic bacteria, fungi and insects) (Walker et al., 2003).

In natural systems, roots are in continual communication and quickly recognize and prevent the presence of invading roots. The known effects of compounds on processes related to growth and development of plants suggest for many hormonal substances such as auxin, gibberellic acids, ethylene, jasmonic acid and salicylic acid, that the compounds should have activity. Allelopathic compounds could interact by inhibiting synthesis, accumulation or utilization of energy rich compounds such as fatty acids or triacylglycerols (Seigler, 2006).

The *Sorghum* genus includes plants whose roots remove sorgolone, a poisonous substance that blocks the respiration and photosynthesis of the plants that come into contact with.

Among grain crops, poppy seeds germinate only in the presence of wheat, while rye has the gift of preventing the germination of certain types of weeds.

Common wormwood inhibits the growth of plants such as lovage, caraway, basil, lemon balm or common sage.

PLANT ALLELOPATHY IN AGRICULTURE

Allelopathy as a natural ecological phenomenon it has been known and used in agriculture since ancient times (Zeng, 2008, 2014). The purposes of research on allelopathy include the application of the observed allelopathic effects to agricultural production, reduction of the input of chemical pesticides and consequent environmental pollution and provision of effective methods for the sustainable development of agricultural production and ecological systems (Macias et al., 2003; Li et al., 2010; Han et al., 2013; Jabran et al., 2015). The suitable application of allelopathy toward the improvement of crop productivity and environmental protection through environmentally friendly control of weeds, insect pests, crop diseases, conservation of nitrogen in crop lands and the synthesis of novel agrochemicals based on allelochemicals has attracted much attention from scientists engaged in allelopathic research.

In agriculture the use of allelopathic crops is currently being realized, as components of crop rotations, crop mixtures or intercropping, as cover crops or as green manure (Cheng, Cheng, 2015).

Aiming for an ecological, sustainable and successful agriculture is also by making crop rotations, crop mixtures, intercropping, which has little harmful impact on environment conditions and maintains soil productivity over a long period of time maintaining the soil fertility, keeping the pest under control and reducing soil sickness problem.

Crop rotations - maintains and even improves the soil fertility, prevents the buildup of pest and soil sickness as compared to monoculture (Arnon, 1972) provides sustainability to agriculture by reducing the requirement of chemical nitrogenous fertilizers and thereby decreasing environmental pollution by substituting them with biologically fixed nitrogen of legumes (Narwal et al., 2000).

Crop mixtures or intercropping it is more productive in term of land use (Willey, 1979).

In soils with poor fertility, the cultivation of cereals with legumes improves both total yields and reduces the nitrogen requirement of cereal component. Besides, the legume biomass may be used as mulch/green manure. Both the crop rotations and intercropping systems or crop mixtures through inclusion of legumes maintains or improves soil fertility (Reigosa et al., 2006).

Biomass - soil fertility management in ecological sustainable agriculture gives much reliance on the use of biomass (crop residues and other organic wastes) to maintain the status of organic matter in the soil and to meet the nutrients requirement of the crops. The crop residues release allelochemicals through volatiles, leaching and during microbial decomposition (Reigosa et al., 2006). The production of allelochemicals in soil affects germination, growth and yield of crops depending on plant residue type, amount and depth of placement and length of decomposing period. The allelochemicals may either be inhibitory or stimulatory to the succeeding crops (Rice, 1984; Waller, 1987; Narwal et al., 2000).

Weed management - Several types of allelopathic plants can be intercropped with other crops to suppress weeds, crop cultivars with allelopathic potentials can be grown to suppress weeds under field conditions.

Several studies were elaborated on the significance of allelopathy for weed management. Rye, sorghum, rice, sunflower, rape seed and wheat have been documented as important allelopathic crops. These crops express their allelopathic potential by releasing allelochemicals which not only suppress weeds, but also promote underground microbial activities (Jabran et al., 2015).

Natural herbicides

Many plants make allelochemicals that deter competitors. Allelopathic chemicals interfere with growth of nearby plants. Among the plant products as herbicides - juglone, is an allelochemical produced by black walnut (*Juglans nigra*), isolated from walnut tree has been found effective against redroot pigweed (*Amaranthus retroflexus)*, velvetleaf (*Abutilon theophrasti)* and barnyard grass (*Echinochloa crus-galli*) (Shettel, Abalke, 1983; Spelce, Muselman, 1981; Weston et al., 1987).

Another allelochemical - sorgoleone is produced in *Sorghum bicolor* root hairs and exuded as oily drops; it accumulates in the soil and acts as a pre-emergence herbicide affecting photosintesis in very young seedlings.

Narwal et al., 2000, has exemplify some other important plant products having promising herbicidal activity: Dhurrin (*Sorghum*); gallic acid (spurge); Phlorizin (apple root); trimethylxanthene (coffee) and cinch (eucalyptus). The commercialization and marketing of "Herbiacae" the herbicide from microbial natural product bialaphos in Japan (Hatzios, 1987) has opened up a new era in weed management. Other microbial phytotoxins found to suppress weed growth include anisomycin, tentoxin, biopoloroxin, herbimycin etc.

CONCLUSIONS

This review study on allelopathy role plant interference, exploring the allelopathy in the context of environmental characteristics that control the production and release of allelochemicals in both natural and agricultural systems, clearly demonstrated that allelochemicals play an integral part in synergistic plants interactions. Allelopathy can be considered as a form of communication between plants. Plants are affected by each other positively and negatively. Nutrient sharing and suppression of parasitism and stress cues are the positive effect examples, while for the negative allelopathic effect is the invasive species that can suppress all others. Allelochemicals can suppress plant growth directly or indirectly. *Alliaria petiolate* (garlic mustard) it is one invasive plant example that indirectly suppress growth plant through the inhibition of their mycorrhizal fungal symbionts. Also, from fescue plants roots (*Festuca* spp.) there is *m*-tyrosine which is a non-protein amino acid that inhibits plant growth directly (Bertin et al., 2007).

Nevertheless, over the years the number of reports indicating the improvement in crop production due to allelopathic interactions had increased. The use of allelopathic crops can be achieved as components of crop rotations, crop mixtures or intercropping, weed management, etc. The use of allelopathy for controlling weeds could be either through directly utilizing natural allelopathic interactions, particularly of crop plants or by using allelochemicals as natural herbicides. For a sustainable agriculture allelopathy has achieved great success in weed management. Utilization of water extracts of allelopathic crop combined with reduced doses of herbicides can be a promising strategy for sustainable weed management and environment health (Amb, Ahluwalia, 2016).

Allelopathy determines the dynamics of plant species interaction in different environments influencing the agroecosystem. Because of these interactions, allelopathy is a complex phenomenon with limited field repeatability (Trezzi et al., 2016).

Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides. The structure of allelochemicals can be used as an analogue for the synthesis of new pesticides which will be less harmfull for the environment as compared to synthetic agrochemicals.

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INVESTIGATION ON THE YIELD AND GRAIN QUALITY OF COMMON WHEAT (*Triticum aestivum* L.) CULTIVARS GROWN UNDER THE AGROECOLOGICAL CONDITIONS OF CENTRAL BULGARIA

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Abstract

The field experiment was held in Slatina, Bulgaria during the period 2012 - 2015. The test was performed by using blocking with four repetitions; experimental field area - 15 m^2 with sunflower predecessor. The varieties common wheat Enola, Kristal, Pirineo, Bononia and Kapo, were studied. The growing of plants was performed in compliance with the standard technology. The aim of the present research was to carry out a comparison study of the yield and quality of some common wheat cultivars grown in Central Bulgaria. The analysis of the results showed that the highest grain yield was obtained from Pirineo variety – 7700 kg/ha, followed by Enola – 7567 kg/ha and the lowest one – from Kapo variety 6550 kg/ha. Among the studied common wheat cultivars, the highest values of thousand kernel weight and the wet gluten content was reported for Pirineo (51.7 g and 33.3%, respectively) and the highest values of test weight were reported for Bononia (84.7 kg).
The lowest value of the thousand kernel weight was recorded for Kapo variety (43.0 g); the lowest value of the test weight and the wet gluten content was reported for Kristal variety (78.7 kg and 26.1%, respectively).

Key words*: wheat, grain yield, thousand kernel (grain) weight, test weight, gluten.*

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is one of the most widely grown and most consumed food crops all over the world. It is also a major field crop in Bulgaria, grown using 11,182,401 decares of the area and producing 5,662,721 tons and 474.8 kg per decare^{-1} grain yield in 2016. Compared with other cereals, it provides food humans with more calories and proteins in their daily diet, a considerable amount of trade throughout the world, and a lot of other products.
The development of high grain yields potential for good quality and resistance to biotic and abiotic stress factors. In addition, it responds to improved agricultural practices which are the main achievements for bread wheat breeding programmes (Delibaltova, 2016; Delibaltova, Kirchev, 2016).
In the last few decades, efforts taken by wheat breeders have resulted in successful development of bread wheat varieties possessing higher grain yielding potential, improved resistance to pest and diseases and better quality parameters.
The gluten content of wheat is a critical factor in bread making and high gluten content of wheat is associated with good bread making characteristics. It is genetically controlled but it may vary widely on the variety, location, climatic conditions, soil fertility and the complex interactions between these factors. In general, high gluten flours generate better results since they have a high loaf volume potential with higher water absorption. Genotype-by-environment interactions and the negative correlation between grain yield and grain gluten content of wheat had been established in different studies (Williams et al., 2008).
If a genotype has a high stability and shows low interactions with the environment are the desirable conditions in plant breeding.
Studies of a number of authors show that the amount of grain yield is closely related to the cultivar, the use of farming machinery and the soil and climate of the region (Dallev, Ivanov, 2015; Dimitrov et al., 2016; Ivanova et al., 2010). Therefore, in order to use the full productive potential of the cultivar, the proper choice of suitable cultivars for each

agroecological region is a decisive factor for obtaining high yields. That necessitates systemic studies of the cultivars in the different regions of the country (Ilieva, 2011; Yanchev, Ivanov, 2012).

The aim of the study was to establish the grain yield and quality of some common wheat cultivars grown in Central Bulgaria.

MATERIALS AND METHODS

A field experiment with common wheat was carried out on an experimental field in Slatina, Bulgaria during the period 2012 - 2015. The test was performed by means of a block method with four replications; the experimental field area was 15 m^2, with sunflower predecessor. The following varieties were tested: Enola, Kristal, Pirineo, Bononia and Kapo. All the stages of the established technology for wheat growing were followed.

Soil tillage included single disking (10-12 cm) after harvesting of the previous crop, and double disking after the main fertilization. The area was treated with N120P80 and the whole quantity of the phosphorous fertilizer and 1/3 of the nitrogenous fertilizer were applied before main soil tillage.

The remaining amount from the nitrogen norm was applied before the beginning of permanent spring vegetation. Triple superphosphate and ammonia nitrate were used. Sowing was completed within the agrotechnical term optimal for this region at sowing norm 550 germinating seeds/m^2.

Control of weeds, diseases and pests was done with suitable pesticides when necessary. Harvesting was done at full maturity. The grain yield is determined with standard grain moisture of 13%.

The indices grain yield (kg ha^{-1}): thousand kernel weight (g), test weight (kg), wet gluten content (%) were determined.

For the purpose of determining the quantity dependence between the studied indicators, the experimental data was processed according to the Anova Method of dispersion analysis, and the differences between the variants were determined by means of the Dunkan's Multiple Range Test (Dunkan, 1995).

The period of the research (2012-2015) is characterized with variety of temperatures and rainfall conditions which enables to evaluate the reaction of the studied varieties in accordance with their yields and quality characteristics under different climate conditions.

Rainfalls in autumn and during the critical spring period are decisive for the development of the wheat plants. The mean annual precipitation sums during October - March, which formed the autumn-and-winter moisture reserves in soil during the experimental years 2012-2013, 2013-2014 and 2014-2015 were higher with 77.7, 64.2 and 119.5 mm, respectively, than the mean sums of the long - term period. During April-May when plants were at stages booting and heading, the mean annual precipitation sum in 2013 and 2014 was higher, while in 2015 this sum was lower than the mean long - term value.

In June and July (during grain filling-maturation) rainfalls in harvest years 2013, 2014 and 2015 were higher with 61.6, 45.6 and 47.2 mm, respectively, than the mean sums of the long - term period.

The most favourable for plant growth and development was the first experimental year (2012-2013), followed by the second (2013-2014), while the third year (2014-2015) of the experiment was unfavourable, having an effect on yield and grain quality of common wheat.

RESULTS AND DISCUSSIONS

The results obtained were presented in Table 1 and they show that for both, by years and in average, for the experimental period variety Pirineo surpassed in grain yield all the other varieties included in the study.

The highest grain yields were obtained in the favourable for wheat year 2013 when the temperature values and the precipitation sum fully met the plant requirements for warmth and moisture throughout the whole vegetation period. The yields obtained reached up to 8500 kg/ha in variety Pirineo. Referring to grain yield that variety surpassed the varieties Enola, Bononia, Kristal and Kapo by 1.2%, 3.7%, 7.6% and 21.4%, respectively, the differences being statistically significant.

Table 1. Grain yield, kg/ha

Variety	Years of study			Average for the period kg/ha
	2012-2013 kg/ha	2013-2014 kg/ha	2014-2015 kg/ha	
Enola	8400 ^{d}	7300 ^{c}	7000 ^{c}	7567
Kristal	7900 ^{b}	6800 ^{b}	6700 ^{b}	7133
Pirineo	8500 ^{e}	7500 ^{e}	7100 ^{d}	7700
Bononia	8200 ^{c}	7400 ^{d}	7000 ^{c}	7533
Kapo	7000 ^{a}	6450 ^{a}	6200 ^{a}	6550
Mean values for Years	8000 ^{c}	7090 ^{b}	6800 ^{a}	

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test.

In the second experimental year (2014) the grain yields obtained varied between 6450 kg/ha and 7500 kg/ha, i.e. they were by 11.4% lower in average in comparison to 2013. Mathematical processing of data showed that varieties Bononia, Enola and Kristal signify-cantly fell behind Pirineo by 100, 200 and 700 kg/ha, respectively. The lowest yields were realized by variety Kapo - 6450 kg/ha.

In the third experimental year of the study the meteorological conditions during the variety vegetation were unfavourable and the plants were not able to attain their biological potential.

The grain yields obtained were within the limits of 6200 to 7100 kg/ha. Statistically proven, the lowest ones were those of variety Kapo and the highest - Pirineo.

During the period of study (2012-2015) Pirineo variety realized the yield of 7700 kg/ha in average and it surpassed the varieties Enola, Bononia, Kristal and Kapo by 1.7%, 2.2%, 7.9% and 17.6%, respectively.

The results from the multifactor analysis of variances showed the independent effect of the investigated factors, as well as their interaction (Table 2). Years with their climate conditions had highest statistic influence on the seeds yield - η 98, followed by variety - η 88.

Table 2. Analysis of variance for grain yield for the period 2012-2015

Source of Variation	Sum of Square	DF	Mean Square	Sig of F	η^2
Variety	10087099.90	4	2521775	.000	88
Years	15596239.90	2	7798119.89	.000	98
2- Way Interactions	999200.60	8	124900.07	.000	74
Residual	242331.25	45	5385.14		

Interaction - Years x Variety - η 74 was also significant for grain yield.

The results of the quality characteristics of the studied varieties are presented in Table 3. The thousand kernel weight is a cultivar specific trait influenced by the agroecological conditions and the growing technology.

The results show that thanks to the favourable climatic conditions during the wheat vegetation period in the third year, the values of that characteristic was significantly higher compared to the other experimental years.

In 2012-2013 Pirineo variety produced the largest grains (53.0 g of 1000 grains), followed by Enola and Kristal (48.0 g), while Kapo had the smallest grains (45.0 g). The differences between the varieties were statistically significant. The thousand kernel weight of Enola and Kristal variety had similar values and the difference was statistically insignificant.

Drought weather combined with high air temperatures at the stage of grain formation and ripening in 2014-2015 had an effect on grain weight. The lowest weight of thousand kernel (grain) was reported for Kapo variety (42.0 g). The varieties Pirneo and Bononia surpassed in weight of thousand kernel (grain) the varieties Kristal and Enola by 20.9% and 4.6%, respectively, the differences being significant.

The largest grains in average for the period 2012-2015 were reported for Pirineo variety (51.7 g), followed by the varieties Bononia (46.0 g) , Kristal and Enola (45.3 and 45.0 g). The lowest values of that characteristic were established for Kapo variety.

Table 3. The results of the quality characteristics of the studied varieties

Index	Variety	Years of study			Average for the period (2012-2015)
		2012-2013	2013-2014	2014-2015	
Thousand kernel (grain) weight, g	Enola	48.0 ^{c}	44.0 ^{b}	43.0 ^{b}	45.0
	Kristal	48.0 ^{c}	45.0 ^{b}	43.0 ^{b}	45.3
	Pirineo	53.0 ^{d}	50.0 ^{d}	52.0 ^{d}	51.7
	Bononia	47.0 ^{b}	46.0 ^{c}	45.0 ^{c}	46.0
	Kapo	45.0 ^{a}	43.0 ^{a}	41.0 ^{a}	43.0
	Mean values for Years	48.2 ^{c}	45.6 ^{b}	43.8 ^{a}	
Test weight, kg	Enola	80.0 ^{c}	81.0 ^{b}	82.0 ^{b}	81.0
	Kristal	78.0 ^{a}	78.0 ^{a}	80.0 ^{a}	78.7
	Pirineo	81.4 ^{c}	82.5 ^{c}	83.0 ^{b}	82.3
	Bononia	85.0 ^{d}	84.0 ^{d}	85.0 ^{c}	84.7
	Kapo	79.0 ^{b}	82.0 ^{c}	82.1 ^{b}	81.0
	Mean values for Years	80.7 ^{a}	81.5 ^{a}	83.4 ^{b}	
Wet gluten, %	Enola	26.0 ^{b}	28.5 ^{b}	28.4 ^{b}	27.6
	Kristal	25.0 ^{a}	26.0 ^{a}	27.0 ^{a}	26.1
	Pirineo	34.0 ^{e}	33.0 ^{d}	34.0 ^{e}	33.3
	Bononia	33.0 ^{d}	32.0 ^{c}	30.0 ^{c}	32.0
	Kapo	32.0 ^{c}	33.5 ^{d}	31.4 ^{d}	32.3
	Mean values for Years	30.0 ^{a}	30.6 ^{a}	30.2 ^{a}	

* Means within columns followed by different lowercase letters are significantly different ($P<0.05$) according to the LSD test

Test weight is a commercial indicator showing grain quality and it plays an important role in determining the market price. That characteristic of the studied varieties in the years of the experiment varied between 78.0 and 85.0 kg.

The highest test weight of wheat grain, in average for the period of the study, was reported for Bononia variety (84.7 kg.), followed by Ririneo, Kapo and Enola (82.3 kg, and 81.0 kg, respectively), while the lowest values were reported for the variety Kristal (78.7 kg).

In the experimental years the wet gluten content of the studied varieties obtained varied from 25.0% to 34.0%. It showed that the grain of these varieties is considered to be good for flour producing.

The highest wet gluten content, in average for the whole study period 2012-2015, was esta-blished in Pirineo variety (33.3%) and the lowest - in Kristal (26.1%).

CONCLUSIONS

Under the conditions of Central Bulgaria the highest yield, in average for the experimental period 2012-2015, was obtained from Pirineo variety - 7700 kg/ha, followed by Enola - 7567 kg/ ha and the lowest one - from Kapo variety 6550 kg/ha. Pirineo variety produced by 133, 167, 567 and 1150 kg/ha higher grain yield than the variety Enola, Bononia, Kristal and Kapo, respectively.

Among the studied common wheat variety, the highest values of thousand kernel weight and the wet gluten content were reported for Pirineo (51.7 g and 33.3% respectively) and the highest values of test weight were reported for Bononia (84.7 kg)

The lowest value of the thousand kernel (grain) weight - for Kapo variety (43.0 g); of the test weight and of the wet gluten content was reported for Kristal variety (78.7 kg and 26.1%, respectively).

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APPLICATION OF THE COEFFICIENT OF USING THE ACTIVE SUBSTANCE FOR THE EVALUATION OF THE EFFECT OF CHANGES OF NOZZLE OPERATING PARAMETERS ON THE SPRAYING PROCESS

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Abstract

The aim of the research was to determine the coefficient of using the active substance ($K_{s.a.}$), which was used to assess the quality of the spray. The quality of spraying was determined by the amount of copper deposited used for spraying from two different preparations. Two standard nozzles, flat fan XR 110-02 and double flat fan DF 120-02, were used for testing, which operated at a constant speed of 0.86 $m \cdot s^{-1}$ and two pressures of 0.2 and 0.28 MPa. The foliar fertilizer containing copper and a preparation containing the nano copper particles were used for spraying. The deposition tests were performed in the "Aporo1" spraying chamber. Mineralization was carried out in order to determine the deposition of copper on winter rape plants, and then the concentration of the copper element in the tested plants was measured using a spectrometer. After obtaining the deposition results the coefficient of using the active substance ($K_{s.a.}$) was calculated. It was observed that the $K_{s.a.}$ coefficient increases with the growth of the plant regardless of the spraying process parameters used.

***Key words**: nano copper, copper, foliar fertilizer, nozzle, spraying.*

INTRODUCTION

The quality of the plant spraying treatment depends on many technical and technological factors, as well as the weather conditions under which the spraying is carried out. According to many authors, the quality of spraying depends largely on the selection of the right nozzle. Therefore, the right type of a nozzle, its size and operating parameters (pressure, working height, spacing, setting) should be selected for the treatment. Choosing the right nozzle limits the entry of excess plant protection products into the environment and is a prerequisite for the proper course of spraying, and consequently results in a higher degree of coverage and spray deposition on the sprayed surfaces (Szewczyk et al., 2012; De Souza Christovam et al., 2010a; Hołownicki et al., 2002; Lipiński et al., 2007; Godyń et al., 2008; Kierzek, 2007). The selection of a nozzle is important during the performance of every protection treatment of the field, orchard and covered crops (Hoffmann, Hewitt, 2005; Özkan et al., 2012). The quality of spray deposition on the sprayed plants depends, among others, on the spray characteristics of the liquid stream (drop spectrum, mean droplet diameter and maximum droplet diameter). The efficiency of use of the active substance, and hence the biological effectiveness and the amount of spray liquid depends on the quality of spraying the liquid stream (Douzals, 2012). In the presented work, the authors sought to describe the spraying process of plants, determining its effectiveness with the help of the author's coefficient of using the active substance ($K_{s.a.}$).

The aim of the study was to determine the coefficient of using the active substance ($K_{s.a.}$). This parameter was used to evaluate the process of spraying winter rape in three development phases depending on the selected parameters of the spraying process.

MATERIALS AND METHODS

The experiment of the copper deposition on the sprayed plants was carried out under greenhouse conditions at the Institute of Soil Science and Plant Cultivation - National Research Institute - in the Department of Herbology and Crop Cultivation Techniques in Wroclaw (Poland). The winter rapeseed of the DK EXTROVERT F1 variety was used for testing, which was sown in pots with a 15 cm diameter in the amount of 5 pcs./pot. Ultimately, one plant was left for spraying in the pot. A modified phytotest of the first generation was used to establish the experiment (Sekutowski, 2011). The sowing surface was a peat-mineral mix with a pH = 6.5 and sand with a diameter of 0.6-0.8 mm - mixed in a ratio of 2:1. Winter oilseed rape plants were sprayed in three different development stages: 12, 14 and 16, according to the BBCH scale. Each development phase of the plant was sprayed in triplicate. The absolute control without spraying was also included in triplicates for each development phase of the plant.

The foliar fertilizer Mikrovit Copper 80 was used for the deposition of the Intermag company along with the preparation containing nanoparticles of copper oxide (II) of the < 50 nm size of the Sigma Aldrich Company. Both preparations were used at a dose of 160 g $Cu \cdot ha^{-1}$, whose were dissolved in 250 l of water per hectare at a pressure of 0.20 MPa and 300 l of water per hectare at a pressure of 0.28 MPa. The plants were sprayed in the "Aporo1" spraying chamber, at a constant operating speed of 0.86 $m \cdot s^{-1}$. The spraying treatment was conducted using two standard nozzles: XR 110-02 (flat fan nozzle) and DF 120-02 (double flat fan nozzle), and two operating pressures: 0.20 and 0.28 MPa. The height of the spray tips from the sprayed plants was equal to 0.5 m. The laboratory air temperature during spraying was 20°C, and the humidity was 60%. The plants were cut 24 h after the spraying operation, then dried and subjected to the mineralization process.

The winter oilseed rape mineralization was carried out in a laboratory located at the Institute of Biology (Department of Hydrobiology and Aquaculture) at the Faculty of Biology and Animal Breeding of the Wroclaw University of Environmental and Life Sciences (Poland). The technique of the microwave “wet” dissolution was performed in order to determine the copper deposition on the plants using the nitric acid (V) (69.0-70.0%, Sigma Aldrich). 0.5 g of material was weighed out of each sample, and then 5 ml of the HNO_3 acid was poured and mineralized in a Mars 5 microwave digestion system (CEM Corporation, USA). The content of the studied element (Cu) was measured by atomic absorption using the FS220 spectrometer of the Varian Company. The correctness of the determinations was verified with the reference material ERM-CD281 Rye Grass at the level of 97%.

After obtaining the results of the copper deposition on the studied plants, the coefficient of using the active substance ($K_{s.a.}$) was calculated, which was described by the authors with the formula 1:

$$K_{s.a.} = \frac{V_{s.a.}}{V_w} \times g \text{ [-]} \quad (1)$$

where:

$V_{s.a.}$ - the amount of active substance assimilated by the plants during the treatment (deposition) $[kg_{s.a.} \cdot kg_{s.m.}^{-1}]$; s.m.- dry mass; s.a.- active substance;

V_w - the amount of active substance being splashed onto a given surface (dose) $[kg_{s.a.} ha^{-1}]$;

g - density of plants $[kg_{s.m.} \cdot ha^{-1}]$.

The plant density was calculated according to formula 2:

$$g = i \times m_{s.m.} \; [kg_{s.m.} \cdot ha^{-1}] \quad (2)$$

i - the number of plants on a given area, 400000 $[plants \cdot ha^{-1}]$ were adopted for calculations;

$m_{s.m.}$- mass of one dry plant $[kg_{s.m.} \cdot plant^{-1}]$; s.m.- dry mass.

The formula 1 is subject of the patent application (no. P. 423023).

The statistical analysis of the test results was performed using the Statistica 12.5 program. On the basis of conducted normality tests (Shapiro-Wilk) and homogeneity of variance (Levene), it was shown that the conditions for the applicability of multivariate analysis of variance are not met. Therefore, the non-parametric Mann-Whitney U test was used to determine the effect of the factors. The tests were carried out at the significance level $\alpha=0.05$. The assessment of the impact of the nozzle, preparation and pressure on the coefficient of use the active substance ($K_{s.a.}$) was analyzed.

RESULTS AND DISCUSSIONS

The results of the study of the coefficient of using the active substance ($K_{s.a.}$), for winter oilseed rape in the development phases of the plant: 12, 14 and 16 BBCH and the examined spraying process parameters are presented in figures 1-3. The highest values of the coefficient of using the active substance ($K_{s.a.}$) were noted for the development stage of winter oilseed rape 16 BBCH for all tested parameters of the spraying process. While the lowest values of the $K_{s.a.}$ coefficient were noted for the development phase 12 BBCH, except for the values obtained using liquids containing nano copper for spraying, where slightly lower values were noted for both types of tested nozzles for the 14 BBCH development phase. Differences of these values for the pressure of 2.0 MPa were equal to: 0.001 (flat fan nozzle XR 110-02) and 0.002 (double flat fan nozzle DF 120-02). The lowest value of the $K_{s.a.}$ coefficient (0.015) was observed for the 14 BBCH development phase when spraying winter oilseed rape with a double flat fan nozzle DF 120-02 and spray liquid containing nano copper. Increasing the working pressure to 0.28 MPa increases the value of the $K_{s.a.}$ coefficient for both types of nozzles and both spray liquids used. The exception was the higher value of the $K_{s.a.}$ coefficient by 0.006 at a lower pressure (0.20 MPa) for winter oilseed rape plants in development phase 14 BBCH at spraying with the XR 110-02 nozzle and liquid containing nano copper.

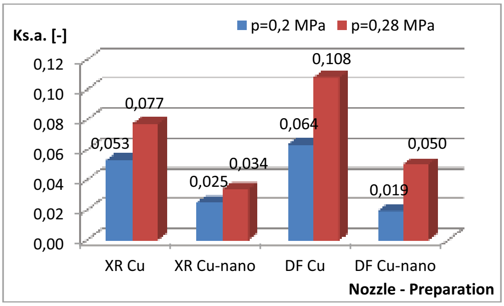

Figure. 1. Coefficient of using active substance ($K_{s.a.}$) for winter oilseed rape in development stage 12 BBCH and the selected parameters of the spraying process (XR - flat fan nozzle XR 110-02, DF - double flat fan nozzle DF 120-02, Cu - fertilizer Mikrovit Copper 80, Cu-nano - preparation containing nano copper)

Based on the data presented in figure 1, it was observed that the pressure of 0.2 MPa and the spray performed with the foliar fertilizer Mikrovit Copper 80, the value of the coefficient $K_{s.a.}$ is by 0.011 higher for the double flat fan nozzle DF 120-02 compared to the flat fan nozzle XR 110-02. However, when the pressure increased to 0.28 MPa, the increase in the value of $K_{s.a.}$ was noted for the double flat fan nozzle DF 120-02 o 0.031. We can conclude that, with lower pressure, both flat fan and double flat fan nozzles can be used for spraying, while at increased pressure it is more beneficial to perform spraying with a double flat fan nozzle. A similar situation was observed for the spray liquid containing nano copper.

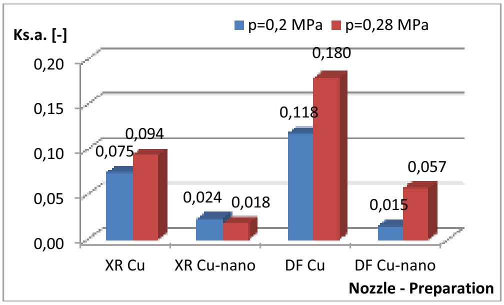

Figure 2. Coefficient of using the active substance ($K_{s.a.}$) for winter oilseed rape in the development stage 14 BBCH and the selected parameters of the spraying process (XR - flat fan nozzle XR 110-02, DF - double flat fan nozzle DF 120-02, Cu - fertilizer Mikrovit Copper 80, Cu-nano - preparation containing nano copper)

Based on the analysis of the data included in Figure 2, it was observed that the values of the $K_{s.a.}$ coefficient obtained for winter oilseed rape during the 14 BBCH development phase are definitely higher in the case when the spraying treatment was performed using the foliar fertilizer Mikrovit Copper 80.

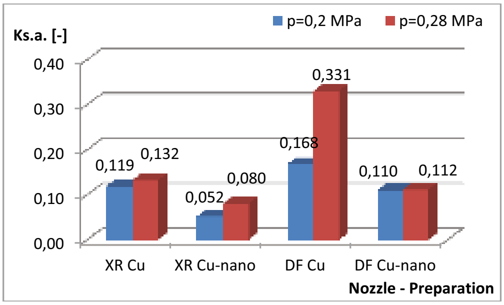

Figure 3. Coefficient of using the active substance ($K_{s.a.}$) for winter oilseed rape in the development stage 16 BBCH and the selected parameters of the spraying process (XR - flat fan nozzle XR 110-02, DF - double flat fan nozzle DF 120-02, Cu - fertilizer Mikrovit Copper 80, Cu-nano - preparation containing nano copper)

For a double flat fan nozzle DF 120-02 and liquid pressure of 0.2 MPa a 0.103 higher $K_{s.a,}$

coefficient value was obtained, when spraying with the foliar fertilizer Mikrovit Copper 80, than when spraying plants with a preparation containing nano copper (at a pressure increased to 0.28 MPa this value was higher by 0.123).

In the case of spraying the winter oilseed rape in the development phase 16 BBCH (Figure 3), similar values of the $K_{s.a.}$ coefficient were obtained, for the XR 110-02 nozzle and the foliar fertilizer Mikrovit Copper 80 for both tested pressures. Similarly, for the DF 120-02 nozzle and the preparation containing nano copper a similar value of the $K_{s.a.}$ coefficient was noted for both tested pressures. There was also a significant increase in the value of the $K_{s.a.}$ coefficient (by 0.163) for the double flat fan nozzle DF 120-02 and the fertilizer Mikrovit Copper 80 with the pressure increased up to 0.28 MPa.

The results of the statistical analysis (assessment of the impact of the nozzle, preparation and pressure on the coefficient of use the active substance $K_{s.a.}$) are presented in Table 1.

Therefore, in 2010, in Romania there were just 45% of dairy cows compared to the year 1990. Taking into account the dynamics of cattle and dairy cows stock, the share of dairy cows in the cattle stock has recorded a similar decreasing trend. In the year 1990, dairy cows represented 59.46% of the cattle livestock and in the year 2010, they registered just 53.73% (Table 1).

Table 1. Mann-Whitney U test results for $K_{s.a.}$ coefficient and individual development phases of winter oilseed rape

Factors	Value p		
	Development phase of winter oilseed rape		
	12 BBCH	14 BBCH	16 BBCH
Nozzle	0.370845	0.174854	0.022577
Preparation	0.002437	0.000077	0.007260
Pressure	0.008616	0.340779	0.260237

The data presented in Table 1 shows that the type of preparation used for spraying plants had a significantly statistical effect (at the significance level of α = 0.05) on the value of $K_{s.a.}$ for all studied development stages of winter oilseed rape. The data presented in Table 1 shows that the type of preparation used for spraying plants had a significantly statistical effect (at the significance level α = 0.05) on the value of $K_{s.a.}$ coefficient for all development phases of winter oilseed rape examined. There were no statistically significant impacts of the nozzle type (plant development phase 12 and 14 BBCH) and pressure (plant development phase 14 and 16 BBCH) on the value of the $K_{s.a}$ coefficient.

For the research of the usable spray deposition, samplers in the form of filter papers are used most commonly, while the researchers use the following, among others, as markers: fluorescein, nigrosine, BSF, copper oxychloride or tartrazine (Sánchez-Hermosilla et al., 2012; Godyń et al., 2011; Hołownicki et al., 2012, De Souza Christovam 2010a and b; Celen 2009; Larsolle, 2002). The authors used the foliar fertilizer Mikrovit Copper 80 and the preparation containing nano copper in own studies, while the deposition tests were carried out performing the mineralization of whole plants and checking the amount of copper element absorbed by them.

According to some scientists, the factor influencing the spray deposition indicator on the sprayed trees is their phenological phase. Świechowski et al. (2012) examined the effect of the liquid dose and the type of the nozzle on the spray deposit in the crown of apple trees in various phenological phases. Two types of nozzles were analysed: hollow cone nozzles TR 80-01 and air-injector nozzles ID 90-01. Larger deposition of the liquid was obtained by the authors in the phase of establishing and full fruit development for the hollow cone nozzles TR 80-01. De Souza Christovam et al. (2010a) performer studies aimed at determining the impact of various spraying techniques on the deposition of copper on the soybean plants. Standard flat fan nozzles TeeJet XR8002 were analysed, which operated at a pressure of 0.287 MPa, speed of 2 $m \cdot s^{-1}$and a liquid dose of 130 $l \cdot ha^{-1}$ and rotary nozzles LVO (low volume oily), which operated at the pressure of 0.621 MPa, operating speed of 1.5 $m \cdot s^{-1}$and a liquid dose of 40 $l \cdot ha^{-1}$. Both nozzles operated at different air velocities (0, 2.5 and 8 $m \cdot s^{-1}$) produced by the fan of the nozzle with the auxiliary air stream. These authors have stated that the air velocity has no effect on the amount of copper deposition on the leaf surfaces in the upper part of the soybean plants. However, in the presented own research, the authors used two nozzles producing fine droplets (XR 110-

02 and DF 120-02). Own research shows that higher values of the $K_{s.a.}$ coefficient were obtained for the double flat fan nozzle DF 120-02, regardless of the pressure and liquid used to spray the winter oilseed rape.

Many authors analysed the effect of various parameters of the spraying process on the value of spray deposit on the sprayed plants. In the presented own research, the author's coefficient of using the active substance $K_{s.a}$ was proposed, checking the influence of the parameters of the spraying process on its value. Similarly to other researchers, it was observed that the type of the nozzle used and the pressure value influence the deposition of the spray liquid and the value of the $K_{s.a.}$ coefficient.

It was observed that the value of the $K_{s.a.}$ coefficient also depends on the development phase of the plant, the bigger the plant the bigger the value of the $K_{s.a.}$ coefficient. The coefficient of using the active substance $K_{s.a.}$ presented by the authors is used as the description of the plant spraying process in terms of its effectiveness.

CONCLUSIONS

1. The highest values of the coefficient of using the active substance ($K_{s.a.}$) were recorded for winter oilseed rape plants in the development stage 16 BBCH, with all the spraying parameters used.
2. The value of the $K_{s.a.}$ coefficient increases with the growth of the plant, which is a consequence of the greater mass (surface) of the plant, allowing greater absorption of the preparation.
3. Taking into account the $K_{s.a.}$ coefficient when comparing the nozzles used in the research, it can be concluded that the best results were obtained for the double flat fan nozzle DF 120-02, at a pressure of 0.28 MPa and using the foliar fertilizer Mikrovit Copper 80 for the spraying treatment, for all analysed development phases of the winter oilseed rape.

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EVALUATION OF SOME MORPHOLOGICAL, CHEMICAL PARAMETERS AND ANTIOXIDANT CAPACITY OF POMEGRANATE

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Abstract

The aim of this study is the evaluation of some morphological and compositional characteristics of three pomegranate fruit samples. For pomegranate juice samples (PJ), total soluble solids (TSS) varies between 14.6 and 16.3°Brix, titratable acidity (TA) between 0.28 and 1.13(%), total phenolic contents (TPC) from 221 to 323.3 (mg/100 mL) and DPPH radical scavenging activity shows EC50 values between 35.2 and 48.3 (mL PJ/g). For peel methanol extracts (PE), TPC was 198.2-279.8 and EC50 3.7-5.6 (µg/mL).

***Key words**: pomegranate, phenolic contents, DPPH.*

INTRODUCTION

Punica granatum L. (*Punicaceae*) has been used for centuries in the folk medicine of many countries (Kumar et al., 2013) for the prevention and treatment of a wide number of health disorders such as inflammation, diabetes, diarrhea, dysentery, dental plaque and to combat intestinal infections and malarial parasites (Ismail et al., 2012, Rosenblat et al., 2006).
Pomegranate fruit juice, peel and leaf extracts have been reported to possess strong antioxidant activity (Zhang et al., 2008), and can help prevent or treat various disease risk factors including high blood pressure, high cholesterol, oxidative stress (Aviram et al., 2001), hyperglycemia, inflammatory activities (Lansky and Newman, 2007) and disorders of the digestive tract (Seeram et al., 2005).
The scientific studies on the antioxidant activities, bioactive constituents, and pharmacological properties of pomegranate have increased considerably in the last decade (Kalaycıoglu and Erim, 2017).
Figure 1 shows the number of studies on pomegranate recorded in Science Direct between 2008 and 2017.
The main objective of this research was to characterize three pomegranate samples with various origins, to quantify phenolics content in pomegranate juice and methanolic extract and to evaluate free radical scavenging activity.

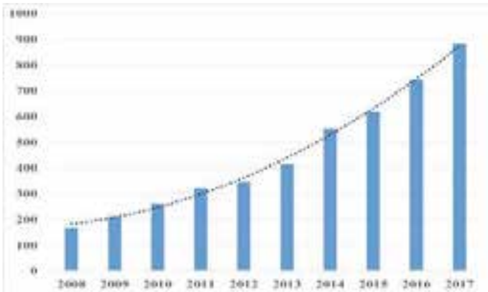

Figure 1. Number of publications recorded in Science Direct between 2008-2017 for „pomegranate"

MATERIALS AND METHODS

Plant material

We used three pomegranate (*Punica granatum* L.) fruits samples weighing about 2 kg each. Two samples (S1 and S2) were purchased from local markets (imported from Turkey), the third sample (S3) being obtained from Spain.
From each sample were selected healthy fruits with no visible external cuts or spoilage. The

fruits were rinsed with tap water and cut at the equatorial zone. Arils were manually extracted and squeezed through a metal sieve. The resulting juice was filtered through filter paper.

Pomegranate peel extraction. Pomegranate peels were dried to brittleness (hot air oven 45^{o}C, 40 h) and powdered to 40 mesh (Grindomix GM200 knife mill). Peel powder (20 g) was extracted for 4 h with MeOH solution (MeOH: water 4:1) in a Soxhlet extractor (Buchi B811). Methanol extracts were concentrated under reduced pressure (Rotavapor Bucchi R215) and lyophilized.

TSS and TA measurements. The total soluble solids (TSS) were determined with a digital refractometer (Mettler-Toledo, 30 PX). The titratable acidity (TA) was obtained by titration with NaOH 0.1 N to pH 8.2 (g citric acid/100 mL). The maturity index was calculated as the ratio of TSS/TA.

Determination of total phenolic content (TPC). TPC of the extracts were estimated using the Folin-Ciocalteu colorimetric method reported by Singleton et al. (1999). After appropriate dilution, the samples were mixed with 1.0 mL of 10-fold diluted Folin-Ciocalteu reagent and 0.8 mL of a 7.5% sodium carbonate solution. The mixture was kept for 30 min at room temperature and after that, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (JASCO V 630). The results were expressed as mg of gallic acid equivalents (GAE) per g of powder extract.

DPPH radical scavenging activity. The free radical scavenging activity was determined using DPPH (2,2-diphenyl-picryl-hydrazil) test (Blois,1958). A DPPH solution (0.1 mM in ethanol, 4 mL) was mixed with 1 mL sample, containing different concentrations of extract. After 30 min, absorbance at 517 nm was recorded. The antiradical activity (%) was calculated using the relation:

$$\begin{pmatrix} DPPH\ radical \\ scaveging\ activity\ \% \end{pmatrix} = \frac{A_c - A_s}{A_c} \cdot 100$$

A_c- absorbance of DPPH solution;
A_s- absorbance of sample.

The value corresponding to 50% inhibition (EC50) was obtained from the graph of anti-oxidant activity (%) *vs*. extract concentration.

All results (mean ± standard error) were the mean of three determinations.

RESULTS AND DISCUSSIONS

The weights of the three pomegranate samples as well as their diameter, arils weight and juice volume are presented in Table 1. As it can be observed, there are no significant differences between S1 and S2 samples for any of the studied parameters.

The S3 sample presents significant differences versus S1 sample considering the fruit weight, arils weight proportion and the volume of the juice obtained.

Our results correspond with those presented for Turkish cultivars by Gözlekçi et al. (2011) (arils %: 42.3-52.85; juice volume %: 37.16-48.69), Durgaç et al. (2008) (arils %: 36.9-59.4). For Spanish cultivars Martinez (2006) has obtained for pomegranate juice values between 50.25% and 64.17%.

Table 1. Morphological parameters of pomegranate fruits

Sample	Total weight (g)	Equatorial diameter (mm)	Arils (%) (g/ 100 g FW)	Juice (mL/100 g FW)
S1	296.94± 13.67^{a}	81.0± 4.0^{a}	52.4± 6.8^{a}	31,4± 3.2^{a}
S2	317.9± 19.9^{a}	83.0 ±4.0^{a}	56.1± 4.8^{a,b}	38.6± 5.0^{ab}
S3	377.3± 19.4^{b}	85.7± 4.04^{a}	66.2± 4.3^{b}	46.4± 4.5^{b}

In each column, values with the same letter are not significantly different (Tukey simultaneous tests for differences between means - P ≤ 0.05).

Because pomegranate fruit external skin color does not indicate the extent of ripening degree or its readiness for consumption (Holland et al., 2009), another parameter such as color of aril, total soluble solids, titratable acidity, maturity index are usually considered for fruit quality assessment (Martinez et al., 2006).

Sugar content determined as total soluble solids (TSS) varies between 14.6 and 16.3^{o}Brix, pH value between 3.12 and 4.10, titratable acidity between 0.28 and 1.13.

The results of the chemical analyzes for pomegranate juice presented in Table 2 clearly distinguish the three samples. Similar results were communicated by Hernandez (1999) for

Spanish cultivars, pH: 2.89-4.42, TSS: 13.48-16.51, TA: 0.23-2.03, and by Nuncio-Jáuregui et al. (2014), pH: 3.55-5.42, TSS: 14.80-16.53, TA: 0.23-2.14.

The polyphenols content of pomegranate juice varied between from 221 to 324 mg gallic acid equivalents per 100 mL juice and from 198.2 to 279.8 mg gallic acid equivalents per g extract.

Table 2 Chemical analysis of the juice from the pomegranate fruits

Sample	TSS (^{o}Brix)	pH	TA (g/100 mL)	Maturity index
S1	14.6± 0.11^{a}	4.10± 0.02^{a}	0.28± 0.01^{a}	52.1
S2	15.3± 0.20^{b}	3.12± 0.01^{b}	1.13± 0.01^{b}	12.4
S3	16.3± 0.05^{c}	3.49± 0.03^{c}	0.69± 0.00^{c}	23.6

In each column, values with the same letter are not significantly different (Tukey simultaneous tests for differences between means - P ≤ 0.05).

Table 3. Total phenolic content (TPC) and DPPH radical scavenging activity (EC50) in juice and peel methanol extracts

Sample	Juice		Peel Extract	
	TPC (mg/100 mL)	EC50 (mL PJ/g)	TPC (mg/g)	EC50 (µg/ mL)
S1	221± 3.0a	48.3± 0.9a	198,2± 3.4a	5.6± 0.3 a
S2	243± 2.0b	35.2± 1.1b	248,6± 4.5b	3.7± 0.2 b
S3	323.3± 2.1c	40.2± 1.4c	279,8± 4.1c	4.2± 0.4 b

In each column, values with the same letter are not significantly different (Tukey simultaneous tests for differences between means - P ≤ 0.05).

Data presented in Table 3 prove that no relation can be clearly established between the total content of phenolic compounds and the free radical scavenging activity. As the antioxidant activity increases with the decrease of EC50 value, it would has been expected the S3 sample, having the highest value for polyphenols, 324 mg gallic acid equivalents per 100 mL juice, to have the lowest value for EC50, but our experimental results showed that the lowest EC50 value was obtained for the S2 sample with a medium value for TP, 243 mg /100 mL. Future experiments will be conducted to verify this result and to get additional evidence.

It could be also observed that samples with high polyphenol content in the juice, will have also have a high phenolic content in peels. High majority of the authors of the scientific papers in the area used for the determination of the phenolic compounds content the Folin-Ciocalteu regent method. For this reason, the results reported by different teams can be easily compared. Özgen et al. (2008) reported values starting from 124.5 to 207.6 mg GAE/100 mL for the concentration of phenolic compounds in six cultivars grown in Turkey, while Çam et al. (2009) in experiments conducted with eight cultivars, obtained for the same characteristic, values between 208.3-343.6 mg GAE/100 mL. The experiments done by Çalıskan and Bayazıt (2012) with 76 accesions grown in Turkey revealed values of the content of phenolic compounds between 1080 - 9449 mg GAE/kg. Similar results were also communicated by other scientists for Spanish cultivars: 150-450 mg GAE/100 mL (Mena et al., 2011), 267.4-421 mg GAE/100 mL (Nuncio-Jáuregui et al., 2014), 113.62-358.11 mg GAE/100 mL (Vegara et al., 2014). On the other hand, the values of the antioxidant activity are more difficult to be compared with data from the scientific literature, as this parameter is determined through different analytical methods.

However, we can mention the results presented by Kulkarni et al. (2005), who obtained a value EC50 of 8.33 µg/mL for a methanolic extract of peels, working with a Ganesh variety, cultivated in India, or the results of Fernandes et al. (2015) reporting an EC50 value of 16.33 µg/mL for the methanolic extract of peels for the variety Mollar de Eiche.

In scientific publications (Fawole et al., 2013, Mphahlele et al., 2014; Hmid et al., 2016) it is demonstrated that the chemical parameters of the pomegranate juice depend on cultivar, geographic origin, harvest time and post-harvest practices. For these reasons, the values obtained by us would not be considered as characteristic for pomegranate varieties. However, these results are important because they represent characteristics of the fruit reached on the consumer's table.

CONCLUSIONS

Based on the results experimentally obtained, one can conclude that the only use of morphological parameters (fruit weight, aril weight, juice volume) does not permit the sample differentiation. The chemical characteristics (TSS, pH, TA, Maturity Index), on the other hand, are different enough to make a difference between the three analyzed samples. All three samples have high values for both TPC and free radical scavenging activity. The samples with high concentration of polyphenolic compounds in juice, also present high YPC values in the methanol extract. However, we cannot establish so far a direct correlation between TPC values and the antioxidant activity expressed as free radical scavenging activity.

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EFFECT OF BIOCYCLIC HUMUS SOIL ON YIELD AND QUALITY PARAMETERS OF SWEET POTATO (*Ipomoea batatas* L.)

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Abstract

A field experiment was conducted on sweet potato (Ipomoea batatas L.) at the Agricultural University of Athens during the growing summer season 2017 to evaluate the effects of biocyclic humus soil on plant growth, yield as well as chemical constituents and quality parameters. The experiment was laid out in completely randomized design (CRD) with three replications of three treatments (untreated, inorganic fertilizer and biocyclic humus soil). A two-leaf cutting was placed into the treated soil to make a sweet potato plant. The highest sweet potato yield was obtained by using biocyclic humus soil with average total yield (35.6 t/ha) and average marketable yield (24.3 t/ha). There were no statistically significant differences between the treatments for the compression (Max Load 0.622-0.780 kN) and the penetration (Max Load 0.0439-0.0447 kN) tests on sweet potato tubers. Furthermore, measurements were implemented for the total nitrogen content of tubers with no statistical significant differences between treatments. The big difference in yield between sweet potato grown in humus soil and sweet potato treated conventionally probably is related to the fact that the structure of soil which is a clay loam soil was too compact for the cultivation of sweet potato, a disadvantage which has been compensated by using humus soil as substrate while substituting soil.

***Key words**: biocyclic humus soil, Biocyclic Vegan Standard, sweet potato, Ipomoea batatas* (L.), *productivity and quality.*

INTRODUCTION

The total global sweet potato production in 2016 was more than 105 million tonnes (FAO-STAT, 2018) with China having the biggest share in production (about 66%). China and the U.S. are the fastest growing exporters. Though sweet potato is a traditional crop for many countries of the world (China, Mexico etc.) cultivation and consumption in European countries became more popular in the last decades. (Loebenstein, 2009). The European production is offered by four countries: Portugal, Spain, Italy and Greece with a total production of 52 thousand of tonnes in 2016. In the same year Greek sweet potato production was 3.3 thousand tonnes cultivated in an area of 164 ha (FAO-STAT, 2018).

Sweet potato origins are found in Central or Northwestern South America due to the occurrence of Tropical Forest root crop agriculture (O'Brien, 1972). It was shown that after an analysis of sweet potato genotypes with RAPD markers the dispersal of sweet potato has been achieved more through the Central/Caribbean genopool (Gichuki et al., 2003).

The scientific name of sweet potato is *Ipomoea batatas* (L.) Lamk and belongs to the *Convolvulaceae* family. *Ipomoea batatas* can be adjusted in many climate zones such as warm humid tropics or mild sub-temperate zones also at an altitude of 2000 meters. The plant of sweet potato prefers a sandy loam ground and is cultivated often on mounds or ridges (Lim, 2016).

The edible parts of the sweet potato plant are the roots or tubers but also the sweet potato leaves and green tips. Sweet potato flour or starch is also used as an ingredient for secondary food products. As the sweet potato is considered to be one of the most healthiest food because of its health-giving additives it seems likely it could play an important role in a balanced and healthy human diet (Padmaja, 2009).

Global organic food and drink sales are increasing in scope year by year with an annual growth of 10% for 2015 with 81.6 Billion U.S. dollars sales (Amarjit, 2017). But also, the number of producers has increased by 7% in 2015 (Lernoud, 2017).

To control and compare all these organically grown products several standards and regulations have been developed over the past decades worldwide, especially in 2016 there were 87 national organic standards. All these recognized standards should follow the international approved guidelines of the Codex Alimentarius. Following this Codex Guidelines ensures the comparability of all these different standards (Huber, 2017).

A call is made to all stakeholders in the organic sector to participate in the renewal and the advancement of organic visions through the idea of IFOAM's Organic 3.0. The movement of Organic 3.0 promotes a more sustainable management through the hole agricultural sector with innovation being the driving force (Arbenz, 2016).

As shown in the study of Bilalis et al. (2017) organic agriculture could play a pivotal role in the introduction of innovative crops into the Mediterranean area as part of the implementation of measures for adaptation to climate change. In this context further field studies are needed to support an integrated approach of cultivations with a high interest in organic production such as sweet potato under the special Greek-Mediterranean conditions.

Even though, the consumption and the interest in cultivation of sweet potato to cover the needs of both the local market and exports is becoming bigger no scientific research has been done in Greece yet to cover this growing agricultural sector. With this experiment a first step is done in this direction.

In several researches the positive effect of organic matter through mulching or organic fertilization in the improvement of sweet potato production has been shown (Janssens et al., 2014; Nwosisi et al., 2017).

The organic cultivation of sweet potato in this experiment was practiced according to the Biocyclic Vegan Standard to meet the special needs and particular conditions of Greek agriculture.

The Biocyclic Vegan Standard became as a global standard a full member of the IFOAM's Organic Family of Standards in December 2017. This standard based on the German pioneer Adolf Hoops (1932-1999) was developed and practiced under the Greek Mediterranean conditions. From the very beginnings the biocyclic idea included a vegan aspect which were brought into play in the past years after combining initiatives from the organic vegan movement in agriculture. The Biocyclic Vegan Standard promotes a more lifecycle orientated organic agricultural model by excluding animal production (as known in the form of husbandry) and animal products from the hole agricultural production chain. The constant rise of soil fertility by increasing soil organic matter or humus is of high-priority. This is done by using mature compost or biocyclic humus soil together with green manuring and mulching (Biocyclic-Vegan Network, 2018).

Humus soil is characterized as a mature compost which has gone through a post-maturing process. The stability of this material empowers the possibility even to grow directly young plants and seedlings as it is very root-friendly. According to the Biocyclic Vegan Standard all materials used for the production of humus soil should be of plant origin (Biocyclic-Vegan Network, 2018)

The purpose of this study was to evaluate the effects of biocyclic humus soil application on total yields, texture through compression and puncture test and the content of some nutrients of sweet potato tubers.

MATERIALS AND METHODS

From May till September 2017 a field experiment was conducted at the organic certified experimental field of the Agricultural University of Athens (Latitude: 37°59′ 1.70″ N, Longitude: 23°42′ 7.04″ E, Altitude: 30 m above sea level) in Votanikos, Athens. Mean temperature and precipitation for the growing season are shown in Figure 1.

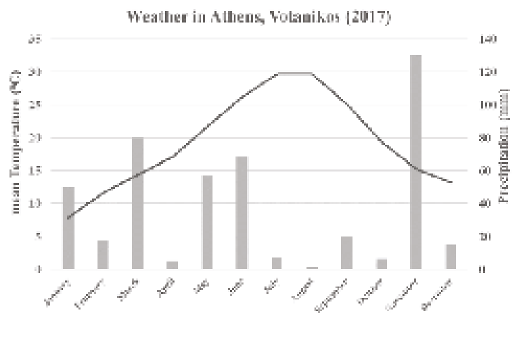

Figure 1. Meteorological data (mean temperature and precipitation) recorded by the weather station of Agricultural University of Athens

The soil was clay loam (29.8% clay, 34.3% silt and 35.9% sand) (Bouyoucos, 1962) with pH (1:1 H_2O) 7.29, nitrate-nitrogen (NO_3-N) 12.4 mg kg^{-1} soil, available phosphorus (P) 13.2 mg kg^{-1} soil, available potassium (K) 201 mg kg^{-1} soil, 15.99% $CaCO_3$ and 1.47% organic matter (Wakley, Black, 1934).
In this experiment olive pomace (30%), olive-tree leaves (50%) as a by-product of olive-mills equipped with two-phase centrifugal decanters, grape marc (10%) and ripe humus soil (10%) were used to derive a mature compost of rotting degree V (Table1). An industrial windrow composting procedure like that described by Manios (2004) was followed. Materials were placed in a windrow of 3 m wide by 1.5 m high and 150 m length and watered at 60% of their water holding capacity weekly for the first 3 months and every 4 weeks for the next 2 months of composting. To obtain a soil-like state beyond substrate maturity a post-maturing process followed for the next 3 years. In this stadium humus soil can be used especially in horticulture for direct planting.
The three treatments biocyclic humus soil (HS), Inorganic fertilizer 42-0-0 (IN) and the untreated control (CON) of the trial were arranged in a Completely Randomized Design with three replications. Each of 9 plots had a size of 2.0 m x 3.6 m. On the 8th of Mai 2017 6 sweet potato slips were planted in every plot in a distance of 1.2 m x 0.66 m. As a slip is a rooted sweet potato cutting described. Each slip had been produced from a two-leave sweet potato cutting and were planted into pots with biocyclic humus soil for one week to produce roots before planting in the end field position. The leaves of the cuttings were shortened and misted every 5 hours for the first 3 days and then regularly irrigated.
Soil was prepared by ploughing at a depth of about 0.25 m. Small round embankments were prepared in each planting position. A day before planting, inorganic fertilizer was applied by hand and the biocyclic humus soil was incorporated into final planting positions. 340 g of inorganic fertilizer in form of urea 42-0-0 was applied on every plot of 7.2 m^2 which corresponds to 200 kg N/ha.
15 liters of biocyclic humus soils was applied in every planting position to obtain the direct contact of the plant roots with the material. Irrigation was done every 3 to 4 days by hand and weeding was necessary every 3-4 weeks. No plant protection application was needed.
The sweet potato tubers were harvested and the vines emmoved by hand 137 days after planting on the 22th of September. The roots were stored for one week for curing (28°C, 80-90% relative humidity) and then stored for one month (15°C, 80-90% relative humidity) before doing measurements.

Table 1. Analysis of biocyclic humus soil on dry basis and in water extraction

Analysis description	On dry basis	Extraction with 600 ml deionised water from 360 g humus soil
Total Nitrogen (N), g/100 g	2.8	0.015
P_2O_5 soluble in inorganic acids (total), g/100 g	0.8	0.002
Total Potassium (K), g/100g	0.6	0.034
Electrical Conducitivity (1:5), pH units	7.6	
Cation Exchange Capacity (C.E.C.), meq Na/100 g	91.9	

Sweet potato tubers and vines were weighed after harvest to determine total yields with digital scale (Kern TB, Kern & Sohn GmbH, Balingen-Frommern, Germany).

Harvested tubers were classified according to weight of tubers in marketable sweet potato tubers (>100 g) and the non-marketable sweet potato tubers (<100 g)

Cylindrical samples with diameters of Ø 2.5 cm were cut from the central region of sweet potato tubers using a cork borer of the same diameter and then trimmed with a stainless cutting knife to a height of 2 cm. For each experiment a sweet potato cylindrical sample was placed on the bottom parallel plate and compressed. Compression experiments were performed in 3 replications.

The test was carried out between the standard Instron stainless steel polished platens (upper plate: 4 cm in diameter and designed for fracture testing) of a Instron Universal Testing Machine (Instron Model 1011, Canton, MA, USA) using a 5 kN load cell and a flat 15 mm diameter compression plunger. During compression experiments cross-head speed was 6 mm/min. Maximum force to break (N), displacement at maximum force (mm), and the initial slope (N/mm) of the curves were chosen as parameters to evaluate texture.

To measure puncture (penetration) whole sweet potato samples were placed on the stainless-steel platform of a Instron Universal Testing Machine (Instron Model 1011, Canton, MA, USA). A puncture probe with 4 mm diameter was used to punch the sweet potato using a constant speed of 6 mm/min and a 5 kN load cell. Puncture experiments were performed in 3 replications. As in compression test maximum force to break (N), displacement at maximum force (mm), and the initial slope (N/mm) of the curves were chosen as parameters to evaluate texture.

Two samples of stored sweet potato tubers were cleaned with fresh water and peeled and then sliced to 3 mm thickness and then mixed. The samples were dried in a hot air oven at a temperature of 60°C for 24 h. The dried samples were milled in attrition mill to obtain fine flour used for chemical analysis. Nitrogen (N) concentration was obtained using the Kjeldahl method (AOAC, 2006),

Potassium (K) was determined by a flame Spectrophotometer. Calcium (Ca), and Iron (Fe) were determined by atomic absorption spectrometry.

RESULTS AND DISCUSSIONS

Figures 2 and 3 shows average total and marketable yield, average tuber number per plant, but also the maximum load for compression and puncture tests and the total content of nitrogen in potato tubers of all three treatments.

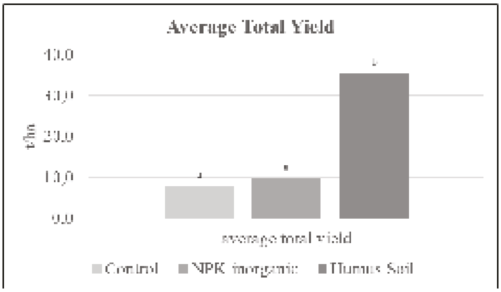

Figure 2. Average total yield of sweet potato (5.5 plants per m^2)

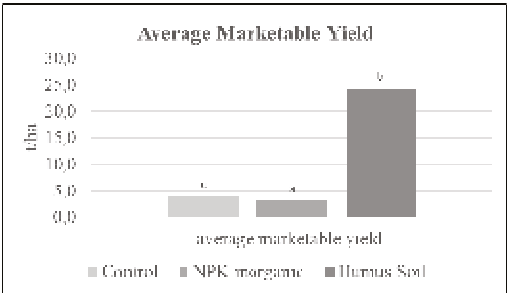

Figure 3. Average marketable yield of sweet potato (5.5 plants per m^2)

Analysis of the yield responses showed statistical significant differences ($P < 0.05$) of total and marketable yield as well as for the average potato tuber number per plant with treatments with humus having the biggest rates. Average total yield were 35.6 t/ha and 9.9 t/ha measured for a density of 5.5 plants per m^2 and average marketable yield 24.3 t/ha and 3.2 t/ha for treatments with humus soil and inorganic fertilizer respectively. Sweet potato yields depend a lot on the cultivated variety but also on the location of the site. For example, the average yield of sweet potato for the United States was 21.2 t/ha for 2008 ranging from 35.9 t/ha in California and 18.5 t/ha in North Carolina (Smith et al., 2009). The average yield for Greece was 18.6 t/ha in 2006 and 20 t/ha for 2016 (FAOSTAT, 2018). The average sweet potato yield in the world has been doubled from 7.3 t/ha to 13.87 t/ha from 2001 to 2006 (Srinivas, 2009). According to Nwosini et al. (2017) a field experiment in the USA in Nashville was performed to evaluate yield performance of organic sweet potato varieties in various mulches which indicated that the average marketable yield was between 20 and 28 t/ha due to different mulching techniques and between 4 and 40 t/ha affected by the sweet potato variety. The big difference in yield between sweet potato grown in humus soil and sweet potato treated conventionally probably is related to the fact that the structure of soil which is a clay loam soil was too compact for the cultivation of sweet potato, a disadvantage which has been compensated by using humus soil as substrate while substituting soil.

The average sweet potato yield in the world has been doubled from 7.3 t/ha to 13.87 t/ha from 2001 to 2006 (Srinivas, 2009). According to Nwosini et al. (2017) a field experiment in the USA in Nashville was performed to evaluate yield performance of organic sweet potato varieties in various mulches which indicated that the average marketable yield was between 20 and 28 t/ha due to different mulching techniques and between 4 and 40 t/ha affected by the sweet potato variety. The big difference in yield between sweet potato grown in humus soil and sweet potato treated conventionally probably is related to the fact that the structure of soil which is a clay loam soil was too compact for the cultivation of sweet potato, a disadvantage which has been compensated by using humus soil as substrate while substituting soil.

The mean values of maximum load needed in the compression test ranged from 0.623 to 0.787 but there was no significant difference found for the different treatments (Table 2). No statistical significant differences were found also for maximum load value performed in the puncture test. Total nitrogen content varied from 0.192% for the control to 0.304% for the inorganic treatment but with no statistical significant difference ($P < 0.05$).

As shown in Table 2 there were no found correlations between yield parameters and physicochemical properties.

Table 2. Effect of fertilization on qualitative and quantitative parameters of sweet potato tubers

Treatment	Average total yield (t/ha)	Average marketable yield (t/ha)	Average tuber number per plant	Compression Max Load (kN)	Puncture Max Load (kN)	Total content of N (%)
Control	8.2 a	4.1 a	3.6 a	0.665 a	0.0443 a	0.192 a
NPK inorganic	9.9 a	3.2 a	4.0 a	0.623 a	0.0439 a	0.304 a
Humus Soil	35.6 b	24.3 b	6.4 b	0.787 a	0.0447 a	0.229 a

Table 3. Correlation coefficient between yield parameters and physicochemical properties. Marked correlations are significant at $p < 0,05000$ N=9

	Average total yield (t/ha)	Average marketable yield (t/ha)	Average tuber number per plant	Compression Max Load (kN)	Puncture Max Load (kN)	Total content of N (%)
Average total yield (t/ha)	1.0000 p= ---					
Average marketable yield (t/ha)	0.9772 p=0.000	1.0000 p= ---				
Average tuber number per plant	0.9157 p=0.001	0.8212 p=0.007	1.0000 p= ---			
Compression Max Load (kN)	0.6026 p=0.086	0.6078 p=0.083	0.4882 p=0.182	1.0000 p= ---		
Puncture Max Load (kN)	0.0932 p=0.812	0.1349 p=0.729	0.0519 p=0.894	-0.2091 p=0.589	1.0000 p= ---	
Total content of N (%)	-0.1087 p=0.781	-0.1045 p=0.0789	-0.1611 p=0.679	-0.4873 p=0.183	0.2906 p=0.448	1.0000 p= ---

To identify factors affecting the level of Average marketable yield multiple regression analysis was performed to build the regression equation as shown below:

Average marketable yield = 5.52202 + 1.3943*(Average total yield) – 0.4556*(Average tuber number per plant)			
St. error:	(2.24837)	(0.10954)	(0.10954)
P(level)	(0.049)	(0.000014)	(0.005949)
Std. Error of estimate: 1.3366 F (2.6) = 254.95			

The regression equation shows the existence of two predictors: The Average total yield which has a positive correlation relation to Average marketable yield and the Average tuber number per plant which has a negative correlation relation to Average marketable yield. This means that as the Average tuber number increases the Average marketable yield is affected into the opposite way.

CONCLUSIONS

Yield components as well as quality parameters of sweet potato tubers grown into biocyclic humus soil were determined in comparison with tubers grown conventionally.
Total and marketable yield as well as the tuber number per plant were significant higher using biocyclic humus soil (photos 1 and 2).

Photo 1. Sweet potato tubers
(inorganic fertilizer)

Photo 2. Sweet potato tubers
(humus soil)

Marketable yield in this study was 24.3 t/ha for biocyclic humus soil which is comparable to the average marketable yields of sweet potato grown in Greece 20 t/ha.
Though, other textural parameters and the total nitrogen content didn’t show any statistical difference. Because of the clay loam soil of the experimental site the conventional yield and plant development was not sufficient.
There is a potential for using humus soil as a growing substrate to compensate adverse growing conditions for certain crops like sweet potato in an inadequate soil environment.

This experiment shows us that it is worth developing further research to generate more knowledge about both, the usage of humus soil in agriculture and the characteristics of humus soil as such.

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DETERMINATION OF THREHSING PERFORMANS OF NEW DESING THRESHING UNIT FOR SAGE•

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Abstract

The Sage (Salvia officinalis), Lamiaceae family, most of the Mediterranean countries are spread. Sage is one of the countries with the most collected are up to 90 species of sage in Turkey. Salvia officinalis species will be used in the scope of the research. Sage plants usually start to flower from May. Harvest time is the end of budding or flowering head.

In this study, the threshing system performances required for Sage (Salvia officinalis) have been determined. The threshing efficiency, work efficiency, power requirement and specific energy consumption values of the threshing unit developed were determined. In order to determine the threshing performance of the prototype, experiments were made at 3 different moisture ranges, 3 different drum speed, 3 different drum-concave open and 3 different feeding rates. Each experiment was performed in 3 replicates. As a result, a total of 243 experiments were carried out for sage plant in the threshing experiments.

According to the study results, threshing efficiency for sage (Salvia officinalis) in the study have changed between 48.92% and 94.58%. Work efficiency of threshing unit has been changed between 1.43 kg/h and 11.87 kg/h. Power requirements and specific energy consumption of threshing unit have been determined as 0.202- 0.972 kW and 0.04-0.53 kWh/kg respectively.

***Key words**: sage (Salvia officinalis), threshing, design, aromatic plant.*

INTRODUCTION

The genus *Salvia* (sage) of the family *Lamiaceae* com-prises nearly 900 species spread widely throughout the world, which correspondingly display marked morpho-logical and genetic variations according to their geo-graphical origin (Chalchat et al., 1988). Sage (*Salvia officinalis* L.) is widely used as a savory food flavoring as the dried leaves or concentrated as the essential oil (Heath, 1978). Sage (*Salvia officinalis* L.) and its products, such as EOs and oleoresins have been widely used as food flavourings and health promoting agents (Perry et al., 1999; Perry et al., 2003). It is also used in cosmetics, perfume and medicine (Tucker et al., 1980; Chalchat et al., 1988).

Numerous studies have carried out on sage plants, but work on the mechanization of the sage plant is limited. Harvesting, threshing and cleaning medical aromatic plants is very important. The sage plants have been wildly collected and processed from the nature. In recent years, however, studies carried out on cultivating some species have provided higher productivity and quality production. During the processing of these plants such as threshing of the products some problems are emerged and lead to the yield loss and damages on products. In order to overcome this problem, it is necessary to know the operating conditions and performance values of the machines designed according to the plant. In the course of determining of threshing unit performance threshing efficiency, seed damage, unbroken capsule percentage, specific energy consumption, power requirement are used (Sudanjan et al., 2002).

In this study, the threshing parameters and the system performance such as threshing efficiency, work efficiency, power requirement and specific energy consumption of a threshing unit designed and developed for sage (*Salvia officinalis*) have been determined.

•This study is contained some of the TUBITAK-3501-National Young Researchers Career Development Program (CAREER) Project named "Determination of Some Threshing and Separation Parameters of Medical and Aromatic Plants and Development of Prototype (111O179)"

MATERIALS AND METHODS

The specific threshing unit designed for sage has been used during the performance experiments.
The threshing unit consists of 2 rasp bar type threshing cylinders, 2 gear motors, main frame of threshing unit, torque meter for power measurement, cylinders distance mechanism (Figure 1).
For this study, sage (*Salvia officinalis*) plants were harvested by hand from the experimental field in Suleyman Demirel University, Isparta, Turkey.

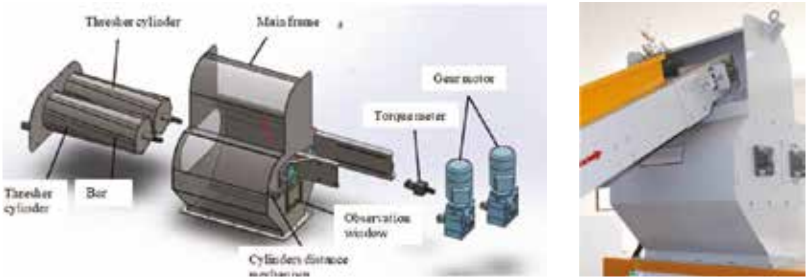

Figure 1. The threshing unit used in the experiments

In order to calculate the torque and the power consumed by the threshing unit a torque meter connected between the reducer electric motor and the drum shaft.
Sage (*Salvia officinalis*) plants have been dried in the rooms at 35°C after harvesting. In order to determine the threshing performance of system for sage plant the experiments have been conducted at 3 different moisture contents as 8.8%, 10.1% and 14.5% d.b.
The threshing cylinders (drum) speeds of the unit have been determined as 100, 250 and 400 rpm. Three different drum-concave open for the threshing unit have been adjusted as 15, 18 and 20 mm.
The product feeding rates have been determined as 190, 280, 570 kg/h. Each experiment has been performed in 3 replicates.
The operating parameters of the threshing unit for sage plant carried out at 3 different moisture contents have been given in Table 1.

Table 1. The operating parameters of the threshing unit for sage plant

Drum Speed (rpm)				Drum-Concave Opening (mm)		
Cylinder 1			Cylinder 2	1	2	3
1	2	3				
100	250	400	35	15	18	20

RESULTS AND DISCUSSIONS

Because of the experiments conducted depending on the moisture content of the sage, drum-concave opening, feeding rate and drum speed of the threshing unit, the threshing efficiency values have been range from 48.92% to 94.58% d.b.
The threshing unit efficiency for sage plant depending on the three different moisture contents are given in Figure 2.

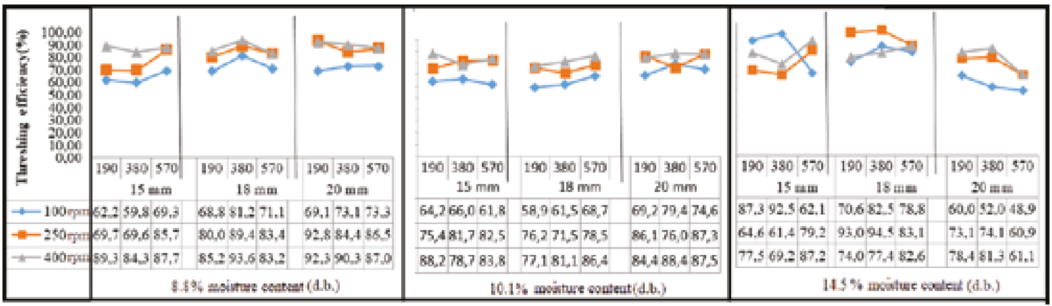

Figure 2. The effect of drum-concave opening × feeding rate× drum speed on the threshing efficiency at different moisture contents

As a result of the threshing experiments depending on the moisture content of the sage plant, the threshing efficiency have been decreased as the moisture content have been increased. The triple interaction of drum-concave opening × feeding rate × drum speed on the threshing efficiency at 8.8%, 10.1% and 14.5% d.b. moisture contents has have been found statistically significant ($p < 0.05$).

The highest threshing efficiency value has been found at 14.5% d.b. moisture content, 18 mm drum-concave opening, 250 rpm drum speed and 380 kg/h feeding rate. On the other hand, the lowest efficiency value has been found as. 48.92% d.b at the same moisture content, 20 mm drum-concave opening, 100 rpm drum speed and 570 kg/h feed rate.

According the result of the study conducted depending on the moisture content of the sage, drum-concave opening, feeding rate and drum speed of the threshing unit, the work efficiency values for sage plant was given in Figure 3.

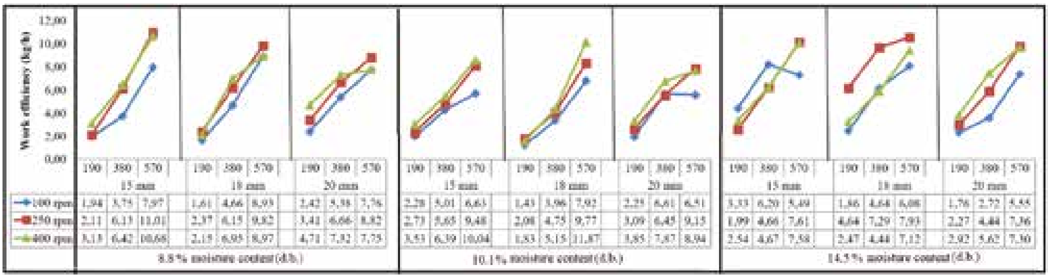

Figure 3. The effect of drum-concave opening × feeding rate × drum speed on the work efficiency at different moisture contents

According to the result of the study depending on the moisture content of the sage plant, the work efficiency has decreased with increasing moisture content. The triple interaction of drum-concave opening × feeding rate × drum speed on the work efficiency at 8.8%, 10.1% and 14.5% d.b. moisture contents has been found statistically significant ($p < 0.05$).

The work efficiency values changed between 1.43 kg/h to 11.87 kg/h. The highest and lowest work efficiency values have been found as 10.1% d.b. at same moisture content and as 18 mm at the same drum-concave opening. Whereas the lowest work efficiency value has been determined at 100 rpm drum speed and 190 kg/h feed rate, the highest values have been observed at 400 rpm drum speed and 570 kg/h feed rate.

Power requirement is one of the most important design parameters for threshing unit and it should be known for system performance. The power requirement values of the threshing units carried out at 3 different moisture contents are presented in Figure 4.

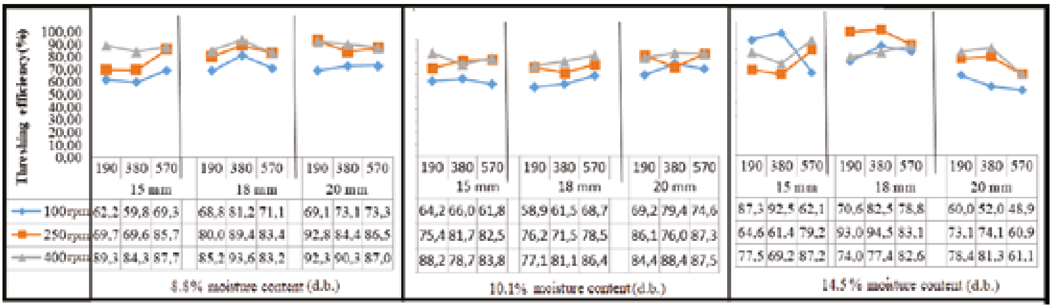

Figure 4. The effect of drum-concave opening × feeding rate × drum speed on the power requirement at different moisture contents

The power requirement values of the system according to the measured values depending on the moisture content of the sage, drum-concave opening, feeding rate and drum speed of the threshing unit, varied between 0.028 kg/h to 0.534 kg/h.

The triple interaction of drum-concave opening×feeding rate×drum speed on the power requirement at 8.8%, 10.1% and 14.5% d.b. moisture contents have been found statistically significant ($p<0.05$). Power requirement decreased as moisture content increased from 8.8% to 14.5% d.b.
While the lowest and highest power requirement of the threshing unit for sage plant have been determined as 0.028 and 0.534 kW at same moisture content of 8.8% d.b., respectively, the lowest and highest values have been obtained at 100 and 400 rpm drum speed, respectively. The drum-concave opening and feed rate values for minimum power requirement for threshing of sage plant is determined as 18 mm and 570 kg/h.
Specific energy consumption of sage plant must be known for the proper system performance of threshing unit.
The specific energy consumption values of the threshing unit carried out at three different moisture contents were varied from 0.028 to 0.534 kWh/kg and presented in Figure 5.

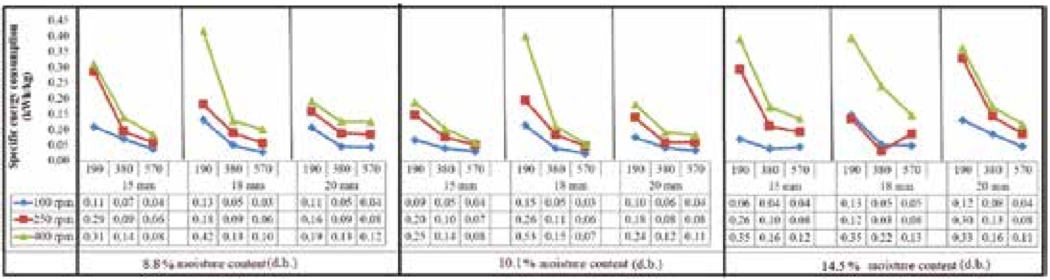

Figure 5. The effect of drum-concave opening × feeding rate × drum speed on the specific energy consumption at different moisture contents

The triple interaction of drum-concave opening×feeding rate×drum speed on the specific energy consumption at 8.8%, 10.1% and 14.5% d.b. moisture contents have been found statistically significant ($p < 0.05$).
The lowest specific energy consumption value of threshing unit for sage plant has been determined at 8.8% moisture content and the highest value has been observed at 10.1% d.b. While specific energy consumption of threshing unit for sage plant has been lowest at 18 mm drum-concave opening, 570 kg/h feeding rate and 100 rpm drum speed, it has been highest at 18 mm drum-concave opening, 190 kg/h feeding rate and 400 rpm drum speed.

CONLUSIONS

In this study, threshing unit performance values and working parameters have been determined for the sage plant, which has important cultivating areas for our country and Mediterranean region.
The threshing efficiency, work efficiency, power requirement and specific energy consumption values of threshing unit for the sage plant have been determined.
As a result, a total of 243 experiments have been carried out for the sage plant in the threshing experiments.
It is recommended to operate the threshing unit with 8.8% d.b. moisture content at 18 mm drum-concave opening, 380 kg/h feeding rate and 400 rpm drum speed in terms of threshing efficiency. On the other hand, it is suggested that for the high work efficiency, threshing unit can be performed with 10.1% d.b. moisture content, 18 mm drum-concave opening, 570 kg/h feeding rate and 400 rpm drum speed.
The working parameters must be selected as 8.8% moisture content, 18 mm drum-concave opening, 570 kg/h feeding rate and 100 rpm drum speed for the minimum power requirement and specific energy consumption.
The moisture content values for threshing of the sage plant are 8.8% and 10.1% d.b. The drum-concave opening can be selected as 18 mm and drum speed values is recommended as 400 rpm because of the sage leaf density.

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This study is included a part of the project TUBITAK-3501-National Young Researchers

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DETERMINATION OF THE EFFECTS OF GENOTYPE AND SOWING TIME ON PLANT GROWTH AND DEVELOPMENT IN CAMELINA [(*Camelina sativa* L. (Crantz)]

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Abstract

Appropriate sowing time and effects on agricultural and technological properties of sowing time is one of the most important issues. Therefore, this research has been carried out to determine plant growth and development pattern which effects on changes in agricultural characteristics which are important for growing and adaptation of genotype and sowing time in camelina. Research was carried out according to split-split plot design with three replications that four different sowing times (1 May 2017, 11 May 2017, 21 May 2017 and 31 May 2017) using two camelina genotypes (Ames-26665 and PI-304269).

As a result, it was determined that the longest plant length for Ames-26665 genotype was reached at the 1st sowing time, while the highest plant height at PI-304269 genotype was reached at the 4th sowing time. The maximum amount of total dry matter: Ames-26665 genotype was obtained at the first sowing time whereas PI-304269 genotype was obtained at the third sowing time. In addition, the maximal relative growth rate was obtained at the 1st sowing time in Ames-26665 genotype and 3rd sowing time in PI-304269 genotype.

Key words: *camelina, total dry matter, relative growth rate, sowing time.*

INTRODUCTION

Camelina (*Camelina sativa* L. Crantz), a member of the *Brassicaceae* family, has been attracting researchers and industry in recent years due to its adaptability, reasonable seed yield and good oil quality for many biological based applications. Camelina seed oil content ranges from about 35 to 45% and is suitable for biodiesel production (Fröhlich, Rice, 2005). It has recently been used as an excellent raw material for renewable aviation fuels (Shonnard et al., 2010). Another important characteristic of camelina plant is the low cost of agricultural input (Gesch, 2014).

Camelina plant is an alternative plant that can be planted in summer or winter. The most important factors determining the sowing time, which have different effects on plant growth and development, are the climate conditions of the region and the biology of the plant to be grown. Seed oil content and composition of fatty acids are affected by the plant variety and growth conditions of the plant. When sowing time is determined according to climate factors, the maximum expected yield can be obtained. For this reason, this research has been carried out in order to determine the effect of sowing time on the growth and development of camelina plant in Samsun ecological conditions and to determine the most suitable planting time.

MATERIALS AND METHODS

This research was carried out in Samsun Province, Alaçam District, Geyikkoşan location in the summer of 2017. The altitude of the experimental area is 4 meters. The soil characteristics of the experimental area are the following: clay, lime, salt-free, pH is slightly alkaline, soil organic structure is medium, phosphorus is medium and potassium is high.

When the climate is evaluated as the average of the growth season of the experimental area and the multiannual average, the data are the followig: the amount of rainfall is 708 mm in the growing season of 2016-2017, which is higher than the multiannual average (581 mm). The average temperature range is 15.5°C during the growing season of 2016-2017, which is higher than the multiannual average (14.5°C). The average relative humidity is 65.15% in the 2016-2017 growing season and is lower than the multiannual average (72.1%).

Two camelina genotypes (Ames-26665 and PI-304269) were used as plant material in the study. Field experiment was arranged in a split-split plot design with three replicates. Seed of the camelina cultivars were drilled at a rate of 600 plants per square meter 4 different times (1 May, 11 May, 21 May and 31 May). Plots were 3x1 m and comprised five rows with 3 m length at 20 cm spacing. During the experiment the necessary standard maintenance procedures were carried out. Diamonyum phosphate at a rate of 100 kg/ha was applied on June 13, 2017. Irrigation was carried out at field capacity on 1 July, 2017. Weeds were controlled by hand on 9 June, 2017 and 29 June, 2017.

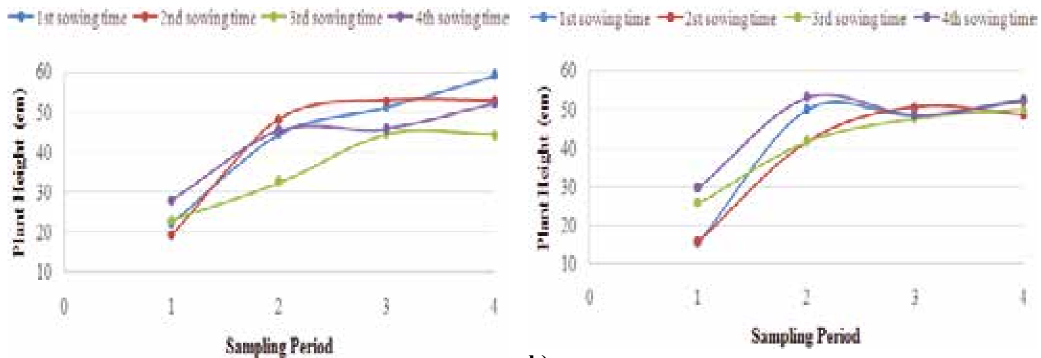
Plant samples comprising 2x50 cm lengths of adjacent centre rows were taken for growth analysis before flowering, during flowering period, after flowering and at harvesting time. After washing, ten plants were randomly selected for determination of stem length, leaf number and area and component dry weights. The plant was divided into root, stem and leaf parts and dried at 80°C for 48 hours and dry weight was determined. Statistical analysis of data was done by analysis of variance and significance assessed by calculation of least significant difference at P < 0±05 or lower.

RESULTS AND DISCUSSIONS

Plant Height

As a result of the research, it was determined as the statistically significant difference (P<0±05) between the plant height at the 1^{st} sampling time and the plant height at the 2^{nd}, 3^{rd} and 4^{th} sampling time. For Ames-26665 genotype, the longest plant height was obtained at the 4^{th} sowing time (28 cm) in the first sampling period. At the 2^{nd} and 3^{rd} sampling periods, the longest plant height was obtained at the 2^{nd} sowing time (48 and 52 cm, respectively). In the 4^{th} sampling period, the longest plant height was obtained at the 1^{st} sowing time (59 cm) (Figure 1a). For PI-304269 genotype: the longest plant height was obtained from the 4^{th} planting time (29 and 53 cm, respectively) in the 1^{st} and 2^{nd} sampling periods. In the third sampling period, the longest plant height was obtained from the second sowing time (50 cm). In the 4^{th} sampling periods, the longest plant height was determined as the first planting time (52.4 cm) (Figure 1b). In both genotypes, the plant height was found to increase faster from the outlet to the 2^{nd} sampling period. After the 2^{nd} sampling period, it was determined that the growth in the plant height continued to slow down and the harvest time reached maximum.

Although some previous studies in camelina have reported that the plant varies from 30 to 90 cm (Putnam et al., 1993; Mc Vay, Lamb, 2008; Francis, Worwick, 2009). Sadhuram et al. (2010) reported that the length of the plant is between 47.25-51.50 cm, Katar et al. (2012) between 47,88-71,22 cm, Koç (2014) is of 43.39 cm and Ayışığı (2015) varied between 30.1 and 36.6 cm. These reported results are consistent with the results obtained in this study and are in parallel.



a) b)

Figure 1. Variation of plant height depending on sampling periods: a) Ames-26665; b) PI-304269

Total Dry Matter

The increase of total dry matter was high at the early stages of plant growth but increased slowly with the advancement of plant age in all the genotypes. The cause of rapid increase of total dry matter at early stages was possibly due to emergence of considerable number of new leaves per plant and branhes per plant. Significantly higher total dry matter production was noticed in first sowing time in Ames-26665 genotypes compared to the other sowing times (Figure 2a). In addition, the lowest total dry matter accumulation was obtained in the 1st sampling period at the 2nd sowing time of the genotype PI-304269 (Figure 2b). Ahmed et al. (2005) stated that variety had significantly different effect on dry matter production. Pavlista et al. (2012) report that the total dry matter accumulation in the camelina is 0.096 gram per decare. These findings are parallel to the findings obtained from this research.

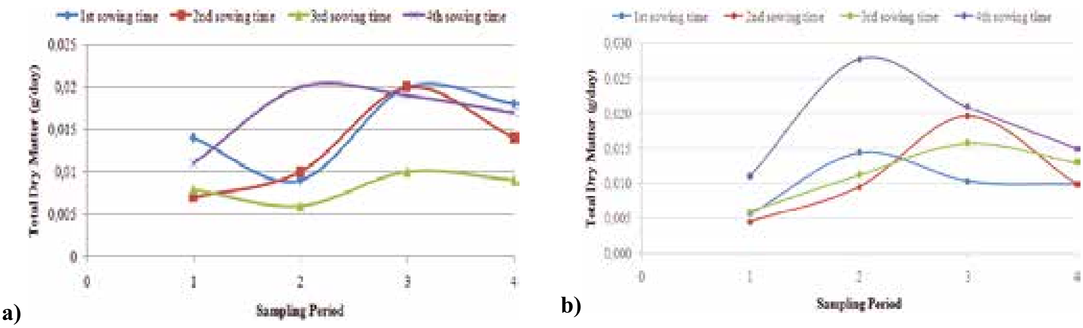


Figure 2. Variation of total dry matter depending on sampling periods: a) Ames-26665; b) PI-304269

Relative Growth Rate

As a result of the research, it was determined that there was a statistically significant difference between the 1st sampling period with the 2nd and 4th sampling periods, between the 3rd and 4th sampling periods and between the 2nd and 3nd sampling periods in terms of the relative growth rate. Relative growth rates increased in each sampling period. The greatest increase was observed in Ames-26665 genotype with a relative growth rate of 11.90% between 3rd and 4th sampling periods at the 1st sowing time. The lowest relative growth rate was found to be 0.50% growth rate between the 2nd and 3rd sampling periods in Ames-26665 genotype at the 3rd sowing time.

Higher relative growth rate was noticed at early stages of growth in all the varieties (Figure 3). These results are in agreement with the findings of Rahman (2004) in wheat. The reasons for higher relative growth rate values at early stages of growth is possibly due to the juvenility of the plants and less effects on accumulation of dry matter. Relative growth rate values decreased steadily with the advancement of plant age due to less dry matter accumulation. Decrease after flowering, capsule formation and filling of the grains are started. Finally, there is a decline in the rate of active growth during this period.

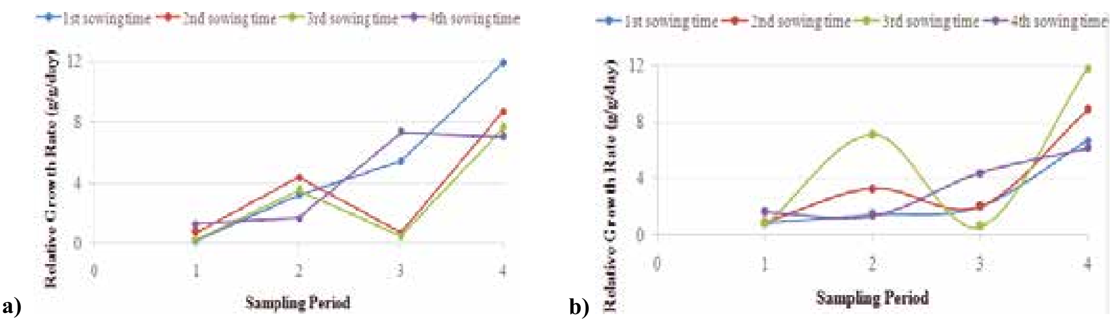


Figure 3. Variation of relative growth rate depending on sampling periods: a) Ames-26665; b) PI-304269

In the genotype Ames-26665, a statistically significant relationship was found between plant length and total dry matter amount (Figure 4a). It was determined that the plant length was the greatest at the first sowing time and the plant length decreased as the sowing time was delayed. It was also determined that when the plant height is the longest, the total amount of dry matter is the greatest, and that the total dry matter amount decreases due to the reduction in plant length. In PI-304269 genotype, the relation between plant length and total dry matter was negative and statistically insignificant (Figure 4b). The relationship between plant length and total dry matter arises differently in the two genotypes; the genetic characteristics are different and the two genotypes are affected differently from the environmental factors.

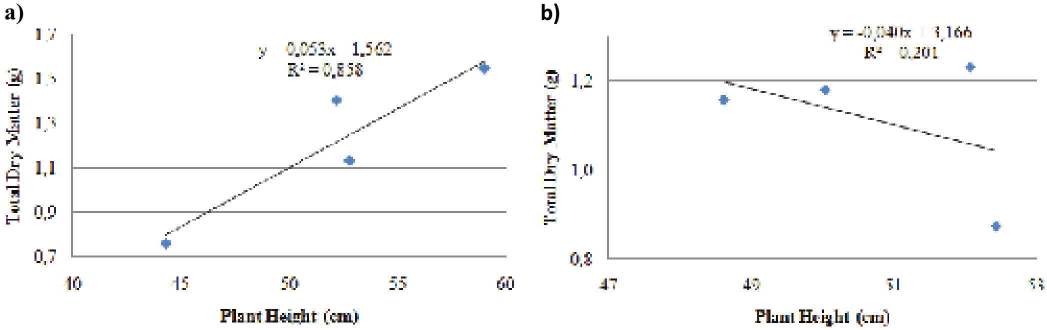


Figure 4. Relations between plant height (cm) and total dry matter (g): a) Ames-26665; b) PI-304269

CONCLUSIONS

As a result, it was determined that the longest plant length for Ames-26665 genotype was reached at the 1st sowing time, while the highest plant height at PI-304269 genotype was reached at the 4th sowing time. The maximum amount of total dry matter: Ames-26665 genotype was obtained at the first sowing time whereas PI-304269 genotype was obtained at the third sowing time. In addition, the maximal relative growth rate was obtained at the 1st sowing time in Ames-26665 genotype and 3rd sowing time in PI-304269 genotype.

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WEEDS MAPPING FROM WHEAT CROPS

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Abstract

The study of weed flora dynamics in agricultural crops is a complex and never-ending process which requires the continuous inventory and monitoring of problem weeds that cause the greatest damages on crops, according to which the most effective fighting strategies are established. The mapping action was carried out in fields cultivated with winter wheat in crop rotation and in monoculture crop from Ilfov, Giurgiu and Constanta counties. Weed files have been drawn up for each fields and they have shown the density, participation and constancy of each weed species. The analysis of the data shows that the dominant species in the fields where the wheat was cultivated in crop rotation system were: Veronica hederifolia, Veronica persica and Setaria pumila in Giurgiu county, V. hederifolia and V. persica in Ilfov county, V. hederifolia and Polygonum convolvulus in Constanta county. In monoculture system the number of weeds was higher and several species appeared, including weeds on the list of invasive species: Ambrosia artemisifolia and Coniza Canadensis. Regarding the distribution of weed species by botanical groups, the largest group was the one of the annual dicotyledonous and among the perennial dicotyledonous the most important species were Cirsium arvense and Convolvulus arvensis. From the group of annual monocotyledonous, the most important species were those of the genre Setaria and broad-leaved cockspur (Echinochloa crus-galli), species that invades all cultures in our country and is found in all areas and is also considered to be an invasive species. The dominant species in both crop rotation and monoculture system belong to the Veronica genre, exceeding an average density of 100 plants/m².

Key words: *dominant species, invasive species, mapping, weeds.*

INTRODUCTION

Damages caused by weeds to agricultural crops are diverse, large and irrecoverable by quantitative and qualitative reduction of the crop yields, disturbing maintenance works, increasing the cost price, toxic effects for humans and animals, favoring and transmitting insect pests and disease pathogens and extra costs due to the spreading of weeds as well, especially the invasive ones.(Sarpe et al., 1976; Chirila, 2001; Berca, 1996, 2004). Weeds infestation of agricultural crops is a dynamic process and represents the qualitative and quantitative expression of the influence of the soil seeds stock, the changes in plant cultivation technology and the weed control management. Changes in the dynamic of segetal flora are more evident if they are studied for a long time and on large agricultural surfaces, as they allow conclusions to be drawn regarding the occurrence of new weed species, the increasing importance of some of the existing ones, or the reduction in importance of others, or the disappearance of certain species from agricultural crops (Berca, 2004). Many studies, observations and conclusions were presented by many researchers (Sarpe et al., 1976; Berca, 1996, 2004; Budoi, Penescu, 1996; Ellenberg, 1998; Hanegraaf et al., 1998; Chirila, 2001; Lososova et al., 2004) showing that, as a general rule, the number of weed species decreases, so as the number of weeds/m², but that new species appear or the importance of the already existing ones changes by time. There have been concerns about the dynamics of segetal florain Romania, from the first synthesis information (Prodan, 1939) to the present, but especially between 1973-2000 when a nationwide study known as "weed mapping" was developed in order to establish the structure and the degree of qualitative and quantitative weed infestation at the level of common areas, villages, farms, plots and crops as well as to determine the changes in the segetal flora dynamics. Weed mapping is a necessary measure to determine the degree of infestation and the prognosis of weed emergence in all agricultural crops, and allows differential weed control proceedings to be applied in each plot, avoiding unnecessary application of the herbicides and to elaborate short and medium forecasts of the evolution of weed infestation. As a result, the paper presents the data obtained by mapping the wheat crop weeds in 3 counties from southeastern Romania.

MATERIALS AND METHODS

Weed mapping was conducted in 10 fields cultivated with winter wheat included in a suitable crop rotation and in 10 winter wheat cultivated fields in monoculture from the counties of Ilfov, Giurgiu and Constanta during the period 2015-2017.The numerical method of weeds assessments was used to which the phenophase and the average height of each plant were added. There were 8 samples made for each 1 hectare surface.

The winter wheat field in which the assessments were made were cultivated by private farmers according to their own technologies. For each field, landfills of weed infestation have been done and they present both general data on the location, the soil type, the previous crop and specific data on the density, participation and constancy of each weed species, class (monocotyledonous or dicotyledonous) as well the life period of each weed (ephemeral, annual, biennial, or perennial). For sampling and weeds inventory, the metric frame (1 m^2) was used. Each sampling point was established by going through the field in 1-2 diagonals according to the number of specified samples. At the final stage, the data obtained from all the analysed samples from one year, as well as from the three years in which determinations were made, were centralized. The data obtained on weed infestation of winter wheat crop cultivated in rotation crop system and in monoculture reveals the ecological characteristics of the weed flora existing at a certain moment in the studied fields. Finally, the weeds were distributed in density categories in descending order to identify the dominant species and the problem weeds, the results obtained by mapping being an important tool in taking the most appropriate weed control measures in a crop.

RESULTS AND DISCUSSIONS

Winter wheat crops in Romania are yearly weed-infested, especially in monoculture, with segetal species belonging to diverse, annual, biennial and perennial botanical families, some of them very difficult to fight against. Thus, in wheat monoculture crop from Constanta county 32 weed species with an average density of 263.4 plants/m^2 were identified (Table 1). Among the weeds found in a crop, we were concerned about only 5-10 species because they excel by the large number of individuals. These species are usually dominant and are important because they cause important losses on crops. From a practical point of view, these species have been called problem-weeds. These, due to their specific biology (Sagar, Hawson, 1994), by their density (Spitters, 1983; Wilson, Wright, 1990) and relative resistance to control measures, concern the farmers about the damages they can produce. Among the problem weeds, some are considered target species, according to which specific weed control decisions are taken in a crop. (Ionescu et al., 2016). In order to identify the problem weeds and establish the target species, the studied weeds were divided into 4 density categories. The first category, the most dense with 20-40 plants/m^2, included the species: *Veronica hederifolia* L., *Polygonum convolvulus* L., *Chenopodium album* L. and *Echinochloa crus-galli* (L.) Pal. Beauv. The second category had a density of 10-20 plants/m^2, being found the following species: *Convolvulus arvensis* L., *Cirsium arvense* (L.) Scop., *Solanum nigrum* L. and *Veronica persica* Poir. From the third group, with 5-10 plants/m^2, there were identified: *Sinapis arvensis* L., *Apera spica-venti* (L.) Pal. Beauv., *Setaria viridis* (L.) Pal. Beauv., *Matricaria inodora* L. and *Papaver rhoeas* L. The fourth group with 1-5 plants/m^2 was the largest, including 20 species, the most important being as follows: *Capsella bursa-pastoris* (L.) Medic., *Taraxacum officinale* Weber., *Lamium amplexicaule* L., *Cardaria draba* (L.) Desv., *Ambrosia artemisiifolia* L. etc.

Table 1. Weeds in wheat monoculture, Constanta county

Wheat Monoculture (2 years)			
Species	Growth stage/height (cm)	Average no./m^2	K (%)
Veronica hederifolia	A-B-C 6-10	40.5	100.0
Polygonum convolvulus	A 25-30	35.0	100.0
Chenopodium album	A 10-25	25.5	87.5
Echinochloa crus-galli	A 3-15-20	22.5	87.5
Cirsium arvense	A 12	12.5	62.5
Solanum nigrum	A 15-20	12.0	50.0
Veronica persica (I)	A-B-C 10-12	10.5	87.5
Convolvulus arvensis	A-B 25	10.2	50.0
Sinapis arvensis	A15	8.6	37.5
Apera spica-venti	A 15	8.5	37.5
Setaria viridis	A 8-20	8.4	50.0
Papaver rhoeas	A 10-25	8.0	37.5
Matricaria inodora	A-C 20-25	6.0	37.5
Pplygonum aviculare	A 10-15	5.0	25.0
Capsella bursa-pastoris	A 10	4.5	25.0
Taraxacum officinale	A-C 10-15	4.4	12.5
Lamium amplexicaule	A-B-C10-14	4.2	25.0
Cardaria draba	A 20	4.0	25.0
Ambrosia artemisiifolia (I)	A 10-15	3.5	12.5
Thlaspi arvense	A 25-30	3.5	12.5
Galeopsis tetrahit	A 10	3.2	12.5
Descurainia sophia	A 12-15	3.2	12.5
Anagalis arvensis	A 10	3.0	12.5
Bromus secalinus (I)	A 20-25	2.4	25.0
Raphanus raphanistrum	A 15-20-30	2.2	12.5
Sonchus arvensis	A 25	2.2	12.5
Stachys annua	A 12-15	2.0	12.5
Veronica arvensis	A10	2.0	25.0
Viola arvensis	A-B-C 6-10	2.0	12.5
Agroryuron repens	A 20-30-50	1.0	12.5
Sonchus oleraceus	A10-18	1.0	12.5
Cirsium vulgare	A15-20	1.0	12.5
Conyza Canadensis (I)	A20-40	1.0	12.5
TOTAL		**263.4**	

Legend
A-5-7 leaves unfolded/shoot; B-flower buds/ Gramineous-bootstage; C-Flowering
P% = Proportion in which each species was involved to general weed infestation
K% = Proportion in which each species was found in observation points
MP = perennial monocotyledonous; MA = annual monocotyledonous
DP = perennial dicotyledonous; DA = annual dicotyledonous; I = invasive species

Generally, the species that infest the wheat crops are part of the group of weeds that grow in the spring and systematically they are usually annual and perennial dicots (Anghel et al., 1972; Mortimer, 1996; Slonovoski et al., 2001). Regarding the distribution of weed species on botanical groups, in wheat monoculture from Constanta county predominated the annual dicots with 19 species (Table 2), which had an average density of 182.9 plants/m^2 and a rate of 69.3% in the general weed infestation process. When the wheat was included in an appropriate crop rotation, having had the winter oil rape as previous crop, only 14 species of weeds with an average density of 116 plants/m^2 were determined in Constanta County, of which 10 annual species are dicotyledonous, 1 perennial dicotyledonous species and 2 species of the annual monocotyledonous class (Figure 1 and Figure 2).

Table 2. Weeds in wheat rotation, Constanta county

Wheat Rotation (previous crop: winter oilseed rape)			
Species	Growth stage/ Height (cm)	Average no/m^2	K %
Veronica hederifolia	A- B-C 6-8	30.0	100.0
Polygonum convolvulus	A 15-20	24.0	87.5
Chenopodium album	A 15	14.0	62.5
Solanum nigrum	A 15	10.5	50.0
Echinochloa crus-galli	A 8-10	10.0	50.0
Veronica persica (I)	A- B-C 6-8	8.0	75.0
Sinapis arvensis	A 15	5.0	50.0
Cirsium arvense	A 10-15	5.0	37.5
Papaver roheas	A 12-15	2.5	25.0
Fumaria schleicheri	A 13	2.5	12.5
Setaria viridis	A12	1.5	12.5
Matricaria inodora	A-B 18-30	1.0	12.5
Hibiscus trionum	A15	1.0	12.5
Ranunculus sardous	A16	1,0	12.5
TOTAL		**116**	

In the wheat monoculture crop in Constanta county (Table 1), there were also identified four invasive species compared to wheat in crop rotation system where only one species was identified (*V. persica*) (Table 2).

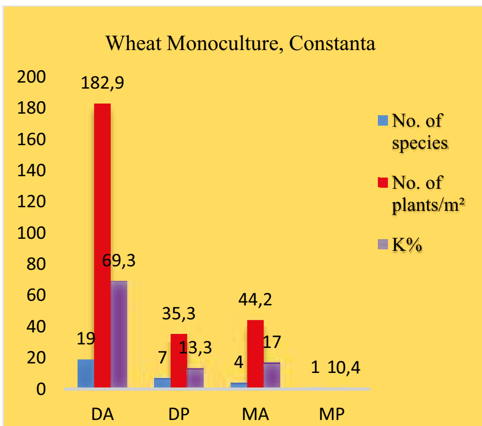

Figure1. Weeds distribution by botanical group in monoculture, Constanta

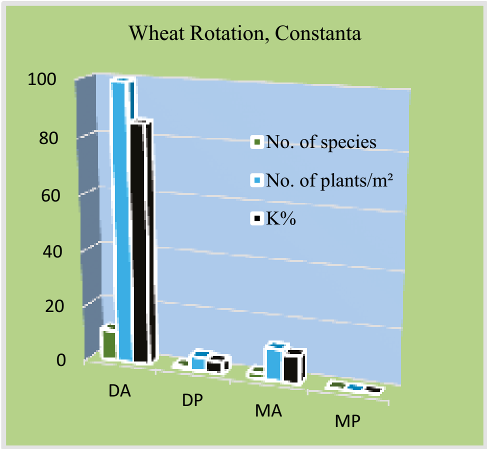

Figure 2. Weeds distribution by botanical group in crop rotation system, Constanta

In Ilfov County, due to the monoculture during 3 years, the degree of weed-infestation exceeded 304.6 plants/m^2 (Table 3), compared to the wheat included in rotation system, having corn as previous crop, at which the degree of weed-infestation was 116.4 plants/m^2 (Table 4).

Table 3. Weeds in wheat monoculture, Ilfov county

Wheat Monoculture (3 Years)			
Species	Growth stage Height (cm)	Average no./m^2	K %
Veronica hederifolia	A- B-C 5-8-18	48.0	100.0
Veronica persica (I)	A- B-C 6-16	37.0	100.0
Galium aparine	A 10-20	31.0	87.5
Convolvulus arvensis	A-B 15-20	28.5	75.0
Polygonum convolvulus	A 20-30	22.0	62.5
Cirsium arvense	A 10-20	20.4	50.0
Echinochloa crus-galli	A 5-10-20	20.0	75.0
Veronica arvensis	A-B-C 5-12	16.5	62.5
Matricaria inodora	A-B 12-20	12.0	37.5
Sttelaria media	A 15	10.0	25.0
Lamium amplexicaule	A-C 8-14	10.0	25.0
Chenopodium album	A 3-20	8.5	25.0
Papaver roheas	A-B 12-40	8.0	12.5
Setaria pumila	A 5-10	7.0	25.0
Capsella bursa-pastoris	A 12-15	6.5	12.5
Setaria viridis	A 3-15	5.2	25.0
Sinapis arvensis	A15	5.0	12.5
Matricaria chamomilla	A-B 12-24	3.5	12.5
Centaurea cyanus	A 30-35	2.5	12.5
Descurainia sophia	A 20	2.0	12.5
Lolium multiflorum (I)	A10-20	1.0	12.5
TOTAL		**304.6**	

Due to the high density of weeds per m^2, they were divided into 5 density categories.

The group with the highest density ranging

Table 4. Weeds in wheat rotation, Ilfov county

Wheat Rotation (Previous Crop: Corn)			
Species	Growth stage/ height (cm)	Average no./m^2	K %
Veronica hederifolia	A-B-C 6-8	28.0	100.0
Veronica persica (I)	A-B-C 8-10	20.8	87.5
Galium aparine	A15-20	14.4	50.0
Stellaria media	A20	10.2	25.0
Chenopodium album	A10-15	10.0	37.5
Convolvulus arvensis	A –B 20	8.0	25.0
Matricaria inodora	A-B 10-12	8.0	25.0
Capsela bursa-pastoris	A10	6.0	12.5
Cirsium arvense	A 6	3.0	12.5
Papaver rhoeas	A10-15	3.0	12.5
Lamium amplexicaule	A-B-C10-12	2.0	12.5
Apera spica-venti	A 25-30	2.0	12.5
Poa annua	A 20	1.0	12.5
TOTAL		**116.4**	

from 30 to 50 plants/m^2 was dominated by *Veronica* species along with *Galium aparine (*L.), followed by the group of species with a density ranging from 20 to 30 plants/m^2 consisting of: *C. arvense* (L.) Scop., *P. convolvulus* (L.), *C. arvensis* (L.) and *E. crus-galli* (L.) Pal. Beauv.

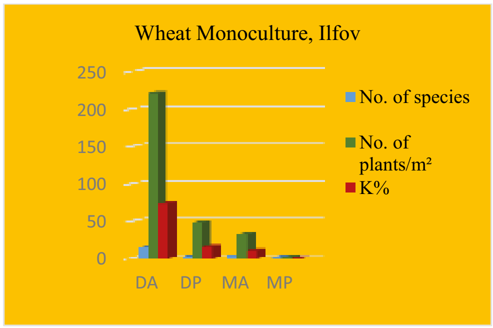

Figure 3. Weeds distribution by botanical group in monoculture, Ilfov

The third group, with 10-20 plants/m^2, included the following species: *Veronica arvensis* (L.), *M. inodora* (L.), *Stellaria media* (L.) Vill. and *L. amplexicaule* (L). The fourth group with 5-10 plants/m^2, had as more important species: *C. album* (L.), *P. rhoeas* (L.), *Setaria* species, *C. bursa-pastoris* (L.) *and S. arvensis* (L.). The fifth group with densities ranging from 1.0-5.0 plants/m^2, included only 4 species with a low density that do not raise any particular problems in weed control.

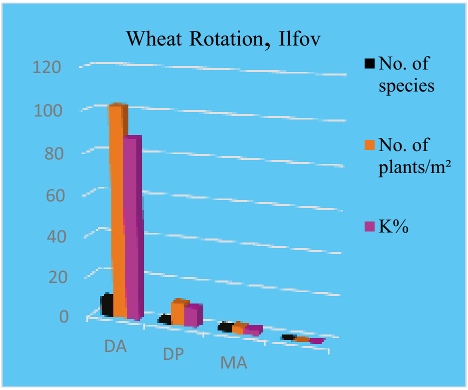

Figure 4. Weeds distribution by botanical group in crop rotation system, Ilfov

In the Ilfov county, in wheat rotation only 13 weed species with an average density of 116.4 plants/m^2 were recorded, of which 9 annual species were dicotyledonous, 2 perennial dicotyledonous species, 2 annual species monocotyledonous species and one was invasive species (Table 3 and Figure 4).

In wheat monoculture crop in Giurgiu county 34 weed species were identified, with an average density of 259.2 plants/m² (Table 6). These species were divided into four categories of density and data analysis showed that in both monoculture and crop rotation system, *Veronica* species prevailed over 40 plants/m^2, being accompanied by the perennial species *C. arvensis* (L.). In Giurgiu county, 2 invasive species *V. persica* Poir. and *Xanthium italicum* Moretti were identified in wheat monoculture, and only one species (*V. persica.)* was identified in the crop rotation system.

The analysis of the obtained data shows that the distribution of weed species by botanical groups was specific and demonstrates the high degree of weed-infestation in the wheat crop as well as it establishes the clear way of action to find the strategic solutions for weed control.

The dominant species from the fields where winter wheat was cultivated in crop rotation system were: *Veronica hederifolia* (L.), *Veronica persica* Poir. and *Setaria pumila* (Poir.) Roem.

In Giurgiu county, *V. hederifolia* and *V. persica* Poir. In Ilfov county, *V. hederifolia* (L.) *and Polygonum convolvulus* (L.) in Constanta county.

Table 6. Weeds in wheat monoculture, Giurgiu county

Wheat Rotation (2 Years)			
Species	Growth stage /height (cm)	Average no./m^2	K %
Veronica hederifolia	A-B-C 6-10	40,0	100.0
Veronica persica (I)	A-B-C 7-8-12	20.5	100.0
Convolvulus arvensis	A-B 20-25	20,0	87.5
Polygonum convolvulus	A 5-25	18,0	50.0
Setaria pumila	A 3-5	16,0	75.0
Echinochloa crus-galli	A 4-8	15.0	75.0
Matricaria inodora		10,0	50.0
Chenopodium album	A3	13.5	50.0
Galium aparine	A 10	12,6	50.0
Cirsium arvense	A10	12,0	50.0
Sinapis arvensis	A5-15	8.5	62.5
Papaver rhoeas	A-B-15-20	8.0	37.5
Lamium amplexicaule	A-C 8-12	7.4	25.0
Setaria viridis	A2-6	7.0	37.5
Capsella bursa-pastoris	A14	6.6	37.5
Centaurea cyyanus	A 12	4,5	25.0
Papaver roheas	A 10	4,5	25.0
Anagallis arvensis	A18-22	4,0	25.0
Matricaria chamomilla	A-B-C 16-20	3,5	25.0
Bifora radians	A14	3.2	25.0
Cardaria draba	A16	3.0	25.0
Erodium cicutarium	A22	2.8	12.5
Lithospermum arvense	A20	2.6	12.5
Myosotis arvensis	A6	2.2	12.5
Plantago major	A 20	2.2	12.5
Polygonum persicaria	A16-24	2.0	12.5
Senecio vulgaris	A10	1.8	25.0
Spergula arvensis	A14	1.4	25.0
Viola tricolor	A-B10	1.4	12.5
Amaranthus retroflexus	A 5-10	1.0	12.5
Sonchus arvensis	A 12	1.0	12.5
Helianthus annuus	A 20-25	1.0	12.5
Xanthium italicum (I)	A 20	1.0	12.5
Agropyron repens	A15-25	1.0	12.5
TOTAL		**259.2**	

Table 7. Weeds in wheat rotation, Giurgiu county

Wheat Rotation (previous crop: winter oilseed rape)			
Species	Growth stage/ height (cm)	Average no./m^2	K %
Veronica persica (I)	A-B-C 7-12	24.0	100.0
Veronica hederifolia	A-C 6-10	20.0	87.5
Convolvulus arvensis	A 5	15.5	75.0
Polygonum convolvulus	A 8	12.5	62.5
Galium aparine	A 10	10.0	50.0
Lamium amplexicaule	A-C 8	8.6	37.5
Chenopodium album	A10	8.2	37.5
Setaria pumila	A5	8.0	37.5
Sinapis arvensis	A7	4.5	25.0
Matricaria inodora	A 10	4.0	12.5
Capsella bursa-pastoris	A 12	3.0	12.5
Papaver rhoeas	A-B 16-18	3.0	12.5
Polygonum aviculare	A10	2.0	12.5
Thlaspi arvense	A10	1.5	12.5
Hibiscus trionum	A8-12	1.0	
TOTAL		**125.8**	

Regarding the distribution of weed species on botanical groups (Figure 5), the largest group was the one of the annual dicotyledonous and among the perennial dicotyledonous, the most important species were *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* (L.).
From the annual monocotyledonous group, the most important species were *Setaria* species and *Cockspur grass (Echinochloa crus-galli* (L.) Pal. Beauv.), a species that invades all crops in our country and is found in all areas and is also considered to be an invasive species (Dihoru, 2004).

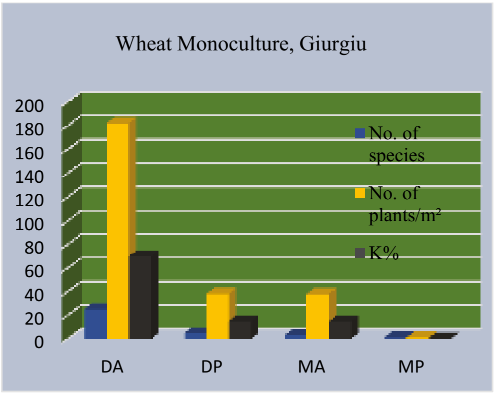

Figure 5. Weeds distribution by botanical group in monoculture, Giurgiu

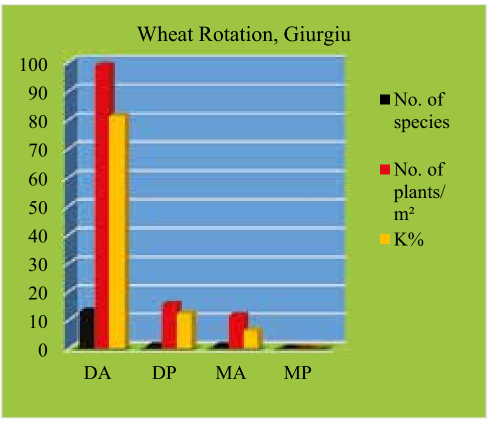

Figure 6. Weeds distribution by botanical group in crop rotation system, Giurgiu

The dominant species in both crop rotation system and monoculture belong to the genus *Veronica*, with 3 species having exceeded an average density of 100 plants/m^2 in the monoculture of winter wheat in Giurgiu county (Table 7).

CONCLUSIONS

Weed mapping is a necessary measure to determine the degree of infestation and the prognosis of weed emergence in agricultural crops.
By centralizing the data according to density and constancy and by separation by botanical groups, the weed control strategy is established. Wheat crops in Romania are infested every year, especially in monoculture with segetal species belonging to diverse, biennial and perennial botanical families, some of them being difficult to combat. By mapping, there were identified in Constanta county 32 species in wheat monoculture, of which 11 were problem weeds, 8 target species and 4 invasive species: *Veronica persica, Ambrosia artemisiifolia, Bromus secalinus* and *Conyza canadensis.*
In the winter wheat cultivated fields from Constanta county, there were determined 14 species, among 5 of them were problem weeds, 3 target species and 1 invasive species (*Veronica persica*).
In Ilfov county, in monoculture there were identified 21 species - 12 were problem weeds, 7 target species and 2 invasive species: *Veronica persica* and *Lolium multiflorum.*
In the wheat cultivated with wheat in Ilfov county, 13 species were determined, out of which 6 were problem weeds, 4 target species and 1 invasive species (*Veronica persica*).
In wheat crop rotation fields in Ilfov county we found 13 weed species - 6 problem weeds, 4 target species and 1 invasive species. (*Veronica persica*).
In the wheat monoculture in Giurgiu county, 34 species were identified, out of which 13 were problem weeds, 7 target species and 2 invasive species: *Veronica persica* and *Xanthium italicum.*
When the wheat crop was included in a rational crop rotation system in Giurgiu county, 15 species were identified, out of which 5 were problem weeds, 4 target species and 1 invasive species (*Veronica persica*).
The dominant species both in crop rotation system and monoculture belonged to the Veronica species, exceeding an average density of 100 plants/m^2 in wheat monoculture in Ilfov county.

By applying the crop rotation the degree of weed-infestation is significantly reduced compared to monoculture.

The distribution of weed species on botanical groups shows that in addition to the known annual weeds (annual and perennial dicotyledonous), monocotyledonous annuals (*Setaria* and *Echinochloa*), other species of the *Bromus, Avena* and *Lolium* species appear to be more damaging by time.

In order to achieve the control of these weed species in the winter wheat crop, it is recommended to apply 1-2 treatments with herbicides with the widest effectiveness, usually a treatment for dicotyledonous weeds and another separate treatment for monocotyledonous weeds.

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ANALYSIS OF SOME QUALITY COMPONENTS TO FEW AMPHIDIPLOID LINES OF WHEAT

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Abstract

Data presented in this experiment was conducted to evaluate some quality components of 10 amphidiploid lines of wheat comparative to their parental forms in order to identify new desirable genotypes or sources of variability. Two consecutive years (2015-2017) was carried out field trials in South Romania. Results for analyzed characters (protein and starch content, hectoliter mass, humidity, grain hardness and water absorbtion) were registered as average for experimentation period. Standard deviation and the coefficient of variability analysis for the mentioned characters revealed that for protein, there is significant variation. Most of the amphidiploid lines of wheat possessed higher levels of protein or starch comparative with parental forms. Highest protein content recorded E35-A line, while higher values of starch presented E19-A line. Also, higher values for hectoliter mass presented E1-A and E5-A lines while most of them registered same values as parental forms for grain hardness or smaller. As a conclusion, E35-A line combined successfully almost all characters and the present study demonstrated high quality characteristics of the amphidiploid lines of wheat in combination with other agronomic desirable characters.

***Key words**: quality components, wheat, amphidiploid lines, variability.*

INTRODUCTION

The increase of the global population and the continuous demand for food requires also an increase in the rate of progress for breeding gains in major food crops like wheat. Its popularity is caused by wide adaptation to diverse environmental conditions and presence of a strong storage protein complex. Genetic improvement of wheat has received considerable attention over the last years and it seems that doubled haploid technology succeeded to bring considerable increases in the efficiency of the breeding programs by returning fully homozygous lines from priority crosses within a single year comparative with conventional one.

Practical aspects of double haploids breeding have been demonstrated by the production of valuable breeding lines and new cultivars. Wheat genotypes developed by this method, both by anther-culture and maize induction systems have been released for cultivation in all continents. The first DH wheat cultivars with haploid origin from Romania were Faur and Glosa, registered in 2004, respectively 2005 (Săulescu et al., 2012). Glosa occupy around 35 percent from total cultivated area with wheat (http://agrointel.ro).

The hard work made by NARDI Fundulea researchers leaded to the creation of new material using DH technology in winter wheat. In modern research of genetics, haploid and DH populations are biological materials for a large area of domains: induction of mutations to haploid level, genomic and proteomic studies, introgression of useful genes from wild germplasm, genetic transformation to haploid level etc. (Verzea et al., 2007).

Research in crop genetics as concern improvements in quality it stared long time ago and plant breeding continues to make use of genetics and new molecular techniques in order to boost crop performance. There were made some effort to identify and utilize molecular markers related with the industrial quality characters such as grain hardness and protein content etc. (Giroux, Morris, 1998). Bread wheat should have high protein concentration and quality. Wheat grain protein concentration can range from 8 to 20% depending upon genotype and environment (Wieser, Kieffer, 2001).

The objective of this experiment was to evaluate the behavior of 10 amphidiploids lines as concern some quality components.

MATERIALS AND METHODS

Ten amphidiploids of winter wheat lines were cultivated in the experimental field from Agricultural Research and Development Station Caracal of University of Craiova (44^0 06' N, 24^0 21' E and 98 m altitude) in 2015-2017. Sowing was made in last decade of October and the harvest was made in the first 10 days of July. A completely randomized block design was used in three repetitions and standard agronomic practices were followed. These lines represent diverse agronomic types of DH wheat and under geographical origin of these biological material, these became from National Agricultural Research and Development Fundulea.

Quality parameters were analyzed as follows: protein and starch content, grains hardness and water absorbtion were determined by an Inframatic Analyzer. The determination of hectoliter mass was made using hectoliter balance. From the statistical parameters which characterizes well the variability, it were calculated the average values, standard deviation, limits of variation and variability coefficient. The obtained results for the variation amplitude for the two years are presented in tables and figures.

RESULTS AND DISCUSSIONS

Gil et al., 2011, and Hristov et al., 2010, says wheat variety is the most important factor which influences wheat quality parameters. So that careful selection and appropriate use of the currently available genotypes may be an effective way to improve wheat quality (Zhang et al., 2004b).

One of the most important wheat quality characteristics is the protein content of the grains. From the table 1 it can notice that the variation of the protein content presented values between 10.70% and 17.20% with an average of 14.69% for the 10 experienced amphidiploid lines while the parental forms limits ranged from 12.20% to 13.00% with the average of 12.60%.

The coefficients of variability, by their values, indicate a medium variability of the amphidiploid lines or small for parental forms in terms of protein content. The distribution of the registered values by the experimented winter wheat genotypes as concern protein content is shown in figure 1.

By comparing the variability parameter values for amphidiploid genotypes with those registered by parental forms, it can see that there is an important variability of genotypic nature for the protein content at lines level. The magnitude of this variability is illustrated by the wider variation amplitude for the 10 lines comparative with parental forms.

The useful portion of this variability is located between the average protein content and the upper limit. From this portion, valuable lines for protein content have been identified. Thus, in 2015-2016, among the lines with the highest protein content are: E35-A, E25-A, E24-A with protein content of 15.6%; 14.8% and respectively 14.6%, while in 2016-2017 high protein content (17.2%, 16.9% and 16.6%) were: E32-A, E24-A and E35-A lines. It highlights the E35-A line with high content in protein content in both years of experimentation (Figure 1).

A similar variability for this character was reported by Dobre et al. (2016) by studying 524 mutant/recombinant DH wheat genotypes and their parental forms. They obtained variability for protein content between 11 and 19.5%. Pasha et al. (2010) sustain that the major determinants of wheat quality are endosperm texture and protein content. Endosperm texture has a profound effect on milling, baking and end-use quality.

Starch is the most important cereal polysaccharide (Parker, Ring, 2001). In wheat, starch is the most abundant component present in the grain endosperm (Lineback, Rasper, 1988). It is also a useful component, constituting a source of calories for the body; all cereals have a high content (56-76%) of starch. The amount of starch is influenced by climatic conditions and nitrogen in the soil, while the quality of starch is determined by genetic factors. Table 1 show that the variation of starch content recorded values between 68.20% and 79.60% with an average of 71.24 for the 10 experimental lines and for the parental forms, the limits ranged from 70.60% to 74.90% with an average of 72.93%. The variability coefficients indicate a small variability both for amphidiploid lines and the parental forms with respect to the starch content. The distribution of the values obtained by the experimented autumn wheat genotypes for starch content is shown in Figure 2. By comparing the variability parameter values for amphidiploid genotypes with those of parental forms, it can see that at the level of the lines there is a rather small variability for the starch content. The variability coefficient values are 3.53% for the amphidiploid lines and 2.43% for the parental forms.

In 2015-2016, the lines with the highest starch content include: E19-A, E32-A, E24-A, with a starch content of 79.6%; 74.1, 72.8% , while in 2016-2017 starch high strand lines (72.2%, 71.6% and 70.5%, respectively) were: E17-A, E7-A and E6-A. It highlights E17-A line with high stability in starch content in both years of experimentation (Figure 2).

Table 1. Parameters of variability for protein and starch content in the amphidiploid lines and parental forms (2015-2017)

		Content of proteins (%)		Content of starch (%)	
Population		10 synthetic amphiploids	2 parental forms	10 synthetic amphiploids	2 parental forms
Average		14,69	12,60	71,24	72,93
Standard deviation		1,63	0,37	2,52	1,77
Amplitude of variation	Minim	10,70	12,20	68,20	70,60
	Maxim	17,20	13,00	79,60	74,90
Coefficient of variability		11,10	2,90	3,53	2,43

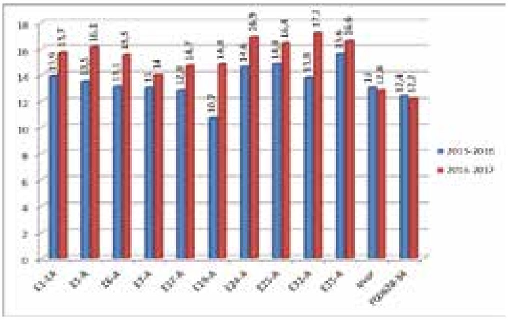
Figure 1. Variability of protein content (%)

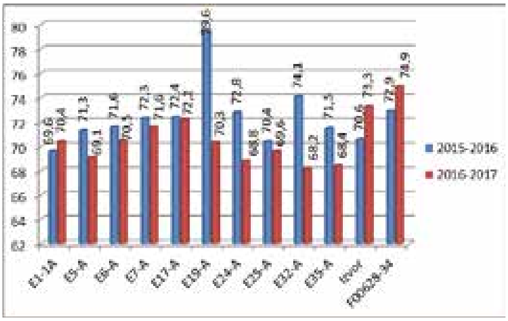

Figure 2. Variability of starch content (%)

Climate variability and extreme events can be also an important factor for yield quality. Protein content of wheat grain has been shown to respond to changes in the mean and variability of temperature and rainfall (Porter, Semenov, 2005).

High-temperature, extremes during grain filling can affect the protein content of wheat grain (Hurkman et al., 2009).

Both conventional and molecular improvement will be valid means of increasing wheat quality, which can be helped by efficient quality analysis technologies such as infrared spectrophotometric technology and molecular marker technology for gene analysis inflates quality. Quantitative location mapping (QTL) associated with grain characteristics and flour quality at wheat lines grown under contrasting environmental conditions could be a genetic potential for use of quality control (Sun et al., 2010; Zhao et al., 2010).

The hectoliter mass is another studied character. It is ranked as an element of appreciation of grain quality and a high hectoliter mass indicates a quality seed (Table 2).

Some lines have exceeded the average of experience for this character, while other lines have fallen below its value. The variability parameters ranged from 68.70 to 78.70 kg/hl. Thus, the average of the experience was 74.51 kg/hl with a standard deviation of 2.75. The coefficient of fairly small variability (3-4%) indicates a more narrow variability for this character. The data in the table 2 indicates variability in the volumetric weight from 76.90 to 80.70 kg/hl for parental forms.

These results on the variability of the hectoliter mass in the 10 amphidiploid lines, respectively the parental wheat forms, are inferior to those obtained by Dobre et al., 2016 in some mutant/recombinant wheat lines and similar to those reported by Girma Fana et al., 2012, which studied the response of quality parameters at different experimental locations of some durum wheat cultivars subjected to different rates of nitrogen and phosphorus fertilization.

The best time to harvest wheat is at full maturity, when grain humidity has reached 14-15%. Thus, the harvesters work without the loss of grains in the vegetal remains, the grains do not break, their cleaning can be easily adjusted and can be stored in good conditions, without the need for drying interventions. The optimal harvesting time of wheat is about 5-8 days (http://www.agrotechjdr.ro).

Wheat grain moisture is negatively correlated with the degree of protein storage (Johansson et al., 2008). Dry cereal moisture varies between 10-20%, with a normality threshold of about 14%; the importance of moisture is maximal in the storage phase.

The variability parameters for parental patterns ranged between 12.00 - 13.30%, with an average experience of 12.68% and a standard deviation of 0.54. The coefficient of variability is rather small (4.24%), indicating a reduced variability for this character. For experimental amphidiploid lines, the variability parameters for harvest humidity ranged between 10-12.20% and an average of 10.88% and a standard deviation of 0.67. The coefficient of variability showed small value of 6.16%. It is noted that the average amphidiploid line is lower than that of parental forms, which shows that these lines had very dry grains at the time of harvesting. Figure 4 presents the values obtained for this character and it can see that in the second year these are much lower than the first year and this is due to the much higher temperatures in June 2016 that forced the grain to mature.

Table 2. Parameters of variability for hectoliter mass and humidity at harvest for the amphidiploid lines and parental forms (2015 - 2017)

		Hectoliter mass (kg/hl)		Humidity (%)	
Population		10 synthetic amphiploids	2 parental forms	10 synthetic amphiploids	2 parental forms
Average		74,51	79,20	10,88	12,68
Standard deviation		2,75	1,62	0,67	0,54
Amplitude of variation	Minim	68,70	76,90	10,00	12,00
	Maxim	78,70	80,70	12,20	13,30
Coefficient of variability		3,69	2,04	6,16	4,24

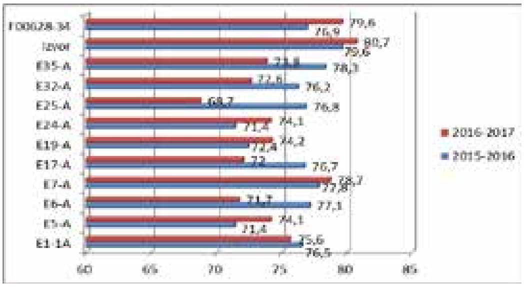

Figure 3. Variability of hectoliter mass (kg/hl)

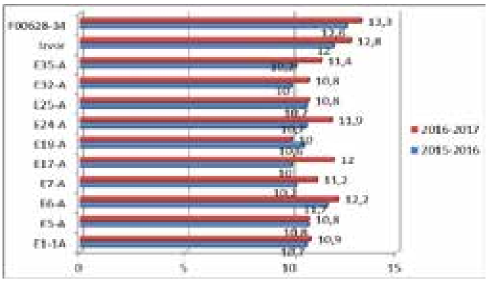

Figure 4. Variability of humidity at harvest (%)

Grain strength or hardness is a property that is indicative of how cereals resist handling, preparation and processing operations. Grain hardness is also an important criterion for assessing the energy demand for the grinder.

The variability parameters for parental patterns ranged from 80.00 to 83.00% with an average experience of 81.50% and a standard deviation of 1.29. The coefficient of variability is rather small (1.58%) which indicates a low variability for this character. For experimental amphidiploid lines, variability parameters for grain hardness ranged between 78.00 - 82.00% and an average of 80.00% and a standard deviation of 0.97. The coefficient of variability showed a small value of 1.58%. It is found that the average amphidiploid lines is lower than that of parental forms, which shows that these lines have less harsh grains compared to parental forms (Table 3). From the Figure 5 it is observed that in the agricultural year 2015-2016, the values obtained for this character are much lower than those of the agricultural year 2016-2017.

Hardness or softness of wheat grain is inherited and controlled by a single locus referred to as hardness (Ha), which comprises three genes (Pin a, Pin b and Gsp-1) within a region of about 82.000 bp (Chantret et al., 2005). The hard wheat possess the recessive or mutated form (ha) while soft wheat have the prominent or wild type form (Ha) (Gazza et al., 2005; Bhave, Morris., 2008a). The registered values for hardness obtained in this experience are similar to those obtained by Ai-Ling Choy et al., 2015, which analized 84 varieties of wheat in different areas as concern the relationship between milling yield and grain hardness. Pasha et al., 2010, said that wheat with high content of protein tend to be hard. In this way line E35-A combine well the mentioned characteristics.

Parameters of water absorption variability in the two years of experimentation for parental forms ranged between 60.30 - 64.20%, with an average experience of 61.90% and a standard deviation of 1.85. The coefficient of variability is quite small (2.99%) which indicates a reduced variability for this character. Amphidiploid lines variability for water absorption ranged between 59.40 - 63.30% and an average of 61.39% and a standard deviation of 1.23. Variability coefficient had a reduced value of 2.01%. It is found that the average of amphidiploid lines is lower than that of parental forms (table 3). Figure 6 shows that in the agricultural year 2015-2016, the values obtained for this character are higher than those of the agricultural year 2016-2017, with few exceptions.

Table 3. Parameters of variability for a grains hardness and water absorbtion
for the amphidiploid lines and parental forms (2015 - 2017)

		Grain hardness (%)		Water absorbtion (%)	
Population		10 synthetic amphiploids	2 parental forms	10 synthetic amphiploids	2 parental forms
Average		80,00	81,50	61,39	61,90
Standard deviation		0,97	1,29	1,23	1,85
Amplitude of variation	Minim	78,00	80,00	59,40	60,30
	Maxim	82,00	83,00	63,30	64,20
Coefficient of variability		1,58	1,58	2,01	2,99

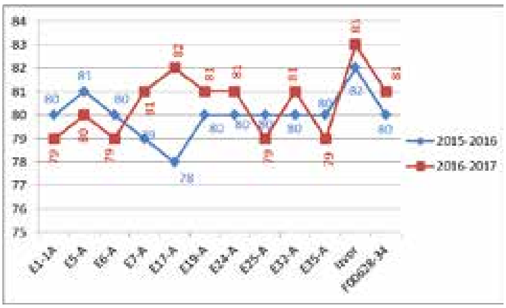

Figure 5. Variability of grains hardness (%)

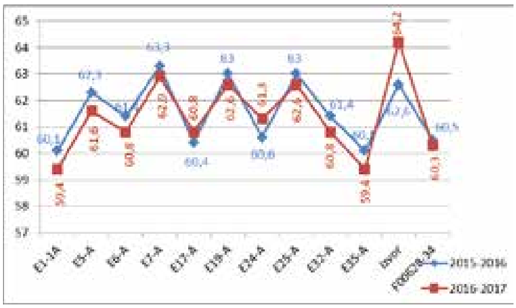

Figure 6. Variability of water absorbtion (%)

CONCLUSIONS

During this experiment some amphidiploids lines produced by NARDI Fundulea, which are actually synthetic common winter wheat, E35-A, A19-A, E1-A, E5-A, with higher values for some of the analyzed parameters and their potentially useful variation could represent valuable gene source which can be incorporated into future wheat breeding programs and other genetic studies.

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DRY MATTER YIELD AND DIGESTIBILITY OF SECOND CROP SILAGE CORN CULTIVATED AFTER CEREALS UNDER ESKISEHIR ECOLOGICAL CONDITIONS

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Abstract

Silage corn has a potential as the second crop in the irrigated lands of the Central Anatolia Region. The research was conducted in 2014 and 2015 years to determine dry matter yield and digestibility of the second crop silage corn after barley and wheat harvest and sown using direct and conventional methods with five cultivars (Ada, Cadiz, Donana, Sagunto, Sakarya). Dry matter yield did not vary significantly between years. Similarly, dry matter yield of the plant sown after wheat and barley harvest varied slightly but the difference was statistically nonsignificant. In conventional sowing, dry matter yield was higher (29.96 t ha^{-1}) than direct sowing (19.83 t ha^{-1}) and the highest dry matter production was estimated in Cadiz (27.23 t ha^{-1}) while the lowest was in Sakarya (22.30 t ha^{-1}). Digestible dry matter ratio was higher in 2014 (70.37%) than 2015 (68.17%). Silage corn that sown after barley harvest had slightly higher digestibility and it was 69.33% and 68.63% after barley and wheat harvest respectively. The digestible dry matter ratio was higher in directly sown plants (71.26%) than conventionally sown (67.28%). The highest and the lowest dry matter digestibility were estimated in Donana (71.02%) and Sakarya (67.42%) cultivars, respectively. Results indicated that sowing should be carried out conventionally using Donana cultivar after barley harvest. In the conditions of extended main crop harvest, Donana cultivar could be an alternative because direct sowing may be required if wheat harvest delayed and the cultivar could produce sufficient dry matter that has a high digestibility.

***Key words**: dry matter, yield, digestibility, second crop, silage corn.*

INTRODUCTION

Central Anatolia is a semi-arid region where annual rainfall is 350-450 mm. Main cultivation pattern of the region is cool-season cereals and sugar beet but the area has a potential for second crop cultivation after cereals harvest under irrigated conditions. This potential could be utilized by cultivating forage crops in the second crop season because forage production has to be increased to overcome good quality forage shortage in the area and animal breeding could be carried out economically only if forage costs reduced. Summer fallow (fallow cropland) period of the region is adequate for second crop production in terms of duration and temperature sum in irrigable areas but dry matter (DM) yield of annual forage crops are not satisfactory except some warm-season crops as silage corn and sorghum (Geren, Kavut, 2009; Carpici et al., 2010).

Silage corn is a proper plant for both second crop production in the region and silage making. High dry matter yield, fermentable carbohydrates, energy content and low buffering capacity make the plant a unique silage material (Coors et al., 1994; Barnes et al., 1995; Acikgoz, 2001). Due to data generated from TUIK (2016), silage corn has cultivated in 425,775 ha area of Turkey in the 2016 year. Second crop production of the plant is widening every year in the irrigable lands of Central Anatolia. Hence, cultivation conditions should be monitored and optimized in every region separately for silage production from the plant. In silage, dry matter production of the plant is crucial because feeding value decreases and silo loss increases if silage made with the low dry material (Acikgoz, 2001). Digestibility is another important factor that indicates silage quality. According to Di Mario et al. (2002) digestibility of corn inversely correlated with the development of the plant because starch accumulation increases in the grain as the plant maturity increase.

Residues of preceding cereals may affect positively or negatively the germination, growth, yield, and quality of second crop silage corn in terms of allelopathy (Dhima et al., 2005), changing soil moisture and nutrition characteristics (Ma et al., 2003) and reduced disease (Reid et al., 2001). Therefore, the effects of preceding cereals on dry matter yield and digestibility of second crop silage are non-negligible. For example, Singer and Cox (1998) reported that sowing second crop corn after wheat harvest increased the dry matter yield. In Central Anatolia, wheat and barley are commonly cultivated cereals and second crop silage corn cultivation usually carried out after cereals harvest in the region. Sometimes the period from cereal harvest to second crop sowing time is very limited due to climatic conditions and therefore, researchers investigate the benefits of soil cultivation before sowing second crop (Bayhan et al., 2006; Yalcin, Cakir, 2006; Zuber et al., 2017; Li et al., 2018).

Tillage practices are carried out especially for weed management before sowing second crop in the region because weeds are a major factor limiting crop production together with ecological conditions. In second crop cultivation, stubble burning method is applied also by local producers but living soil surface protects the soil from erosion, structural decomposition, and loss of moisture (Garibay et al., 1997). Energy conservation and erosion control are another factors which affect tillage practices. Thus, some researchers suggest minimum tillage practices (Koller, 2003; Yalcin, Cakir, 2006). Dam et al. (2005) stated that dry matter yield of corn decreased in direct sowing method considering conventional sowing but especially in conditions of elongated main crop harvest due to ecological reasons, producers tend to direct sowing without any plowing because overdue sowing significantly decreases dry matter yield of corn (Darby et al., 2002). Moreover, the effect of tillage practices could be different in various regions due to changes in ecology and production patterns. Therefore, proper tillage practices should be determined for different regions and necessity of direct or conventional sowing methods should be evaluated and in terms of dry matter yield and digestibility of second crop silage corn.

Characteristics of the plant are determined by the genetic potential of cultivar and therefore, cultivar selection is significant for productive cultivation. Dry matter yield and digestibility of the cultivars should be examined in different ecological conditions and cultural applications. This study was planned to determine the dry matter yield and digestible dry matter (DDM) of the second crop silage corn cultivars sown after barley and wheat harvest using direct and conventional sowing methods.

MATERIALS AND METHODS

This study was conducted in the experimental field of Eskisehir Osmangazi University, Faculty of Agriculture in 2014 and 2015 years. Climatic data of the experimental area belong to 2014 and 2015 years were given in Table 1. The area has a typical Central Anatolia climate which explained as continental climate and has cold and moist winter while summer is hot and dry. Generally, experimental area was observed to have higher averages of precipitation, humidity, and temperature than long-term averages (Table 1).

The experiment was laid out in randomized complete block design with three replications in split-split plot arrangement keeping two cereals (namely wheat and barley) in the main plot, two sowing methods (namely direct and conventional) in sub-plots and five silage corn cultivars in sub-subplots. Sowing carried out in direct and conventional methods after main crops harvest. In the direct method, sowing carried out directly into main crops stubble but in the conventional method, plowing carried out conventionally using rotator after main crop harvest and then sowing was done. Irrigation applied by controlling the requirement of the plants using sprinkler as three times in both years and weed control was carried out mechanically. Ada, Cadiz, Donana, Sagunto and Sakarya cultivars were used in the experiment.

Every plot was arranged in 8.4m^2 area (4 m x 2.1 m) and row spacing was 0.7 m. Phosphorus and nitrogen fertilization were applied as Acikgoz (2001) suggested and it was 80 kg ha^{-1}

and 160 kg ha^{-1} respectively. Nitrogen was applied by dividing into two parts. The first part was applied by sowing and the second when the plants reached the v3 stage (Ciampitti, Vyn, 2011).

Table 1. Climatic data of the experimental area for 2014 and 2015 years and long-term average

	Precipitation (mm)			Humidity (%)			Temperature (°C)		
Months	2014	2015	1970-2011	2014	2015	1970-2011	2014	2015	1970-2011
July	7.5	0.0	13.1	58.6	60.3	51.9	22.6	22.1	22.1
August	27.0	37.2	9.2	59.8	64.3	53.6	23.0	22.7	21.8
September	82.7	3.1	18.1	70.7	63.3	58.4	17.4	20.9	16.7
October	42.9	34.0	32.8	78.9	77.1	64.7	12.2	13.1	11.7

Harvest was carried out from the mid-line of the plots to prevent edge effect. Silage corn was harvested in dough stage considering the suggestion of Acikgoz (2001) and chopped into small pieces to accelerate the drying process. Plant parts were air-dried for 2 days in the open air in sunlight received storage and then oven-dried at 70ºC for 48 h until they reached to constant weight (Cook, Stubbendieck, 1986). Dry matter yield was estimated from the dry matter weight of harvested area and digestible dry matter content was determined from the formula given by Barnes et al. (1995). Dry sample was grounded in the mill to pass through a 2 mm sieve and acid detergent fiber (ADF) content of the samples was determined using the method suggested by Van Soest et al (1991) to estimate digestible dry matter.

$$Digestible\ Dry\ Matter = 88.9 - (0.779\ x\ ADF)$$

The data of dry matter yield and digestible dry matter ratio were analyzed using general linear model ANOVA in SAS 9.3 statistical software package (SAS, 2011) and the means were compared with TUKEY Multiple Range Test.

RESULTS AND DISCUSSIONS

Dry matter yield of second crop silage corn was not affected significantly from years and main crops but it was significantly varied between sowing methods (P<0.01) and among cultivars (p<0.05). Three-way and four-way interactions were significant due to ANOVA results except for year x main crop x tillage (Table 2). Dry matter yield was lower in direct sowing method (19.83 t ha^{-1}) compared to conventional sowing method (29.96 t ha^{-1}). Average DM yield was 24.95 t ha^{-1} and the highest DM yield was observed in Cadiz (27.23 t ha^{-1}) while Sakarya was the lowest (22.30 t ha^{-1}).

Climatic variation between years could greatly influence dry matter yield of corn (Dam et al., 2005) but years did not cause any significant differences on dry matter yield in our study (Table 2). DM yield of second crop silage corn, which sown after barley harvest was slightly lower than sown after wheat, but the changes caused by preceding cereals was statistically non-significant. Carter et al. (2002) also stated that forage yield of maize was not affected by preceding cereals.

Direct sowing greatly decreased dry matter yield compared to conventional sowing method and this difference was higher especially in the second year (Figure 1). Some researchers indicated the forage yield of second crop silage corn is not affected by different tillage practices including direct and conventional sowing methods (Carter et al., 2002; Korucu, Arslan, 2009) but Barut et al. (2011) stated that yield of corn was lower in direct sowing methods as similar with our findings. DM yield was generally similar in conventional sowing for both preceding crops but in direct sowing, it was lower after barley harvest (Figure 2). This could be due to the inhibitory effect of barley because residual of the preceding cereal remains at the surface in direct sowing as straw and surface water as precipitation or irrigation causes leakage of allelochemicals. Researchers indicated that barley has a strong allelopathic effect due to released allelochemicals from residual of the plant (Kremer, Ben-Hammouda, 2009; Bouhaouel et al., 2015). Moreover, Zhang et al. (2015) determined that corn intercropped with barley produced lower biomass than intercropped with wheat. Allelochemicals could inhibit the vegetative growth and photosynthesis area of the plant and therefore dry matter accumulation could decrease. In conventional sowing, plowing carried out for residue and weed management. Therefore, early growth of the plants could be better and consequently might cause a higher dry matter yield in conventional sowing method.

Table 2. Dry matter yield and digestibility of second crop silage corn

	DM Yield (t ha^{-1})	Digestible DM (%)
Year (Y)		
2014	21.54	70.37^{a}
2015	28.22	68.17^{b}
Main Crop (MC)		
Barley	23.25	69.93^{a}
Wheat	26.47	68.63^{b}
Sowing Method (T)		
Direct sowing	19.83^{b}	71.26^{a}
Conventional sowing	29.96^{a}	67.28^{b}
Cultivar (C)		
Ada	23.57^{ab}	70.40^{ab}
Cadiz	27.23^{a}	67.99^{c}
Donana	26.52^{ab}	71.02^{a}
Sagunto	25.16^{ab}	69.31^{b}
Sakarya	22.30^{b}	67.42^{c}
Mean	*24.95*	*69.23*
ANOVA		
Y	ns	**
MC	ns	**
T	**	**
C	*	**
Y*MC	ns	ns
Y*T	ns	ns
Y*C	ns	**
MC*T	*	**
MC*C	*	ns
T*C	ns	**
Y*MC*T	ns	ns
Y*MC*C	**	**
Y*T*C	*	**
MC*T*C	*	**
Y*MC*T*C	**	ns

(ns: nonsignificant *:P<0.05, **:P<0.01)

Cultivars significantly varied in terms of dry matter yield possibly due to their different genetic potentials. Cadiz had the highest DM yield but the difference among Donana (26.52 t ha^{-1}), Sagunto (25.16 t ha^{-1}) and Ada (23.57 t ha^{-1}) were not significant statistically. DM yield of cultivars was not significantly varied after barley harvest in the first year but Cadiz produced higher dry matter especially in 2015 that sown after barley harvest (Figure 3). Cultivars sown after wheat showed different responses in both years with regard to DM yield. Sagunto had the same value in both years while the DM yield of other cultivars increased in the second year after wheat harvest (Figure 3). After barley harvest, the highest DM yield was observed in Donana if sowing carried out conventionally and Cadiz had better result in direct sowing (Figure 2) but after wheat harvest, Cadiz produced more dry matter in both sowing methods.

Digestible dry matter (DDM) was significantly varied between years (P<0.01), main crops (P<0.01), sowing methods (P<0.01) and cultivars (P<0.01) and three-way interactions were significant except year x main crop x tillage (Table 2). DDM was higher in 2014 (70.37 %) than 2015 (68.17 %) and plants sown after barley harvest had higher DDM (69.93 %) than sown after wheat (68.63 %). Direct sowing method increased the DDM (71.26 %) compared to conventional sowing method (67.28 %). An average of 69.23 % DDM was estimated but there were significant variances among cultivars in terms of DDM and it was the highest in Donana (71.02 %) while it was the lowest in Sakarya (67.42 %) together with Cadiz (67.99 %).

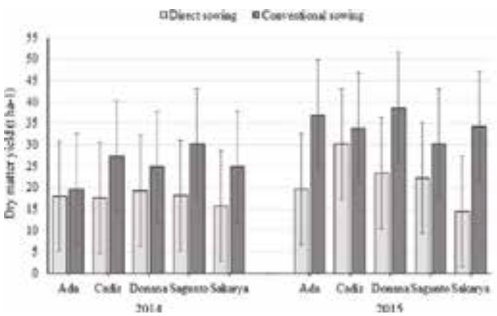

Figure 1. Dry matter yield of the cultivars sown using direct or conventional methods in 2014 and 2015

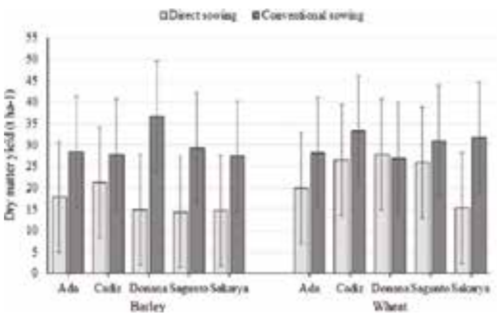

Figure 2. Dry matter yield of the cultivars sown after barley or wheat harvest using direct or conventional sowing methods

Digestible dry matter has a crucial importance in energy gain of ruminants (Hanley et al., 1992) and therefore determination of DDM ratio of the forages should be beneficial for sustainable animal production. The value was estimated from the ADF content of the plant and it can be considered that if any factor increases ADF value, it decreases DDM and total digestible nutrients (Abrams, 1988; Bingol et al., 2007). In the research, DDM value significantly varied among the experimental years. There were precipitation differences between the years but precipitation has low probability to cause variance because irrigation carried out during the research. Different temperature regime of the years possibly caused a significant variance of DDM and it decreased in 2015.

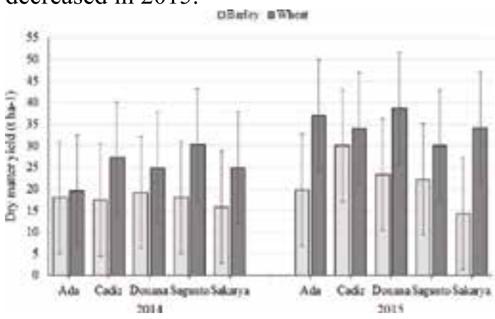

Figure 3. Dry matter yield of the cultivars sown after wheat or barley in 2014 and 2015

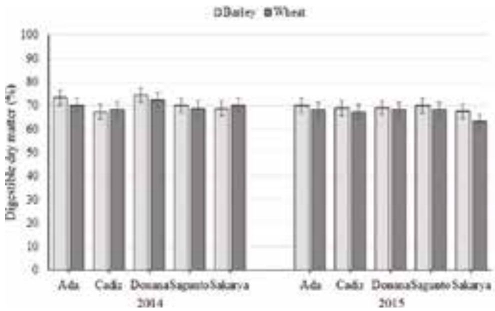

Figure 4. Digestible dry matter of the cultivars sown after wheat or barley in 2014 and 2015

Changes in DDM caused by main crops was 1.3% but this difference was statistically significant. Wheat increased the ADF value of the second crop silage corn and therefore DDM content of the plants sown after wheat harvest was lower than which sown after barley. The value was more regular in the second year especially in the plants sown after wheat harvest (Figure 4).

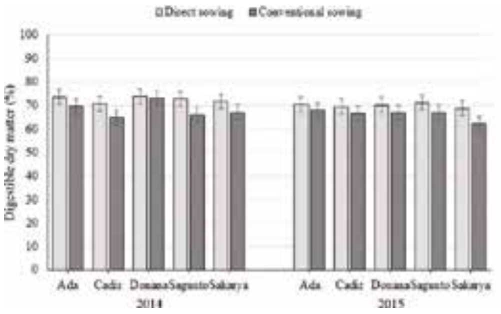

Figure 5. Digestible dry matter of the cultivars sown using direct or conventional methods in 2014 and 2015

Sowing methods significantly influenced the DDM of second crop silage corn and it was 3.98% higher in plants sown directly. Digestibility of corn decreases with advanced maturity (Weaver et al., 1978) and plants had about 10 days more vegetation period in conventional sowing compared to direct sowing method. Although plants reached to harvest maturity simultaneously, 10 days longer vegetation period could decrease DDM because as the maturity increase, starch accumulated in the ear and cell wall content increases, which cause a decrement of digestibility (Kruse et al., 2008). Therefore, digestibility was higher in directly sown plants.

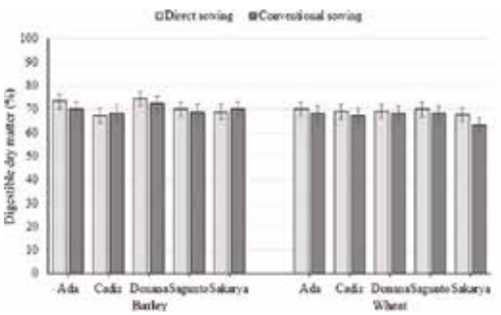

Figure 6. Digestible dry matter of the cultivars sown after barley or wheat harvest using direct or conventional sowing methods

Maturity of cultivars are generally different and these difference could cause changes in the ADF content as the cultivars varied significantly in terms of DDM. Average DDM was 69.23% and the highest value was estimated in Donana (71.02%) together with Ada (70.40%) while Sakarya was the lowest (67.42%). Adelana and Milbourn (1972) determined that the DDM of the grain maize in silage harvest stage was 62%. Wilson (1976) stated that DDM of leaves belongs *Panicum maximum* var. *trichoglum,* which is another C_4 plant, was between 77.3% and 65.0%. Bingol et al. (2007) estimated DDM in some vetch varieties between 58.58% and 69.20%. Second crop silage corn cultivars had higher DDM after barley harvest in both years but the difference was slight in Donana between main crops in 2014 (Figure 5). DDM of the cultivars were higher in direct sowing either the main crop was wheat or barley but Cadiz and Sakarya sown after barley harvest showed different responses to sowing methods than other cultivars and DDM was lower in these cultivars when sown directly after barley harvest (Figure 6), possibly due to different impact of barley to these cultivars.

CONCLUSIONS

Second crop silage corn cultivation could be carried out economically in irrigated lands of Central Anatolia but the effects of main crops on the DM and DDM which are the main factors for high-quality silage making should be determined. Moreover, proper sowing methods should be evaluated to conduct effective and economical production. In our research, higher dry matter yield was observed in the plants sown after wheat harvest and the conventional sowing provided the higher dry matter production of the second crop silage corn. Although DDM was slightly higher in direct sowing method, DM yield was prominently increased with the conventional sowing. Therefore, we recommend Cadiz cultivar after wheat harvest using conventional sowing method for second crop silage corn cultivation but if the main crop was barley, Donana cultivar can be selected to cultivate by sowing conventionally in the region.

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RESULTS REGARDING YIELD COMPONENTS AND GRAIN YIELD AT SUNFLOWER UNDER DIFFERENT ROW SPACING AND NITROGEN FERTILISATION CONDITIONS

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Abstract

The elements that are participating in the yield formation, respectively the so called yield components, and the yielding capacity are specific features to the cultivated variety, but these are influenced by the growing conditions which are given by the environmental and technological factors. From this perspective, the aim of this paper is to present the results regrading yield components and grain yield obtained at two sunflower hybrids cultivated at different row spacing and nitrogen fertilisation conditions. In this respect, a field experiment was performed in the climatic condition of 2016 on a reddish preluvosoil from South Romania. The field experiment consisted in sowing two sunflower hybrids under four nitrogen fertilisation conditions (N_0; N_{50}; N_{100}; N_{50+50}) and at two row spacing conditions (70 and 50 cm). The following yield components were determined: head diameter, number of grains per head, grain weight per head, and thousand grain weight (TGW). The grain yield was calculated at 9% moisture content and was expressed in kg.ha^{-1}. Under the specific experimental growing conditions, nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg.ha^{-1} increased the grain yield and the yield components, except TGW which tended to be more related to the sunflower hybrid. The average grain yield was higher for row spacing of 70 cm than that registered at row spacing of 50 cm. The two studied sunflower hybrid reacted in their own way to the nitrogen fertilisation conditions as well as to the row spacing conditions.

Key words: *sunflower, yield components, grain yield, nitrogen fertilisation, row spacing.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a temperate zone crop, which can perform well under a variety of climatic and soil conditions (Canavar et al., 2010), and fits well into various cropping systems (Pattanayak et al., 2016). Combining a high yield potential with a great adaptation capacity (Agele, 2003), this is one of the most important oil crop in the world, with a harvested area which increased from 6.6 million ha in 1961 to 26.2 million ha in 2016, according to FAO data (FAOSTAT database).

For Romania, sunflower represents the most important oil crop, with a harvested area which increased spectacularly over the last century, respectively from 672 ha in 1910, when sunflower appeared for the first time in statistics in Romania, to about one million ha per year in the period 2012-2017. In present, Romania has the highest sunflower harvested area in European Union. This is explaining by the favourable growing conditions Romania has for sunflower crop, with a real potential for developing further this crop by increasing first of all the yield (Ion et al., 2013).

The elements that are participating in yield formation, respectively the so called yield components, and the yielding capacity are specific features to the cultivated variety, but these are influenced by the growing conditions which are given by the environmental and technological factors.

The yield of a sunflower hybrid is conditioned by its capacity to use efficiently the environmental variables in different phenophases (Gonzáles et al., 2013). From this perspective, sunflower seed yield is a very complex trait and it is very dependent on environmental conditions (Jockovic et al., 2015).

Agronomic practices in addition to high yielding varieties are important items for higher productivity of the sunflower crop (Beg et al., 2007). Among the technological factors with an important contribution upon the values of the yield components and, finally, on the grain yield, there are counted the nitrogen fertilisation conditions, respectively the nitrogen rate and eventually its splitting in different moments of application, and row spacing which for a given plant population is determining the shape of the nutritional space.

Sunflower yield could be increased by fertilizer application, particularly nitrogen, which is an essential major mineral nutrient for plant growth and development (Ali et al., 2012), which has the greatest impact on seed size, leaf size and number of leaves, test weight and yield (Toosi, Azizi, 2014).

Nitrogen fertilisation and increasing of nitrogen rate increase the sunflower yield due to the increasing of the values of yielding components (Gholinezhad et al., 2009). However, sunflower yield response to increasing nitrogen rate varies with different environmental variables, including weather, soil type, residual fertility (especially nitrate), soil moisture, and cultivar (Killi, 2004).

Sunflower crop can be grown over different row spacing conditions. The experimental results show that different planting patterns sometimes produced higher yield, but not always (Zarea et al., 2005). Narrow rows make sunflower plants able to use in an efficient way the growing resources, respectively the solar radiation, water and nutrients, but this seems to be influenced by the specific environmental factors (Ion et al., 2015).

The aim of this paper is to present the results regrading yield components and grain yield obtained at two sunflower hybrids cultivated at different row spacing and nitrogen fertilisation conditions.

MATERIALS AND METHODS

Researches were performed in a field experiment under rainfed conditions in the year 2016. The field experiment was located within Moara Domnească Experimental Farm (44°29' N latitude and 26°15' E longitude), belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania.

The field experiment was performed on a reddish preluvosoil with the following characteristics: humus content between 2.2 and 2.8%; clay loam texture; pH between 6.2 and 6.6.

The year 2016 was characterised by the following climatic conditions for the period April-August: 20.1°C the average temperature; 284 mm the sum of rainfall. As multiannual average values, the climatic conditions for the period April-August are the following: 18.5°C the multiannual average temperature; 313.2 mm the multiannual average rainfall. By reporting the climatic conditions of the year 2016 to the multiannual average values in the studying area, it results that the year 2016 can be characterised as being warmer and drier than normal years.

The field experiment consisted in sowing two hybrids which are representative for sunflower cultivation in Romania (Performer and P64LE19) under the conditions of two row spacing (70 cm and 50 cm) and four nitrogen conditions (N_0 = 0 kg ha^{-1}; N_{50} = 50 kg ha^{-1}; N_{100} = 100 kg ha^{-1}; N_{50+50} = 50+50 kg ha^{-1}). Nitrogen fertilisation was performed immediately after sowing (1st of April 2016), except for the experimental variant N_{50+50}, which consisted in applying half of nitrogen rate (50 kg ha^{-1}) just after sowing and the other half of nitrogen rate (50 kg ha^{-1}) in the growth stage of six leaves, respectively the BBCH code 16 (27th of May 2016). The used fertilizer was the ammonium nitrite with a nitrogen content of 33.5%.

The field experiment was organised in split plots with 16 experimental variants (2 hybrids x 2 row spacing x 4 nitrogen fertilisations). Each experimental variant consisted in six lines with a length of 8 m.

The preceding crop was maize. The soil tillage consisted in ploughing performed on 30th of October 2015, one harrow work performed on 18th of March 2016 followed by one combinator work performed on 28th of March 2016. Sowing was performed by the help of a manual planter on 1st of April 2016, and the plant density was of 60,000 plants ha^{-1}. The weed control was performed by two manual hoeing.

In the stage of full maturity, the heads (capitulum) from one square meter (six heads) in each experimental variant were analysed. There were performed the following determinations of the yield components: head diameter (cm), number of grains (achenes) per head, grain weight per head (g), thousand grain weight - TGW (g). The grain moisture content was determined using a moisture analyser. Based on average grain weight per head, plant population and grain moisture content, the grain yield was calculated at 9% moisture content and was expressed in kg.ha^{-1}. Obtained data were statistically processed using the analysis of variance (ANOVA). The variants with the nitrogen rate of 0 kg ha^{-1} were taken as control variants for each row spacing and sunflower hybrid.

RESULTS AND DISCUSSIONS

Head diameter. In the studied area and in the climatic conditions of 2016, the head diameter registered values between 11.2 and 14.4 cm, according to sunflower hybrid, row spacing and nitrogen fertilisation conditions (Figure 1).

Nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased the head diameter, but statistically differences compared to N_0 variant were registered only for the variant N_{100} at Performer hybrid.

Splitting the nitrogen rate of 100 kg ha^{-1} in two applications of 50 kg ha^{-1} leaded to a further increase in the head diameter for Performer hybrid at row spacing of 70 cm, while for P64LE19 hybrid this leaded to an important decrease of the head diameter regardless the row spacing, respectively it determined the smallest values of the head diameter.

Regarding the row spacing, the average head diameter was 12.9 cm for the row spacing of 70 cm and 12.7 cm for the row spacing of 50 cm. As concerning the hybrid, the average head diameter was 13.1 cm for the Performer hybrid and 12.5 cm for P64LE19 hybrid.

Number of grains per head. In the studied area and the climatic conditions of 2016, the number of grains per head registered values between 674 and 1252, according to sunflower hybrid, row spacing and nitrogen fertilisation conditions (Figure 2).

Nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased the number of grains per head, with statistically differences compared to N_0 variant registered for the variant N_{100} as well as for the variant N_{50+50} regardless hybrid and row spacing. Moreover, statistically differences were registered also for variant N_{50}, but only for row spacing of 70 cm at P64LE19 hybrid and only for row spacing of 50 cm at Performer hybrid.

Splitting the nitrogen rate of 100 kg.ha^{-1} in two applications of 50 kg.ha^{-1} leaded to a decrease in the number of grains per head for Performer hybrid regardless the row spacing, while for P64LE19 hybrid this leaded to a decrease in the number of grains per head for row spacing of 70 cm and to an increase in the number of grains per head for row spacing of 50 cm.

Regarding the row spacing, the average number of grains per head was 965 for the row spacing of 70 cm and 936 for the row spacing of 50 cm. As concerning the hybrid, the average number of grains per head was 844 for the Performer hybrid and 1056 for P64LE19 hybrid.

Grain weight per head. In the studied area and the climatic conditions of 2016, the grain weight per head registered values between 41.5 and 72.7 g, according to sunflower hybrid, row spacing and nitrogen fertilisation conditions (Figure 3).

Nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased the grain weight per head, but statistically differences compared to N_0 variant were registered only for the variant N_{100} regardless row spacing and sunflower hybrid.

Splitting the nitrogen rate of 100 kg ha^{-1} in two applications of 50 kg ha^{-1} leaded to a further increase in the grain weight per head except for variant with row spacing of 50 cm at Performer hybrid.

Regarding the row spacing, the average grain weight per head was 59.9 g for the row spacing of 70 cm and 57.3 g for the row spacing of 50 cm. As concerning the hybrid, the average grain weight per head was 55.6 g for the Performer hybrid and 61.6 g for P64LE19 hybrid.

Thousand grain weight (TGW). In the studied area and the climatic conditions of 2016, TGW registered values between 54.1 and 74.6 g, according to sunflower hybrid, row spacing and nitrogen fertilisation conditions (Figure 4).

Nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased

TGW in the case of Performer hybrid and decreased TGW in the case of P64LE19 hybrid. There was registered a negative statistically difference compared to N_0 variant for P64LE19 sunflower hybrid at nitrogen rate of 50 kg ha^{-1} and row spacing of 70 cm.

Splitting the nitrogen rate of 100 kg ha^{-1} in two applications of 50 kg ha^{-1} leaded to a further increase in TGW in the case of variant with row spacing of 70 cm regardless the hybrid.

Regarding the row spacing, the average TGW was 62.1 g for the row spacing of 70 cm and 61.6 g for the row spacing of 50 cm. As concerning the hybrid, the average TGW was 65.2 g for the Performer hybrid and 58.5 g for P64LE19 hybrid.

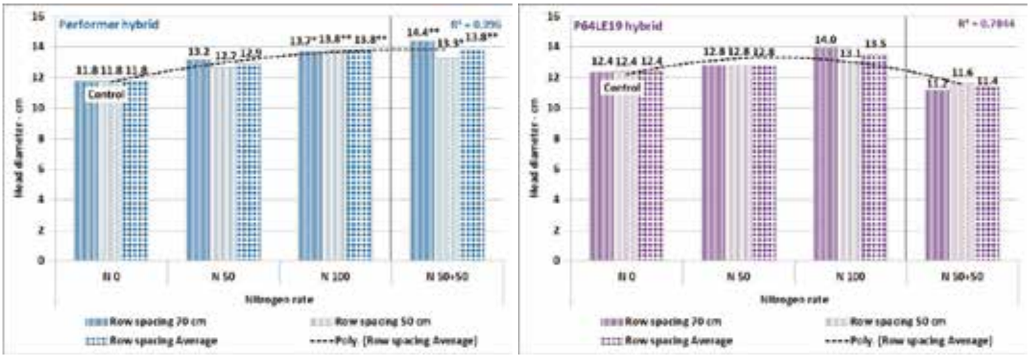

Figure 1. Head diameter at different sunflower hybrids, row spacing and nitrogen fertilisation conditions

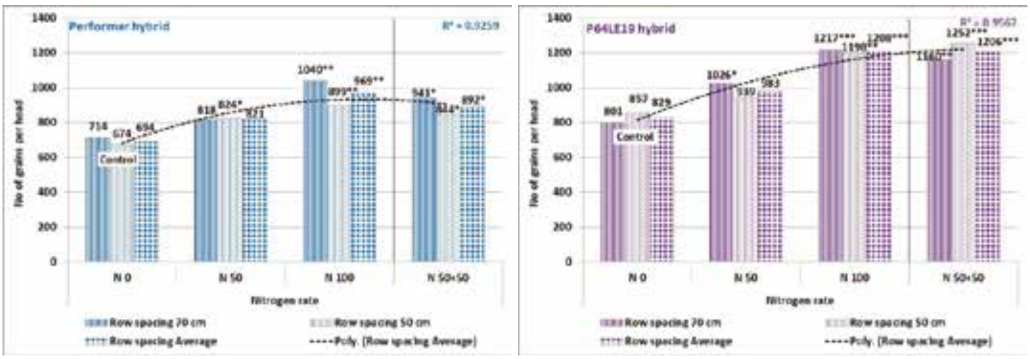

Figure 2. Number of grains per head at different sunflower hybrids, row spacing and nitrogen fertilisation conditions

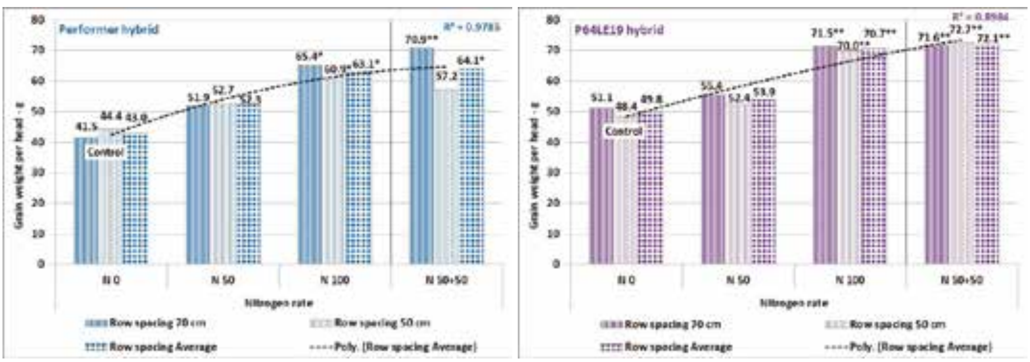

Figure 3. Grain weight per head at different sunflower hybrids, row spacing and nitrogen fertilisation conditions

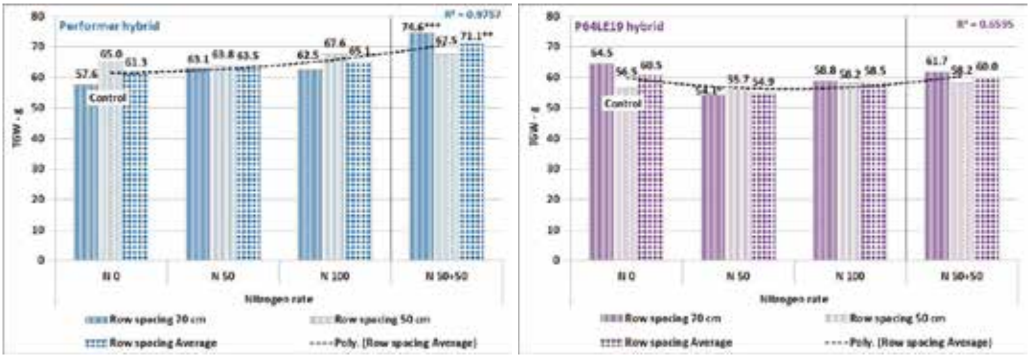

Figure 4. Thousand grain weight (TGW) at different sunflower hybrids, row spacing and nitrogen fertilisation conditions

Grain yield at 9% moisture content of grains. In the studied area and the climatic conditions of 2016, the grain yield registered values between 2585 and 4501 kg ha^{-1}, according to sunflower hybrid, row spacing and nitrogen fertilisation conditions (Figure 5).

Nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased the grain yield with statistically differences compared to N_0 variant registered for the variant N_{100} for both hybrids and row spacing (Figures 5 and 6.a).

Splitting the nitrogen rate of 100 kg ha^{-1} in two applications of 50 kg ha^{-1} leaded to a further increase in grain yield in the case of variant with row spacing of 70 cm at Performer hybrid and in the case of variant with row spacing of 50 cm at P64LE19 hybrid, this experimental variant giving the highest grain yield, with statistically differences compared to N_0 variant (Figure 5).

In average for the field experiment, the highest grain yield was registered in the case of splitting the nitrogen rate of 100 kg ha^{-1} in two applications of 50 kg ha^{-1} (Figure 6.a).

Regarding the row spacing, the average grain yield was 3710 kg ha^{-1} for the row spacing of 70 cm and 3556 kg ha^{-1} for the row spacing of 50 cm (Figure 6.b). As concerning the hybrid, the average grain yield was 3450 kg ha^{-1} for the Performer hybrid and 3816 kg ha^{-1} for P64LE19 hybrid (Figure 6.b).

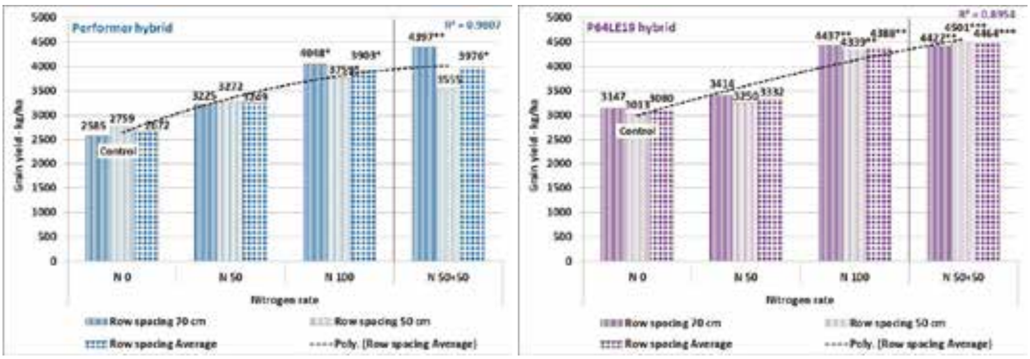

Figure 5. Grain yield at 9% moisture content of grains at different sunflower hybrids, row spacing and nitrogen fertilisation conditions

The values of the yield components and grain yield are smaller for the studied area because of the climatic conditions of the year 2016, which can be characterised from this point of view as being warmer and drier than normal years. Also, it has to be mentioned that the plant density of 60,000 plants ha^{-1} is at the upper threshold for the rainfed conditions in the studied area.

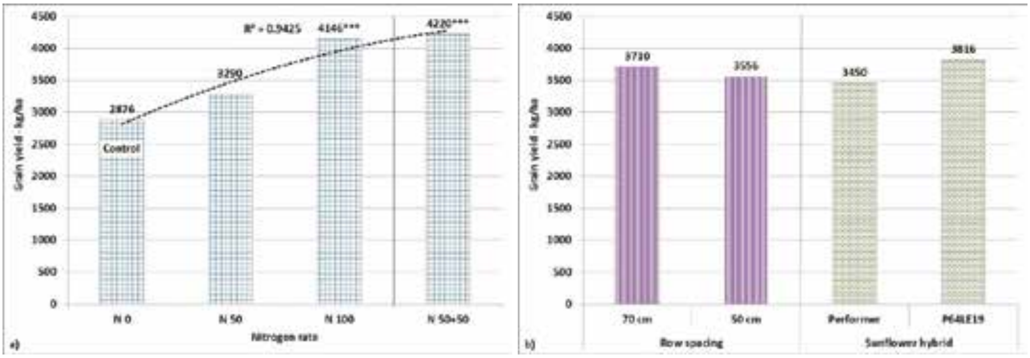

Figure 6. Average grain yield at different nitrogen fertilisation conditions (a)
and at different row spacing and sunflower hybrids (b)

Nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased the grain yield and the values of the following yield components: head diameter, number of grains per head, grain weight per head.

These findings are according to those find out of other authors. Thus, Escalante Estrada et al. (2015) found in a field experiment, which was performed at sunflower in Montecillo in Mexico, that grain yield, grain number and the capitulum area showed significant increases due to nitrogen application. Ali et al. (2012) found in a field experiment, which was performed at sunflower in the years 2010 and 2011 at Sargodha in Pakistan, that nitrogen application markedly enhanced growth and yield by affecting plant height, head diameter and thousand grains weight. Baig et al. (2016) found in a field experiment, which was performed at sunflower in the years 2012 and 2013 at Islamabad in Pakistan, that the number of achenes per head, thousand grains weight, and achene yield increased with increased nitrogen application. Similar results were found out at confectionary sunflower in field experiments performed in Turkey by Killi (2004) as well as by Day and Kolsarici (2016).

Unlike that Ali et al. (2012), Baig et al. (2016), Killi (2004), and Day and Kolsarici (2016) found that nitrogen increased the thousand grains weight (TGW), our results showed that nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg.ha^{-1} increased TGW in the case of Performer hybrid and decreased TGW in the case of P64LE19 hybrid. So, in our study, the sunflower hybrids registered different reactions of the yield components and grain yield, but specially concerning the TGW. Ali et al. (2012) found also that sunflower hybrids showed significant differences in case of head diameter.

In fact, each sunflower hybrid is defined by specific traits which determine a specific reaction to a certain growing factor. In our case, Performer hybrid is a mid-late hybrid while P64LE19 is a mid-early hybrid, each of them reacting in its own way to the nitrogen fertilisation conditions as well as to the row spacing conditions.

Despite the fact that Performer hybrid had larger heads than P64LE19 hybrid, the number of grains (achenes) per head was smaller for Performer hybrid than P64LE19 hybrid. That means that the P64LE19 hybrid had a better central area of head filled with grains. Also, the Performer hybrid had TGW with higher values than P64LE19 hybrid, which could be explained by the negative correlation between the number of grains per head and TGW value, which means that for smaller number of grains per head the TGW value is higher. But, despite the P64LE19 hybrid has smaller TGW values, because of the higher number of grains per head it registered higher grain weight per head, which leaded finally, for the same plant population, to a higher grain yield expressed in kg ha^{-1}.

The two experimented sunflower hybrids reacted quite different at splitting the nitrogen rate of 100 kg ha^{-1} in two applications, respectively 50 kg ha^{-1} nitrogen rate applied just after sowing and 50 kg ha^{-1} of nitrogen rate applied in the growth stage of six leaves. Splitting the nitrogen rate of 100 kg ha^{-1} in two applications leaded to the highest grain yields, but while the highest grain yield at Performer hybrid was registered at the row spacing of 70 cm, for the P64LE19 hybrid the highest grain yield was registered at the row spacing of 50 cm. In average for the whole field experiment, the highest grain yield was registered in the case of splitting the nitrogen rate of 100 kg ha^{-1} in two applications.

As concerning the row spacing, generally the yield component values were in favour of row spacing of 70 cm compared to row spacing of 50 cm, which leaded to an average grain yield higher for row spacing of 70 cm than that registered at row spacing of 50 cm. As in the case of the nitrogen fertilisation conditions, the two sunflower hybrids reacted in a specific way to the row spacing conditions.

In the same area, in the field experiments performed in 2013 and 2014, the results showed that the optimal row spacing depended on growing conditions, the highest yields being obtained at row spacing of 75 cm under favourable growing conditions and at narrow rows under less favourable growing conditions, especially at row spacing of 50 cm (Ion et al., 2015). Taking into account these findings and the results we have obtained in 2016, one makes us conclude that there are necessary further studied and experiments related to the effect of row spacing upon grain yield and yield components at sunflower.

There are authors who obtained higher grain yields at row spacing of 75 cm than at row spacing of 50 cm (Diepenbrock et al., 2001; Kazemeini et al., 2009) or obtained higher grain yields at row spacing of 60 cm than at row spacing of 45 or 30 cm (Nawaz et al., 2001), while other authors obtained higher grain yields at narrow rows (Zarea et al., 2005).

CONCLUSIONS

For the specific growing conditions from South Romania, in the climatic conditions of 2016 and on a reddish preluvosoil, nitrogen fertilisation and increasing the nitrogen rate from 50 to 100 kg ha^{-1} increased the grain yield and the values of the head diameter, number of grains per head, and grain weight per head, while TGW tended to be more related to the sunflower hybrid (TGW tended to increase at Performer hybrid and to decrease at P64LE19 hybrid).

Splitting the nitrogen rate of 100 kg ha^{-1} in two applications leaded to the highest grain yields for the two studied sunflower hybrids, but according to row spacing, respectively at row spacing of 70 cm for Performer hybrid and at row spacing of 50 cm for P64LE19 hybrid.

Generally, the yield component values were in favour of row spacing of 70 cm compared to row spacing of 50 cm, which leaded to an average grain yield higher for row spacing of 70 cm than that registered at row spacing of 50 cm, but these studies need further experiments for clarifications.

The two studied sunflower hybrids reacted in their own way to the nitrogen fertilisation conditions as well as to the row spacing conditions.

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VARIATION OF CURRENT MORPHOLOGICAL CHARACTERS IN WINTER BARLEY, *Hordeum vulgare* L.

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Abstract

Various studies on morphological, biochemical, molecular (Martinez, Esparza-Fostre, 1998) could be used in the progress of improvement in new barley varieties. Board genetic dowry and barley culture conditions usually result in the characteristic expression of plant morphology. In the case or winter barley, there were some differences in the two forms: two rows (distichon-D) and six rows (hexastichon-H). By comparison the average values were 62-76 cm (D-H) straw length, 10.5 cm/3.2 mm-11.1 cm/3.8 mm length/ diameter of the basal internode, 28-36 cm length of the apical internode, 7.5 cm/1.45 g-7.5 cm/ 2.51 mm length/ weight of the ear, 25/10.6 mm-56/11.3 mm number and length of the spikelets, 2.8-14.5 number of sterile spikelets in a ear, 15.9-14.7 cm awn length, 22.5/1.18 g- 41.9/2.05 number and grain weights in an spike (ear), 52.3-48.6 g thousand grains weight (TGW) and 8.63/3.96 mm-9.13/3.75 mm grain sizes (length/thickness/width). Between the main morphological caries studied, significant positive correlations were obtained, which demonstrates the good adaptability of the plant to the crop area, both two- and six- rows barley.

Key words: *ear, Hordeum vulgare, spikelet, straw, variation.*

INTRODUCTION

Having a long history, barley (*Hordeum vulgare* L., pro syn *H. sativum* Jessn., common barley, cereal barley, barley) is an important crop plant (Pickering, Johnson, 2005). *Barley* comes from Old English *baere* (similar to *farina- flour*) derived from adj. *barley*, originally from North Scotland. It is expressed as *beer* in the description of the specific form of the six-row barley (the one growing in the region). As a surface into the World, barley follows maize, rice and wheat, and is met in a multitude of conditions (Delhaize et al., 2004). The purpose of its cultivation is the production of feed (as vegetable material and grains), for the production of beer (*ale* beer from two-rows barley, and *lager* beer from six-rows barley), seed production and human food. The grains contain starch (59-65%), crude protein (9-11%), fats (2-3%), cellulose (4-7%), ash (2-3%) (Bîlteanu, Bărnaure, 1989), and a variety of other phenolic compounds (*caffeic acid*, *p-coumaric acid*), *ferulic acid*, *8,5-diferulic acid*, flavonoids (*catechin-7-O-glucozide, saponarin, catechin*, *procyanidin* B_3 and C_2, *prodelphinidin* B_3), and alkaliods (*hordenine*) (Friedrich, Galensa, 2002). In the course of time, the plant has evolved through various forms and characteristics (Strelchenko et al., 1999; Mayer et al., 2012). Thus, barley is considered one of the most diverse cereal from a genetic point of view, with winter and spring types, with two and six rows, with both grained and an-grained fruits. In fact, the genome consists of seven pairs of choromosomes (type 1H, 2H, 3H, 4H, 5H, 6H and 7H) with 2n = 14. The existence of a gene, *vrs1*, which is responsible for the transition from two-row to six-row forms of barley (Komantsuda et al., 1999; Tano et al., 2002) has recently been demonstrated. The inflorescence of the plant is an ear consisting of spikelets attached directly to the central shaft (rachis). At each node of the rachis are three spikelets colled triplets. They alternate in opposite places of the ear. Each spikelet has up to two glumes in the form of bracts, palea and lemmas. Depending on the variety, the lemma expands like a awn and it is rarely like a hood. In same varieties glumes of sterile grains can be awness. Varieties of awnless barley are not well known. When the palea and lemma stick to the grain, it gets dressed. At six-rows barley, the entire set of triplets is fertile and thus grows grains. The central grain is usually round and the lateral ones are slightly asymetrical. In barley two-rows only the central spikelet is fertile, while the two sides pieces are reduced, with rudimentary stamens, ovary and stigma. Due to the fact that the two collars spikelets are sterile and only the central grain is formed, the ear has the flat aspect. Each ear can produce 16-30 grains in the form of two-rows and 25-60 grains in the form of six-rows. The plant generally forms stems with heights between 60 and 120 cm, and their appearance in the crop is characteristic (Figure 1 and Figure 2).

The research carried out to determine the variation of plant characters of the two barley forms consisted of: i) the strain by the total length of the straw, the length of the apical internode, the length and thickness of the basal internode; ii) the length and weight of the ear; iii) the number and length of spikelets, the number of sterile spikelets, awn legth; iv) the number of grains, their weight, the thousand grain weight (TGW) and the size of grains.

Figure 1. Two-rows barley plants, 'Trasco' variety

Figure 2. Six-rows barley plants, 'Sistem' variety

MATERIALS AND METHODS

The variants have been cultivated over the past two years with two-row barley 'Trasco' variety and six-row barley 'Sistem' variety, like winter forms. The experience set up by the block method has the variants of 25 m^2 in 4 replicates. The crop technology used was the one recommended by the resort. At the full maturity, 25 plants/stems from each replicates (total 100 stems) were randomply picked and brought to the laboratory. The 100 strains were measured and determined the total length of the straw, the length and thickness of the down internode, the length of the apical internode, the length and weight of the ears, the number of spikelets in the ear and their length, the awn length, the number of grains from ear and their weight, thousand grains weight (TGW), the size of grains: the length and thickness.

The morphological characters obtained were analyzed by the histogram method (frequency polygons, %, FP). In their expression were used the class ranges established according to the specific values obtained. The study revealed several aspects, namely: i) the modal values (with the highest frequencies); ii) the limits of the variability ranges of the studied characters; iii) the specificity of each character of the barley ecotypes in the analyzed area. Graphs have a comparative character between the two forms of autumn barley, with 2-rows and 4-rows of grains in each ear.

Between the analyzed characters the correlations were established, with the help of which they were able to observe their tendencies within the studied ecotypes.

The Excel program was used to express the values. The significance of the correlation coefficients obtained was compared to the r_{max} values (Erna Weber, 1961) for the 5%, 1% and 0.1% levels of transgression probabilities.

In the statistical calculation of the all values obtained we used variance analysis (Anova test) on the variation rows.

Statistical parameters were calculated using the formulas:

$\bar{a} = \frac{\Sigma x}{n}$, where: ā = media of determinations,

x = the values;

S^2 (variance) $= \frac{1}{n-1}\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]$, S (standard error) = $\sqrt{S^2}$, and S % (variation coefficient) = $\frac{S}{\bar{a}}100$.

RESULTS AND DISCUTIONS

Variability of barley straw sizes. The stalk/straw of barley consists of several internodes (usually 5-7) with increasing lengths to the ear. Generally, the straw has lengths between 50 (60) cm and 100 cm. At harvest maturity, the plants exibit heights considered modest due to the necessarily manifested appearance. The measurements showed that the straw of two-row barley was between 49 and 80 cm. The high frequency had straws of 61-64 cm (29%), followed by 57-60 cm (27%). Smaller and larger lengths togheter had 10% of the strains, the other being intermediate (Figure 3). In six-row barley the straw ranged from 61 to 92 cm, with a higher frequency between 73-80 cm (26% at 73-76 cm and 26% at 77-80 cm). The graph shows the obvious difference between straw lengths/stems between the two winter forms. The internode under the ear (apical) is usually the longest. Thus, in the two-row barley it was between 17 and 41 cm, and in the six-row barley between 23 and 47 cm. The highest frequencies were 29-32 cm (32% in two-row and 35-38 cm (29%) in six-row (Figure 4). The internode at the base (considered the third at the top), being much shorter, had dimensions between 7 and 15 cm in both forms. However, the higher frequency was 10-11 cm (30%) for two-row and 11-12 cm (28%) for six-row (Figure 5). The diameter of the base internode was instead different. At the two-row it ranged from 2.1 to 4.4 mm, with a higher frequency at 3.0-3.2 mm (55%). Six-row diameter of this internode was 3.0-5.0 mm, with high frequency at 3.9-4.1 mm (39%) (Figure 6).

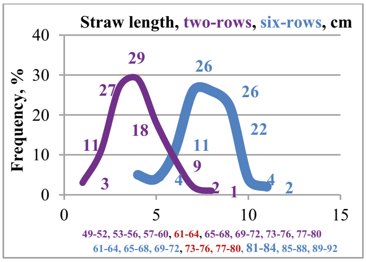

Figure 3. Frequency of straw lengths, cm

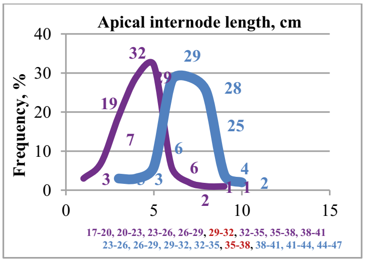

Figure 4. Frequency of apical internode lengths, cm

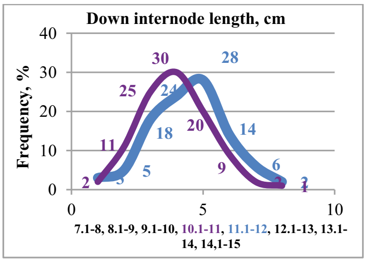

Figure 5. Frequency of down internode lengths, cm

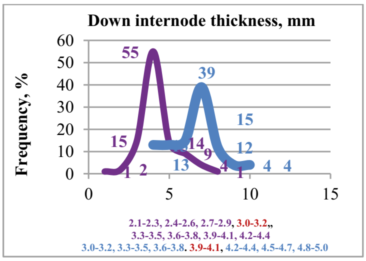

Figure 6. Frequency of down internode thickness, mm

Variability of barley ear/spikes. The appearance and dimensions of the barley spike are characteristic. Thus, its length was between 5 and 10 cm. In the two-row, the lengths of 7.1-8 cm (49%) were dominated, while in six-row the highest values were at 6.1-7 cm (30%), but with high values close to 8.1-9 cm (29-26%) (Figure 7). The awns that the spikelets form have the obvious characteristic length. Between the two forms were found some differences in length. Thus, the two-row awns ranged from 12 to 21 cm, with a maximum of 15.7-17.4 cm (46%), while the six-row were between 8.5 and 22.8 cm, with a maximum of 13.9-15.6 cm

(34%). Given the modal value of the two forms, it was found that the two-row were slightly longer than the six-row type (Figure 8). The number of spikelets in a ear/spike is different due to the configuration of the two forms. Thus, in two-row ear they formed between 15 and 34 spikelets. Higher frequency was obtained at 25-29 spikelets (58%). Barley six-row ranged from 35 to over 70 spikelets. The higher frequency was obtained at 50-54 (24%), although spikes with 60-64 spikelets were numerous (20%) (Figure 9). From the determinations it appeared that in the autumn barley ears, regardless of form, were found sterile spikelets. The two-row form was between 3 and 12 sterile spikelets/ear. There were those under 3 sterile spikelets (51%) dominated, followed by 3-7 sterile (45%) spikelets. In the form of six-row, between 3 and 37 sterile sikelets were found. There were dominated those with 13-17 sterile spikelets (30%) and those with 8-12 sterile spikelets (29%) (Figure 10).

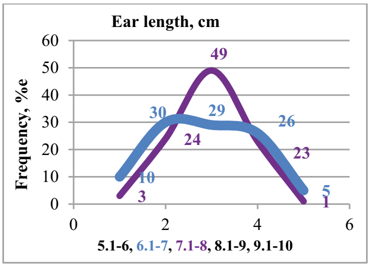

Figure 7. Frequency of barley ear lengths, cm

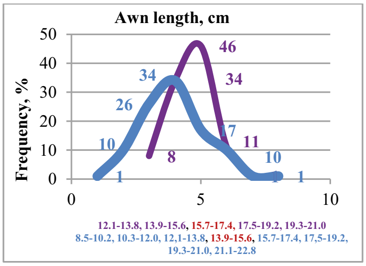

Figure 8. Frequency of barley awn lengths, cm

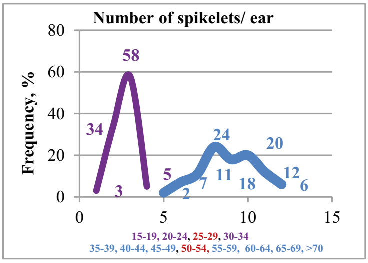

Figure 9. Frequency of spikelets number/ear

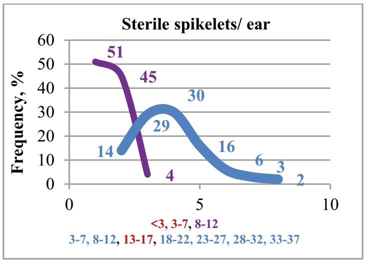

Figure 10. Frequency of sterile spikelets/ear

Figure 11. Two-rows barley characters, 'Trasco' variety

Figure 12. Six-rows barley characters, 'Sistem' variety

Variability of barley grains. The grains (caryopsis type) that are formed have specific characters: they are dressed in the lower palea and the upper palea (also called the lemma), with dimensions between 8-12 mm in length, 2.0-4.5 mm in thickness, in elongated shape and having a thousand grains weight in wide range: 23-58 g (Fox et al., 2006). From the determinations it appeared that between the two forms there were some differences (Figure 11 and Figure 12).

The weight of the grains formed in a ear ranged between 0.53 g and 1.97 g in barley two-row form. The highest frequency was obtained at

0.90-1.25 g (49%). In barley six-row form, the grains in an ear weighed between 0.90 and 3.71 g. The highest freqoency was between 2.34 and 2.69 g. The data shows the great difference due to the two forms of autumn barley (Figure 13). The thousand grains weight (TGW) showed slight differences. Thus, at two-row, the grains had an TGW of between 35 and 70 g, with a modal value of 50.1-55 g (44%) (Figure 14). The six-row form has a TGW of between 35 and 60 g, with the maximum frequency at 45.1-50 g (40%). From these data it results that the two-row has generally formed grains with TGW larger by about 5 g.

Tha grain sizes also had some characteristics. Thus, the length of the grains in the form of two-row ranged between 7.9 and 9.9 mm, with the modal value at 8.6-9.2 mm (57%). The length of the grains in the form of six-row ranged between 6.5-11.5 mm. The higher frequency was 8.6-9.2 mm (42%) (Figure 15). The thickness of the beans differed between the two forms. The two-row ranged from 3.3-4.4 mm, with a modal value of 3.9-4.1 mm (63%). In the six-row form, tha grain thichness was 3.0-5.0 mm, with modal value at 3.9-4.1 mm (39%) (Figure 16).

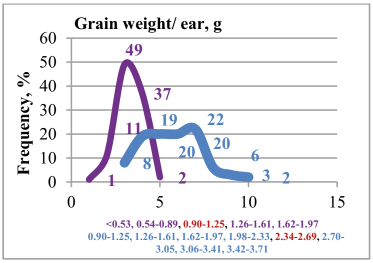

Figure 13. Frequency of grains weight/ear, g

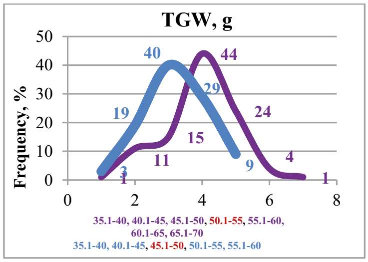

Figure 14. Frequency of barley TGW, g

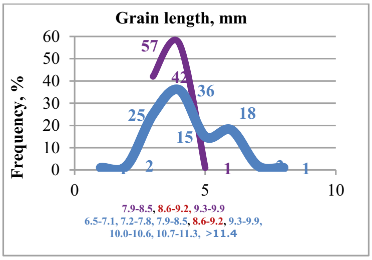

Figure 15. Frequency of grain length, mm

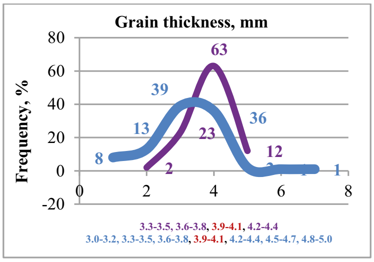

Figure 16. Frequency of grain thichness, mm

Correlations between the main characters If we look at the whole set of correlations between all the analyzed characters, we find mostly positive and statistically significant assurance. The situation is favorable for both the two-row form and the six-row form. In addition, there are small differences between the two forms, but which show nothing but that winter barley, in both forms, is well suited to the crop area.

Thus, at two-row form, the length of the straw correlated best with the number of grains in the ear (r=0.304**). The spike's length was very closely related to the weight of the spike, the number of spikelets in the ear, the length of the spikelet, the number of grains in the ear/spike, the weight of the grains/ ear, the weight of the grains/ ear, the TGW and the size of the grain. The weight of the ear correlated positively with the other characters and correlated negatively with the number of sterile spikelets in a ear (r=-0.323^{000}).

The number of spikelets in an ear correlated positively with the other characters, less obvious with the number of sterile spikelets (r=0.164). Similarly, it was obtained in the case of spikelet length, and the exception was found to correlation with sterile spikelets (r=0.053). The number of sterile spikelets bindings correlated negatively with most of the characters except in the size of the grains (insignificant positive correlations). The awn length has been positively and clearly correlated with all the grain characters. The number of grains in the ear has positively correlated with the length of the grain (r=0.126). The length of the grain in this type of barley correlated positively but insignificantly with the grain thickness (r=0.160), which means that there is only a small dependence between these two characters (Table 1.a).

In the case of the six-row form, the length of the straw correlated positively with the length of the spike, the length of the spikelet, the TGW and the length of the grain. The length of the ear correlated significantly positively with all the other characters analyzed. The ear's weight correlated very positively with most of the characters, less the length of the grain (r=0.247*) and negative with the sterile spikelets ($r=-0.323^{000}$). High positive values of the coefficients were found for the number of spikelets/ ear and the length of the spikelets, except in both cases the connection with the sterile spikelets (r=0.164 and r=0.053 respectively). Negative correlations of different levels were found between the number of sterile spikelets with most characters. However insignificant positive correlations were observed between sterile spikelets with grain dimensions (r=0.087 in length and r=0.014 in the grain thickness). The awn length had positive causal relationships with the other characters, but the closest was the length of the grain (r=0.443***). The number of grains in an ear correlated positively but insignificantly with the length of the grain (r=0.126). Favorable situations were also observed between the weight of the grains/ ear with TGW and with the size of the grains. The thousand grains weight correlated closely with the grain size, especially the grain thickness (r=0.381***), being different from the two-row form. Between the six-row grain dimensions the correlation was low (r=0.160) (Table 1.b), the same as in the two-row form.

Table 1.a. Corelations between different characters of *Hordeum vulgare distichon* form

Character	Straw length	Ear length	Ear weight	Spikelets no./ear	Spikelet length	Sterile spikelets	Awn length	Grains no/ear	Grains weight	TGW g	Grain length	Grain width
Straw length,cm	1	.169	.178	**.208***	-.130	**$-.191^{0}$**	.028	**.304****	**.211***	-.024	.180	.158
Ear length, cm		1	**.708*****	**.797*****	**.379*****	.042	.166	**.641*****	**.682*****	**.435*****	**.252****	**.213***
Ear weight, g			1	**.672*****	**.243***	**$-.296^{00}$**	.175	**.767*****	**.930*****	**.699*****	**.365*****	.072
No. spikelets				1	**.249***	.159	.045	**.731*****	**.672*****	**.293****	.196	.077
Spikelet length, cm					1	.044	.170	.176	**.207***	.141	**.370*****	**.228***
Sterile spikelets						1	.172	**$-.550^{000}$**	**$-.432^{000}$**	-.057	**$-.215^{0}$**	.007
Awn length, cm							1	-.079	.088	**.295****	**.232***	.065
No.grains/ear								1	**.863*****	**.282****	**.328*****	.045
Grains weight/ear									1	**.718*****	**.363*****	.087
TGW, g										1	**.256****	.133
Grain length, mm											1	.147
Grain width, mm												1

Table 1.b. Corelations between different characters of *Hordeum vulgare hexastichon* form

Character	Straw length	Ear length	Ear weight	Spikelets no./ear	Spikelet length	Sterile spikelets	Awn length	Grains no/ear	Grains weight	TGW g	Grain length	Grain width
Straw length,cm	**1**	**.385*****	**.203***	.071	**.277****	.157	.123	.089	.155	**.194***	**.432*****	.090
Ear length, cm		**1**	**.655*****	**.836*****	**.374*****	**.266****	**.307****	**.547*****	**.549*****	**.271****	**.284****	**.274****
Ear weight, g			**1**	**.778*****	**.328*****	**$-.323^{000}$**	**.289****	**.888*****	**.962*****	**.663*****	**.247***	**.358*****
No. spikelets				**1**	**.266****	.164	**.240****	**.757*****	**.726*****	**.302****	**.212***	**.268****
Spikelet length, cm					**1**	.053	**.562*****	**.195***	**.253****	**.265****	**.664*****	**.225***
Sterile spikelets						**1**	-.100	**$-.521^{000}$**	**$-.473^{000}$**	-.152	.087	.014
Awn length, cm							**1**	.141	**.200***	**.219***	**.443*****	**.204***
No.grains/ear								**1**	**.941*****	**.362*****	.126	**.223***
Grains weight/ear									**1**	**.656*****	**.213***	**.318****
TGW, g										**1**	**.287****	**.381*****
Grain length, mm											**1**	.160
Grain width, mm												**1**

Statistical analysis of the variability of morphological characters in barley. The results obtained in the morphological analysis of some characters in winter barley have shown specific aspects. Thus, straw length measured 62 cm for two-row barley and 76 cm for six-row barley (Table 2.a). Variability demonstrated medium coefficients at two-row (16.6%), and low coefficients at six-row (8.4%).

The internode at the base measured 10.5 cm in two-row and 11.1 cm in the six-row form. The coefficient of variability (CV) was medium and close in both cases (11 to 15%). The diameter of the basal segment was between 3.1 mm at the two-row and 3.8 mm at six-row. The variability in this character was similar, at about 11%.

The internode under ear (apical) measured 23.9 cm at two-row and 35.6 cm at six-row. The variability of character was over 20% in two-row and 12% in six-row.

The ear's length was about 7.5 cm in both forms, but with character variability between 10% (two-row) and 15% (six-row).

The ear weight was characteristic of the two forms: 1.45 g with 14% in two-row and 2.51 g with 26% in six-row variability.

In a similar way the number of spikelets/ ear was 25.4 with 5% in two-row barley variability, and 56.4 with 15% in six-row barley variability. The length of the spikelets was 10.6 mm at the two-row (6% CV) and 11.3 mm at the six-row (7% CV).

Sterile spikelets consisted of 2.8 of medium two-row barley and 14.5 per six-row barley. The variability of this character was 78% in the first case and 46% in the second case (Table 2.b). The awn length was 15.9 cm at the two-row and 14.7 cm in the six-row.

The character had variability of 9 to 15%. The number of grains formed in an ear was 22.5 for two-row and 41.9 for six-row. Character variability shows values of 14% in the first case and 24% in the second case.

The grain weight of an ear was 1.18 g for two-row barley, with the variability of 19%, and 2.05 g for six-row barley, with 28% variability.

The thousand grain weight was 52.3 g in the two-row form and 48.6 g in the six-row form. The variability was similar, about 10%. The grains had average dimensions of 8.63/ 3.96 mm at the two-row barley and 9.13/ 3.75 mm in six-row barley. The variation in grain size of the two forms was small (less than 10%).

Table 2.a. Statistical indices of *Hordeum vulgare* plants

Indices	Straw length, cm	Base straw segment, cm		Up straw segment, cm	Ears		Spikelets	
		length, cm	width, mm		length, cm	weight, g	no./ear	length, mm
Winter barley *distichon* form								
Media, ā	**62.08**	**10.52**	**3.183**	**28.14**	**7.510**	**1.450**	**25.41**	**10.59**
Variance, s^2	27.12	1.379	0.118	45.06	0.599	0.042	1.739	0.409
Standard error, s	5.208	1.174	0.344	6.712	0.774	0.205	1.319	0.640
Variation coef, s%	**8.39**	**11.16**	**10.81**	**23.85**	**10.31**	**14.10**	**5.19**	**6.04**
Winter barley *hexastichon* form								
Media, ā	**76.06**	**11.10**	**3.844**	**35.63**	**7.470**	**2.514**	**56.35**	**11.32**
Variance, s^2	159.64	2.679	0.189	18.99	1.279	0.428	74.19	0.582
Standard error, s	12.625	1.637	0.435	4.358	1.131	0.654	8.613	0.763
Variation coef, s%	**16.60**	**14.75**	**11.33**	**12.23**	**15.14**	**26.01**	**15.29**	**6.74**

Table 2.b. Statistical indices of *Hordeum vulgare* plants

Indices	Sterile spikelets	Awn length, cm	No. grains/ ear	Grains weight /ear, g	TGW, g	Grains, mm	
						length	width
Winter barley *distichon* form							
Media, ā	**2.820**	**15.87**	**22.49**	**1.182**	**52.33**	**8.630**	**3.962**
Variance, s^2	4.796	2.135	10.03	0.048	**30.03**	0.086	**0.031**
Standard error, s	2.190	1.461	3.167	0.220	**5.480**	0.292	**0.176**
Variation coef., s%	**77.66**	**9.21**	**14.08**	**18.61**	**10.47**	**3.38**	**5.74**
Winter barley *hexastichon* form							
Media, ā	**14.47**	**14.71**	**41.88**	**2.051**	**48.58**	**9.129**	**3.747**
Variance, s^2	43.48	5.055	99.08	0.337	21.98	0.762	0.096
Standard error, s	6.594	2.248	9.954	0.581	4.689	0.873	0.309
Variation coef., s%	**45.57**	**15.28**	**23.76**	**28.32**	**9.65**	**9.56**	**8.25**

CONCLUSIONS

The morphological characters of autumn barley were specific to the two forms: two-row and six-row. Thus, the strain/ straw had dominant lengths of 61-64 cm in the two-row barley and 73-80 cm in six-row barley. At the height of the plant obviously contributed the apical internode, with lengths of 29-32 cm in the two-row and 35-38 cm in six-row. The basal internode has closely spaced lengths (10-12 cm), but with different diameters: 3.0-3.2 mm in two-row barley and 3.9-4.1 mm in six-row barley. The ear had close lengths (6-8 cm), with longer awns at two-row form (2 cm more). Spikelets from an ear were on average between 25-29/two-row and 50-54/six-row. Sterile spikelets were found less than 3/two-row and 13-17/six-row. The grains in an average ear weighted 0.90-1.25 g/two-row and 2.34-2.69/six-row. TGW was higher at two-row barley: 50-55 g, vs. 45-50 g/six-row barley. The grains were similar in size: 8.6-9.2 mm in length and 3.9-4.1 mm in thickness.

Between all these characters were established simple correlations (r), with some specific differences to the two forms. Most of these were positive and statistically assured. The length of the two-row barley straw obviously correlated with the number of grains in the ear (r=0.304**) and the lower correlations with ear length (r=0.169). The six-row barley have strong correlated in the straw length with grain length (r=o.432***), and with ear length (r=0.385***). The correlations obtained showed the level of adaptability of the two forms of winter barley in the southern area of territory.

The statistical indicators have demonstrated the average values obtained for each character studied. On the one hand, values were obtained that could characterize the morphology of the two-row barley and of the six-row barley. At the same time, the variability coefficients obtained had mostly low and medium values. There was only one exception to the high variability of the grain weights of a six-row barley (CV: 28.32%).

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SALINITY EFFECTS ON SWEET CORN YIELD AND WATER USE EFFICIENCY UNDER DIFFERENT HYDROGEL DOSES

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Abstract

The research was carried out at 2015 to determine the effects of the irrigation water salinity and different hydrogel doses which controlled releasing fertilizer and soil conditioner on soil as PAM hydrogel source on sweet corn (Zea mays saccharata Sturt.) yield under greenhouse condition. The experiment was conducted 4 different irrigation water salinity level (S_1: 0 dS m^{-1}; S_2: 1.5 dS m^{-1}; S_3: 3.0 dS m^{-1}; S_4: 5.0 dS m^{-1}) and 4 different hydrogel doses (H_1: 0 gr pot^{-1}; H_2: 0.5 gr pot^{-1}; H_3: 1.0 gr pot^{-1}; H_4: 1.5 gr pot^{-1}). The experimental design was randomized factorial design with three replications. According to the results; significant and negative relationship was observed between the water salinity and dry and wet plant weight. The highest wet and dry weights was observed for irrigation water salinity and hydrogel doses at the S_1 as 170.94 gr $pot^{-1}$and 22.77 gr $pot^{-1}$and the H_2 as 179.75 gr pot^{-1} and 26.47 gr pot^{-1} respectively. Hydrogel effect decreased when the salinity level increase. Irrigation water use efficiency was obtained the highest value belong to S_1H_2 with the value of 0.26.

Key words: *corn, hydrogel, irrigation water salinity, yield.*

INTRODUCTION

The quality of water resources around the world is getting worse each year (Jimenez and Asano, 2008). For this reason, it is necessary to use lower quality waters in order to increase crop water productivity and economic value of the productions. Especially in arid and semi-arid regions, the use of new methods and technologies is becoming popular to make more effective use of water resources (such as green manure, animal fertilizer, hydrogel). For this purpose, in addition to organic resources, polymer substances have been developed as soil conditioners. However, due to the economic reasons at the cost of production, these materials did not see the need to be concerned. In the recent years, the efficiency of the polymers has been increased and higher effective polymers have been produced with lower costs and they have started to be used in agricultural areas. Hydrogels, which were developed to increase the water holding capacity of amended media, have been used to aid plant establishment and growth in dry soils (Al-Sheikh and Al-Darby, 1996). They have the potential to absorb water many times their weight, retain it and supply it to plant roots during water stress, thereby enhancing plant survival and growth (Agaba et al., 2010). Due to the wide variety of such products there is not enough technical information about the effects of irrigation water quality. This study aimed to determine the effects of different hydrogel doses on yield and development of corn (*Zea mays saccharata* Sturt.) under saline irrigation water.

MATERIALS AND METHODS

The experiment was carried out at 2015 in 2.5 kg plastic pots with a diameter of 25 cm and a height of 20 cm under the plastic greenhouse conditions in Aksu district of Antalya. The soil texture was Silty Clay Loam, field capacity (FC) 27 %, permanent wilting point 17%, bulk density 1.38 gr cm^{-3} and electrical conductivity (EC) 1.04 dS m^{-1}. According to soil fertility analysis results for basal fertilizer was applied to the pots. Polyacrylamide (PAM) polymer was used which was a water absorption capacity as high as 400-500 times the hydrogel weight. It is insoluble in water and organic solutions. Sweet corn (*Zea mays saccharata*) of Merit F_1 variety was used in this study and this genotype was moderately sensitive to the salt stress. Five seeds were planted in each pot and irrigated to FC according to weight base.

The experimental design was factorial randomized block design. The treatments of the experiment was 4 different irrigation water salinity with **S_1** - 0 dS m^{-1}, **S_2** - 1.5 dS m^{-1}, **S_3** - 3 dS m^{-1}, **S_4** - 5 dS m^{-1} and 4 different hydrogel doses with **H_1** - 0 gr pot^{-1}, **H_2** - 0.5 gr pot^{-1}, **H_3** - 1.0 gr pot^{-1}, **H_4** - 1.5 gr pot^{-1}. NaCl was used while preparing different salinity level of irrigation water. Pots were weighed regularly and irrigation treatments were made when soil moisture content decreased below the 30% field capacity. The plants were harvested 46 days after the saline water application. After harvesting plant wet weights were measured. The plants were dried until the plants reached a constant weight at 65°C and the values obtained were reported as dry weight. Results were analysed by Duncan test of variance analysis using SPSS 18.0 statistical program and evaluated according to the principles given by Yurtsever (1984).

RESULTS AND DISCUSSIONS

Effects of irrigation water salinity on wet and dry weight of corn

The average wet and dry weight of corn and Duncan classes was given at Table 1.

Table 1. Wet and dry weight of corn (gr pot^{-1})

Treatments	Wet weight	Dry weight
S_1	170.94^{a*}	22.77^{a}
S_2	140.67^{b}	21.88^{a}
S_3	118.92^{b}	18.61^{b}
S_4	93.06^{c}	16.18^{c}

*Duncan class

According to results the highest yield (as wet and dry weight) was obtained the lowest salinity level. Increasing the salinity of the irrigation water caused the yield to decrease. There was negative and important relationship between plant dry and wet weight and irrigation water salinity. Determination coefficients (R^2) were 0.98 and 0.96 for dry and wet weight, respectively (Figure 1). Similar results have been reported by Ashraf and O'leary (1997), Turan et al. (2009), Tekeli and Kale Celik (2017).

Relative yield values were calculated on the basis of S_1 treatment which was non saline water. It was presented in Table 2.

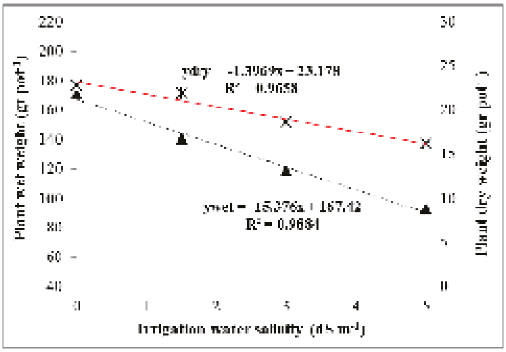

Figure 1. Irrigation water salinity and crop wet and dry weight relationship

Table 2. Relative percentage and variation of yield at different salt doses

Treatments	Wet weight		Dry weight	
	RP	RD	RP	RD
S_1	100.0	0.00	100.0	0.00
S_2	82.29	17.70	96.09	3.90
S_3	69.57	30.40	81.73	18.20
S_4	54.44	45.50	71.06	28.90

RP: Relative persentage, RD: Relative difference.

The wet weight reduction was around 50% at the highest salinity level (S_4). It was 30% for dry weight.

Effects of different hydrogel doses on wet and dry weight of corn

The average wet and dry weight of corn for different hydrogel doses and Duncan classes was given at Table 3.

Table 3. Wet and dry weight of corn (gr pot^{-1})

Treatments	Wet weight	Dry weight
H_1	170.94^{b}	22.77^{b}
H_2	179.75^{a}	26.47^{a}
H_3	175.46^{a}	25.39^{a}
H_4	171.53^{b}	24.34^{a}

*Duncan class

The wet and dry weight at H_2 treatment was higher than the other treatments. However H_2 and H_3 treatment was the same Duncan classes. There was a negative significant quadratic relationship between hydrogel level and plant wet and dry weight (Figure 2).

The coefficient of determination (R^2) was 0.82 and 0.85 for wet weight and dry weight, respectively.

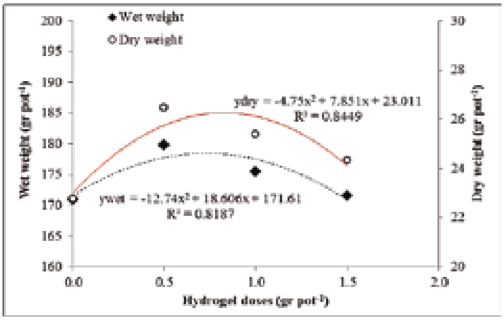

Figure 2. Relationship between wet and dry weight of corn and hydrogel doses

Relative dry and wet weight values were calculated on the basis of H_2 treatment which was obtained the highest yield. It was presented in Table 4.

Table 4. Relative percentage and variation of yield at different hydrogel doses

Treatments	Wet weight		Dry weight	
	RP	RD	RP	RD
H_1	95.10	4.90	86.02	13.98
H_2	100.00	0.00	100.00	0.00
H_3	97.61	2.39	96.92	4.08
H_4	95.43	4.57	91.95	8.05

RP: Relative persentage, RD: Relative difference.

Relative yield and relative differences were 4.90%, 2.39% and 4.57% and 13.98%, 4.08% and 8.05% for H_1, H_3 and H_4 for wet weight and dry weight, respectively.

Irrigation water salinity and hydrogel relationship

The salinity and hydrogel doses were evaluated together and the average wet and dry weight values are given in Table 5.

The highest average wet and dry weight was obtained at S_1H_2 treatment. When the salinity of the irrigation water increased, it was observed that to get a highest yield the hydrogel dose to be used increased up to the H_3 dose. If the salinity level continues to increase more than 3 dS m^{-1}, the hydrogel use dose decreased. As the concentration of salt in the medium increases, the swelling rate of the gel decreases.

Therefore, after a certain level of salinity, the hydrogel function decreases. It can be said that the amount of hydrogel used to reduce the loss of yield caused by the increase of irrigation water salinity should increase up to the determined threshold value. Several studies have suggested that hydrogels are less effective as salinity increases (Shannon 1978; Epstein 1985; Gumuzzio et al., 1985; Ashraf 1994; Aydın et al., 2000; Aydın, Malkoç 2003).

Table 5. Wet and dry weight according to different salt and hydrogel doses of corn

Wet weight (gr pot^{-1})					
Treatments	H_1	H_2	H_3	H_4	Average
S_1	171	180	175	172	174^{a}
S_2	141	149	170	143	151^{b}
S_3	119	121	125	139	126^{b}
S_4	93	96	103	100	97^{c}
Average	131^{c}	136^{a}	142^{b}	151^{c}	
Dry weight (gr $pot^{-1)}$					
Treatments	H_1	H_2	H_3	H_4	Average
S_1	22.77	26.47	25.39	24.34	24.70^{a}
S_2	21.88	23.17	23.89	22.84	22.90a
S_3	18.61	21.12	21.49	21.77	20.70^{b}
S_4	16.18	16.66	17.89	17.15	16.90^{c}
Average	19.68	21.86	22.17	21.53	

Irrigation water use efficiency

The irrigation water use efficiency (IWUE) values calculated for each pot using the amount of dry weight obtained from the experiment and the amount of irrigation water applied (Table 6). The highest water use efficiency is obtained at S_1H_2 treatment (salinity: 0 dS m^{-1} and hydrogel dose: 0.5 gr pot^{-1}) with a value of 0.26.

Table 6. Irrigation water use efficiency (kg m^{-2}mm)

Treatments	H_1	H_2	H_3	H_4	Average
S_1	0.24	0.26	0.24	0.25	0.25^{a}
S_2	0.22	0.25	0.25	0.24	0.24^{a}
S_3	0.20	0.23	0.22	0.23	0.22^{b}
S_4	0.17	0.19	0.19	0.19	0.19^{c}
Average	0.21^{b}	0.23^{a}	0.23^{a}	0.23^{a}	

CONCLUSIONS

The highest average yield was obtained on non-saline irrigation water and 0.5 gr pot^{-1} hydrogel doses. As the salinity of the irrigation water increases, the swelling rate of the hydrogel was decreasing. According to this study results 1 gr pot^{-1} the hydrogel dose can be accepted as a threshold value. If irrigation water salinity up to 3 dS m^{-1} the hydrogel function is decreasing. In order to increase yield and IWUE, using 0.5 gr pot^{-1} hydrogel dose with good quality irrigation water can be recommended.

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EFFECTS OF DIFFERENT SEED SIZES AND SHAPES ON FORAGE YIELD AND QUALITY OF FODDER MAIZE

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Abstract

The aim of this study was to investigate the effects of different seed sizes and shapes on forage yield and quality of some maize (Zea mays L.) varieties grown under ecological conditions of Aydın. Three hybrid maize varieties (Bolson, Simon, Diptic) seeds were used as material in three different sizes (Small, Medium, Large) and two different shapes (Flat, Round) in the study. In this experiment; plant height, fresh forage yield, dry forage yield, crude protein ratio, ADF, NDF and ADL was measured. After the measurements, crude protein yield, digestible dry matter, relative feed value and relative feed quality was calculated. It was determined that the highest forage yield and quality were found in the Diptic maize variety in the direction of the results obtained from the experiment, it was determined that the highest forage yield and quality were obtained from small seeds in terms of size. As a result of the experiment, it has been determined that the shape and size of the varieties besides the selection of varieties have significant effects on the forage yield and quality.

***Key words**: fodder maize, forage quality, seed size, seed shape.*

INTRODUCTION

Forage crops are known to have a positive influence on the physical and chemical properties of the soil and on the yield and quality of the cultivated plants following it, as well as providing the fodder which constitutes one of the most important inputs of animal production (Sürmen, Kara, 2017). Within forage crops, maize is ranked as the third major cereal crop after wheat and rice in world production. Production of maize cultivation area having an important place in Turkey as of 2017, 474,590 ha, the average yield of 4885 kg/ha reached (TUIK, 2018). The crop has a wider range of uses. These include the following: human food, industrial processed food production of starch and used as forage to feed animals. Maize with its large number of cultivars and different maturity periods has wider range of tolerance to different environmental conditions (Purseglove, 1972).

The grain is classified according to its size and shape due to the development of the instructions on the maize cob to the tip. According to the position of the grains on the cob, the small and round seeds are large from the tip of the cob, the rounds are from the bottom of the cob, and the straight seeds are from the middle of the cob (Nielsen, 1996; Chaudhry, Ulah, 2001; Kara, 2008).

This suggests that, except for varietal selection, varieties may produce differences in forage yield and quality in size and shape differences. For this reason, the effects of fodder maize on the yield and quality of seeds of different sizes and shapes are investigated.

MATERIALS AND METHODS

The trial was carried out in the province of Aydın in 2015 and the climate data for the time when the experiment was conducted are given in Table 1.

Three hybrid corn varieties (Bolson, Simon, Diptic) seeds were used as material in two different shapes (Flat, Round) with three different sizes (Small, Medium, Large) in the study. All plots were fertilized as 18 kg/da N and 7 kg/da P_2O_5 (Ergin,1974).

When the plants reached 6-8 leaf turns, intermediate anchor and throat filling operations were performed. Irrigation was carried out 5 times considering the periods of growth and development.

Table 1. Climate data for 2015 and long years of the area where the experiment was conducted

Months	Rainfall (mm)		Avg. Temp. (°C)	
	2015	Long Years	2015	Long Years
January	117.4	126	7.8	8.4
February	166	96	8.7	9.6
March	70.8	71	11.1	11.7
April	5.8	45	14.1	15.9
May	79.6	29	20.9	20.8
June	38.2	14	23.6	24.7
July	2.4	3	27.7	27.8
August	0	3	27.3	27.2
September	29.4	17	24.1	23.7
October	74.4	47	18.7	18.7
November	85.2	74	13.3	14
December	4	139	6.2	10
Total	673.2	664		

Source: ADU Faculty of Agriculure Climate Station for 2015, https://tr.climate-data.org/location/21651/ for long year climate datas.

Formal operations were performed during the lactation period. Herbage yield (kg/da) was measured after harvesting and hay yield (kg/da) was measured by fan drying at 70°C for 48 hours until the weight was fixed (Albayrak et al., 2006).

Crude protein ration (%), ADF (%), NDF (%) and ADL (%) of the samples taken from the experiment was measured by NIRS-FT (Bruker MPA) (Gislum et al., 2004). The crude protein yield (kg/da), digestible dry matter (DDM%), relative feed value (RFV) and relative feed quality (RFQ) were calculated by the obtained data.

The following procedures were used to calculate the relative feed value (Horrocks, Vallentine, 1999; Jeranyama, Garcia, 2004).

DDM% = 88.9 - (0.779 x ADF%) x DMI% (Dry Matter Intake) = 120/NDF%

RFV = (DDM%) x (DMI%) x 0.775

RFQ = (DMI%) x (TDN%)/1.23

In order to compare the results obtained from the study, variance analysis was applied according to randomized blocks trial design with the help of MSTAT-C statistical package program. LSD multiple comparison test was used in comparison of the averages.

RESULTS AND DISCUSSIONS

When the results obtained from the experiment are analyzed, it is seen that the interactions are important in many parameters.

The most remarkable result is that the shape* size interaction is insignificant in terms of relative feed value (Table 2).

Table 2. According to the results of variance analysis, the significance ratings of the parameters

	Parameteres										
İnt.	P.H.	FFY	DFY	CPR	ADF	NDF	ADL	CPY	DDM	RFV	RFQ
Var.	*	*	*	Ins.	**	*	*	*	**	*	*
Shape	Ins.	*	Ins.	Ins.	*	*	Ins.	*	*	*	*
Size	**	*	*	*	**	*	*	*	*	*	*
V*Sh	Ins.	*	*	*	*	*	*	*	*	**	**
V*Si	*	*	*	*	*	*	*	*	*	*	*
Sh*Si	Ins.	*	*	*	Ins.	Ins.	*	*	Ins.	Ins.	Ins.
V*Sh*Si	*	*	*	*	*	*	*	*	*	*	*

*p<0.01, **p<0.05, Ins: Insignificant.

When the results of plant height averages are evaluated, it is seen that the values change between 201.36-225.25 cm.

The highest value was obtained from the seeds of flat and medium sized Simon variety. In terms of varieties average, the highest value is found in Simon variety with 235.95 cm (Table 3). In the researches carried out, average plant height in Bulut (2016), 174-210.5 cm, Akdeniz et al. (2004), 143.7-242.6 cm and Özata et al. (2012), 300.2 cm. The results are generally similar to the experiment.

Table 3. Averages and grouping of plant height (cm)

Plant Height (cm)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	223.60 d	226.13 cd	234.80 b	228.17
	Round	231.53 bc	236.03 b	220.13 de	229.23
	Average	**227.56**	**231.08**	**227.46**	**228.70 b**
Simon	Flat	237.23 b	245.26 a	223.66 d	235.38
	Round	239.16 ab	236.06 b	234.30 b	236.51
	Average	**238.20**	**240.66**	**228.98**	**235.95 a**
Diptic	Flat	210.26 f	210.66 f	211.66 f	210.86
	Round	201.36 g	210.16 f	214.66 ef	208.73
	Average	**205.81**	**210.41**	**213.16**	**209.80 c**
Gen. Ave.		**223.86 b**	**227.38 a**	**223.20 b**	224.81

When we measured fresh average yields, the values were found to be between 2380.95-5283.80 kg/da, the highest value being obtained from round and medium sized seeds of Diptic variety. The highest variety average was 4179.20 kg/da of Diptic variety (Table 4).

In researches, Bulut (2016) was found between 5642-7123 kg/da. This results are higher than the experiment. Özata et al. (2012) were found 4670.2 kg/da, Akdemir et al. (1997) were found between 4834-6706 kg/da. These results are similar to the experiment.

Table 4. Averages and grouping of fresh forage yield (kg/da)

Fresh Forage Yield (kg/da)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	3361.42 h	4379.99 cd	3352.37 hı	3697.93
	Round	4657.61 b	4335.23 cd	3759.04 fg	4250.63
	Average	**4009.52**	**4357.61**	**3555.71**	**3974.28 b**
Simon	Flat	3388.56 h	4159.99 de	3312.37 hı	3620.31
	Round	3551.42 gh	2814.28 j	2380.95 k	2915.55
	Average	**3469.99**	**3487.14**	**2846.66**	**3267.93 c**
Diptic	Flat	3993.33 ef	4000.47 ef	3101.90 ı	3698.56
	Round	4141.42 de	4554.28 bc	5283.80 a	4659.83
	Average	**4067.37**	**4277.37**	**4192.85**	**4179.20 a**
Gen. Ave.		**3848.96 b**	**4040.71 a**	**3531.74 c**	3807.13

Values according to dry weight average ranged from 351.82-1080.68 kg/da and the highest value was obtained from round and small sized seeds of Diptic variety. In the varieties, the highest value was determined with 829.72 kg/da of Diptic variety (Table 5).
In research results, Özata et al. (2012) were found 1455.5 kg/da and Erdal et al (2009) were found 2333 kg/da. These results are higher than the experiment. Akdeniz et al. (2004) were found between 683-1723 kg/da. These results are similar between the experiment.
The mean values of crude protein ratio ranged from 8.15 to 9.64% and the highest crude protein ratio was obtained from round and medium sized seeds of Simon variety. There was no statistical difference between varieties general averages (Table 6). In studies, Erdal et al. (2009) were found 7.5%, Akdeniz et al. (2003) were found between 6.65-6.82%. These results are lower than the study.

Table 5. Averages and grouping of dry forage yield (kg/da)

Dry Forage Yield (kg/da)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	714.24 ef	939.61 b	502.29 gh	718.72
	Round	842.91 bd	594.88 fg	590.05 fg	675.95
	Average	**778.58**	**767.25**	**546.17**	**697.33 b**
Simon	Flat	477.83 gı	836.56 be	772.62 de	695.67
	Round	535.24 gh	451.67 hı	351.82 ı	446.24
	Average	**506.54**	**644.11**	**562.22**	**570.96 c**
Diptic	Flat	900.17 bc	791.23 ce	526.07 gh	739.15
	Round	907.36 bc	1080.68 a	772.80 de	920.28
	Average	**903.76**	**935.95**	**649.43**	**829.72 a**
Gen. Ave.		**729.63 a**	**782.44 a**	**585.95 b**	699.33

Table 6. Averages and grouping of crude protein ratio (%)

Crude Protein Ratio (%)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	8.76 ef	8.62 eg	9.49 ac	8.96
	Round	8.67 ef	8.53 fg	9.30 bc	8.84
	Average	**8.72**	**8.58**	**9.40**	**8.90**
Simon	Flat	9.57 ab	8.91 de	8.15 h	8.88
	Round	8.59 fg	9.64 a	9.34 ac	9.19
	Average	**9.08**	**9.27**	**8.74**	**9.03**
Diptic	Flat	9.20 cd	8.53 fg	8.89 e	8.87
	Round	8.91 de	8.36 gh	9.58 ab	8.95
	Average	**9.05**	**8.44**	**9.23**	**8.91**
Gen. Ave.		**8.95 b**	**8.76 c**	**9.12 a**	8.95

ADF averages ranged from 27.4 to 35.72%, NDF averages ranged from 37.38 to 50.84% and ADL averages ranged from 2.80 to 4.18%. In all three parameters, the lowest values are high quality and the lowest values are obtained from the round and small size seeds of the Diptic variety (Tables 7, 8, 9.). In study about NDF% and ADF%, Özata et al. (2012) were found 53.5%, 32.2%, respectively. The mean values for crude protein yield ranged from 222.55 to 506.54 kg/da, while the highest value was obtained from the round and medium sized seeds of the Diptic variety. The highest value according to the varieties average was determined at the Diptic variety with 373.48 kg/da (Table 10).

Table 7. Averages and grouping of ADF (Acid Detergent Fiber) (%)

ADF (%)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	32.59 bc	28.62 fg	33.39 ab	31.53
	Round	29.13 eg	30.16 cf	33.81 ab	31.03
	Average	**30.86**	**29.39**	**33.60**	**31.28**
Simon	Flat	35.72 a	34.84 ab	28.70 fg	33.08
	Round	27.84 fg	32.42 bc	26.72 g	28.99
	Average	**31.78**	**33.631**	**27.71**	**31.04**
Diptic	Flat	29.87 cf	32.09 be	27.36 fg	29.77
	Round	32.33 bd	29.22 dg	27.11 fg	29.55
	Average	**31.10**	**30.66**	**27.23**	**29.66**
Gen. Ave.		**31.24 a**	**31.22 a**	**29.51 b**	30.66

Table 8. Averages and grouping of NDF (Neutral Detergent Fiber) (%)

NDF (%)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	47.93 ad	48.06 ad	48.42 ac	48.13
	Round	41.70 fg	44.78 df	49.05 ab	45.18
	Average	**44.81**	**46.42**	**48.73**	**46.65 a**
Simon	Flat	50.84 a	48.74 ab	41.87 fg	47.15
	Round	42.33 fg	48.26 ac	39.64 gh	43.41
	Average	**46.58**	**48.50**	**40.76**	**45.28 a**
Diptic	Flat	43.13 ef	43.93 ef	39.37 gh	42.14
	Round	45.92 be	45.01 cf	37.38 h	42.77
	Average	**44.53**	**44.47**	**38.38**	**42.46 b**
Gen. Ave.		**45.31 a**	**46.46 a**	**42.62 b**	44.80

Table 9. Averages and grouping of ADL (Acid Detergent Lignin) (%)

ADL (%)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	4.18 a	3.27 df	3.68 b	3.71
	Round	3.52 bd	3.54 bc	2.98 gh	3.34
	Average	**3.85**	**3.40**	**3.33**	**3.53 a**
Simon	Flat	3.50 bd	2.60 ı	3.52 bd	3.20
	Round	3.53 bd	3.98 a	3.19 fg	3.56
	Average	**3.51**	**3.29**	**3.35**	**3.38 ab**
Diptic	Flat	3.14 fg	3.66 b	3.20 eg	3.33
	Round	3.31 cf	3.45 be	2.80 hı	3.19
	Average	**3.22**	**3.55**	**3.00**	**3.26 b**
Gen. Ave.		**3.53 a**	**3.41 b**	**3.23 c**	3.39

Table 10. Averages and grouping of crude protein yield (kg/da)

Crude Protein Yield (kg/da)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	294.69 hı	377.82 c	318.26 fh	330.26
	Round	403.99 b	370.06 cd	350.02 de	374.69
	Average	**349.34**	**373.94**	**334.14**	**352.47 b**
Simon	Flat	324.28 fg	370.90 cd	270.01 ı	321.73
	Round	305.40 gh	271.29 ı	222.55 j	266.41
	Average	**314.84**	**321.10**	**246.28**	**294.07 c**
Diptic	Flat	367.47 cd	341.54 ef	275.58 ı	328.20
	Round	369.01 cd	380.74 bc	506.54 a	418.76
	Average	**368.24**	**361.14**	**391.06**	**373.48 a**
Gen. Ave.		**344.14 a**	**352.06 a**	**323.83 b**	340.01

The values for digestible dry matter averages ranged from 61.07 to 68.08%, the highest value being obtained from round and small sized seeds of the Simon variety (Table 11).

Relative feed value averages ranged from 111.72-169.66, relative feed quality ranged from 117.20-177.98. Both parameters showed the most economical application in terms of varieties and yields, while these values were obtained from round and small sized seeds of Diptic variety (Tables 12, 13).

It is thought that some studies may have different results because of different seed varieties and cultivation conditions.

Table 11. Averages and grouping of digestible dry matter (%)

Digestible Dry Matter (%)					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	63.51 ef	66.60 ab	62.88 fg	64.33
	Round	66.20 ac	65.40 be	62.55 fg	64.72
	Average	**64.86**	**66.00**	**62.72**	**64.52**
Simon	Flat	61.07 g	61.75 fg	66.54 ab	63.12
	Round	67.20 ab	63.64 ef	68.08 a	66.31
	Average	**64.14**	**62.70**	**67.31**	**64.71**
Diptic	Flat	65.62 be	63.89 cf	67.58 ab	65.70
	Round	63.71 df	66.13 ad	67.78 ab	65.87
	Average	**64.66**	**65.01**	**67.68**	**65.78**
Gen. Ave.		**64.55 b**	**64.57 b**	**65.90 a**	65.01

Table 12. Averages and grouping of relative feed value

Relative Feed Value					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	123.37 eh	128.92	120.84 gh	124.38
	Round	148.01 bc	136.02 cf	118.78 gh	134.27
	Average	**135.69**	**132.47**	**119.81**	**129.32 b**
Simon	Flat	111.72 h	118.55 gh	148.14 bc	126.13
	Round	147.87 bc	122.64 fh	159.74 ab	143.42
	Average	**129.79**	**120.59**	**153.94**	**134.78 b**
Diptic	Flat	141.60 cd	135.40 cf	159.80 ab	145.60
	Round	129.43 dg	136.95 ce	169.66 a	145.35
	Average	**135.52**	**136.18**	**164.73**	**145.48 a**
Gen. Ave.		**133.67 b**	**129.75 b**	**146.16 a**	136.53

Table 13. Averages and grouping of relative feed quality

Relative Feed Quality					
Varieties	Shape	Size			Gen. Ave.
		Large	Medium	Small	
Bolson	Flat	129.43 eh	135.25 dg	126.77 gh	130.48
	Round	155.26 bc	142.69 cf	124.61 gh	140.85
	Average	**142.35**	**138.97**	**125.69**	**135.67 b**
Simon	Flat	117.20 h	124.36 gh	155.40 bc	132.32
	Round	155.12 bc	128.66 fh	167.58 ab	150.45
	Average	**136.16**	**126.51**	**161.49**	**141.39 b**
Diptic	Flat	148.55 cd	142.04 cf	167.64 ab	152.74
	Round	135.78 dg	143.67 ce	177.98 a	152.48
	Average	**142.16**	**142.86**	**172.81**	**152.61 a**
Gen. Ave.		**140.22 b**	**136.11 b**	**153.33 a**	143.226

CONCLUSIONS

One of the important roughage sources today is the maize, which is the day that the forage crops importance is increasing day by day. corn is an annual and high-yielding plant. Variety selection in maize production has an important place. Besides the selection of varieties, seeds with different shapes and sizes can have an effect on yield and quality. According to the results of the research, it has been seen that the varieties, shapes and sizes yield and quality values are different. Especially Diptic variety and small and round seeds of this variety have the highest feed value in the direction of the results obtained from the experiment.

These results also showed that the shape and size differences affect the yield and quality of feed significantly.

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EFFECTS OF NANO SULFUR (S) APPLICATIONS ON YIELD AND SOME YIELD PROPERTIES OF BREAD WHEAT

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Abstract

Research was carried out to determine the effects of different nano-sulfur applications on yield and some plant properties of bread wheat during 2016-2017 winter growing season in Isparta/Turkey. Bread wheat cultivar of 'Koç 2015' was used as a plant material. The nano-sulfur (particle size 20 nm) was supplied by New Systems Petrol Products Import and Export Manufacturing Company. Five different applications control (CA), application to soil (SA), seed coating (SC), seed coating+application of booting stage (SC+BA) and seed coating+application of heading stage (SC+HA) were examined. The experiment was conducted to completely randomized block design with three replications.

Results showed that the effect of nano-S applications were significant in all of examined traits (emergence rate, mean emergence time, plant height, spike length, kernel number per spike, grain yield, protein rate and sedimentation value). Nano-sulfur applications in bread wheat have had positive effects on all the examined traits. Mean values of examined traits varied for emergence ratio 75.0-100.0%, mean emergence times 2.35-2.83 days, plant height 68.7-73.7 cm, spike length 8.37-9.92 cm, kernel number per spike 35.55-39.50, grain yield 3431-3911 kg ha^{-1}, protein ratio 13.29-14.57% and sedimentation values 38.20-48.70 ml, respectively. In particular, nano-sulfur application in seed coating+booting period resulted in 14% grain yield increase compared to control.

***Key words**: Bread wheat (Triticum aestivum), nano-sulfur (S), yield.*

INTRODUCTION

Wheat is in first place in terms of field crops cultivation area and production units in Turkey. The amount of consumption in a year of wheat, which is the main food source of our country, is over 200 kg per person. In addition, our country is one of the leading countries of the world in terms of exporting semi-processed or processed product wheat products. However, in recent years there have been significant reductions in wheat cultivation areas. The wheat cultivation areas, which were about 9.4 million hectares in 2000s, decreased by 7.7 million in 2014. Despite of the number of cultivars of wheat in Turkey (over 200) increases, significant increases in yield and production cannot be achieved. As the phrase goes, wheat production make no headway for 30 years. In addition to the increase in domestic demand for wheat, the increase in foreign demand for wheat also increases the need for high quality wheat. In some years due to adverse climatic conditions, demand cannot be met due to problems in production and quality, and imports were being made. Turkey in terms of self-sufficiency in 2005-2006 had a rate of 120% but in 2014-2015, this rate has dropped to 89%. As a matter of fact, our external procurement amount, which was 2 million tons in 2007, has exceeded 4 million tons in 2016. Finally, in 2017, 230,000 tons of wheat were purchased from the EU to meet the demand for high quality wheat and to balance the price. Efficiency and quality priorities should be included in breeding and production policies of wheat so that Turkey, which has an important geographical point in terms of agricultural potential, can preserve the position of self-sufficient countries in the wheat. After 2011, Turkish Grain Board (TMO in Turkish) has made an innovation in its wheat buying strategies and switched production-based purchasing to quality-based (protein) purchasing wage scale. For example, during the 2016-2017 purchasing period, the purchase price for wheat with 12.5-13% protein rate was 910 tl/ton; protein ratio of wheat over 13% to

949 tl/ton (Current exchange rate of 1 tl is about 0.4 Euro). In order to maintain its position in terms of wheat production and evaluation, Turkey needs to increase the production of wheat.

If we think that wheat sowing areas have declined or reached marginal borders in recent years, for the purpose of sustainable production, it is necessary to increase the yields and to obtain high quality products.

Agricultural land in regions where most of our wheat production is provided requires new technologies as well as variety improvement due to the arid or semi-arid climatic conditions and high pH characteristics.

The vast majority of our wheat production is made in dry soils in regions with arid or semi-arid climatic conditions and high pH. For this reason, new technologies are needed in addition to variety improvement.

Wrong and unconscious use of agricultural inputs (especially chemical fertilizers and pesticide) will cause physical, chemical and biological properties of agricultural soils to be adversely affected. In the long run, the soils may become completely unusable.

For this purpose, especially chemical fertilizer application should be treated very carefully.

Alternative new technologies are needed to reduce the risk of desertification and salinization of agricultural soils. Nano-technological fertilizers in this aspect will gain importance in the future in order to make sustainable plant production. Nanomaterials are defined as one billionth of a physical size, and these products exhibit much higher activity than their normal size.

Due to the fact that the soil of our country is generally calcified and the pH is very high, there are deficiencies in the plants taking of some nutrients.

There are difficulties in absorbing the nutrients from the soil and leaves in the fertilizer which was consisting of macro and micro sized fractions. Especially in the soil with high pH characteristics, in the application of many sulfuric fertilizers, it takes a long time to reduction of sulfur to sulfate and transform useful form for plants, and the plant cannot make effective use of other elements. Nano S can be highly homogeneous in the distribution to the ground with an average particle size of 20 nm. Depending on the surface size, sulfur bacteria work very quickly.

So it can be converted into forms that can be taken by plants within 1-2 days. Also, depending on the nano size, the absorption from the leaf is fast. Nano-S allows the plants to efficiently utilize many nutrients by rapidly reducing the soil reaction (pH) and cause increase in yield and quality.

The use of Nano-S prevents the use of excess fertilizer and negatively affects the environment and human health of fertilizers. As a result, nearly 100% efficiency is obtained.

This study was conducted to determine the effects of different Nano-S applications on yield and some yield characteristics of wheat.

MATERIALS AND METHODS

The experiment was conducted in the Research-Application Farm of Agriculture Faculty, University of Süleyman Demirel, Isparta in 2016-2017 growing season. Bread wheat cultivar (‘Koç 2015’ cv.) was used as a plant material. The material of nano-sulfur (particle size 20 nm) was supplied by New Systems Petrol Products Import and Export Manufacturing Company.

The soil of experiment area is texturally tinny, alkaline (pH value 8.1), cation exchange capacity 36% and total salt content 0.025%, rich in regard to lime (255 g/kg), suitable phosphor (199 mg/kg P_2O_5), rich in terms of potassium (75.4 kg/da K_20), inadequate in terms of nitrogen (0.14% N) and organic material (13.4 g/kg).

Vegetation season was semi-arid, slightly moisture, cool winter and hot climate.

The experiment was conducted to completely randomized block design with three replications. In research, 5 different Nano-S (particle size 20 nm) applications and control were examined and details of these were given at Table 1.

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Table 1. Application subjects, time and doses

Treatments	Dose and time of application
1. **CA**	Only water applied
2. **SA**	Soil application with 1% **Nano-S** before sowing
3. **SC**	Seed application of 10% **Nano-S** before sowing
4. **SC+BA**	Seed application of 1% **Nano-S** before sowing+ leaf application at booting stage
5. **SC+HA**	Seed application of 1% **Nano-S** before sowing + leaf application at heading stage

The fields were prepared with standard wheat production practices, such as land preparation, fertilizer application, herbicide application, and seed rate of 450 grain/m^2 was used and it was planted in plots that had four 6 m length rows with 20 cm between rows. Fertilizer (80 kg ha^{-1} P_2O_5 and 70 kg ha^{-1} N) were applied as recommended rates.

There was no irrigation during the growth period. The five applications used in the study were prepared as described fallow. Only water was sprayed to control parcels **(CA).**

The seeds were thoroughly soaked with 10% Nano S solution prepared the day before sowing for seed coating **(SC)** and seeds left to dry again. The dried seeds were kept in the refrigerator until the next sowing day.

In the case of soil application **(SA),** the solution of 1% Nano-S prepared and it was applied to the parcels by using a mini atomizer (SEKAK VETA 16 A) 16 liters, low pressure 1 mm plaque diameter rechargeable motorized back sprayer), at the rate of 200 liter water/ha before seeding. Leaf applications were performed according to the label of the Nano-S solution.

Prepared 1% Nano-S solution were sprayed at booting stage **(SC+BA)** of wheat at the dose of 200 L/ha and some dose of Nano-S sprayed at heading stage of wheat **(SC+HA)**. In leaf applications most care has been taken to homogenized spraying the whole green component of the wheat plants. Prior to the application, the device was calibrated so that each plot can be supplied with liquid at a rate of 200 liters water/ha.

The control plots are only sprayed with water. The pH of the water used in was 7.6, the total dissolved solids content was 292.6 mg/L, a salinity grade of C2S1 which has no salinity hazard.

Emergence ratio (%), mean emergence time (day), plant length (cm), spike length (cm), kernel number per spike (grain), grain yield (kg/ha), protein ratio (%) and sedimentation value (ml) were examined in the experiment. Protein ratio was found according to Kjeldahl method (Kacar, 2010) and sedimentation value was found according to Zeleny method (Willams et al., 1986).

All data were statistically analyzed by using TOTEMSTAT package program and differences between the applications were compared with LSD test.

RESULTS AND DISCUSSIONS

Analysis of variance showed that the effects of Nano S applications on emergence ratio, mean emergence time, spike length, kernel number per spike, grain yield, protein ratio and sedimentation value were significant in 0.01 levels and plant length was significant in 0.05 levels. The mean values and difference groupings for the characteristics studied are summarized in Table 2.

Application of Nano-S to soil **(SA)** resulted in the highest field emergence ratio (100%) and this application was followed by control application **(CA)** with 78.3% (Table 2).

Keşli (2009) and Bejandi et al. (2009) were reported that increasing doses of sulfur was increased in emergence ratio as compared to control. Other applications caused decrease field emergence ratio but these reductions were not significant as compared to control treatments.

Application of Nano-S in bread wheat caused decreased mean emergence time.

In other words, field emergence was faster than control application. While the fast emergence time was determined in application of SA with 2.35 days, the slowest emergence time was in CA with 2.83 days. This application was followed by SC, SC+BA and SC+HA with the value of 2.53 days, 2.55 days and 2.57 days, respectively (Table 2).

Table 2. Means of yield and some plant characteristics in applied different Nano-S bread wheat

	Means		
Treatments	Total Emergence Ratio (%)	Mean Emergence Time (day)	Plant Height (cm)
CA	78.3 b	2.83 a	68.7 b
SA	100.0 a	2.35 c	73.4 a
SC	75.0 b	2.53 b	71.6 ab
SC+BA	75.0 b	2.55 b	73.7 a
SC+HA	76.3 b	2.57 b	71.2 ab
LSD	6.04	0.11	3.32
	Spike Length (cm)	Kernel Number per Spike	Grain Yield (kg/ha)
CA	8.37 b	35.55 c	3431 b
SA	9.92 a	39.50 a	3835 a
SC	9.66 a	38.03 b	3558 b
SC+BA	9.59 a	38.57 b	3911 a
SC+HA	9.47 a	38.57 b	3846 a
LSD	0.58	0.71	140.5
	Protein Ratio (%)	Sedimantation values (ml)	
CA	13.3 c	38.2 d	
SA	14.6 a	46.6 b	
SC	14.0 b	48.7 a	
SC+BA	14.3 ab	45.6 b	
SC+HA	14.3 ab	42.1 c	
LSD	0.46	1.16	

It is determined that the highest plant height was 73.7 cm obtained from SC+BA Nano S application. This application was followed by application to SA with 73.4 cm, SC with 71.6 cm and SC+HA with 71.2 cm, respectively. The lowest plant length was determined in CA with 68.7 cm. The lowest spike length of 8.37 cm was determined in CA, and the highest spike length of 9.92 cm was determined in SA. SA applications were increased spike length of 13.14-18.52% compare to CA. The lowest mean values in terms of kernel number per spike were found in control plots (35.55) and this was followed by seed coat, seed coat+bolting period and seed coat+heading period application of Nano S. The best kernel numbers per spike with 39.5 in all of applications was Nano S application to soil. Eraslan (2006) was reported that increasing doses and application methods of S were positively affected and increased plant length, spike length and kernel number in spike in two wheat cultivars. It was reported by meant earlier researchers that increased the plant height in some crop plants such as rapeseed (Rehman et al., 2013), fenugreek (Tunçtürk et al., 2011; Verma et al., 2014) sunflower (Demir, 2009), chickpea (Togay et al., 2008; Kamiloğlu, 2008).

The lowest grain yield of 3431 kg/ha was obtained in CA but this was not significantly lower than SC (Table 2). The highest grain yield of 3911 kg/ha was determined SC+BA application. This grain yield increases was 14% higher than the CA. Although the highest grain yield was obtained from the application of Nano S in seed coat+booting period plots; three applications (SA, SC+BA, SC+HA) were also economically suggestible because they were in the same statistical group. İnal et al. (2003) were indicated that sulphur applications significantly contribute to yield and yield factors in wheat. A lot of researchers were found to increased grain yield with application of Sulfur (Jackson, 2000; Khan, Samiullah, 2005; Eraslan, 2006; Zhao et al., 2008; Tonguç et al., 2017).

All of the Nano-S applications have significantly increased protein ratio of bread wheat. The highest protein ratio was determined in SA, which was increased protein ratio about 10% (Table 2). The minimum protein ratio of 13.3% was observed in the CA. Since protein ratio is an important factor in terms of flour quality and unit price in wheat purchase scale, it is very important to determine the applications of increasing protein ratio. Eraslan (2006) reported similar results regarding to protein ratio. Nitrogen and Sulfur were compounds of proteins and so, a balance between N and S is very important in bread quality of wheat (Randall, Wrigley, 1986). Ryant and Hrivna (2004) were indicated that sulfur does not only affect nitrogen use and protein quality, but also plays an important role in cooking quality. Singh (2003), was reported that S deficiency in cereal crops is a limiting factor not only on plant growth and yield, but also on the poor quality of crops. Because sulfur is taken place in a lot of main compounds some of structure such as cysteine, methionine, coenzymes, thioredoxin and sulfolipids, it is so important that sufficient amount or beneficial form of S need to be applied or need to be found in the plant growth environment. Sulfur application, amino acid composition was changed especially sulfur-containing cysteine and methionine ratios. Ali et al. (1990) were reported sulfur had an essential role in the synthesis of proteins and of a wide variety of metabolites that are critical for plant growth.

Gluten content and sedimentation value are important in terms of flour quality in bread wheat. All of Nano-S applications was significantly increased the sedimentation value. Nano-S applications were also positively influenced sedimentation value as compared to control plots. As a matter of fact, the lowest sediment value of 38.2 ml was determined in the CA and it was ranked in the middle class sedimentation value (middle class is value between 20-40 ml). The highest sediment value of 48.7 ml was determined in SC and it was taken place very strong class in terms of sediment value in flour (Table 2). The lowest sedimentation value was observed in CA. Kınacı and Kınacı (2004) found the highest sedimentation value (33-36 ml) with $ZnSO_4$ applications rather than other chemical fertilizers. Dizlek et al. (2013), were reported 30 kg/ha S application was increased sedimentation value. These results demonstrate that Nano-S applications in terms of flour quality may have adverse effects decreased sunn sucking and the preparation can be used effectively in struggle against the sun.

CONCLUSIONS

When the results of the research were evaluated collectively the application of Nano-S in all the examined traits resulted in significant increases as compared to the control plots. Generally the CA was followed by the SC, while other Nano S applications had the highest average. In most of the traits, the SA has the highest traits values; especially grain yield and protein ratio properties. For this reason, to increase of grain yield and grain quality of bread wheat SA and SC+BA treatments were applicable. If it is possible SC+HA treatments were also suggestable.

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EFFECT OF EXTRA POTASSIUM SUPPLY ON AMINO ACID COMPOSITION OF CORN SEED UNDER THE DEFICIT IRRIGATION CONDITIONS: (SECTION C)

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Abstract

The study investigates the influence of extra potassium application and deficit irrigation on amino acid composition of corn seed. The field experiment was conducted with split plot design with three replications in Aydın location of Turkey in 2013. Parcels of the experiment was established with three different supplies as non-fertilization, standard fertilization [210 kg.ha^{-1} pure N, 60 kg.ha^{-1} pure P, 60 kg.ha^{-1} pure K, 60 kg.ha^{-1} (NH_4NO_3, P_2O_5 and K_2O with 15-15-15 composite before planting and H_2NCONH_2 - 150 kg.ha^{-1} with urea before first water)] and extra potassium (standard fertilization +60 kg.ha^{-1} K_2SO_4) and three irrigation doses (500 mm, 400 mm and 300 mm) during development stages [(8 leaf stage (V8), before Tasseling (VT), after blister, milk stage and dough stage)]. 31G98 corn variety, identified as having the highest yield in previous our paper published Scientific Papers Series A. Agronomy, was used. Amino acids rates (essential, conditionally essential and non-essential) of the corn seeds obtained from the different treatments parcels were measured with HPLC.

According to the results of the experiment, significant differences among treatments and irrigation doses were found. Almost all essential amino acids were increased with standard fertilization and extra potassium supply compared to non-fertilization. Just methionine and threonine in this group were irregular with these treatments. Similarly, almost all essential amino acids values except for histidine, valine and phenylalanine were increased with 400 mm compared to 300 mm, whereas these groups were decreased with 500 mm irrigation dose. As for conditionally essential and non-essential amino acids, glutamic acid and serine were increased with standard fertilization while were decreased with extra potassium supply. Contrary to glutamic acid and serine, glycine was decreased with standard fertilization while was increased with extra potassium supply. The other amino acids in the group were increased with both standard fertilization and extra potassium supply compared to non-fertilization. In terms of irrigation doses, tyrosine and serine values were increased with raising irrigation doses. Proline, arginine, aspartic acid and glutamic acid were increased with 400 mm compared to 300 mm, whereas the amino acids were decreased with 500 mm irrigation dose.

Key words: *corn, amino acid, potassium supply, lysine, HPLC.*

INTRODUCTION

Proteins are one of the most necessary component of the body. They are abundant component after water and they play significant role in the whole cells of body both structural and functional. For example muscle cells make up over 40% of our body protein (Grimble et al., 1992). Moreover hemoglobin contains 16% (Vinton et al., 1986). So we can say that protein has an essential function in every cell including in membranes, transporters, enzymes, components of the immune system, and is also a precursor to hormones (Jerlich et al., 2000).

Proteins are macromolecules, which are constructed in the body from chains of amino acids. Using the 20 primary amino acids, the body constructs an overwhelming abundance of protein chains, each with a different order coded by our genetic code (Raiha et al., 1996). These chains interact and fold into specific three dimensional shapes to give specialized functionality. Throughout human life, there is some speedy growth periods especially in infancy, childhood and teenage (Lourenço, Camilo, 2002), so there are increases in length, mass, development and maturation of function (Vinton et al., 1986). Similarly, some other periods like pregnant or lactating, there are also the demands for net tissue deposit or milk formation. In each of these periods, the needs are for a pattern of amino acids that matches the material being deposited, including extracellular proteins, DNA, RNA, cell membranes etc. (Jerlich et al., 2000). There is good evidence that the pattern of amino acids that is needed to meet these demands is different from that in the basal state. There are some studies about different practices like planting date, genotype selection, reduced tillage, and diversification of crop, organic matter and mineral application to increase defense against drought (Carr, 2010; Allen, 2012). Potassium application is the one of treatments for maximum seed yield and quality against the effects of drought. Because potassium plays a vital role in control of ionic balance and regulation of stomas for water use (Rafat et al., 2012). Besides potassium is a primary regulator for osmotic potential while maintaining low water potential of crop. Therefore, accumulating K^+ in their tissues may play an important role for crops under drought condition (Zare et al., 2014). The study were investigated to influence of standard (60 $kg.ha^{-1}$ K_2SO_4) and extra potassium fertilizer (double potassium rate) on amino acids composition under deficit irrigation conditions.

MATERIALS AND METHODS

The research was carried out in Aydin with typical Mediterranean climate (hot summer and mild winter), located in west Turkey at 37º 44' N 27º 44' E at 65 m above sea level; and was conducted during 2013. Initial result of soil analysis is shown in Table1 (Bouyoucos G.J., 1962; Ayers, Westcot, 1989; Walkley, Black, 1934).

Table 1. Soil texture and chemical analysis

Soil texture[1] (%)			pH[2]	Organic mater[3]
Sand	Silt	Clay		(%)
72.0	16.7	11.3	8.4	1.2

Method of: [1]Bouyoucos; [2]1: 2.5 Saturasyon; [3]Walkley-Black.

The experimental soil in studied field contains with sandy loamy structure with alkaline characteristic and it mixed with quite low organic matters.

Monthly temperature, total rainfall and long term (1975-2013) values in Aydin shown in Table 2. The temperature of 2013 was higher than long term means expect for June and July. Rainfall data analysed that May, July and August were showed lower value than long term means.

Table 2. Monthly temperature and total rainfall during corn growth period and long term mean (1975-2013) in Aydin

Months	Temperature (°C)		Precipitation (mm)	
	2013	Long term	2013	Long term
April	16.1	15.7	42.6	45.5
May	23.2	20.9	1.0	33.5
June	25.3	25.9	18.4	14.0
July	27.9	28.4	2.4	3.5
August	27.8	27.2	0.0	2.2
September	22.6	23.2	22.8	14.4

Experimental design

The experiment was set up as split block experimental design with 3 replications. All parcels were sowed April 26, 2013 and the first seed emergence observation was conducted on May 13, 2013. Each plot area was 28 m^2 (5 m x 5.6 m) and consisted of 8 rows. Distance between rows was 70 cm and intra row spaces were 18 cm. P31G98 which is hybrid (F1) and has single cross corn (*Zea mays* L.) cultivar was used as the crop material. The variety produced by Pioneer Turkey Seed Distribution and Marketing Co. Ltd was identified as having the highest yield and high thousand seed weight in previous our paper published Scientific Papers, Series A, Agronomy. Treatment factors were created out with non-fertilizer, standard fertilizer and extra potassium application. There was not been any applications fertilizers form to non-fertilizer parcels. Standard fertilizer application from soil was applied as 210 $kg.ha^{-1}$ pure nitrogen (NH_4NO_3) (60 $kg.ha^{-1}$ with 15-15-15 composite was applied immediately at the beginning of cultivation - 150 $kg.ha^{-1}$ with urea (H_2NCONH_2) before first water), 60 $kg.ha^{-1}$ phosphor (P_2O_5) and 60 $kg.ha^{-1}$ potassium (K_2O). Extra potassium application was formed to by being added to 60 $kg.ha^{-1}$ K_2SO_4 on to the standard fertilizer application. In other words, it has been said that extra potassium application had only double potassium rate compared to standard fertilization. So we hope that effects of double potassium application on amino acids composition of corn seed under deficit irrigation conditions.

Soil from the field experiment [(0-30 cm, 30-60 cm and 60-90 cm depth (Rd)] was put into pots. The water content of the soil after being saturated by irrigation and allowed to drain is called field capacity (FC). Crop can no longer take up water from the soil is referred wilting point (WP). The water held by the soil between field capacity and the permanent wilting point is considered available water. Corn is capable of using 50% of the available water (AW). Irrigation water requirement (100 mm) was calculated with the following formulas (Martin, Gilley, 1993; Lamm et al., 1994).

$$AW = Rd\ (FC-WP)/100$$

Irrigation doses was formed as standard (5x100 mm) during 5 times (V8, before VT, after blister, milk stage and dough stage), as deficit irrigation (4x100 mm) during 4 times (V8, before VT, after blister and milk stage) and as more deficit irrigation (3x100 mm) during 3 times (V8, before VT and after blister) at corn growth period. All irrigation applied and time were given in Table 3.

Table 3. Irrigation times in corn growing season

Irrigation time	Irrigation rate per plot		
	300 mm	400 mm	500 mm
20th June 2013	**X**	**X**	**X**
04th July 2013	**X**	**X**	**X**
12th July 2013	**X**	**X**	**X**
25th July 2013	-	**X**	**X**
06th August 2013	-	-	**X**
10th September 2013	Harvest time		
X: Applied 100 mm water			

The analysis of amino acid composition of corn seed

On the basis of needs from the diet for nitrogen balance or growth, amino acids were traditionally classified as nutritionally essential or non-essential for humans and animals (Lupi et al., 2008). Essential amino acids are defined as amino acids whose carbon skeletons cannot be synthesized by the body relative to needs (El Idrissi, 2008). Conditionally essential amino acids normally can be synthesized in adequate amounts by the organism, but must be provided from the diet to meet optimal needs under conditions where rates of utilization are greater than rates of synthesis. Non-essential amino acids can be synthesized in adequate amounts by the body to meet optimal requirements (Novelli, Tasker, 2008; Phang et al., 2008).

Sample Preparation

Grain samples from the experiment corn samples were milled and weighed 0.1 g. 5 ml 6 N HCl was added to the sample and respectively 250 µl 2 mM phenol and 0.1 g Na_2SO_3 was added. The sample was placed in an oven at 110°C 24 h and after the time pH level of the sample was adjusted to 6.7-7.3 with 5 N NaOH. The supernatant was centrifuged with 4000 rpm in 5 minutes and then filtered through a filter and fitted to vials.

HPLC Determination

The whole amino acid content of the samples was performed with HPLC system, consisting of a Shimadzu Nexara XR (Shimadzu Corporation, Kyoto, Japan) with Zorbax Eclipse AAA (15 cm x 4.6 mm x 3.5 µm) coloumn. The mobile phase A which consisted of 40 mmol $NaH_2PO_4.2H_2O$ adjusted to pH 7.8 with 5 N NaOH. The mobile phase B was composed of acetonitrile-methanol-water (45: 45: 10, vol. %). Briefly the hydrolyzed samples and solutions the standard amino acid mixture was automatically derivative with OPA and FMOC. Coloumn temperature was 40°C and injection volume was 9 µl. The amino acids were achieved by calibrating with a standard mixture of amino acids. Peak integration accuracy was enhanced by manual establishment of peak baselines using software.

Statistical analysis

The amino acids data collected from the experiment were subjected to analysis of variance (ANOVA) using TOTEM STAT statistical software (Acikgoz et al., 2004). Means among treatments were compared using Least Significant Difference (LSD) at $P \leq 0.05$ probability (Stell et al., 1997).

Table 4. Change of essential amino acids with potassium treatment on deficit irrigation conditions

Treatment (A)	Irrigation Doses (B)	HIS	VAL	MET	THR	ILE	LEU	LYS	PHE
Non-fertilization	300	0,218	0,294	0,198	0,234	0,203	0,606	0,179	0,303
	400	0,218	0,281	0,191	0,213	0,207	0,616	0,183	0,307
	500	0,242	0,305	0,178	0,234	0,210	0,641	0,197	0,316
	average	0,226	0,293	0,189	0,227	0,207	0,621	0,186	0,309
Standard fertilization	300	0,246	0,333	0,176	0,242	0,244	0,782	0,205	0,374
	400	0,245	0,314	0,152	0,282	0,253	0,769	0,236	0,347
	500	0,251	0,307	0,183	0,239	0,225	0,776	0,175	0,354
	average	0,247	0,318	0,170	0,254	0,241	0,776	0,205	0,358
Extra Potassium supply	300	0,243	0,340	0,178	0,243	0,247	0,774	0,224	0,366
	400	0,277	0,374	0,224	0,278	0,267	0,839	0,234	0,385
	500	0,246	0,367	0,229	0,230	0,290	0,766	0,198	0,368
	average	0,255	0,360	0,210	0,250	0,268	0,793	0,219	0,373
Average of Irrigation Doses	300	0,236	0,322	0,184	0,240	0,231	0,721	0,203	0,348
	400	0,247	0,323	0,189	0,258	0,242	0,741	0,218	0,346
	500	0,246	0,326	0,197	0,234	0,242	0,728	0,190	0,346
LSD A*B (0,05)		0,009	0,023	0,011	0,015	0,013	0,047	0,018	0,021

HIS: Histidine, VAL: Valine, MET: Methionine, THR: Threonine, ILE: Isoleucine, LEU: Leucine, LYS: Lysine, PHE: Phenylalanine

Table 5. Change of conditionally essential and dispensable amino acids with potassium treatment on deficit irrigation conditions

Treatment (A)	Irrigation Doses (B)	GLY	PRO	ARG	TYR	CYS	ASP	GLU	SER
Non-fertilization	300	0,290	0,558	0,360	0,253	0,359	0,416	1,056	0,327
	400	0,283	0,548	0,369	0,258	0,282	0,454	1,068	0,378
	500	0,306	0,592	0,394	0,257	0,315	0,484	1,146	0,377
	average	0,293	0,566	0,374	0,256	0,319	0,451	1,090	0,361
Standard fertilization	300	0,314	0,623	0,410	0,300	0,342	0,538	1,384	0,439
	400	0,262	0,629	0,405	0,290	0,357	0,510	1,313	0,378
	500	0,289	0,597	0,378	0,291	0,257	0,477	1,297	0,430
	average	0,288	0,616	0,398	0,294	0,319	0,508	1,331	0,416
Extra Potassium supply	300	0,311	0,625	0,399	0,303	0,370	0,480	1,243	0,324
	400	0,353	0,680	0,459	0,314	0,419	0,594	1,451	0,455
	500	0,249	0,650	0,411	0,334	0,346	0,482	1,106	0,407
	average	0,304	0,652	0,423	0,317	0,378	0,519	1,267	0,395
Average of Irrigation Doses	300	0,305	0,602	0,390	0,285	0,357	0,478	1,228	0,363
	400	0,299	0,619	0,411	0,287	0,353	0,519	1,277	0,404
	500	0,281	0,613	0,394	0,294	0,306	0,481	1,183	0,405
LSD A*B (0,05)		0,025	ns	0,026	0,013	0,044	0,038	0,088	0,053

GLY: Glycine, PRO: Proline, ARG: Arginine, TYR: Tyrosine, CYS: Cysteine, ASP: Aspartic acid, GLU: Glutamic acid, SER: Serine

RESULTS AND DISCUSSIONS

Least Square means of amino acid parameters was calculated through variance analysis. According to The results of variance analysis for treatment factors (different fertilizations) and water doses (deficit irrigations) and their interaction are presented in terms of essential, conditionally essential and dispensable amino acids. Irrigation dose x treatment factor interaction was found to be significant in all parameters except for proline. All essential, conditionally essential and dispensable amino acids values were tabulated in Table 4 and Table 5. Moreover LSD (0.05) values about Irrigation dose x treatment interaction were given under the tables.

Histidine, valine, methionine, threonine, isoleucine, leucine, lysine and phenylalanine values were shown as essential amino acids in Table 4. Almost all essential amino acid averages were increased with standard fertilization compared to non-fertilization. Just methionine average under standard fertilization condition was decreased. Similarly, almost all essential amino acid averages were increased with extra potassium supply compared to standard fertilization. Only threonine under extra potassium supply condition was decreased. So we can say that a dose potassium (60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively almost all essential amino acids.

According to irrigation doses results, some essential amino acid averages were increased with increasing irrigation dose but, others were decreased or didn't change. Valine, methionine and isoleucine averages were increased with increasing irrigation doses (only isoleucine stable under 500 ml). Histidine, threonine, leucine and lysine averages were increased with 400 ml and then the averages decreased with 500 ml. just phenylalanine value was decreased with increasing irrigation doses. Thus, we can say that drought stress (300 ml) affected negatively almost all essential amino acid averages expect for phenylalanine. And increase water doses once (400 ml) or twice (500 ml) into irrigation period could block the negative effect on some essential amino acid averages in some degree.

Glycine, proline, arginine, tyrosine, cysteine, aspartic acid, glutamic acid and serine values were shown as conditionally and non-essential amino acids in Table 5. Almost all conditionally essential and non-essential amino acid averages were increased with standard fertilization compared to non-fertilization. Just glycine was decreased and cysteine wasn't change under standard fertilization condition. Similarly, almost all conditionally essential and non-essential amino acid averages were increased with extra potassium supply compared to standard fertilization. Only glutamic acid and serine averages were decreased under extra potassium supply condition. So we can say that a dose potassium (60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively or nerveless almost all conditionally essential and non-essential amino acids.

In accordance with irrigation doses results, some conditionally essential and non-essential amino acid averages were increased with increasing irrigation dose but, others were decreased or didn't change. Serine and tyrosine were increased with increasing irrigation doses. Proline, arginine, aspartic acid and glutamic acid were increased with 400 ml and then the averages decreased with 500 ml. Glycine and cysteine values were decreased with increasing irrigation doses. So, we can say that drought stress (300 ml) affected negatively on almost all conditionally essential and non-essential amino acid averages expect for glycine and cysteine. And increase water doses once (400 ml) or twice (500 ml) into irrigation period could comparatively block the negative effect.

CONCLUSIONS

Our results from the study given below:

- Except for methionine and threonine, all essential amino acids were increased with treatments (standard fertilization and extra potassium supply). So we can say that a dose potassium (60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively almost all essential amino acids;
- Except for phenylalanine, drought stress (300 ml) affected negatively all essential amino acid averages. And we can say that increase water doses either once (400 ml) or twice (500 ml) into irrigation period blocked the negative effect on essential amino acid averages in some degree;
- There were found different results about potassium treatments effects on conditionally essential and non-essential amino acid averages. Glycine and cysteine averages under standard fertilization and glutamic acid and serine averages under extra potassium supply decreased. So we can say that a dose potassium

(60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively or nerveless almost all conditionally essential and non-essential amino acids;

- Expect for glycine and cysteine, drought stress (300 ml) affected negatively on almost all conditionally essential and non-essential amino acid averages. Increase water doses either once (400 ml) or twice (500 ml) into irrigation period could comparatively block the negative effect.

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PHOSPHINE RESISTANCE OF RUSTY GRAIN BEETLE *Cryptolestes ferrugineus* (*Coleoptera: Laemophloeidae*) POPULATIONS IN TURKEY

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Abstract

Rusty grain beetle Cryptolestes ferrugineus (Coleoptera: Laemophloeidae) is one of the most important pests of stored grains. It is known that long term use of phosphine against the pests causes to resistance development in the pests. In this study, 16 C. ferrugineus populations from grain warehouses of 11 provinces were collected. The resistance to phosphine of the populations was determined by modifying the FAO method to obtain LC_{50} values. Resistance ratios were calculated by comparing the LC_{50} values of the populations to the LC_{50} value of the sensitive population. The C. ferrugineus population obtained from İzmir province died at 36 ppm discriminating dose and LC_{50} value was calculated as 16.82 ppm/20 hrs. This population was considered susceptible and used in LC_{50} comparisons to determine the resistance ratios. The LC_{50} was not calculated for the six populations which died at the discriminating dose. In the 10 populations which LC_{50} were calculated, resistance ratios were found between 3.0 and 392.6 times. According to the results, phosphine resistance of C. ferrugineus was common and generally high in Turkish populations.

***Key words**: Cryptolestes ferrugineus, phosphine resistance, Turkey.*

INTRODUCTION

Among the 23.9 million hectares of arable land, 49% is the largest share of grains. In total cereal fields, wheat is the first with 67% share. Wheat is followed by barley with 24% share, maize with 6%, rice, rye and oat with 1%. Turkey's grain productions in the year 2016 have been realized as 225 thousand tons; 20.6 million tons of wheat, 6.7 million tons of barley, 6.4 million tons of corn, 920 thousand tons of rice and 300 thousand tons of rye, oats (Anonymous, 2016).
Cereals are subjected to weight, technological value and seed loss due to body residues, scum and nettings and similar substances that have been secreted by many harmful pests. One of the most important pest of the stored grain in our country is the Rusty Grain Beetle *Cryptolestes ferrugineus* (Stephens) (*Coleoptera: Laemophloeidae*). *C. ferrugineus* larvae and adults are fed on seeds and endosperm in wheat, barley, rye, triticale, oats and sometimes milled crops, causing fungal spores to spread throughout the warehouse (Canadian Grain Comission, 2014). This pest is known commonly in Turkey (Aydın, Soran, 1987; Coşkuncu, 2004; Bağcılar et al., 2014). Cereals should not be consumed in a short time and special conditions such as natural disasters and war should be stored on suitable conditions for the preparation. It is important to combat insect species that lead to product loss in the prevention of quality and weight losses that can occur during storage of cereals.
In the world, methyl bromide and phosphine gas were widely used to fight against stored product pests. 157 tons of aluminum phosphide and 12 tons of magnesium phosphide imports were carried out to Turkey in 2011 (Anonymous, 2012). Only phosphine (PH_3) is used as a fumigant by removing it from the use except methyl bromide quarantine applications.
Phosphine mechanism of action on insects; reducing the effect of the enzyme acetylcholinesterase in the nervous system, inhibiting the enzyme glycerophosphate dehydrogenase, and neutralizing the Complex IV region in mitochondria. Furthermore, because of a direct redox relationship between phosphine and cysteine in reactive disulfide, phosphine disrupts the enzyme glutathione reductase and breaks down sulphide bonds in cysteine (Nath et al., 2011). It is important to determine the resistance status of this pest to phosphine when taking into consideration that

it is a long-lasting and intensely single fumigant phosphine used in the challenge with *C. ferrugineus*. The World Health Organization defines resistance as the development of a specific dose of a toxic substance found to have killed a majority of individuals in a normal population, the ability to gain tolerance in other affected individuals. The major disadvantage is that the ability of the pests to develop resistance to this chemical but phosphine has many advantages such as ease of use, low cost and no significant residue problem.

Warehouse pests have been found to develop resistance to phosphine in more than 45 countries (Benhalima et al., 2004; Zettler and Keever, 1994; Pimentel et al., 2010). Phosphine is preferred because of its low cost, rapid spreading in air and low residue (Champ and Dyte, 1976; Benhalima et al., 2004).

Therefore, it is important to develop resistance management models by protecting the efficacy of phosphine against stored product pests, the sustainability of its use, and the rate of resistance to phosphine in the pests.

In this study the phosphine resistance levels against *C. ferrugineus* populations collected from grain storage in various regions of Turkey was determined.

MATERIALS AND METHODS

In the experiments, 16 *C. ferrugineus* populations obtained from grain warehouses in Adana, Burdur, Konya, Isparta, İzmir, Samsun and Şanlıurfa (Figure 1). *C. ferrugineus* were cultured in 500 ml glass jars at 30°C and 60% relative humidity under constant conditions with 5 g yeast in 100 g oatmeal.

From this culture, in which the adults were kept in the medium for 10 days, the eggs were transferred to a new culture medium daily. Toxicological studies were carried out on 2-3 week old adults (Flinn and Hagstrum, 1998).

In order to determine the resistance ratios of the cultured individuals to the phosphine in the laboratory, discriminating dose applications were performed primarily.

The discriminating dose for *C. ferrugineus* is the application of 0.05 mg/l phosphine for 20 hours. LC_{50} and LC_{99} studies have been carried out for the populations in the case of live individuals. In the absence of live individuals, the population was considered sensitive (FAO, 1975; Anonymous, 2013).

Figure 1. Provinces across Turkey where *Cryptolestes ferrugineus* populations collected

In the case of live individual/s, the LC_{50} and LC_{99} studies were carried out in 5 different doses and 3 replications, determined preliminarily. Live-dead insect counts were subjected to probit analysis to obtain lethal concentration values (LC_{50} and LC_{99}), slope and confidence limits. For probit analysis, POLO-PC 1987 and PoloPlus 2002-2009 (LeOra Software Inc., Berkeley, CA, USA**)** programs were used. Resistance ratios were obtained by dividing the LC_{50} values of the land population by the LC_{50} values of the sensitive population (FAO, 1975; Pimentel et al., 2008).

The gaseous phosphine (PH_3) used in the studies was produced by mixing aluminum phosphite pellets in water containing 5% H_2SO_4 (FAO, 1975). The PH_3 gas collected at the top of the gas generator was taken from the septum by gas syringe.

In experiments, gas-tight custom desiccants with a volume of 3 liters and ATI-Porta Sense II gas meter to measure the amount of phosphine were used. In each desiccator 25 randomly selected adult individuals were placed in 3 x 3 x 3 cm PVC containers with 2 g nutrients. Various amounts of phosphine gas were injected from the septum of the desiccator and the required gas concentrations in the desiccator were provided and then the gas meter and the gas inlet-outlet valves were closed.

The desiccator was placed into incubator at 25°C and 65±5% humidity (FAO, 1975; Kahraman, 2009; Opit et al., 2012). After 20 hours of fumigation application and transfer of adult individuals into the food containing jars in incubators (25°C and 65±5% humidity) for

14 days, live individuals were counted for both the discriminating dose and the LC_{50}-LC_{99} determinations.

RESULTS AND DISCUSSIONS

The studies were carried out in 2016-2017. In this study, resistance against phosphine in *C. ferrugineus* populations collected from different regions of Turkey was investigated. After reaching enough numbers to be assayed in the laboratory, the cultures were subjected to a 20-hour treatment with a concentration of 0.05 mg/l (35.71 ppm) phosphine, the discrimiating dose for *C. ferrugineus*. It has been determined that populations resist at varying rates. As a result of the application, live individuals were observed in the populations of K1, K2, K5 and E4, and LC_{50} and LC_{99} values were determined by bioassay experiments. The İ5 population was accepted as sensitive population because of the low lethal concentration of phosphine, LC_{50} and LC_{99} values were calculated and assessed for comparison (Table 1).

In the bioassay experiments with K2, K5 and E4 populations, up to 80% deaths were not detected despite the increase the dose of phosphine. The exact LC_{50} values for the three populations mentioned could not be determined because testing of higher doses than our phosphine measurement device limits had the risk of subsequent flammability.

Considering the LC_{50} values, the resistance ratios obtained by proportion of the populations examined to the sensitive population were 11.73 for A3, 43.88 for E11, 19.73 for E12, 75.32 for F3, 3.68 for G2, 3.38 for H3, 66.71 for İ1, 279.62 for E4, 358.9 for K2 and eventually 392.65 times more resistant for K5 populations. The most resistant population was found in samples taken from Adana Mustafabeyli TMO warehouse with 392.65 times.

Table 1. Of phosphine resistance ratios of *C. ferrugineus* populations across Turkey

Population	n	Slope ± SE	χ2(df)	LC50 (ppm) (Confidence intervals)	LC99 (ppm) (Confidence intervals)	RR
İ5 (Susceptible) Samsun	375	5.09 ± 0.45	15.89	16.82 (14.95- 18.65)	48.14 (40.08- 62.91)	1
H3 Burdur	375	3.19±0.31	10.04	56.91 (49.22- 64.06)	303.87 (238.97-429.25)	3.38
G2 Isparta	375	3.33 ±0.35	9.42	62.05 (53.81 -70.28)	309.77 (237.52- 457.78)	3.68
E12 Konya	375	5.79±0.63	4.93	325.87 (291.24-356.72)	821.93 (710.2- 1017.56)	19.73
İ2 Samsun	375	7.47 ±1.56	15.36	459.18 (390.30 - 501.20)	940.64 (762.38 - 1701.57)	27.29
E11 Konya	375	3.23±0.3	35.36	738.22 (603.75- 908.40)	3864.1 (2463.1- 9161.5)	43.88
İ1 Samsun	375	5.43±0.47	7.22	1122.21 (1048.28 -1198.53)	3005.96 (2582- 3708.86)	66.71
F3 Şanlıurfa	375	5.35 ±0.55	18.24	1265.53 (1166.79- 1373.13)	3438.66 (2758.43-4977.97)	75.32
E4 Konya	375	6.68±1.04	1.79	4703.21 (4354.68-5333.53)	10480 (8170.8-16675)	279.62
K2 Adana	375	7.47±2.05	2.16	6036.73 (5173.89-9797.06)	12365 (8313-46013)	358.9
K5 Adana	375	7.09±2.34	2.92	6604.5 (5388±16247.7)	14046 (8587.7- 135995)	392.65

n = number of *Cryptolestes ferrugineus*
RR = Resistance ratio (LC_{50} value of the examined population/LC_{50} value of the sensitive population)

It has been found that 10% of the storage pest samples collected in the world-wide studies conducted within the FAO in 1972-1973 were found to be phosphine-resistant. Considering the cases of phosphine resistance, it was found highest in *R. dominica* (F.), *Tribolium* spp. and *Sitophilus* spp. (Champ, Dyte 1976; Taylor, 1989). There are a number of studies showing phosphine resistance in *C. ferrugineus* in China (Liu, 2004; Lu et al., 2005). Madhumathi et al. (2007) indicate that the resistance rate of the population of *C. ferrugineus* collected from different regions in India between 1995 and 1997 was 210 to 230 times.

Nayak et al. (2010) found that there was a need for 0.5 mg/l dose-30 days and 1 mg/l dose-24 days in their study to provide resistance management in Australia's highly resistant population of *C. ferrugineus*.

In Australia, Nayak et al. (2013), in the *Rhyzopertha dominica* population known to be resistant to phosphine, 100 % mortality was observed at 1.4 mg/l during the 144 hours application period, while in the same application this dose was found to be 3.62 mg/l in the *C. ferrugineus* populations with strong resistance. Same research indicated absolute death at 25°C in the strongly resistant *Sitophylus oryzae* population and 0.56 mg/l at 72 hours of exposure, while absolute death for *C. ferrugineus* was observed at the dose of 0.7 mg/l for 30 days.

Konemann et al. (2017), report that resistance were 7.3, 636.4, and 968.6 ppm, respectively, over a 3-d exposure period. The most resistant population, was 133.5 times that of the susceptible laboratory strain.

Duong et al. (2016) collected 176 populations from 125 regions of Vietnam. High frequency resistance has been detected from *C. ferrugineus* (37 and 58%, n = 19), *R. dominica* (1.5 and 97%, n = 65), *S. oryzae* (34 and 59%, n = 82) and *T. castaneum* (70% and 30%, n = 10), respectively.

Konemann et al. (2017) estimated discriminating dose by examining susceptible *C. ferrugineus* populations under 56.2 ppm PH_3/20 hours exposure. They used this discriminating dose to determine PH_3 resistance in 19 *C. ferrugineus* populations from Oklahoma/USA. They determined that all tested populations were resistant to phosphine. However, they have calculated only five populations LC_{99} values because the resistance frequencies of these populations are greater than 90%. The LC_{99} of the susceptible population was found to be 7.33 ppm/3 days, the highest two populations were 636.4 and 968.6 ppm/3 days. They reported that the resistance level of the most resistant population was 133.5 times more resistant than the susceptile laboratory population.

CONCLUSIONS

As a result of the study, *C. ferrugineus* populations of Turkey is widespread in grain storages, which are key pests and over the years have been able to develop high resistance against phosphine. The presence of high levels of resistance (8 of 10 populations), shows that Turkish *C. ferrugineus* populations have high resistance (Avg. 80%) against phosphine. When it is considered that the sampled granaries are generally commercial deposits, it can be easily seen that *C. ferrugineus* does not have a no resistance zone in the reserves using phosphine. To ensure the use of phosphine of sustainable, phosphine resistance related results showed that, the researchers, phosphine traders, practitioners and managers must get togather to shifting to phosphine-use management in Turkey and the determination of national phosphine resistance management strategies and implementation requirements.

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IMPACT OF SOIL TILLAGE REDUCTION ON CULTIVATION PRODUCTIVITY OF WHEAT, BARLEY AND SOYBEAN

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Abstract

Field experiments were done to evaluate the impact of soil tillage reduction on energy efficiency and labour productivity in cultivation of winter wheat, spring barley and soybean. Besides the conventional tillage (CT), in non-conventional tillage methods the following implements were used: RT1 - chisel plough, disc harrow, multi-tiller, seed-drill; RT2 - shallow chisel, seed-drill; NT - no-till seed-drill. As the efficiency indicators of different tillage methods, the following parameters were measured: work rate, energy requirement and yield. It was observed that tillage systems greatly differed regarding energy and labour requirements. Substitution of mouldboard plough with chisel in primary tillage provided a substantial specific energy efficiency improvement. Grain yields varied depending on soil tillage method and growing season. The differences in yield were not significant in winter wheat and soybean cultivation, while the yield of spring barley was significantly higher in RT2 and NT variants then in CT. Cereals such as winter wheat and spring barley responded well to the reduction of tillage, and no-till system might be the best option for these crops cultivation.

Key words: *conservation tillage, energy efficiency, work rate, grain yield.*

INTRODUCTION

Reduced soil tillage is a broad concept that requires elaborate methods and machines suitable for specific crop cultivation, as well as soil and climate conditions. Direct sowing or no-tillage represents the highest level of reduction in soil preparation. Between no-tillage and traditional tillage using the plough for primary tillage, there are various other possibilities of combining operations and tools aiming an optimum soil preparation for planting. Energy consumption, working time and costs of mechanization are different for each of these systems.

More than half of direct energy (or fuel consumption) utilised from soil preparation to harvest was accounted to the soil tillage when conventional tillage method is practised, in which case the primary tillage requires up to 65% of total energy utilised before seeding (Pellizzi et al., 1988).

The long-term application of conventional tillage showed significant environmental and economic drawbacks. According to Tebrüge and Düring (1999), conventional tillage requires 434 kWh ha^{-1} of energy and 4.1 h ha^{-1} of human-machine work. In contrast, reduced tillage systems can save up to 30-40% of fuel/energy and human-machine work, and direct sowing as much as 90%, compared with conventional tillage (Kosutic et al., 2006).

Main disadvantages of conventional tillage are increased soil compaction caused by excessive number of machinery passes, systematic reduction of soil organic matter (humus content) as a result of intensive and frequent tillage and the greater the susceptibility to soil erosion (Birkás et al., 2014).

The world leading agricultures in substitution of conventional soil tillage with different variations of the reduced tillage and direct sowing are United States and Canada in North America and Brazil, Argentina, Uruguay and Paraguay on the South, where conservation tillage and no-tillage systems are applied to more than half of total arable crop area (Derpsch, Friedrich, 2009).

According to FAO statistics, in the period 1960-2015, there were approximately 157 million of hectares worldwide under conservation soil tillage (FAO, 2018). The share of agricultural land in Europe under some system of reduced tillage has not been increased significantly until recent, and it is estimated that is still less than 5% in EU-28 (ECAF 2017).

Numerous studies on reduced tillage and no-tillage have been presented in the literature, but most studies were based on biological factors such as grain yield, yield components, soil structure, weeds, and pests, but few are directed towards soil tillage methods from the standpoint of energy consumption and human labour.

Previous studies suggest that reduced tillage is favourable for high density crops such as wheat, barley and canola, while much worse option for row crops such as corn and soybean (Kisic et al., 2010; Spoljar et al., 2009). Reduced tillage systems, specific to sustainable agriculture, require productivity at least equal to that of conventional technology, optimized energy efficiency and, at the same time, diminished environmental impact (Rusu and Moraru, 2013).

Considering these requirements, the main objective of this study was to determine the impact of soil tillage reduction on energy and labour requirements and to evaluate the opportunities productivity improvements in cultivation of winter wheat, spring barley and soybean.

MATERIALS AND METHODS

Field experiments were done at agricultural company “Njive” (45°30' N, 18°06' E) in Slavonia region. Soil type on the location was Gleyic Podzoluvisol (Škorić, 1986) and its texture in ploughed layer belongs to silty clay loam (Table 1). The climate in this area is semi-humid with a total annual precipitation of 806 mm and an average annual temperature of 11.0°C (Meteorological and hydrological institute of Croatia).

Table 1. Soil particle size distribution

Depth (cm)	Particle size (mm), percentage				Texture[1]
	0.2-2	0.05-0.2	0.002-0.05	<0.002	
0-10	0.80	28.80	44.60	25.80	L
10-20	2.20	8.60	69.40	19.80	SiL
20-30	1.00	10.20	58.00	30.80	SiCL

[1] L = Loam, SiL = Silty loam, SiCL = Silty clay loam

Experimental field consisted of 12 plots with dimension 100 m x 30 m each, organized as randomized blocks with three replications. Test crops were winter wheat (*Triticum aestivum* L.), winter barley (*Hordeum vulgare* L.) and soybean (*Glycine max* L.). Cultivars and sowing rates are given in table 2.

Table 2. Cultivars and sowing rates of test crops

Crop	Cultivar	Sowing rate (kg ha^{-1})
Winter Wheat	Renan	220
Soybean	Anica	135
Spring Barley	Prestige	200

Soil tillage methods and implements used were:

1. Conventional tillage (CT): mouldboard plough, disc harrow, seedbed implement, seed-drill;
2. Reduced tillage 1 (RT1): chisel plough, disc harrow, multi-tiller, seed-drill;
3. Reduced tillage 2 (RT2): shallow chisel, seed-drill;
4. No-tillage (NT): no-till seed-drill.

Ploughing depth in CT was 30 cm in average and chisel plough in RT1 was adjusted at the same working depth. Tillage with shallow chisel in RT2 was done at 12.5 cm depth in average. There were two passes with disc harrow in CT and one pass in RT1.

A 4WD tractor with engine power of 140 kW was used for all tillage operations. The working width of the tillage implements (Table 3) was chosen according to the pulling capacity of the tractor.

Table 3. Tractor-implement aggregates in soil tillage

Field operation	Implement	Working width (m)
Ploughing	Rabewerk Supertaube 180 MX	1.58
Deep chiselling	Pegoraro Mega Drag	3.50
Shallow chiselling	Horsch Terrano 5 FX	4.00
Disc-harrowing	OLT Neretva-68	4.00
Seedbed preparation	Lemken System Korund 750L	7.00

Energy requirement of each tillage method was determined based on the tractor’s fuel consumption. Energy equivalent of 38.7 MJ L^{-1} was presumed (Cervinka, 1980). The amount of fuel consumed was measured for each implement during tillage and sowing on each plot. Specific energy requirement was calculated as the ratio of input energy from fuel in relation to crop yield. Labour requirement was determined by measuring the time for finishing single tillage operation at each plot of the known area. Yields were determined by weighing grain mass of each harvested plot and recalculated according to storage grain moisture content. Fertilization and crop protection were uniform in all variants, determined by crop specific nutrient requirements and pest occurrence. In the first vegetation year of this research has been previous crop was onions after which the soil surface remained free of weeds and with very little residue.

Statistical data analysis was done with the SAS software (SAS Institute, 2002). The signify-cance of differences between the mean values of measured parameters was assessed by analysis of variance (ANOVA).

The Fisher's least significant difference test (LSD) was used to compare the means and those differences were considered as significant at the level of probability $p < 0.05$.

RESULTS AND DISCUSSIONS

Conventional soil tillage (CT) was expectedly most fuel/energy consuming tillage system. Average fuel consumption in CT was 65.57 L ha^{-1} in which the primary tillage with mouldboard plough stands out with 34.08 L ha^{-1} or 52% of total fuel consumption for tillage and sowing. This is in accordance with experiences of Zimmer et al. (2014), who stated that ploughing required 25-35 L ha^{-1}, depending on soil types, field conditions and machinery and equipment used.

Substitution of mouldboard plough with chisel plough in RT1 and omission of one pass with disc harrow, enabled 42.5% fuel savings compared to CT.

Average fuel consumption in RT1 was 37.71 L ha^{-1} out of which 16.29 L ha^{-1} or 43.2% was utilised in primary tillage with chisel plough. Reduction of tillage depth only to the shallow seeding layer in RT2, resulted in further decrease in fuel consumption, 16.22 L ha^{-1} in average or 75.3% less than in CT. In No-tillage (NT), only 10.3% of fuel required in CT was consumed.

Values of fuel consumption in individual tillage methods for each crop cultivation are presented in Table 4. A significant decrease in fuel consumption is observed with reduction of soil tillage intensity.

Table 4. Yields, energy requirement and productivity of different soil tillage methods

Crop	Tillage	Yield (Mg ha^{-1})	Fuel (L ha^{-1})	Specific energy (MJ Mg^{-1})	Productivity (ha h^{-1})	Productivity (Mg h^{-1})
Winter wheat	CT	5.689	76.79 a	522.4 a	0.354 c	2.017 c
	RT1	6.059	35.10 b	224.2 b	0.636 c	3.853 c
	RT2	6.629	15.46 c	90.3 c	1.516 b	10.047 b
	NT	6.726	6.33 d	36.4 d	3.413 a	22.956 a
Soybean	CT	2.398	62.50 a	1008.7 a	0.417 c	1.001 c
	RT1	2.136	40.56 a	734.9 a	0.677 c	1.447 c
	RT2	2.973	14.15 b	184.2 b	1.335 b	3.970 b
	NT	2.402	5.92 c	95.4 b	2.987 a	7.175 a
Spring barley	CT	3.391 c	57.42 a	655.3 a	0.436 c	1.478 d
	RT1	4.227 b	37.46 ab	343.0 b	0.648 bc	2.741 c
	RT2	5.101 a	19.05 bc	144.5 bc	1.094 b	5.581 b
	NT	5.122 a	8.10 c	61.2 c	3.327 a	17.041 a

[1] Different letters within a crop growing season indicate significant differences at p < 0,05 level.

Soil tillage did not have a significant impact on grain yield in winter wheat nor in soybean cultivation. The highest average yield of winter wheat was achieved in NT (7.726 Mg ha^{-1}) and soybean in RT2 (2.973 Mg ha^{-1}).

In spring barley cultivation, however, yields were significantly different across tillage methods.

The highest average yield was again recorded in NT (5.122 Mg ha^{-1}), which was 51% more than in CT. RT1 also provided significantly lower yield than RT2 and NT, but higher than CT. That may be a consequence of cumulative soil degradation caused by consecutively applied conventional tillage (Birkas, 2008).

Specific energy requirement (MJ Mg^{-1}) for different soil tillage methods varied due to wide range of crop yields, but a decrease of energy demands with reduction of soil tillage is clearly noticeable and those differences were in most cases statistically significant.

In conventional tillage system the specific energy requirement was 728.8 MJ Mg^{-1} in average for all crops. The lowest specific energy requirement was in NT with 64.3 MJ Mg^{-1} or 91.2% less than in CT. RT1 with 434.0 MJ Mg^{-1} and RT2 with 139.7 MJ Mg^{-1} were 40.5% and 80.8% less demanding than CT, respectively.

Productivity of soil tillage methods have been calculated both considering the machine work rate (ha h^{-1}) and in respect to obtained yields (Mg h^{-1}). Conventional tillage showed the lowest overall productivity with average work rate 0.402 ha h^{-1}.

Primary tillage with mouldboard plough accounted for 58% of total time spent for soil tillage and sowing in CT.

Replacing a mouldboard plough with chisel plough for primary tillage had positive impact on work rate in RT1 which was 0.654 ha h^{-1} or 62.7% higher than in CT.

It should be noticed that in RT1 only one pass with disc harrow was done in secondary tillage, but chisel plough itself had twice the work rate of mouldboard plough.

Further reduction of tillage depth and aggregate passes in RT2 resulted with 3.27 times higher work rate (1.315 ha h^{-1}) than in CT.

The highest work rate was achieved in no-tillage system, 3.242 ha h^{-1} or 8.1 times higher than CT. Similar relations are noticeable in productivity per ton of grain yield.

Average productivity of conventional tillage for all three test crops was 1.499 Mg h^{-1}. In RT1 productivity has increased to 2.680 Mg h^{-1}, or 78.8% higher than CT.

In RT2 and NT productivity was to 6.533 Mg h^{-1} and 15.724 Mg h^{-1} or 4.36 and 10.5 times higher than in CT respectively.

Greater variations in results between test crops were present here (Table 4) due to wide range of different crops yields, but there was a statistically significant increase in productivity with decrease of specific energy requirement as a result of soil tillage reduction (Figure 1). Coicu (2010) and Jug et al. (2007) also highlighted a significant increase in labour productivity with degree of soil tillage reduction, realised through adequate tillage systems where yields were not impaired.

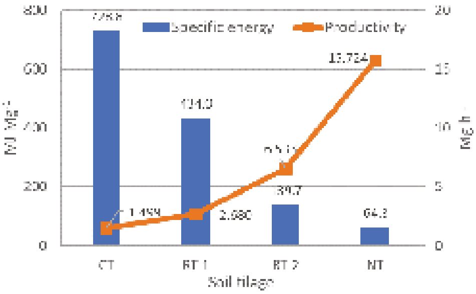

Figure 1. Average values of specific energy requirement and productivity of different soil tillage methods

CONCLUSIONS

The results of this research revealed some important advantages of non-conventional tillage methods over the conventional tillage in wheat, barley and soybean production. Utilization of reduced soil tillage methods has enabled significant fuel/energy saving in field operations prior to sowing. Reduction of soil tillage did not cause a significant decrease of grain yield of test crops. On the contrary, in most tillage methods obtained yields were higher than with conventional tillage. Therefore, the reduced tillage or no-tillage method could be an important tool to improve energy efficiency and labour productivity in arable crop production. In the selection of preferred soil tillage method, assuming uniform levels of yield, the advantage should be given to a method with lower level of tillage intensity, not only to reduce energy requirements and soil degradation, but also because of the simpler production organization due to less machine and human labour.

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A BIBLIOMETRIC REVIEW OF RESEARCH TRENDS IN SALICYLIC ACID USES IN AGRICULTURAL AND BIOLOGICAL SCIENCES: WHERE HAVE BEEN STUDIES DIRECTED?

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Abstract

Plants encounter numerous biotic and abiotic stress factors during their life cycle, either simultaneously or at different times. For the exogenous chemicals applied to improve stress induced perturbations, salicylic acid was found to be mostly preferred and used as a growth regulator. The objective study was to examine the profile of original and review articles under the topic of salicylic acid using bibliometric analysis. The VOSviewer tool was used to visualize the results. The documents including salicylic acid keyword but limited to agricultural and biological sciences were extracted from Scopus database. In this context, 1.422 documents relevant and available peer-reviewed publications were analysed. According to the systematic analysis, 4688 authors, 2880 keywords and 84 countries were determined. The annual trends of publications in the field of research, considering the number of documents, number of authors, levels of collaborations among authors and countries, year publications and core publishing journals were analysed. Finally, the explanations regarding with the improved response via salicylic acid in plant systems were briefly represented in the present study.

***Key words**: salicylic acid, agricultural and biological sciences, bibliometric analysis.*

INTRODUCTION

People have always been tied to plants in order to be the source of the food. With the development of modern and industrial life, there has been a significant increase in both the quantity and quantity of plant products used by humans. The rapid increase in world population (150 babies/ min) has further increased the need for plant products. It has been estimated that people have been struggling with plant growth, yield and quality for a long time for various purposes such as nutrition, clothing, medicine and energy. To control and improve plant growth, yield and quality, plant scientists offer significant contributions to solve the problems of the rapidly increasing world population. Whether future generations can raise their standard of living or continue at all as it is, it will be possible only through the further development of plant physiology and the application of modern methods to agriculture, forestry and industry. The main objectives are the development of methods by which plant scientists can easily and broadly apply the main principles they have gained from various experiments in economics and agriculture. For example; increase in fruits and flower yield, modernization of fertilization and irrigation techniques, storage, transportation and marketing of agricultural products are very important for the healthy and prosperous sustainability of our daily life (Bozcuk, 2004). Numerous and diverse laboratory, pot and field experiments have been set up to solve these and similar problems. In addition to agricultural applications, different harvesting times and post-harvest practices have been carried out to obtain plants at the desired level and quality. In addition, plants are exposed to biotic and abiotic stress factors and the changes that occur in plants are examined. A variety of external practices are being implemented to eliminate the negative consequences of stress. In this study, salicylic acid (SA), which is often used as an exogenous growth regulator, was discussed. The structures of salicylic acid, the mechanism of action, the forms in which it is applied, its concentration and the study trends in the world have been reviewed.

Chemistry of salicylic acid

SA is a group of plant phenols with an aromatic ring carrying a hydroxyl group or a functional derivative thereof and has been reported to play an important role in the regulation, growth and development of plant growth like other phenolic compounds (Özeker, 2005).

Salicylic acid (SA) as a signal molecule in plant systems

As a response to unfavourable conditions, signal molecules are synthesized and subsequently activate a range of signal transduction pathways in complex plant system. Furthermore, the plausible protective roles of the some signal molecules are known in helping plant to cope with the stress (Ganesan and Thomas, 2001). Calcium, jasmonic, ethylene and ethylene and salicylic acid are of the identified signalling molecules (El-Tayeb and Ahmed, 2010). Of the identified molecules, the protective roles of salicylic acid as a defence signal transducer or messenger under stress conditions has been well-reported (Raskin, 1992; Klessig and Malamy, 1994; Ganesan and Thomas, 2001). Greater economic yield of plants under water stress have been observed when plants were treated with SA (Gomez, 1993; Fayez and Bazaid, 2014; Kulak, 2016). Not limited to the response against abiotic stress factors, SA is considered as a likely signal disease resistance in plants (Raskin, 1992).

Why is the salicylic acid most preferred growth regulators? What is the advantage factor of salicylic acid over other growth regulators?

In recent years, many studies have intensively focused on SA due to SA's function as an endogenous signal mediator of local and systemic plant defence responses against biotic factors (Rivas-San Vicente and Plasencia, 2011). Also, for the exogenous chemicals applied to improve environmental induced perturbations, salicylic acid was found to be mostly preferred and used as a growth regulator. Of many signal molecules, SA is described as a hormone-like and phenolic-structured molecule and exhibiting vital role in the regulation of plant growth and development. This endogenous plant growth regulator is synthesized in low amounts. Note that SA should not be narrowly discussed as endogenous molecule but also defined, described and discussed as a potent molecule vital to plant systems once used an exogenous regulator under optimal conditions including optimal concentration, mode of applications, appropriate application time etc. for plants. Furthermore, the concentration in plant tissues is dynamic in response to any environmental stimuli. Its defence roles against biotic and abiotic stresses have been well-documented (Yalpani et al., 1993; Szalai et al., 2011). Regulation of physiological processes in plants such as stomatal closure, nutrient uptake, chlorophyll synthesis, protein synthesis, transpiration and photosynthesis has been partially attributed to the SA (Raskin, 1992).

What about the optimal concentration of salicylic acid? How important the concentration is? What about the application mode of salicylic acid? Foliar, priming or rooting media? At what intervals should salicylic acid be applied?

In the study by Kulak (2016), the role of SA priming against water stress in basil (*Ocimum basilicum* L.) was investigated. For determination of optimum SA-concentration for the subsequent water stress treatment, the basil seeds were soaked in different concentrations of SA including 0.001, 0.05, 0.10, 0.25, 0.50, 1.00, and 2.00 mM in dark at room conditions for 24 hours. Then the seeds were let germination for one week in Petri dishes filled with perlite. Salicylic acid concentrations that showed growth and development in the positive direction relative to the control group (0.00 mM) were used in water stress experiments at the next stage of study. SA concentrations to be used in the study were determined according to the germination percentage, seedling length and age weights of the seeds. According to the findings obtained for the determination of the optimum salicylic acid concentration, 0.05 and 0.1 mM salicylic acid concentration was obtained from the applications. The higher concentration caused adverse effects and consequently not used for the subsequent treatments (Table 1). The similar pre-treatment concentration determination by Kang et al. (2012), the concentration ranging from 0,1 to 3,00 mM was tested in wheat seedlings for 3 days. It was reported that 0.5 mM SA accelerated seedling growth and that other concentrations had adverse effects, particularly high concentrations (2.0 and 3.0 mM). The plant growth and development parameters are-in general-positively influenced with the application of the salicylic acid but at an optimal concentration. It is worthy to note that for each plant species, cultivars or genotypes, preliminary studies concerned with optimal concentration should be conducted and the toxic concentration must be removed for the subsequent processes of the work. Secondly, application time of the chemicals might also be taken into consideration.

Along with the studies listed in Table 2, the concentration range for exogenous applied SA against abiotic stress conditions is about (0.01-0.05 mM), (0.1-0.5 mM) and >1 mM. While pre-treatment with 0.01-0.05 mm exogenous SA is sufficient for significant increase of cold tolerance for wheat and tomato (Ding et al., 2002), the optimal concentration level for increasing stress tolerance usually ranges from 0.1 mm to 0.5 mm for most low-level-SA plants (reviewed by Yuan and Lin, 2008). However, it may not be correct to generalize the results from studies because of the fact that the experimental parameters such as plant species, developmental stages of the investigated plant, duration of the experiment, mode of SA-application, frequency of applications, stress conditions (stress type, severity, frequency, duration or combination of more stress) should be considered, not ignored.

Table 1. Changes in basil seeds primed with salicylic acid*

	Germination (%)		Length (cm)		Fresh Weight (mg)	
SA (mM)	Mean	Change (%)	Mean	Change (%)	Mean	Change (%)
0	89.98b	100	5.07c	100	98.68b	100
0.05	100.00a	111.14	7.0 a	138.66	115.68a	117.23
0.1	94.44ab	104.96	6.13b	120.91	91bc	92.22
0.25	67.78c	75.33	4.00d	78.9	80.68cd	81.76
0.5	58.89d	65.45	3.6d	71.01	76.33d	77.35
1	52.22de	58.04	2.17e	42.8	62.67e	63.51
2	45.55e	50.62	1.97e	38.86	58.00e	58.78

*The results were retrieved from the study by Kulak (2016); Values were mean. Data with different letters in the same column indicate a significant difference at $P < 0.05$ level.

How are the protective mechanisms of salicylic acid?

It has been well-documented that plants exhibit different defence mechanisms including enzymatic and non-enzymatic antioxidant systems in order to cope with stressors or sustain their normal life span. Plants themselves like other livings are perpetual factories and their health and sustainability are dependent on the anabolic and catabolic processes. The proper or improper functions of those processes are consequences of antioxidant systems coupled with antioxidant enzymes and through substances other than antioxidant enzymes. SA-induced suppression of antioxidant enzyme system in plants exposed to Cd-toxicity has been reported. However the protective roles of SA were also highlighted. The protective activity in response to the Cd-toxicity was considered as through substances other than antioxidant enzymes (Metwally et al., 2003). For the sequestration or detoxification of Cd in plants, synthesis of proteins or differing abundance of primary and secondary metabolites may be regarded as protective or inhibitory mechanisms of plants. In order to find unhidden answer for plant systems, many approaches, especially preliminary studies, are milestones directing the way of experiments or pioneering the experimental parameters or tests for the answers through new perspectives in order not to report the repeated results.

One more important issue is about the transportability of SA in plant systems. The questions “In which parts of the plant is salicylic acid produced? When and where is salicylic acid transferred when the plant is exposed to the stress factors?” are great interest for the researchers. In excellent review by Raskin (1992), it has been reported that no clear direct evidence to be used for proving the transportability of SA because of the fact that SA could be transported, metabolized and/or conjugated in complex plant systems due to its physical properties. Furthermore, what happens to the exogenously applied SA and endogenous content of SA? The exogenously applied SA is carried away from its initial application to the other plant tissues for plant response generation.

Table 2. Some studies concerned with the salicylic acid applications

Study	Plant species	Developmental stage	Duration of experiment	Mode of application	Frequency	Conc. (mM)	Experiment
Ananieva et al. (2002)	Barley	12-days-old seedlings	1,2,3, and 6 hours	Rooting	Once	0.5	Paraquat tolerance
Kang et al. (2003)	Banana	11-cm high seedling	3-days treatment	Pre-spray and rooting	Once	0.5	Chilling tolerance
Waseem et al. (2006)	Wheat	Germinated seeds	45-days treatment	Rooting	Once	0.036; 0.072	Drought tolerance
Taşgın et al. (2006)	Wheat	7-days-old seedling	38-days treatment	Spray	Once	0.01	Cold tolerance
Jing et al. (2007)	Rice	Seventh leaf developed	20-days treatment	Rooting	Once	0.1	Lead stress tolerance
Popova et al. (2009)	Pea	Seed	12-days treatment	Priming	6 hours	0.5	Cd- toxicity
El Tayeb and Ahmed (2010)	Wheat	Seed	2- weeks treatment	Priming	6 hours	0.5	Drought tolerance
Szepesi et al. (2011)	Tomato	Seedling	3-weeks treatment	Rooting	Once	0.1	Hardening processes
Nazar et al. (2011)	Mungbean	15-days-old seedling	15-days treatment	Spray	Once	0.1; 0.5;1.0	Salinity tolerance
Dong et al. (2011)	Cucumber	18-days-old seedlings	20-days treatment	Rooting	3, 6, 9, 12 days	0.01; 0.05;0.1	Salinity tolerance
Mirabi and Hasanabadi (2012)	Tomato	Seed	14-days treatment	Priming	48 hours	0.43	Priming effect
Kang et al. (2012)	Wheat	2-weeks-old seedlings	3-days treatment	Rooting	Once	0.5	Salinity tolerance
Sharafizad et al. (2013)	Wheat	seed	7-days treatment	Priming	24 hours	0.7;1.2; 2.7	Drought tolerance
Saidi et al. (2013)	Bean	4-days-old seedling	10-days treatment	Rooting	Once	0.01; 0.05; 0.1	Cd-toxicity
Orabi et al. (2013)	Faba bean	45-days and 60 day-old seedling	120-days treatment	Spray	Twice (45 and 60 days)	1.0; 2.0	Salinity tolerance
Guo et al. (2013)	Bluegrass	Seed	25-days treatment	Priming	12 hours	0.5	Cd-toxicity
Fayez and Bazaid (2014)	Barley	3-weeks-old seedling	2- weeks treatment	Spray	Once	0.05	Drought and salinity tolerance
Agami (2013)	Maize	Seed	60-days treatment	Priming	12 hours	0.1	Salinity tolerance

Does endogenous SA content vary with exogenous applied SA or change with biotic and abiotic stress factors?

The endogenous regulatory roles of SA as an important plant hormone regarding with local and systemic disease resistance and an induced of pathogenesis-related (PR) proteins have been hypothesized and proven using many approaches. The induced increase in resistance against virus and PR-1 proteins were positively correlated with the tobacco tissue levels of SA (Yalpani et al., 1993). Moreover, endogenous SA content was increased in whitefly-infested plants upon Agrobacterium inoculation. Augmented SA content involved in whitefly-derived plant defence against *Agrobacterium* (Song et al., 2015).

Salicylic acid-accumulating mutants of *Arabidopsis* (acd6 and cpr5) were more tolerant to drought stress than wild-type plants and drought tolerance related genes were more expressed in mutant plants. The results suggest that accumulation of endogenous SA confers drought tolerance to *Arabidopsis* (Okuma et al., 2014). Chilling tolerance in cucumber seedlings was also correlated with endogenous SA content (Dong et al., 2014).

Involvement of endogenous salicylic acid in iron-deficiency responses in Arabidopsis was reported. Accumulation of SA content coupled with Fe deficiency elevated auxin and ethylene signalling, thereby activating Fe translocation through transcriptional regulation of downstream Fe genes (Shen et al., 2016).

Following exogenous SA treatment, free SA content decreased at most sampling points after application of SA treatment in *Scutellaria baicalensis* (Hu et al., 2017). Endogenous SA level in pre-treated pea seeds increased but the augmented endogenous SA levels in plant tissues did not originate from the exogenous SA. Measured SA content was reported to be product of de novo synthesis, rather than taken up and mobilized by the plants (Szalai et al., 2011). SA pre-treatment improved and controlled the ability of plants to regulate the endogenous levels of SA when exposed salt stress but under suitable nutrient conditions (Kim et al., 2017).

Why bibliometric analysis?

As a methodology, the VOSviewer tool was used to visualize the results. The documents including salicylic acid keyword but limited to agricultural and biological sciences were extracted from Scopus database. Briefly, research was done using salicylic acid keyword in article title and then 46,454 documents were retrieved on December 20, 2017. The second step was limitation of the results to agricultural and biological sciences. In this context, 1422 documents relevant and available peer-reviewed publications were analysed. According to the systematic analysis, 4688 authors, 2880 keywords and 84 countries were determined. After inclusion of some criteria, those numbers were reduced. The annual trends of publications in the field of research, considering the number of documents, number of authors, levels of collaborations among authors and countries, year publications and core publishing journals were analysed.

According to the analysis results, there was an increase trend in number of publications concerned with salicylic acid in agriculture and biological sciences. The highest number of documents was observed in 2017 with a total of 701 publications. The first publication in the investigated field of study was in 1887 published in Transactions of the American Fisheries Society by A. Howard Clark. The study was about fish preservation by the use of acetic, boracic, salicylic, and other acids and compounds.

Plant Physiology, Plant Journal, Plos One, Molecular Plant Microbe Interactions, Frontiers in Plant Science, Plant Cell, Journal of Experimental Botany, Plant Physiology and Biochemistry, Journal of Plant Physiology and Planta are of the core publishing journals.

What do the terms extracted from documents tell us?

According to the systematic analysis, 4688 authors, 2880 keywords and 84 countries were determined. After inclusion of some criteria, those numbers were reduced. Along with the results, two main salicylic acid research clusters according to the most relevant terms were identified. First cluster was composed of abiotic stress terms and related antioxidant activity and enzymes. The first cluster can be considered as biochemistry and abiotic stress. The second cluster was composed of biotic stress factors and related plant immunity terms and molecular approaches. So, the second cluster can be considered as molecular biology and biotic stress (Figure 1).

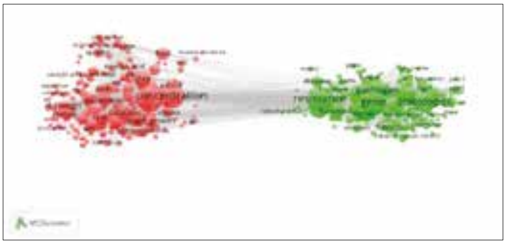

Figure 1. The most relevant and co-occurrence of terms retrieved from the studies

What do the terms proposed by authors in the documents analysed tell us? Can they be pioneer for the future studies?

The simple keyword extraction provides raw information about the research topics but they are assigned to documents to represent the core content of their papers. In this regard, keyword analysis can be used to determine the progress the research frontiers associated with a knowledge (He, 1999), proposing the gap of keyword analysis in SA uses in researches in agricultural and biological studies. Herewith the core results, this section should be considered as the most important contribution of the manuscript. Co-occurrence and author keywords might be considered as one of the factors to provide information on SA and its most uses in many respects in agricultural and biological sciences. In the current study, bibliometric analysis presented nine clusters concerned with keywords proposed by the authors. Of the clusters, economically and nutritionally significant plants (wheat, barley, sunflower, maize, soybean etc.) have been investigated for their tolerance against abiotic stress conditions combined with salicylic acid applications. In this context, proline content, malondialdehyde content and antioxidant enzymes like parameters have been used for assessment of tolerance. In many studies, basic physiological and biochemical assays have been performed. However, of the main clusters, tobacco plant have been extensively used for biotic stress-induced with virus. Along with those studies, local and systemic disease resistance and an induced of pathogenesis-related (PR) proteins have been hypothesized and proven using many approaches. Regulation of PR proteins has been correlated with salicylic acid contents. In those biotic stress studies, molecular approaches, especially gene expression levels, have been highlighted (Figure 2). To conclude, results obtained from abiotic stress researchers are needed and proven using transcriptomic and proteomic approaches.

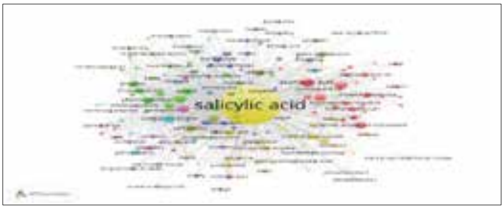

Figure 2. The keywords proposed by the researchers

Top productive countries and authors

China and United States are of the first two-most productive countries. The studies disseminated by researchers from United States have been mostly referenced and total link strength of United States also highest, as well (Figure 3, Table 3). Of the authors, Klessig D.F. (53), Baldwin I.T. (32), Métraux J.P. (31), Pieterse C.M.J. (29), Shah J. (29), Ohashi Y. (28), Seo S. (24), Hwang B.K. (23), Janda T. (23) and Chen Z. (21) are of the most productive authors.

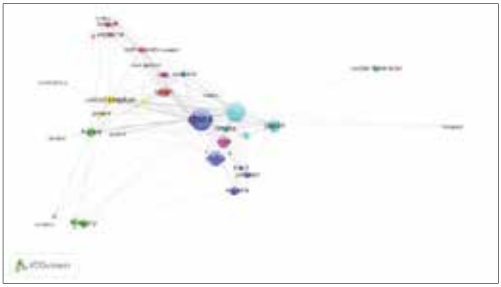

Figure 3. Top productive countries

Table 3. Top ten publishing countries

	Country	Documents	% of Total Documents	Citations	Citation Per Document	Total Link Strength
1	China	303	21.31	5487	18.11	57
2	United States	229	16.10	17485	76.35	144
3	India	144	10.13	2623	18.22	23
4	Japan	105	7.38	4550	43.33	50
5	Iran	93	6.54	794	8.54	11
6	Germany	78	5.49	4481	57.45	71
7	Spain	70	4.92	2532	36.17	35
8	United Kingdom	64	4.50	4733	73.95	64
9	France	52	3.66	2360	45.38	40
10	Egypt	50	3.52	729	14.58	17

Future outlook

In this section, it was focused on the gaps and was given suggestions for future studies associated salicylic acid studies, especially on the abiotic stress and salicylic acid interactions.

1. Biochemical and physiological aspects of SA-triggered increase in tolerance versus abiotic stress factors have been well documented and the studies. The most of the studies are based on the plant species rich for their nutritional and economic values. There are lacking studies concerned with molecular basis of salicylic acid combined abiotic stress conditions. In this context, mechanism-enlightening studies coupled with omic approaches are required.

2. An improvement regarding with increase in tolerance of plants versus stress conditions using salicylic acid have been extensively reported but the studies about secondary metabolite- not only reporting total content of secondary metabolites but also profiling the metabolites essential-are required. Sub-sequential studies may be concentrated on their molecular basis and further desired crop

improvement with high tolerant and quality content.

3. The most favourable conditions including application time, mode of application, application doses or duration of priming should be optimized for each plant species.

Highlights of the study

Along with the present study, it was illustrated a schema as studies regarding to: **i)** the current state knowledge of salicylic acid studies of profiling the key areas of the salicylic acid uses; **ii)** pointing out the stages of development of the studies; **iii)** presenting assessments for the significance of the studies performed; **iv)** giving the results through vote-counting method; **v**) giving suggestions for the key areas for further work.

Limitations of the study

Although this is the first study-up to best survey-to present the salicylic acid containing studies in field of agricultural and biological sciences from the largest existing database using VOSviewer program, we have several limitations in this study. **i**) The data was only extracted from SCOPUS and so documents in non-indexed plant journals have not been considered. **ii**) The search was then restricted for publications that contain the words "salicylic acid" in the title and abstracts as well as the search was then restricted to the agricultural and biological sciences. **iii**) Hence some publications might not contain salicylic acid and related terms in the publications title and abstracts, so it is possible that not all publications for salicylic acid including studies were identified. It is worthy to consider and state that there are many studies deciphering and describing the structure and roles of salicylic acid. The study can be considered as vote-counting review paper.

CONCLUSIONS

The main conclusions of the review have been listed as below:

1. There was an increase trend in number of publications concerned with salicylic acid in agriculture and biological sciences. The highest number of documents was observed in 2017 with a total of 701 publications.

2. China and United States are of the first two-most productive countries.

3. Two main salicylic acid research clusters according to the most relevant terms were identified. First cluster was composed of abiotic stress terms and related antioxidant activity and enzymes. The second cluster was composed of biotic stress factors and related plant immunity terms and molecular approaches.

4. While crop plants such as wheat, barley, sunflower, maize and soybean have been investigated for their tolerance against abiotic stress conditions, tobacco has been extensively used for biotic stress related studies.

Finally, it can be stated that the studies on salicylic acid uses in agricultural and biological studies have been directed in two different ways for stress conditions. Interestingly, abiotic stress studies are mostly limited to the basic physiological and biochemical assays. The similar results have been reported in most of publications. On the other hand, biotic stress researches are mostly concentrated on virus-tobacco interactions and subsequent analysis of transcription levels of pathogenesis-related proteins.

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DETERMINATION OF YIELD AND YIELD COMPONENTS OF SOME CRAMBE GENOTYPES IN THE WORLD CRAMBE COLLECTION

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Abstract

This research was carried out in Samsun ecological conditions, 2016-2017 summer seasons. This study was conducted to determine the yield and some agricultural characteristics of some Crambe hispanica subsp. abyssinica genotypes in the World Crambe Gene Pool, which are the basis for adaptation. In the study, 71 crambe genotypes were evaluated. Research result: the length of the plant is between 51.9 and 90.7 cm; number of branches per plant is between 4.1 and 9.5; the number of seeds per plant is from 57.4 to 376.6; 1000 grain weight was found to vary between 5.13 and 12.24 g and grain yield per plant varied from 0.421 g to 2.717 g. The result of the research: it has been determined that the genotypes evaluated have a superior performance in terms of the characters evaluated in total 13 genotypes including 2, 7, 8, 16, 30, 34, 41, 44, 49, 51, 61, 65 and 66 genotype. As a result, it has been decided that these genotypes can be used as a genitor in the development of crambe varieties suited to the Samsun ecological conditions.

Key words: *Crambe, Crambe abyssinica Hochst, agricultural characters, yield.*

INTRODUCTION

Crambe of the *Brassicaceae* family is originated from the Iranian-Turanian and Mediterranean regions of South-West Asia (Knights, 2002; Özyılmaz et al., 2017). Crambe seeds contain 35-60% oil, 20-40% protein and 12.3% crude fiber. After the oil is removed, the remaining oil cake contains 47.6% fat and 31.6% protein (Van et al., 1990). Crambe is not considered edible oil because it contains high erusic acid. Crambe oil is used in many industrial area such as lubricants, adhesives, plastics, textile industry, synthetic rubber, printer ink, detergent, perfume and motor oil. In addition, crambe is used as an alternative plant in the production of biodiesel, as a renewable energy source. Therefore, crambe is an important plant that contributes to reducing the global warming problem and reducing air pollution caused by fuels (Li et al., 2010).

New oil plants, such as cramble, have to be incorporated into the production system, which is suitable for multipurpose use in order to increase vegetable oil production. When considering climate and soil requirements, there is no factor that prevents the inclusion of crambe plant, which has high adaptability ability, into the agricultural production system. To be included in our agricultural system of crambe plant adaptation experiments in different ecological regions of Turkey must be done. It is extremely important that the suitable crambe genotypes that are eventually identified at the end of these adaptation experiments are introduced into the farm.

For this reason, this research was carried out to determine the yield and some yield components which will be the basis for the adaptation of the crambe genotypes in the "World Crambe Gen Collection" to the Samsun ecological conditions.

MATERIALS AND METHODS

This research was conducted in the Samsun Province, Alaçam District, Geyikkoşan location. The altitude of the experimental area is 4 meters. The soil structure is clay, lime, salt-free, pH is slightly alkaline, organic matter is moderate, phosphorus level is medium and potassium level is high. When the climate data are evaluated as the experiment season and the multiannual average for the experiment area, the data are the followig: the average relative humidity and monthly precipitation during the plant growing season is lower than the multiannual average. Especially the amount of rainfall in July is very low. The average monthly temperature in the plant growing season is approaching the temperature multiannual average.

In this research, as plant material, the 71 crambe genotypes were used (Table 1). The experiment was set up as 4 replications in the Augmented Experimental Design. In the experiment, each line was planted by hand on May 1, 2017, with a length of 2 m, 2 rows, a distance between rows of 30 cm and a distance of 5 cm between plants. Throughout the experiment period, weed control was made manually and mechanically. Because of the inadequate rainfall during the experiment period, irrigation was carried out in the field capacity on 01.07.2017. Harvest was carried out between 26.07.2017 and 12.08.2017, during the period when the varieties reached the stage of physiological maturity. For each lines 10 plants were sampled for analyze for the agronomic characters. Microsoft Computer program was used for evaluating the data and preparing the graphics.

Table 1. *Crambe hispanica* subsp. *abyssinica* lines and their origins

1-PI370747-Turkey	19-NSL74252-USA	37-PI384526-Ames1442-Etiopia	55-PI393515-Russia
2-PI392327-Turkey	20-NSL74251-USA	38-PI384525-Ames1441-Etiopia	56-PI393514-Russia
3-PI392326-Turkey	21-NSL74248-USA	39-PI384524-Ames1440-Etiopia	57-PI393513-Russia
4-PI189139-USA	22-PI378589-USA	40-PI384522-Ames1438-Etiopia	58-PI281736-Russia
5-NSL74278-USA	23-PI533668-USA	41-PI279346Indy-Etiopia	59-PI281735-Russia
6-NSL74272-USA	24-PI533667-USA	42-PI633195-Ames14938-Etiopia	60-PI281734-Russia
7-NSL74270-USA	25-PI533666-USA	43-PI326569BL1067-Etiopia	61-PI281731-Russia
8-NSL74269-USA	26-PI533665-USA	44-PI360893-Sweden	62-PI281730-Russia
9-NSL74267-USA	27-PI514650-USA	45-PI360892-Sweden	63-PI281729-Russia
10-NSL74266-USA	28-PI514649-USA	46-PI360891- Sweden	64-PI392071-Spain
11-NSL74265-USA	29-PI633196-NM 85-USA	47-PI360890- Sweden	65-PI372925-Spain
12-NSL74264-USA	30-NSL77602 PI 305285-USA	48-PI360889- Sweden	66-PI284861-Poland
13-NSL74261-USA	31-PI384533-Ames1435-Etiopia	49-PI305288- Sweden	67-PI311740-Ames1044-Poland
14-NSL74259-USA	32-PI384532-Ames1434-Etiopia	50-PI305287- Sweden	68-PI281737-Ukraina
15-NSL74258-USA	33-PI384531-Ames1433-Etiopia	51-PI305285- Sweden	69-PI337110-Romania
16-NSL74257-USA	34-PI384530-Ames1432-Etiopia	52-PI305284- Sweden	70-PI633198CRA 8/75-Kenya
17-NSL74254-USA	35-PI384529-Ames1431-Etiopia	53-PI305283- Sweden	71-PI633197CR1699-Germany
18-NSL74253-USA	36-PI384527-Ames1429-Etiopia	54-PI247310-Ames1144-Sweden	

RESULTS AND DISCUSSIONS

Plant Height

The distribution of plant lengths of crambe lines evaluated in this study is given in Figure 1. As can be understood from the analyze of Figure 1, average plant height is 68.3 cm; the longest plant height was obtained from genotype 16 with 90.7 cm, while the shortest plant height was obtained from genotype 47 with 51.9 cm. It was determined that as the average of the 71 lines evaluated, 29 genotypes were above the plant length average. These genotypes are; 2, 5, 7, 8, 13, 15, 17, 23, 24, 25, 26, 27, 28, 29, 30, 33, 34, 35, 36, 37, 39, 41, 49, 64, 65, 66 and 68 genotypes. In previous researches in *Crambe hispanica*, plant height was reported to be 40-120 cm (Davis, 1965), 68-128 cm (Weiss, 2000) and 71.40 cm (Tansı et al., 2003). These results are in parallel with the results obtained from this study. However, it is shorter than the reported values of 163.7 cm (Laghetti et al., 1995) and 93.07-103.9 cm (Huang et al., 2013). Differences in plant length are given by the genotype, environmental conditions, sowing time, sowing norm, soil characteristics, cultivation techniques and variety.

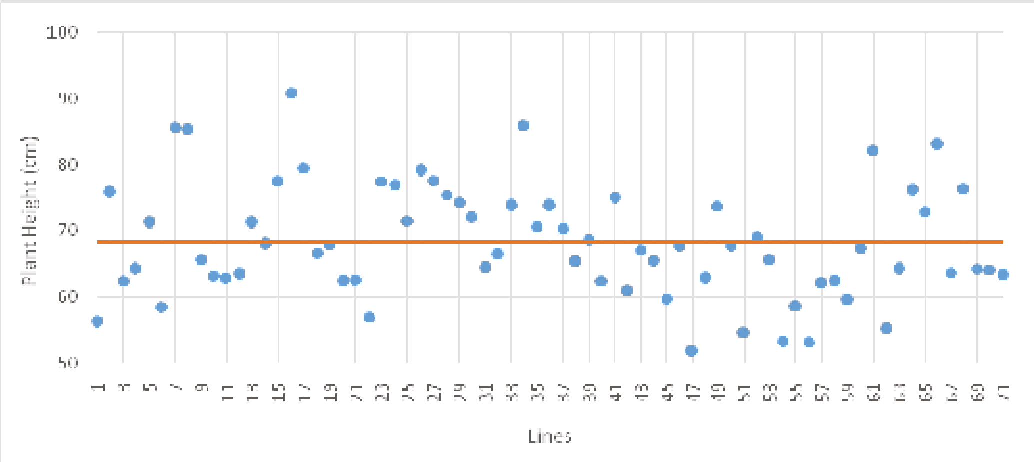

Figure 1. Distribution of plant length data of the lines evaluated in the research

Number of Branches

The distribution of the number of branches per plant in the crambe lines evaluated in this research is given in Figure 2. As can be understood from the analyze of Figure 2, the average number of branches per plant is 6.7. Although the maximum number of branches was obtained from genotype 66 with 9.5, the minimum number of branches was obtained from genotype 54 with 4.1. It was determined that as the average of the 71 lines evaluated, 35 genotypes were above the number of branches average. These genotypes are: 2, 5, 7, 8, 9, 10, 13, 14, 15, 16, 17, 19, 23, 24, 26, 28, 30, 31, 32, 34, 35, 36, 37, 46, 49, 52, 53, 57, 59, 60, 61, 64, 65, 66, 67 and 69 genotypes. The number of branches per plant obtained in this study is higher than the 1.82 (Tansı et al., 2003) reported in previous studies. However, it is lower than 14.1 (Laghetti et al., 1995), 15.0 (Gökçe, 2015) and 16.23-23.8 (Huang et al., 2013). Plant genotype, fertility status of soil, precipitation and number of plants per unit soil determine number of branches per plant.

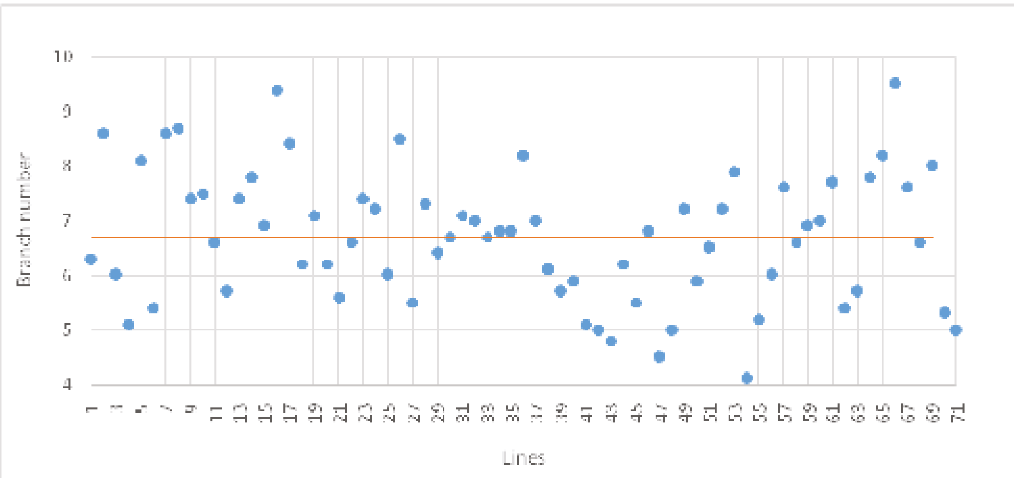

Figure 2. Distribution of data related to branch numbers per plant of the lines evaluated in the research

Seed Number

The distribution of the number of seeds per plant in the crambe lines evaluated in this research is given in Figure 3. As can be understood from the analyze of Figure 3, the average number of seeds per plant is 165.1. Although the maximum number of seeds per plant was obtained from genotype 66 with 376.6, the minimum number of seeds was obtained from genotype 54 with 57.4. It was determined that as the average of the 71 lines evaluated, 29 genotypes were above the number of seeds average. These genotypes are: 2, 5, 7, 8, 10, 13, 14, 15, 16, 17, 18, 19, 32, 33, 35, 36, 41, 49, 50, 53, 54, 57, 58, 61, 64, 65, 66, 68 and 69 genotypes. In previous studies, the number of seeds per plant was reported to be 379 (Köybaşı, 2008), 5250.2 (Tansı et al., 2003) and 1003.71-2397.8 (Huang et al., 2013). These reported values are higher than the values obtained from this research. It has been determined that there is a wide variation in the number of seeds per plant. This variation and the wide range of geographical distribution of cramba lines evaluated is the result of differences in adaptation to growing conditions as well as genetic factors and differences in environmental factors. Besides the environmental conditions, the number of seeds per plant also affects the flowering and branching of the plant. Although thousands of flowers per plant are formed in crambe and mostly self fertilization is observed, fertilization is adversely affected especially due to external factors in summer sowing.

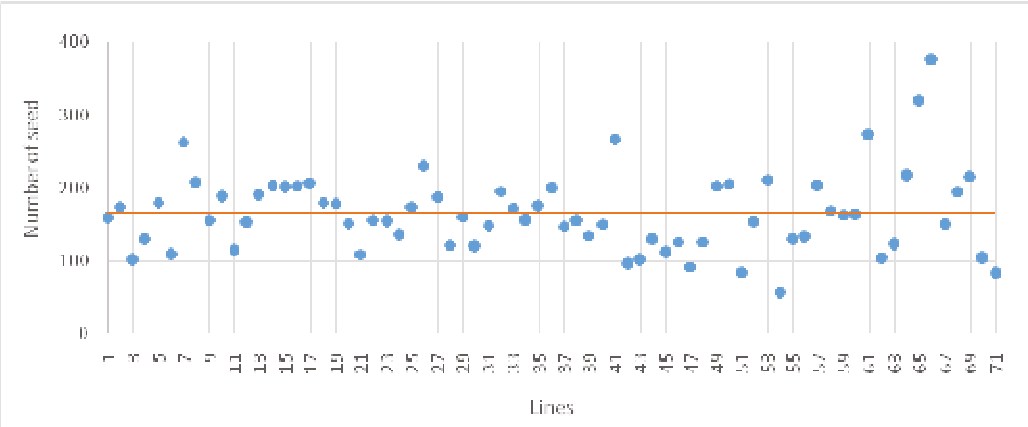

Figure 3. Distribution of data related to seeds numbers per plant of the lines evaluated in the research

1000-Grain Weight

The distribution of the 1000-grain weight in the crambe lines evaluated in this research is given in Figure 4. As can be understood from the analyze of Figure 4, the average 1000-grain weight is 6.93 g. Although the maximum 1000-grain weight was obtained from genotype 30 with 12.24 g, the minimum 1000-grain weight was obtained from genotype 26 with 5.13 g. It was determined that as the average of the 71 lines evaluated, 30 genotypes were above the number of seeds average. These genotypes are: 3, 5, 6, 8, 9, 12, 13, 18, 19, 20, 27, 28, 30, 36, 37, 42, 43, 44, 45, 49, 50, 51, 53, 55, 59, 61, 62, 64, 65 and 66 genotypes. In previous studies, the 1000-grain weight was reported to be 6.8 g (Fontana et al., 1998), 5.7 g (Wang et al., 2000), 6.3 g (Lara-Fioreze et al., 2013), 4.86-6.86 g (Huang et al., 2013) and 2.6-8.5 g (Arslan et al., 2014). These reported values are in parallel with the results obtained in this study.

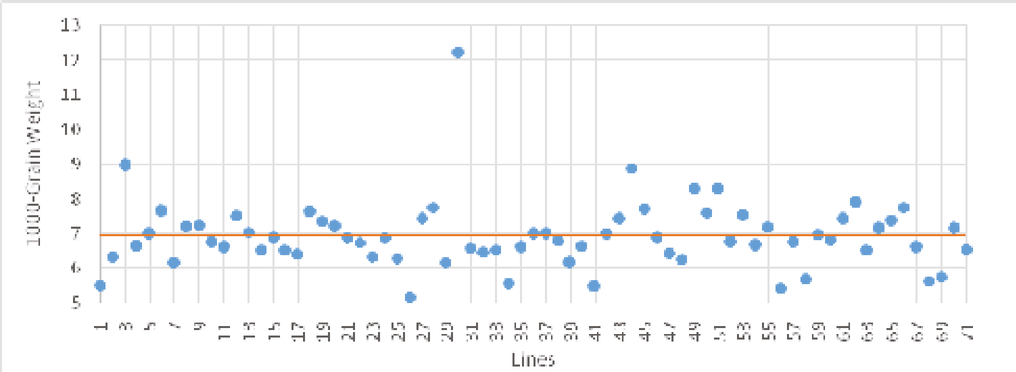

Figure 4. Distribution of data related to 1000-grain weight of the lines evaluated in the research

Grain Yield

In our experiment, the crambe lines showed great variations with respect to grain yield per plant as well as to the agronomic traits. The distribution of the grain yield in the crambe lines evaluated in this research is given in Figure 5. As can be understood from the analyze of Figure 5, the average grain yield is 1.138 g. Although the highest grain yield was obtained from genotype 66 with 2.717 g, the lowest grain yield was obtained from genotype 54 with 0.421 g. It was determined that as the average of the 71 genotypes evaluated, 31 genotypes were above the grain yield average. These genotypes are: 2, 5, 7, 8, 10, 12, 13, 14, 15, 16, 18, 19, 25, 26, 28, 32, 34, 35, 36, 41, 49, 50, 53, 57, 58, 61, 64, 65, 66, 68 and 69 genotypes. The grain yield obtained in this study was lower than the reported value of 4.98-13.91 g (Huang et al., 2013), although it is consistent with the previously reported values of 0.8-5.1 g (Arslan et al., 2014).

Grain yield is the most important herbal character in agricultural terms.

Genotype, environmental conditions and cultivation techniques have direct effects on grain yield.

Therefore, the positive or negative situation that affects any one of these yield components directly affects the grain yield.

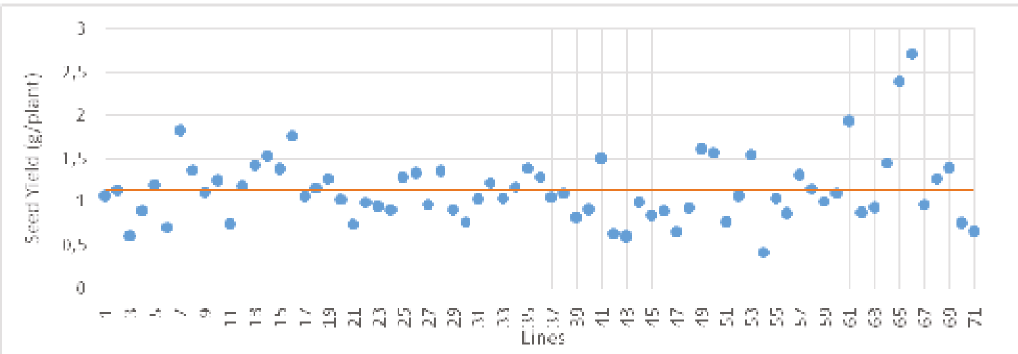

Figure 5. Distribution of data related to seed yield of the lines evaluated in the research

CONCLUSIONS

Research result: the length of the plant is between 51.9 and 90.7 cm; number of branches per plant is between 4.1 and 9.5; the number of seeds in the plant is from 57.4 to 376.6; 1000 grain weight was found to vary between 5.13 and 12.24 g and grain yield varied from 0.421 g to 2.717 g. It has been determined that the genotypes evaluated have a superior performance in terms of the characters examined in total 13 lines including 2, 7, 8, 16, 30, 34, 41, 44, 49, 51, 61, 65 and 66 genotype. As a result, it has been decided that these genotypes can be used as a genitor in the development of crambe varieties suited to the Samsun ecological conditions.

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IMPROVE OF GRAIN YIELD AND QUALITY OF WINTER WHEAT BY NITROGEN INPUTS

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Abstract

*Wheat is an important food crop and is by far the most popular cereal in Europe, Romania being among the six important producers. In Giurgiu County at S.C. AZOCHIM S.R.L farm it was designed a field experiment for investigation of variability of the yield components (yield, plant height, spikes/m^2, number of grains per ear, thousand kernel weight TKW) and variability of quality parameters (wet gluten and crude protein contents) influenced by mineral fertilization and wheat variety. It was developed a bifactorial experiment where **a** factor was wheat variety (Glosa, Miranda) and **b** factor was fertilization. It was adopted three fertilization schemes: starter (NPK 16:16:16) (**V1**), starter (NPK 16:16:16) + CAN (calcium ammonium nitrate) + AN (ammonium nitrate) (**V2**) and starter (NPK 16:16:16) + UAN (urea ammonium nitrate) (**V3**). An efficient nitrogen transfer into grains was obtained by splitting nitrogen fertilization. The results indicated that three applications of liquid fertilizer UAN (V3) increased the proteins levels and produced the highest yields for both wheat varieties. The same trend was recorded for plant height, spikes/m^2, number of grains per ear and for quality parameters. Concerning TKW, fertilization did not lead to significant differences, but it was observed higher values for'Miranda in comparison with Glosa.*

***Key words**: fertilization, nitrogen, wheat, yield.*

INTRODUCTION

Wheat is an important food crop and it is considered that its production accounts for more than 20% of the world's arable land (Liu et al., 2016). In Europe, wheat is by far the most popular cereal, Romania being among the six important producers. In Romania, the average area used for winter wheat culture is about 2 million hectares with total production that ranges between 1-12 million tonnes/year (Bunta et al., 2011).

Balanced fertilization ensure high productivity of wheat and nitrogen is considered as the most influential factor for good quality of grains, protein content and bread-making quality. Accordingly, there are many studies concerning correlations between fertilization and yield components or quality parameters for wheat (Basso et al., 2010; Bunta et al., 2011; Hlisnikovsky et al., 2016; Panayotova et al., 2017).

As nitrogen is one of the most influential factors that control plant development, the extensive use of mineral nitrogen fertilizers has led in the last decade to a significant increase of crop yields, this being the main objective of the farmers. Lately, has surfaced the interest the minimization of harmful effects of application of high doses of mineral fertilizers (contamination of ground waters, eutrophication of surface waters, N_2O emissions) (Basso et al., 2010; Büchi et al., 2016). The important changes in fertilization practices are associated with aforementioned environmental aspects but also with economic ones: fabrication of mineral nitrogen is costly and energy consuming (Büchi et al., 2016).

Other important aspect, subject of many discussions, is related with nitrogen fertilizer type and application manner in order to obtain the best yield and quality parameters. In literature there are inconsistent opinions regarding comparisons between liquid and dry nitrogen sources for wheat crop (Walsh et al., 2016). According to Watson and co-workers (Watson et al., 1992), it appear that liquid products are more efficient (high crop yield and quality) and environmental friendly.

Also, some studies concerning the efficiency of splitting of nitrogen doses on yield indicated that the application of nitrogen in more than two splits increased grain weight per ear

(Abdin et al., 1996). Other authors (El-Agrodi et al., 2011) suggest that split application had beneficial effects on yield and yield components. Therefore, under the same experimental conditions it is recommended to add 120 kg/ha in four splits to obtain the best result of quantity and quality of the wheat.

Taking into consideration the demand for wheat high yields but also the necessity for good quality parameters (wet gluten and high protein content) required for bread-making properties, at Giurgiu County at S.C. AZOCHIM S.R.L farm it was developed a study that aimed with: (**i**) investigation of variability of yield components (yield, plant height, spikes/m^2, number of grains per ear, thousand kernel weight TKW) and (**ii**) study of variability of quality parameters (wet gluten and crude protein contents), both influenced by mineral fertilization and wheat variety.

It was developed a bifactorial experiment where **a** factor was wheat variety (Glosa, Miranda) and **b** factor was fertilization. It was adopted three fertilization schemes: STARTER (NPK 16:16:16) (**V1**); STARTER (NPK 16:16:16) + CAN (calcium ammonium nitrate) + AN (ammonium nitrate) (**V2**); STARTER (NPK 16:16:16) + UAN (urea ammonium nitrate) (**V3**).

MATERIALS AND METHODS

Experimental site

Experimental research was carried out at AZOCHIM SRL located in Călugăreni commune, Giurgiu County (Figure 1).

Figure 1. The position of experimental plots

Wheat varieties

For the experiment were chosen Glosa and Miranda varieties, both obtained at NARDI Fundulea.

Fertilizers

In the experiment were used NPK 16:16:16, calcium ammonium nitrate (CAN) with 27% N, ammonium nitrate (AN) with 33.5% N and liquid fertilizer urea ammonium nitrate (UAN) with 32% N.

Experimental design

It was developed a bifactorial experiment where **a** factor was wheat variety (Glosa, Miranda) and **b** factor was fertilization (Table 1). The experiment consisted in three variants (**V1, V2, V3**) and three replicates.

Table 1. Description of the experimental scheme

a factor = wheat variety	b factor = fertilization
a_1 - Glosa a_2 - Miranda	b_1 – STARTER (NPK 16:16:16) (**V1**) b_2 – STARTER (NPK 16:16:16) + CAN + AN (**V2**) b_3 – STARTER (NPK 16:16:16) + UAN (**V3**)

Soil and plant analyses

A presentation of performed analyses, methods and apparatus are synthesized in Table 2.

Table 2. Analyses, methods and instrumentation

Analyses	Method	Apparatus
Soil		
pH_{H2O} (1:2.5)	potentiometry	Hanna pH-meter
Total soluble salts (1:5)	conductometry	Hach sens Ion 7
Potassium (mobile form), K_{AL}	flame emission spectrometry	Sherwood 410
Phosphorus (mobile form), P_{AL}	spectrophotometry	CECIL 2041 spectrometer
Humus content	Walkley-Black-Gogoașă	-
Plant		
Wet gluten	manual method	-
Crude protein content (on the basis of total nitrogen content)	Kjeldahl method	HACH Digesdahl

P_{AL} - mobile form of phosphorus using for extraction ammonium acetate-lactate (AL);
K_{AL} - mobile form of potassium using for extraction ammonium acetate-lactate (AL).

Fertilization and applied treatments

For both wheat varieties was adopted the same technology and phytosanitary treatments, the difference being represented by the type of applied fertilizer (solid or liquid) and the split of the total dose. Solid fertilizers (**V2**), CAN and AN, were applied in March and April, respectively using a dose of 300 kg/ha composed from 200 kg/ha (CAN) and 100 kg/ha (AN) and totalising 120 kg N/ha. Liquid fertilizer (**V3**), UAN, applied in a dose of 300 kg/ha that was split in three equal fractions that contributed with 128 kg N/ha.

The sowing was done in October, first decade and harvesting in July, the third decade (Table 3).

Table 3. Fertilization (solid vs. liquid fertilizers) and treatments scheme for Glosa and Miranda

Period of time	Fertilizer and phytosanitary treatments		Dose	
October, Ist decade	NPK 16:16:16		200 kg/ha	
March, Ist decade	CAN	*UAN*	200 kg/ha	*100 kg/ha*
April, Ist decade	Gamma Cyhalothrin (insecticide)		0.08 L/ha	
	40 g/L proquinazid + 160 g/L tebuconazole + 320 g/L prochloraz (fungicide)		1 L/ha	
	69 g/L fenoxaprop-P-ethyl + 34.5 g/L cloquintocet-mexyl (herbicide)		1 L/ha	
	250 g/L thifensulfuron methyl + 250 g/L tribenuron methyl (herbicide)		40 g/ha	
April, IInd decade	AN	*UAN*	100 kg/ha	*100 kg/ha*
May, Ist decade	-	*UAN*	-	*100 kg/ha*
May, IInd decade	Plonvit Opty (foliar fertilizer)		3 L/ha	
	Tebuconazole 200 g/L + Trifloxystrobin 100 g/L (fungicide)		1 L/ha	
	Thiacloprid 240 g/L (insecticide)		0.3 L/ha	

CAN - calcium ammonium nitrate, solid fertilizer;
AN - ammonium nitrate, solid fertilizer;
UAN - urea ammonium nitrate, liquid fertilizer.

RESULTS AND DISCUSSIONS

Agrochemical soil analysis (Table 4) indicated that soil reaction was weak acidic for Miranda (0-20 cm) plot, moderately acidic for Miranda (0-40 cm) and Glosa (0-20 cm) plots and very weak acidic for Glosa (20-40 cm) plot. Total soluble salts contents indicate a non saline soil, meanwhile humus contents correspond to medium level. Mobile form of phosphorus (P_{AL}) was classified as medium content for Glosa (20-40 cm) plot and high content for all three other ones. Potassium content (K_{AL}) ranged between 360-540 mg/kg which is considered very high content.

Table 4. Soil agrochemical analysis

Wheat variety plot	pH	Total soluble salts, %	Humus, %	$P_{AL,}$ mg/kg	$K_{AL,}$ mg/kg
Glosa (0-20 cm)	5.77	0.02496	2.87	57.2	460
Glosa (20-40 cm)	6.64	0.02304	2.62	29.2	360
Miranda (0-20 cm)	5.90	0.02528	2.93	68.0	540
Miranda (20-40 cm)	5.72	0.01984	3.12	66.7	400

1. Results concerning yield related to mineral fertilization and wheat variety

The results indicated that using liquid fertilizer (UAN) by splitting the total dose (300 kg/ha) in three equal fractions lead to the highest yields for both wheat varieties (8232 kg/ha and 7404 kg/ha, respectively) (Table 5). Comparing the both wheat varieties it may be noticed that the same fertilization level conducted to higher yields for Glosa. The variance analysis concerning wheat variety influence on yield indicates significant differences for all experimental variants.

Table 5. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on yield (kg/ha)

Yield, kg/ha			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	a6014c	a7452b	a8232a
a_2= Miranda	b4683c	b6247b	b7404a
B constant, A variable: LSD 5%=193* kg/ha ; LSD 1%=308 kg/ha; LSD 0.1%=583 kg/ha A constant, B variable: LSD 5%=214* kg/ha; LSD 1%=311 kg/ha; LSD 0.1%=467 kg/ha			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

2. Results concerning plant height related to mineral fertilization and wheat variety

In comparison with variant **V1** (STARTER), fractionate application of fertilizer and fertilizer type produced significant differences on plant height. Application of liquid fertilizer (UAN) determined the highest height for both wheat varieties, 95 cm for Glosa and 107.5 cm for Miranda.

At the same fertilization level, plant height is higher for Miranda in comparison with Glosa. Also, variance analysis concerning the influence of wheat variety on plant height indicates significant differences for all experimental variants.

Table 6. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on plant height (cm)

Plant height, cm			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	b81.5c	b90.0b	b95.0a
a_2= Miranda	a93.0c	b106.0b	a107.5a
B constant, A variable: LSD 5%=2.10* cm ; LSD 1%=3.40 cm; LSD 0.1%=6.70 cm. A constant, B variable: LSD 5%=2.27* cm; LSD 1%=3.30 cm; LSD 0.1%=4.97 cm.			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

***3. Results concerning spike number/m²* related to mineral fertilization and wheat variety**

Analyzing the influence of mineral fertilization and wheat variety on spike number/m^2, it may be noticed that, in contrast to **V1** variant (STARTER), application of nitrogen fertilizers and their type conducted to significant differences. Application of UAN liquid fertilizer in three equal fractions produced the highest number of spike/m^2, as it follows: 420 spikes/m^2 for Glosa and 410 spikes/m^2 for Miranda (Table 7). Concerning fertilizer type, it may be observed differences between both wheat varieties: 20 spikes/m^2 for Glosa and 25 spikes/m^2 for Miranda.

Comparing both wheat varieties maintaining the same fertilization level it may be observed that number of spikes/m^2 is higher for Glosa. Also, variance analysis concerning influence of wheat variety on number of spike/m^2 indicates significant differences for all experimental variants.

Table 7. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on spikes/m^2

Number of spikes/m^2			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	a358b	a400a	a420a
a_2= Miranda	a342c	a385b	a410b
B constant, A variable: LSD 5%=23.55* no. spikes/m^2; LSD 1%= 46.30 no. spikes/m^2; LSD 0.1%=124.38 no. spikes/m^2 A constant, B variable: LSD 5%= 24.01* no. spikes/m^2; LSD 1%=55.39 no. spikes/m^2; LSD 0.1%= 176.35 no. spikes/m^2			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

4. Results concerning number of grains per ear related to mineral fertilization and wheat variety

The fertilizer type and the split of the fertilizer dose produced yield significant differences, application of liquid fertilizer being more efficient for both wheat varieties: 49 grains per ear for Glosa and 43 grains per ear for Miranda (Table 8). For the same fertilization level it has been found that number of grains per ear was higher for Glosa. Applied fertilizers at Glosa produced an increase with 4 grains per ear in the case of **V2** variant and with 7 grains per ear in the case of **V3** variant in comparison with **V1**. For 'Miranda', the increase was with 6 grains per ear for **V2** and 10 grains per ear for **V3** as against with **V1**. The variance analysis indicates significant differences for all experimental variants. Nitrogen supply will affect the number of grains set on individual ears/spikes determined early from double ridge to floret initiation by the timing of the applied nitrogen (http:www.yara.co.uk/crop-nutrition/crops/wheat/yield/increasing-wheat-grain-numbers-per-ear)

Table 8. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on number of grains/ear

Number of grains per ear			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	a42c	a46b	a49a
a_2= Miranda	b33c	b39b	a43a
B constant, A variable: LSD 5%=6.03* no.grains/ear; LSD 1%= 13.12 no.grains/ear; LSD 0.1%=39.46 no.grains/ear A constant, B variable: LSD 5%= 2.87* no.grains/ear; LSD 1%=4.11 no.grains/ear; LSD 0.1%= 6.28 no.grains/ear			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

5. Results concerning number of thousand kernel weight (TKW) related to mineral fertilization and wheat variety

The value of TKW for the same fertilization level is higher for Miranda as against Glosa. The variance analysis concerning the influence of wheat variety on yield indicates significant differences for all experimental variants. Differentiate fertilization produced increase of TKW with 0.5 g for **V2** variant for Glosa and the same increase for **V3** for Miranda, as against **V1** in both cases. As conclusion, the TKW index is a wheat variety character and it is not influenced by type or fertilization level (Table 9).

Table 9. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on TKW

TKW, g			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	a40.0a	a40.5a	b40.0a
a_2= Miranda	a41.5a	a41.5a	a42.0a
B constant, A variable: LSD 5%=1.87* g; LSD 1%= 3.84 g; LSD 0.1% = 10.85 g A constant, B variable: LSD 5%= 1.21* g; LSD 1%=1.76 g; LSD 0.1%= 2.65 g			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

6. Results concerning wet gluten content related to mineral fertilization and wheat variety

Wet gluten content increased with nitrogen fertilization and fractionate application of UAN determined the highest values: 26.1% for Miranda and 25.8% for Glosa (Table 10). For the same fertilization level, the wet gluten content is higher for Miranda as against Glosa. Also, variance analysis indicates significant differences for all experimental variants. After fertilization it was observed an increase of wet gluten for Miranda with 2.6% for **V2** and with 3.4% for **V3**. For Glosa, the increase was with 2.3% for **V2** and 3.2% for **V3**, all comparisons being made as against **V1**.

Table 10. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on wet gluten content

Wet gluten content, %			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	a22.6c	a24.9b	a25.8a
a_2= Miranda	a22.7c	a25.3b	a26.1a
B constant A variable: LSD 5%=0.67* % ; LSD 1%=1.01%; LSD 0.1%=1.72% A constant B variable: LSD 5%=0.78* % ; LSD 1%=1.14%; LSD 0.1%=1.71%			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

7. Results concerning crude protein content related to mineral fertilization and wheat variety

In comparison with **V1** variant, the fertilizer type and the split of the dose produced significant increase of protein content. Application of liquid fertilizer UAN conducted to the highest contents of protein for both wheat varieties: 13.45% for Glosa and 13.76% for Miranda (Table 11).

For the same fertilization level, crude protein content was higher for Miranda. Variance analysis indicates significant differences for all variants.

The fertilization led to increases of 0.47% for Miranda for **V2** and 1.06% for **V3**, meanwhile for Glosa the increase was of 0.68% for **V2** and 1.13% for **V3**, as against **V1**.

According to literature, high quality flours are characterized by protein content higher than 12%. Moreover, splitting nitrogen fertilization into three or four applications increases yield and protein content as compared to single or dual application (Yara International ASA), situation which is consistent with our results.

Table 11. Influence of wheat variety (a factor) and of mineral fertilization (b factor) on crude protein content

Crude protein content, %			
b= fertilization / a= wheat variety	b_1	b_2	b_3
a_1= Glosa	a12.32b	a13.00a	a13.45
a_2= Miranda	a12.70b	a13.29a	a13.76a
B constant A variable: LSD 5%=0.85* % ; LSD 1%=1.34%; LSD 0.1%=2.48% A constant B variable: LSD 5%=0.95* % ; LSD 1%=1.39%; LSD 0.1%=2.09%			

b_1-STARTER; b_2-STARTER+CAN+AN; b_3-STARTER+UAN
There were made interpretations by LSD 5% indicated in the table by *

CONCLUSIONS

In the field experiment developed at S.C.AZOCHIM S.R.L. in Giurgiu County it was investigated the variability of the yield components (yield, plant height, spikes/m^2, number of grains per ear, TKW) and the variability of quality parameters (wet gluten and crude protein contents) influenced by mineral fertilization and wheat variety. The experimental results allowed obtaining the conclusions presented below:

1. The application of liquid fertilizer UAN in three fractions produced the highest yields for both wheat varieties.

2. Application of UAN produced the highest plant height: 95 cm for Glosa and 107.5 cm for Miranda.

3. Application of UAN liquid fertilizer in three equal fractions produced the highest number of spike/m^2, as it follows: 420 spikes/m^2 for Glosa and 410 spikes/m^2 for Miranda.

4. Concerning number of grains per ear, the application of liquid fertilizer was more efficient for both wheat varieties: 49 grains per ear for Glosa and 43 grains per ear for Miranda.

5. The TKW index is a wheat variety character and it is not influenced by type or fertilization level.

6. Wet gluten and protein contents increased with nitrogen fertilization and fractionate application of UAN.

7. Results of our study suggested that choice of liquid nitrogen fertilizer might be important in winter wheat culture, with positive results obtained with UAN explained by reduced mineralization of these fertilizers due to dry weather conditions in spring inducing better nitrogen availability during protein storage.

8. As general conclusion, application of liquid fertilizer by splitting the total dose in three equal fractions, conducted to the best values for yield components and quality parameters.

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CONTROL OF THE CARROT CYST NEMATODE *Heterodera carotae* BY TANNIN AQUEOUS SOLUTIONS

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Abstract

A field trial was carried out to test the nematicidal activity of chestnut tannin aqueous solutions against the carrot cyst nematode Heterodera carotae. A soil naturally infested by the cyst nematode was subdivided in 2 m x 3 m plots distributed in a randomized block design with five replications/treatment. Plots were treated with tannin aqueous solutions at rates of 25 or 45 g/m^2 in 4 l water/m^2 applied in pre-emergence, 25 or 45 g/m^2 in 4 l water/m^2 applied in pre-emergence and 30 days after carrot emergence. Untreated soil and fenamiphos (60 l c.p./ha) treated plots were used as controls. Number and weight of marketable tap-roots from the central square metre of each plot were recorded at harvest. Cysts and number of eggs and juveniles/100 g soil were also determined. Cysts were extracted from soil samples, collected in each plot, by the Fenwick can. Data were statistically analysed and means compared by LSD's test. On the base of results, the use of tannin should be favourably considered for plant protection against phytoparasitic nematodes although some aspects remain to be investigated.

Key words: *carrot cyst nematode, Chestnut tannin, Heterodera carotae, nematode control.*

INTRODUCTION

The carrot cyst nematode *Heterodera carotae* Jones causes considerable yield losses to the most important European carrot growing areas. The amount of yield losses is related to the soil nematode population density at sowing (Sasanelli, 1994). The use of fumigant (Dimethyl disulphide or 1.3 Dichloropropene, liquid - oxamyl - or granular - fenamiphos) formulations of nematicides can successfully control the nematode (Bealir, 1984; Lamberti et al. 2001; Colombo et al. 2004; Curto et al., 2014). However, the recent European Legislations such as the directive on the sustainable use of pesticides (Directive 2009/128/EC) have deeply revised and restricted the use of pesticides on agricultural crops focusing the attention on environmental safety, human and animal health. Plant protection from phytoparasitic nematodes should therefore rely on alternative control strategies that are both environmentally sound and economically sustainable. Among the alternatives, the more promising ones include the use of soil amendments (D'Addabbo, Sasanelli, 1996), soil solarization alone or in combination with fumigants (Greco et al., 1990, 1992), and more recently the use of microorganism exametabolites or plant-derived formulations (Toderas et al., 2016; D'Addabbo et al., 2008).

Compounds at low environmental impact with nematicidal activity have been reported from many botanical families (Chitwood, 2002; Caboni et al., 2012; Cavoski et al., 2012; Renčo et al., 2014). Plant extract of quillay (*Quillaya saponaria* Molina), neem (*Azadirachta indica* Juss), tagetes (*Tagetes erecta* L.) and sesame (*Sesamum indicum* L.) are already available as commercial formulations. Among the natural products extracted from plants, tannins have been reported in the literature to possess antihelmintic properties especially for gastrointestinal nematodes in ruminants both *in vivo* and *in vitro* experiments (Hoste et al., 2006) and a nematicidal activity against the root-knot nematode *Meloidogyne javanica* (Treub) Chitw. and the potato cyst nematode *Globodera rostochiensis* (Woll.) Barhens

(Maistrello et al., 2010; Renčo et al., 2012). Although plant parasitic nematodes can cause severe yield losses to many agricultural crops, few information is available on the effect of tannins extracted from chestnut plants (*Castanea sativa* Mill.) on the carrot cyst nematode *H. carotae*. Therefore, to verify the possibility to use tannins also against the carrot cyst nematode a trial was carried out on a carrot crop in a field naturally infested by the nematode.

MATERIALS AND METHODS

The field trial was carried out in 2016 at Zapponeta (Province of Foggia, Apulia Region, Southern Italy) (41°.45'N, 15°.96'E) in a sandy soil homogenously and heavily infested by the carrot cyst nematode *H. carotae* (Pi=13.5 eggs and juveniles/g soil) (Figure 1). The field was deeply ploughed, rotavated and subdivided in 2 m x 3 m plots, spaced 0.5 m each other, distributed in a randomized block design with five replications for each treatment (Figure 2).

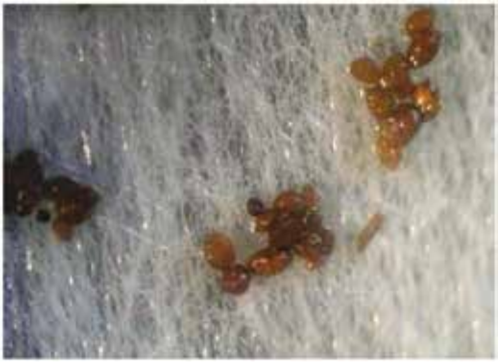

Figure 1. Cysts of *Heterodera carotae*

Figure 2. Experimental field subdivided in 6 m^2 plots

Carrot cv. Presto was sown by a seed drill at the density of 300 seeds/m^2 ten days before chestnut aqueous solution treatments.

The tannins were extracted by vapour from chestnut wood, without chemical solvents, in powder form after dehydration (Saviotan®, Radicofani, Siena Province, Central Italy). Chestnut aqueous solution treatments were: a) 25 g/m^2 in 4 l water/m^2 applied in pre-emergence; b) 25 g/m^2 in 4 l water/m^2 applied in pre-emergence and 30 days later; c) 45 g/m^2 in 4 l water/m^2 applied in pre-emergence; d) 45 g/m^2 in 4 l water/m^2 applied in pre-emergence and 30 days later (Figures 3 and 4). Tannin aqueous solutions were uniformly distributed on plots using a watering-can. Untreated soil and the nematicide fenamiphos (60 L c.p./ha) were used as controls.

Figure 3. Preparation of aqueous tannin solutions

Figure 4. Plots treated with aqueous tannin solutions

During the crop cycle the crop received all the necessary maintenance (irrigation, fertilization, weed control etc.).

At harvest, number and weight of marketable tap-roots from the central square meter of each plot were recorded (Figure 5). Soil samples, each a composite of 20 cores, were collected in the same central area of each plot (Figure 6). Cysts from a 100 g dried sub sample were extracted with a Fenwick can and crushed to count eggs and juveniles (Figure 7).

Figure 5. Square meter representative of the entire plot

Figure 6. Central area useful to collect soil samples

Figure 7. Apparatus of Fenwick for cysts extraction

Data from the experiment were subjected to analysis of variance (ANOVA) and means compared by Least Significant Difference's Test. All statistical analysis were performed using the PlotIT program V. 3.2.

RESULTS AND DISCUSSIONS

In the trial, carrot marketable yield ranged between 22.1 t/ha (untreated control) and 69.2 t/ha (fenamiphos control). Treatments with tannin solutions at 45 g/m^2 applied in pre or in pre and post-emergence significantly increased carrot marketable yield in comparison to the untreated control (Table 1). Among tannin treatments applied at different rates (25 and 45 g/m^2) and application time (in pre and in pre and post-emergence) no significant differences were observed in carrot marketable yield. The highest carrot marketable yield was recorded in fenamiphos treated plots and it was significantly different (P=0.05) from those recorded in all other treatments with the exception of tannin applied in pre-emergence at 45 g/m^2 rate (Table 1).

All treatments significantly increased the average weight of carrots compared to the untreated control excluding the lowest dose of tannin applied before emergence (Table 1). The average weight of carrot, for both applied rates, was not affected by the application time. No significant differences were observed between application in pre-emergence and in pre-emergence and 30 days later (Table 1).

No significant differences were observed in the number of cysts/100 g soil among the different treatments including the fenamiphos and the untreated control (Table 1).

The final nematode population density observed in the untreated control was significantly higher than those observed in all other treatments in which no differences were observed (Table 1).

The highest reproduction rate (ratio between final and initial nematode population density Pf/Pi) was observed in the untreated control (3.1) and it was significantly higher than those in the other treatments, which ranged between 1.2 and 1.6.

On the base of our results it is possible conclude that is not useful to repeat tannin treatments two times in pre emergence and 30 days later because no statistical differences were observed between the two application times.

The highest per cent increase in the marketable yield in comparison to the untreated control was observed in the treatment in which the tannin was applied in pre emergence at the rate 45 g/m^2 (Figure 8).

Figure 8. Untreated and tannin treated (45 g/m^2) plots

A similar trend was observed for the same treatment for the percent reduction of the soil nematode population density (62%) in comparison to the untreated control (Table 2).

The high initial nematode population density (13.5 eggs and juveniles/g soil), higher than the tolerance limit of carrot to *H. carotae* (T= 0.80 eggs and juveniles/g soil), could have masked the possible increase effect on marketable production by the tannin treatments.

Post emergence treatments do not seem to affect the protection of the crop, probably because the most delicate moment is the emission of small roots from the seeds, when the roots are immediately attacked by the nematode juveniles in the soil.

On the mechanisms of action of the tannin on the reduction of *H. carotae* population density in the soil, compared to control, it is possible to formulate hypotheses such as the negative influence of this substance (polyphenols) in the mechanisms of egg embryogenesis or that tannin can act as a larvae repellent or disrupt the juveniles chemoreception towards the carrot radical exudates.

Table 1. Effect of different chestnut aqueous solution treatments on carrot yield and on the soil population density of *Heterodera carotae*

Treatment (aqueous solutions)	Dose (g or ml/m^2)	Application time	Marketable yield (t/ha)		Average weight (g/carrot)		No. cysts/100 g soil		Eggs and J2/g soil		Pf/Pi	
Tannin	25	Pre-emergence	36.8[1]	ab[2]	50.7	ab	85	a	18	a	1.3	a
		Pre-emergence + 30 days later	43.3	ab	61.1	bc	109	a	19	a	1.4	a
Tannin	45	Pre-emergence	52.9	bc	58.4	bc	98	a	16	a	1.2	a
		Pre-emergence + 30 days later	46.6	b	58.5	bc	108	a	20	a	1.5	a
Fenamiphos	6	Pre-emergence	69.2	c	71.8	c	98	a	22	a	1.6	a
Untreated control	---	---	22.1	a	41.3	a	121	a	42	b	3.1	b

[1]Each value is an average of five replications.
[2]Data flanked in each column by the same letter are not statistically different according to Least Significant Difference's test (P=0.05).

Table 2. Effect of aqueous tannin solution treatments, at different doses and application time, on the percent increase of carrot marketable yield and per cent decrease of *Heterodera carotae* soil population density compared to the untreated control

Treatment (aqueous solutions)	Dose (g or ml/m^2)	Application time	% Increase of marketable yield[1]	Significance (P=0.01)	% Decrease of soil nematode population density[1]	Significance (P=0.01)
Tannin	25	Pre-emergence	67		57	*
		Pre-emergence + 30 days later	96		55	*
Tannin	45	Pre-emergence	139	*[2]	62	*
		Pre-emergence + 30 days later	111	*	52	*
Fenamiphos	6	Pre-emergence	213	*	48	*
Untreated Control	---	---	---		---	

[1]In comparison to control.
[2]*Significant at P=0.01.

CONCLUSIONS

The use of tannins offers promising perspectives of practical application in plant protection because it could represent a valid alternative to the use of synthetic nematicides. It could be considered in the context of nematicidal strategies at low environmental impact, not oriented to the eradication of the pest but to a progressive reduction of the level of nematode soil infestation below the damage threshold of the crop, with a lower impact on plant-soil balance compared to synthetic products.

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STUDY OF *Rhinoncus pericarpius* (Linneus, 1758) (*Coleoptera: Curculionidae*) BIOLOGY, AN IMPORTANT PEST OF HERB PATIENCE AND RHUBARB IN ROMANIA

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Abstract

The new agricultural reform policy, which involves an upward trend in the organic farming system of medicinal and aromatic plants, but also plant protection program, raises more and more complicated problems. The protection of organically grown medicinal plants varies according to whether or not the interests of farmers are compatible with the need for introducing these plants grown in the production system. For farmers who cultivate large-grain cereal plants, spontaneous species of the genus Rumex are considered weeds and control measures are taken against them. For those who cultivate Rumex species for food or medical purposes, the protection of these crops raises a number of issues. The common denominator of this controversy is, for some, and for others, the possibility of biological control using biological agents represented by insect species of the Curculionidae family. The flora of Romania includes 25 species and 12 subspecies of the genus Rumex; among them only two species, Rumex patientia L. and R. rugosus Campd, are cultivated in small individual farms. Species of the genus Rumex are widely distributed on Romanian territory, although each species has specific life requirements. Corresponding to the species of the genus Rumex and Rheum are insect species that feed on different plant organs and cause varying degrees of attack. Many of these species are part of two genera of the Curculionidae family: Apion and Rhinoncus. Five species belonging to the Apion genus have been reported in Romania's fauna: Apion frumentarium Payk, A. miniatum Germ., A. cruentatum Steph., A. sanguineum DeGeer and A. rubens Steph. Only one, A. miniatum, which is spread throughout the country, could be used in the biological control of spontaneous Rumex species, or may be considered an important pests for cultivated species. Another important pests belong to the Rhinoncus genus, are three of the eight species occurring on Rumex acetosa L., namely Rhinoncus pericarpius L., Rh. castor F. and Rh. bosnicus Schultze, the last one very rare, was not found in the southern investigation areas. To assess the importance of Rh. pericarpius species both as a harmful species and as a biological control agent, the results obtained in the present paper, refer to the growth of the species under laboratory conditions on natural food. The work brings new data on the feeding trophic range, attack model, incubation duration, fecundity of the female and fertility of the eggs, the active duration of the female and the duration of the growth of the larvae, pupae and diapauses, as well as the spread on the Romanian territory.

***Key words**: Rhinoncus pericarpius L., medicinal and aromatic plants, life cycle, laboratory mass rearing.*

INTRODUCTION

The family of *Curculionidae* (*Coleoptera*) with the 51,000 species and 4,600 genres represent, after Staphylinidae, one of the largest families of beetles from the world. Recently, some of taxonomists had included also the family *Scolytidae* like a subfamily *Scolytinae* into the superfamily *Curculionoidea*. Since the DNA barcoding method revealed that genetically bark beetles are very different from ‘’true weevils’’ the species of this group must be considered like a separated family, morphologically and genetically. From over the 13,000 species mentioned in the Palearctic area, in Romania only around 800 species are mentioned (Petri, 1912). After the Petri study few taxonomists they leaned forward to investigate and to classify into an exhaustive catalogue the fauna of such important species of coleopterans. Except the divers aspects regarding some pest species on the main crops from Romanian agriculture few papers are dealing with taxonomy of *Curculionidae* species (Teodor, 1993; Teodor, Dănilă, 1995; Teodor, Manole, 1996; Manole, Iamandei, 2002). More then 242 species are related to be important in the structure of coleopteran fauna from main agricultural crops (maize, wheat, sunflower, sugarbeet, fodder crops, fruit trees, grasslands and vegetables) (Manole, Iamandei, 2002). One of the remarkable aspect of the species of weevils behavior is the fact that the majority if not all are phytophagous feeding, and that is the explanation, like in the case of majority of *Chrysomelidae* species for which the main important pests are belong to the *Curculionidae* family. Regarding to the host plant of mostly species of weevils the aspects and position of experts are controversial. In the last century a widespread concern of industry from many countries are applied to recovery of a alimentary supplements from wild flora (i.e. medicinal and aromatic plants especially). In the usually community of weeds from main crops some of those plant species are considered weeds and would be target for weeds control methods (herbicides control). In some countries many of those species are cultivated on relatively large surfaces (example for genus Rumex which in China many hybrids of this plant are obtained and cultivated) (Li et al., 2001, 2002, 2003). Another relevant case is considering the invasive plant species. For instance, in Europe and also in Romania the species from genus *Centaurea* (*Asteraceae*) are cultivated in some little farms for many uses in pharmaceutical industry (Alexan et al., 1988; Păun, 1995; Bojor, 2003). In North America those species are invasive and USDA and other American Environmental Services established in the last years some programs for control using insect species like biological control agents (Wilson et al., 2005; Winston et al., 2005, 2010). As I said before in many of my author's scientific works, the problem of weeds control must be mainly well-balanced and environmentally addressed. For those species included in the wild associations of crops weeds, the herbicide control approach may be undesirable. Biological control in agricultural IPM systems became a subject of considerable current interest because of a perceived urgency to develop and adopt safe and efficient methods for managing agricultural pests. Problems associated with pest suppression (including environmental pollution, deleterious effects of pesticide on non-target organisms, pesticide resistance, resurgence of target pests, secondary pest outbreaks, and escalating costs of developing, producing and applying pesticides) all affect the vitality and profitability of agriculture and the well-being of our society. Some few studies carried out in Romania relate to this subject the possibility to use the insects like biological agents for weeds control (Perju, 1982; Perju et al., 1993; Perju et al., 1994; Perju et al., 1995). *Rh. pericarpius* and some species of genus *Rumex*, spontaneous or cultivated are both very important element of wild life in our country. It is in the same time the main pest of herb patience and rhubarb cultures, plants used both in food and in the pharmaceutical industry but in some others cases could be a very efficient agent of biological control of *Rumex* species. Nevertheless, the biology of the species is little known. Our field observations and techniques for mass rearing in laboratory under controlled conditions on natural food permits to obtain a series of new data on species biology, data complemented by observations in the field of species spreading in Romania, occurrence in the field and way of attack. To assess the importance of *Rh. pericarpius* species both as a harmful species and as a biological control agent, the results obtained in the present paper, refer to the growth of the species under laboratory conditions on natural food. The work brings new data on the feeding trophic range, attack model, incubation duration, fecundity of the female and fertility of the eggs, the active duration of the female and the duration of the growth of the larvae, pupae and diapauses, as well as the spread on the Romanian territory. *Rh. pericarpius* is a taxon native on palearctic biogeographically region and belongs to *Curculionidae*, *Ceutorhynchinae*, *Phytobiini*. Genus *Rhinoncus* was established by Schönherr, 1825 after the misidentification by Paykull 1792 and Stephens, 1831 (Huang, Collonelli, 2014). The genus *Rhinoncus* (Schönherr, 1825) is present with 8 species in Europe and 7 in North America from which 3 are invasive (Arnett et al., 2002).

MATERIALS AND METHODS

This study was carried out in the period 2000-2002 at the laboratory of entomology from RDIPP Bucharest and part of field observations were been made at RDIVF Vidra, Giurgiu district and biological material were collected at the country level in the period 1982-2018.

Experimental setup in laboratory

Insects. The insects individuals that formed the starting colony for laboratory gains (G_0) were collected from the field in two localities in the adult stage at the begining of spring when the plants had 2 or 3 leaves growth, in the period of normal appearance of the adults of *Rh. pericarpius* (between 09-15 May 2000). One lot was collected from rhubarb crops (*Rheum officinale* Baill.) from RDIVF Vidra and another lot from a little surface cultivated with *Rumex acetosella* L., at RDIPP Bucharest. The other field observations were been conducted apart of the main study of *Rh. pericarpius* growing to investigate the dispersal of the species population on the Romanian territory. The adults population was introduced in some special plastic recipients with cylindrical shapes and next dimensions: 25 cm Ø and 30 cm height. The bottom of the vessel was covered with a filter paper roll to prevent the moisture on the vessel walls. The adults were feed with leaves of herb patience fresh collected from the field and removed after 72 hours. The insects were daily observed and when the copulation begin the couples of male and female were separated in the other vessels (15 cm Ø and 15 cm height).

Adult growth. The adults were fed until they naturally died and the eggs were collected soon after the female deposit the bunch of eggs on the leaf surface or on the rods of the leaves.

Incubation. The eggs collected from one couple represent a new colony of the insect species and after collection were incubated in plastic Petri dishes with 9 cm diameter at ± 25°C. Inside the Petri dishes, the humidity required for the hatching process was ensured by two methods: a) Petri dishes with daily wetted filter paper; b) Petri dishes with cotton swabs soaked in water and moistened for 48 hours; after hatching the larvae were translated into special glass Petri dishes on the plant food.

Larvae growth. Growth and development of larvae was carried out in Petri dishes of 8 cm, 9 cm and 10 cm Ø. The larvae were fed with the rods of the herb plant (fragments harvested from ribs or young stalks) in which an incision was made for the penetration of neonate larvae. At intervals of 48-72 hours (as the case may be) the vegetal fragments were replaced by fresh ones by two methods: a) direct passage of the larvae one the new fresh stem with a fine paint-brush; b) free migration of larvae after the degradation of the vegetal material to the new fresh stem; In each Petri dish for larvae growth only 2 fragments of plant stem was introduced but the larvae were between 5 and 10 individuals.

Improving and getting adults. After larval development, larvae which reached maturity (L_3) were collected and passed to the hump in two variants: a) Petri dishes on dry filter paper on both lids of the vessel; b) in black plastic plastic sapphires, on filter paper. All stages experimental bioassay for laboratory rearing were carried out in one single variant (2 adults (♀♂) on natural food, leaves and stems from *R.acetosella*) and 10 replications for each stage. The host plant preference test bioassay was performed with 5 variants and 14 replicates. The laboratory conditions were different and variable with the stage of the insect. In the adult case the temperature in the feeding rooms was of 20±2^{0}C and RH of 75-80% at the daylight photoperiod of 16:8 light/dark. The eggs were incubated at 25±2^{0}C in the special conditions of RH mentioned above. The pupal stage was maintained at the lower temperature compared with the soil temperature in the May-June period (i.e. 10-12^{0}C) in special rooms in the dark conditions. The replicates designed for growth period bioassay were also performed with 10 variants including each one couple of adult insects (♀♂).

Experimental setup in the field. Plant host were collected and conserved in laboratory herbarium and for identification Illustrated flora of Romania (Ciocîrlan, 2000) was used. *Rh. pericarpius* feeding on the herb patience were observed in the field to evaluate leaf damage index. A four-degree injury scale was used where (Piesik, Lamparski, 2006):

0 - no injury;
1 - up to 10% injured leaf area;
2 - 11-20% injured leaf area;
3 - 21% injured leaf area;
4 - 31-50% injured leaf area.

Following a five-degree injury scale, leaf damage index was calculated, basing on Townsend and Heuberger equation:

$$IP\% = \frac{\sum_0^i (n*v)}{i\,x N} * 100, \text{ where:}$$

n = number of leaves in consecutive injury-degree;
v = injury degrees from 0 to 1 (the highest in scale);
N = number of examined leaves.

RESULTS AND DISCUSSIONS

The study represent an first attempt to enlarge the knowledge about the biology and behaviour of the species of weevil *Rh. pericarpius*, insect belonging to a group of species very quite widespread in Romania.

In Europe, to which is native, the species is enlarge distributed in all countries (Figure 1). The results obtained on the first step indicate that the colonies of species could be mass reared in controlled conditions to be used in many purposes i.e.like an efficient biological control agent in some cereal crops.

Figure 1. European distribution of species *Rhinoncus pericarpius* L.

Food regimen. *Rh. pericarpius* belong to the family of *Curculionidae* which include only phytophagous insects feeding on a large diversity of plants.

In our study, for the first time in Romania, the host plants (spontaneous or cultivated) of the species had been registered but the insect was, also present on other plant species without detection the sign of consumption or attack on plant organs (Table 1).

In our laboratory experiments bioassay test his feeding preferences were registered (Table 2). *Rh. pericarpius* feed only on a small number of species belonging to the *Polygonaceae* family from genus *Rumex* (*R. acetosella* L., *R. crispus* L., *R. acetosa* L., *R. obtusifolium* L., *R. hydrolapathum* Huds.), *Polygonum* (*P. persicaria* L., *P. lapathifolium* L.), and *Rheum* (*Rh. officinale* Baill., *Rh. rhaponticum* L.). From this point of view his feeding behavior can be framed in oligophagous category. The consumption of the plants organs are: leaves, stems, buds and rods in this order of preferences in the adult stage, only rods or stems fragments in case of larvae.Host plant preference was, after our investigations at the country level (Figure 2), herb patience, *R. acetosella* (with 100% frecquency of presence on this plant). *Rh. officinale* is another prefered host plant but in this case the damages are highest in the rhubarb crops then in herb patience cultures. *R. acetosella* appear to be more resistant to the insect attack. Consecutively, the level of the attack was highest (5.7) then in the case of preferenced host *R. acetosella* (4.6) (Table 2).

Table 1. Localities and plant species where *Rhinoncus pericarpius* L. was collected

LOCALITY	HOST SPECIES	OBSERVATIONS
Bucharest	*Rumex acetosella* L *Rumex acetosa* L. *Rumex crispus* L. *Rumex patientia* L. *Rumex acetosa* L. subsp.*hortensis* Dierb.*	Pairing, eggs deposit, larval hatching, damages of the attack
Vidra	*Rheum officinale* Baill.* *Rheum rhaponticum* L.* *Rheum palmatum* L.* *Rumex acetosa* L. subsp.*hortensis* Dierb.*	Pairing, eggs deposit, larval hatching, damages of the attack
Comana	*Rumex crispus* L. *Rumex hydrolapathum* Huds. *Polygonum persicaria* L. *Polygonum lapathifolium* L. *Rumex aquaticus* L. *Rumex palustris* Sm.	Pairing, eggs deposit, larval hatching, damages of the attack
Arad	*Rumex acetosa* L. subsp.*hortensis* Dierb.*	Pairing, eggs deposit, larval hatching, damages of the attack
Curtici	*Rumex acetosa* L. *Rumex acetosella* L	Pairing, eggs deposit, larval hatching, damages of the attack

Nădlac	*Rumex acetosa* L.	Pairing, eggs deposit, larval hatching, damages of the attack
Timişoara	*Rumex acetosa* L.	Pairing, eggs deposit, larval hatching, damages of the attack
Lugoj	*Rumex obtusifolius* L. *Rheum rhabarbarum* L.*	Pairing, eggs deposit, larval hatching, damages of the attack
Deva	*Rumex obtusifolius* L. *Rumex thyrsiflorum* Fingerh.	Only presence on the plant
Oradea	*Polygonum aviculare* L.	Only presence on the plant
Zalău	*Polygonum persicaria* L.	Only presence on the plant
Blaj	*Rumex obtusifolius* L.	Only presence on the plant
Turda	*Rumex obtusifolius* L.	Only presence on the plant
Braşov	*Rumex alpestris Jacq.* *Rumex alpinus* L. *Rumex confertus* Willd.	Only presence on the plant
Codlea	*Rumex confertus* Willd.	Only presence on the plant
Sibiu	*Rumex acetosa* L. *Rumex acetosella* L	Pairing, eggs deposit, larval hatching, damages of the attack
Tîrgu Mureş	*Rumex confertus* Willd.	Only presence on the plant
Darabani	*Polygonum lapathifolium* L.	Only presence on the plant
Botoşani	*Rumex acetosa* L. *Rumex acetosella* L	Pairing, eggs deposit, larval hatching, damages of the attack
Iaşi	*Rumex obtusifolius* L. *Rumex acetosa* L. *Rumex acetosella* L	Only presence on the plant
Huşi	*Rumex acetosa* L. *Rumex acetosella* L	Only presence on the plant
Roman	*Rumex obtusifolius* L.	Only presence on the plant
Piatra Neamţ	*Euphorbia spp.*	Only presence on the plant
Bicaz	*Rumex palustris* Sm.	Only presence on the plant
Focşani	*Rumex acetosa* L. *Rumex acetosella* L	Only presence on the plant
Măcin	*Rumex obtusifolius* L.	Only presence on the plant
Tulcea	*Rumex tuberosus* L. *Rumex aquaticus* L. *Rumex dentatus* L.subsp. *halácsyi* Rech.** *Rumex maritimus* L.	Only presence on the plant
Sulina	*Rumex tuberosus* L. *Rumex aquaticus* L. *Rumex palustris* Sm. *Rumex maritimus* L.	Only presence on the plant
Medgidia	*Rumex maritimus* L.	Only presence on the plant
Slobozia	*Saponaria officinalis* L. *Brassica oleracea* L.	Only presence on the plant
Ţăndărei	*Rumex acetosa* L. *Daucus carota* L.	Only presence on the plant
Jilava	*Rumex acetosa* L. *Solanum lycopersicum* L.	Only presence on the plant
Giurgiu	*Rumex palustris* Sm. *Saponaria officinalis* L. *Allium cepa* L.	Only presence on the plant
Şimnicul de jos	*Rumex tuberosus* L.	Pairing, small attack
Drobeta Turnu-Severin	*Rumex longifolius* D.C. *Saponaria officinalis* L.	Only presence on the plant
Herculane	*Rumex hydrolapathum* Huds. *Rumex palustris* Sm. *Polygonum lapathifolium* L. *Polygonum persicaria* L.	Pairing, eggs deposit, larval hatching, damages of the attack

*cultivated
**very rare

Regarding to the *Rh. pericarpius* plant hosts there is few contributions in world entomological literature. This wasn't the main purpose of the study but some aspects need to be clarified. For instance, in Romania our study brings for the first time almost exhaustive data about but we can find some most cited host species in other papers. In the world literature Reitter, 1916 cited for host plants only genus *Polygonum* and *Rumex*. Hoffmann, 1954 cited the species *Rh. pericarpius* on *R. obtusifolius* and *R. acetosa* in all France territory. Morris, 1967 had signaled the species *Rh. pericarpius* like common in Ireland on *Rumex* genus species: *Rumex acetosa, Rumex obtusifolius, Rumex crispus, Rumex acetosella, Rumex conglomeratus* Murr. In U.K., Read, 2002 mentioned the weevil species on genus *Rheum*, which are non-native in England. Walsh and Dibb, 1954, noted that only *P. amphibium* was host species for *Rh. pericarpius* and finnally Scherf, 1964, find *Rh. pericarpius* only on *R. hydrolapathum*. In district Nova Scotia (Canada) Majka et al., 2007, signaled the presence of *Rh. pericarpius* like a non-native faunistic element feeding on *R. crispus*, *R. maritimus* and *R. acetosa*. Piesik, 2004, mentioned in his study about using insects like biological control agents host plant for *Rh. pericarpius* the weed *R. confertus* which seems to be a big problem in Poland in pastures as his high amounts of oxalic acid. When consumed in large quantities the lethal poisoning of animals can occur. In China, where the herb patience is cultivated on a large surfaces used in consumption or like medicinal plant, Li et al., 2001, 2002 and 2003, had mentioned the species *Rh. pericarpius* on forage *Rumex* hybrid K-1 in Xinjiang region. Another controversial problem was shows by the studies carried out in Japan by Katsumata et al., 1930, and Harada, 1930, which mentioned the species *Rh. pericarpius* like on the most injurious pest on hemp. No mention in the world literature appear about some species of *Phytobiini* (and we can even say from all *Curculionidae* family) on cultivated or spontaneous hemp plants. In Romania the hemp crop was cultivated (a single species, *Cannabis sativa* L.), with two subspecies *Cannabis sativa* L. subsp. *sativa* Fr., and *Cannabis sativa* L. subsp. *spontanea* Serebr. on a large area in Banat and Transylvania region and even in some localities from southern of country but now the plant were not cultivated yet on the large surface.

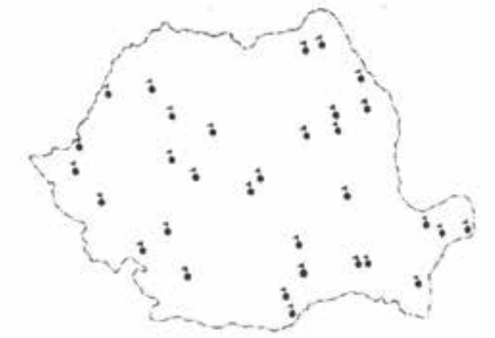

Figure 2. *Rhinoncus pericarpius* L., spread in Romania

Mode of the attack. The attack shape of the adult stage consist in holes punching on the leaves on the entire surface of the leaf limb but concentrated mainly on the centre and on the margins of the leaves. Adults are feeding sometimes (especially on the driest period of weather) with the rods or young stalks causing small cavities on their surface, the way of some pathogens entrance. The larvae feeding behaviour, soon after his emergency was related with the organ were the eggs are deposit. They crunch the surface of the stalks or stems to enter inside of the parenchyma and they dig mining the stem to the roots and to the soil for pupation. The larval gallery could be the entrance way for some plant pathogens and other saprophytic organisms.

Eggs and incubation. After pairing and copulation, which could be after a period of feeding or in some cases during the feeding, the female laid daily the eggs, in small bunches, usually on the young stalks, on the rods or on the leaf petiole (Figure 3).

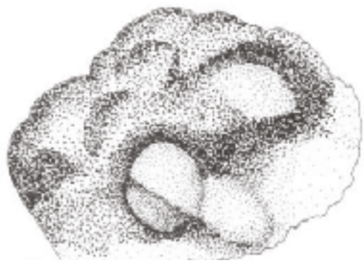

Figure 3. Eggs batch covered with specific glandular agglutinat deposit on *R. acetosella* stem

The eggs were regularly deposit on the leaves petiole at the stem insertion or relatively frequent on the young stalks. In very rare situation (one single case in laboratory) the eggs were laid on the lower face of the leaf. In the field, in conditions of southern part of Romania the female laid the eggs between earlier first week of May and the last eggs were laid on the end of May in very rare cases in the beginning of June. The egg batch had a dark brown colour and count between 5 and 30 eggs agglutinated in the secretion of the female glands situated on the copulatory bursa on the last terga of the abdomen. Eggs have a discoid, flattened shape with length of 1.04±0.39 and width of 0.54±0.1 (Table 5).

The incubation period in the laboratory conditions was registered between 8 and 16 days after deposit on the plant organs. The results are presented in the Table 3.

Table 2. Testing the host preferences of *Rhinoncus pericarpius* L. on spontaneous weeds and culture plants

VARIANT	TESTED SPECIES	FOOD CONSUMPTION		ATTACK DEGREE	OSERVATIONS
		Adults	Larvae		
1 adult ♀	*Rumex crispus* L.	+	++	0.5	Pairing, eggs deposit, larval hatching
1 adult ♂	*Rumex acetosella* L	+++	+++	4.6	Pairing, eggs deposit, larval hatching
2 adults (♀+♂)	*Rumex acetosa* L.	++	++	2.8	Pairing, eggs deposit, larval hatching
10 adults (5♀ + 5♂)	*Rumex hydrolapathum* Huds.	+	-	1.8	Pairing
	Rumex obtusifolium L.	+	-	0.2	Pairing
	Polygonum aviculare L.	+	-	-	-
	Polygonum persicaria L.	+	-	0.6	-
	Polygonum lapathifolium L.	+	-	0.8	-
	Rheum officinale Baill.	++	++	5.7	Pairing, eggs deposit, larval hatching
	Rheum rhaponticum L.	++	++	2.3	Pairing, eggs deposit, larval hatching
	Brassica oleracea L.	-	-	-	-
	Daucus carota L.	-	-	-	-
	Solanum lycopersicum L.	-	-	-	-
	Allium cepa L.	-	-	-	-

Table 3. Eggs incubation duration of species *Rhinoncus pericarpius* L. in laboratory conditions on *Rumex acetosella* L.

Variant / Replicate	1 wetted filter paper	2 cotton swabs soaked
	Eggs incubation duration (days)	Eggs incubation duration (days)
1	10	8
2	13	8
3	10	8
4	16	8
5	10	8
6	11	8
7	11	8
8	14	9
9	13	9
10	13	9
Mean	**12.1**	**8.3**

In the first variant of incubation bioassay, on the wetted filter paper the incubation duration was longer then on the cotton swabs soaked, respectively. In the first variant the incubation period was between 10 and 16 days with the average of 12.1 days. In the second variant the same incubation process were count an average of 8.3 days, between 8 and 9 days maximum. The incubation were influenced by temperature conditions and also by the moisture and relative humidity that could be the explanation of the short period of the incubation process in the second variant.

Female fecundity. On the laboratory conditions the female of *Rh. pericarpius* were laid the number of eggs counting between of 75 and 830 eggs on the entire of the active period (Table 4). The daily average of the eggs deposit in those 10 replicates used in the experiment was between 3.38±1.0 and 18.04±2.1 (Table 4).

Eggs fertility. In laboratory conditions the eggs fertility was relatively high compared with the values obtained in the field. For instance in our study the fertility was between 86.19% and 100.00% compared with the 10 replicates from the field where the fertility reach the values of 45.55 and 70.24% of the hatching larvae. It seems to be some conditions of natural infection from the soil vehiculated by the ants (like gregarine, and some other parasitic organisms) but the small values of the larvae hatching in the field could be explained also by the eggs consumption by some predatory species of ants (*Lasius fuliginosus* Latr., *L. flavus* F. and so on) (Table 4).

Table 4. Prolificity of female and eggs fecundity to species *Rhinoncus pericarpius* L. in laboratory conditions on *Rumex acetosella* L.

Replicate	Prolificity		Fecundity	
	Eggs number	Daily mean	Larvae number	% of hatching
1	105	3.38	105	100.00
2	384	9.60	331	86.19
3	75	3.75	75	100.00
4	98	6.53	92	93.87
5	319	9.66	316	99.05
6	830	18.04	810	97.59
7	188	6.26	188	100.00
8	80	5.33	80	100.00
9	161	5.36	160	99.37
10	132	4.40	131	99.24

Growth development in laboratory conditions.

Larval stage. The larva is grub with length dimensions of 1.8±0.4 in media, apodous, eucephalous type, white in colour, with well-developed head capsule with functional mandibles with act transversely, maxillae stemmata and antennae. Through measure the wide of head capsule of larvae, the larval development covers 3 instars (Table 5). In literature there are few studies regarding the morphology of adult and larvae of the species *Rh. pericarpius*, the only one paper which refer to the larval development were written by Li et al., 2001. The values obtained in this study for head capsule of larvae measurement (L_1 = 0.305±0.0105 mm; L_2 = 0.516±0.0105 mm; L_3 = 0.796±0.0083 mm) was similar with those obtained in the present study. Larval stage duration in laboratory conditions on natural food was covered a period of 6 to 8 days with the average of 6.9 days (Table 7). No other mention in the world literature can be found related to the larval growth and development.

Table 5. Dimensions of cephalic capsule, larvae and egss of *Rhinoncus pericarpius* L., reared in laboratory conditions on natural food

Larvae length (mm)	Head capsule of 3 instars (mm)	Egg (mm)	
		Length	Width
1.8±0.4	0.310±0.01 L_1	0.60±0.3	0.55±0.12
1.9±0.3	0.518±0.01 L_2	0.55±0.2	0.50±0.1
2.0±0.6	0.851±0.08 L_3	0.81±0.3	0.48±0.2
2.1±0.8		0.98±0.2	0.58±0.3
2.3±0.9		1.5±0.8	0.58±0.1
1.6±0.1		1.2±0.5	0.53±0.2
1.8±0.2		1.0±0.2	0.47±0.1
1.6±0.4		1.4±0.4	0.59±0.2
2.0±0.5		1.3±0.5	0.58±0.1
1.5±0.1		1.1±0.5	0.58±0.2
1.8±0.4		**1.04±0.39**	**0.54±0.1**

Adult stage. The adult body of species *R. pericarpius* count after our study measurements between 2 - 3.5 mm with small variations of length between 0.5 - 0.1 mm (no visual sexual dimorphism but generally the female had the body bigger then male) (Figure 4). In the classical studies of taxonomy of *Curculionidae* the species are mentioned by Reitter, 1916, and Hoffmann, 1954, with the body length of 2 - 3.5 mm and Freude et al., 1981, with body length of 2.5 - 3.4 mm. In laboratory conditions the feeding and matting behaviour was studied and confirmed by the field observations.

Soon after his appearance the female, start feeding on the *R. acetosella* leaves and they then was matting. Many observations look like they first start matting and after they begin to feed after or in the same time with copulation.

Figure 4. *Rhinoncus pericarpius* L., adult stage

I think that it could be possible to feed on another plant and to fly after on the new host plant and start matting. In laboratory conditions the active period from female life cycle were rigorous registered. After the matting process which count no more then 2 hours or almost 1 day began the preovipositary period which mean between 2 and 15 days (mean of 6.4 days). After the preovipositary period the female stop feeding and begin to lay eggs on the plant organs closed to the stem or at the insertion of the shells on the stem. The period calls ovipositary period and in conditions of our study this count 15-46 days (with the mean of 29 days) (Table 6).

Table 6. Active period of the female to species *Rhinoncus pericarpius* L. in laboratory conditions on *Rumex acetosella* L.

Replicate	Preovipositary period	Ovipositary period
1	2	31
2	5	40
3	10	20
4	2	15
5	2	33
6	8	46
7	9	30
8	15	15
9	5	30
10	6	30
Mean	**6.4**	**29.00**

Pupal stage. After browsing last instar (L_3) the larvae build a puparium from vegetable tissue debris agglutinated with some special secretions from the last abdominal segment and sterilized in the same time with this secretion paste and inside this pupal lodge the pupal stage was spend. The duration of pupal stage in conditions of our experiment was between 6 and 8 days (mean 6.9 days) (Table 7).

Life cycle duration. In laboratory conditions on natural food the adult life duration was registered contained between 32 and 45 days (mean of 34.1 days). After the pupal stage the adult's emergency and the number of adults obtained from all ten replicates means in percent about 75.26 (similar with the percent observed in field) and 100.00% but the mean value was 92.45% (Table 7). The whole period of life cycle of *Rh. pericarpius* in laboratory conditions beginning to egg stage and ending with the adult dead was contained between 63 and 96 days (mean of 73.6 days). In the field conditions the observations in the field cages were difficult to make because of the insect small size and they had usually the habit to enter in the soil or to hide on the vegetation's from the soil. Only after some estimation could be reasonable to suppose that this duration reach maximum of 75-80 days.

Table 7. Growth duration of the species *Rhinoncus pericarpius* L., in laboratory conditions on natural food

Replicate	Growth duration	Larval stage duration	Pupae stage duration	Adults %
1	43	27	7	93.33
2	45	27	8	75.26
3	33	17	6	93.33
4	32	15	7	81.63
5	40	23	7	94.04
6	34	19	8	96.50
7	36	18	7	95.74
8	34	19	6	100.00
9	44	21	7	96.27
10	34	19	6	98.48
Mean	**34.1**	**20.5**	**6.9**	**92.45**

Using species *Rh. pericarpius* like biological control agent. Beginning with this study the efficacy of insect species in biological control of the host plants from Polygonaceae was revealed. The attack of adults cause significant damages on leaves when the numerical density was more then 5-10 individuals/plant. The most important attack of the adult (especially of the female) is manifest in the flowering and in the period of seeds formation when the adults completely destroy the fructifications. In our previous paper (Manole, Iamandei, 2002) we establish for the first time the important role which he can play in weeds biological control (especially on *R. obtusifolius*). Other than our study form 2002 a much documented review of this subject was published by Herrick and Kok, 2010. The only limit of this study is related with the weeds definition which, in conception of the authors for the study was "an alien plant species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic and/or environmental harm, and/or harm to human health". And in this study it is revealed that the insects from family *Curculionidae* (and *Chrysomelidae*) include the most potential biological control agents against weed plants (Table 8). Several species of beetles in the family *Curculionidae* have played a vital role in the suppression of invasive weeds throughout the world (Julien et al., 1984; Julien, Griffiths, 1998; Coulson et al., 2000). Our study established that over 242 species could be used in Romania like biological control agents but Herrick and Kok, 2010, successfully used as biological control agents in classical biological weed control. In the actual ecological context biological control programs using insects became very desirable. First problem which rises to the scientists all over the world is the problem of alien invasive species, plants including. In many countries that kind of biological control program became very attractive correlated with the ecological gains. Some technical problems need to be resolved in the future according with the costs reduction. First step in this direction consist in development of the systematic studies about some candidates of biological control agents. Present study brings new knowledge's about the biology and ecology of *Rh. pericarpius* which could be mass reared in controlled conditions and released in control of some weeds in horticultural and agricultural crops. In Europe and also in North America, Canada the species *Rh. pericarpius* were used in some programs against the weed plants from *Polygonaceae* family. Piesik (2004) conducted one field research in vicinity of Bydgoszcz and Toruń in period 1997-2001, using some insect's species among those *Rh. pericarpius* against one important weed, *Rumex confertus* Willd. (mossy sorrel). The same mention about this host plant for *Rh. pericarpius* appear in the book written by McPortland et al., 2000. In Asia, Russian Far East, China, Korea and Japan *Rh. pericarpius* is considered invasive species but another weevil belong of Subfamily *Ceuthorhynchinae*, native in the area could be used like biological control agent against some weeds from *Polyganaceae* family. Herrick and Kok, 2010, mentioned that species *Rhinoncomimus latipes* Korot., could be used for biological control of mile-a-minute weed, (*Polygonum persicaria* L., syn. *Persicaria perfoliata* (L.) H. Gross) in Africa. The species had however used already in biological control program in Asia by augmentation practices and in North America by mass rearing and released in the field (Hough-Goldstein et al., 2009; Lake et al., 2011; Paynter et al., 2015). A very intense concern regarding the biological control programs using insects against weeds were to evaluate the effectiveness of the method and implicit the costs of programs. After of that evaluation Herrick and Kok, 2010, mentioned that although the initial investment in a classical biological control programme is expensive, the monetary gain after programme implementation is greater. Classical biological control programme with *Curculionidae* is a viable option for weed control because of its sustainability. Morin et al., 2009, had also made a very documented economic analysis of effectiveness and costs-benefits balance and in conclusion stated that the problem to highlighted the stakeholders is most important to negociate the long-term benefits. The ussual trend is to evaluate the agent effectiveness soon after their release and establishment in the field but practitioners must understand to undertake long-term post-release evaluation. Paynter et al., 2015, concluded in his study in the same manner that is a necessity to make a cost-benefit analysis before implementing the program but the duration of host-range testing agents released is the long-term element for economical analysis.

Table 8. Target plant species worldwide, grouped by habitat, using *Curculionidae* as biological control agents (after Herrick, Kok, 2010, modified)

Habitat type	Plant species targeted worldwide	% of species	Number of insects weevils used
Terrestrial-herbaceous	44	65.7	47
Terrestrial-arborescent	17	25.3	18
Aquatic or semi-aquatic	6	9.0	10
Total	**67**	**100.00**	**75**

CONCLUSIONS

In Romania *Rh. pericarpius* is a well-distributed species associated with some host plants from genus *Rumex* and *Polygonum* which could be used in biological control programme together with other species frequently present in this associations like *Gastroidea polygoni* L., *G. viridula* Deg., *Hypera rumicis* L., *Apion miniatum* Germ., *A. frumentarium* Payk., and *Pegomya nigritarsis*

Zett. In conditions of Romania in the field the life cycle had a single generation/year, diapausing in soil in the adult stage and pairing and oviposing beginning with the first week of May. For the first time new data about ecology and biology of this species were registered. The distribution at the country level and the worldwide status of species was established. The food regimen is phytophagous related with plant associations of *Rumex*, *Polygonum* and cultivated *Rheum* genres in Romania 9 host plant were recorded (*R. acetosella* L., *R. crispus* L., *R. acetosa* L., *R. obtusifolium* L., *R. hydrolapathum* Huds., *P. persicaria* L., *P. lapathifolium* L., *Rh. officinale* Baill., *Rh. rhaponticum* L.). The attack shape of the adult consist in holes punching on the leaves and the larvae were minning the stems. In laboratory conditions the species was reared on natural food on leaves and stems of *Rumex acetosella*. The female had two active period of life cycle: pre-ovipositary which was in our experiment between 2 and 15 days (mean of 6.4 days) and ovipositary period which count 15-46 days (mean of 29 days). Female deposit after copulation daily between 5 and 30 the eggs usually on young stalks or on the leaf petiole. Eggs have a discoid, flattened shape with length of 1.04±0.39 and width of 0.54±0.1. The incubation period in the laboratory conditions was registered between 8 and 16 days after deposit on the plant organs. The larva is grub with length dimensions of 1.8±0.4 in media, apodous, eucephalous type, white in colour, with well-developed head capsule with functional mandibles with act transversely, maxillae stemmata and antennae. Through measure the wide of head capsule of larvae, the larval development covers 3 instars. After browsing last instar (L_3) the larvae build a puparium from vegetable tissue debris agglutinated with some special secretions from the last abdominal segment and sterilized in the same time with this secretion paste and inside this pupal lodge the pupal stage was spend. The duration of pupal stage in conditions of our experiment was between 6 and 8 days (mean 6.9 days). The adult body of species *R. pericarpius* count after our study measurements between 2 - 3.5 mm with small variations of length between 0.5 - 0.1 mm (no visual sexual dimorphism but generally the female had the body bigger then male). In laboratory conditions on natural food the adult life duration was registered contained between 32 and 45 days (mean of 34.1 days). The study establish for the first time the important role which he can play in weeds biological control (especially on *R. obtusifolius*).

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FIRST RECORDS OF NATURAL ENEMIES OF KERMES HERMONENSIS SPODEK & BEN-DOV *(Hemiptera: Sternorrhyncha: Kermesidae)* IN TURKEY

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Abstract

This study was carried out on Quercus infectoria Oliv. (Fagaceae) trees infested with the coccid Kermes hermonensis Spodek & Ben-Dov (Hemiptera: Sternorrhyncha: Kermesidae) between 2013 and 2014, in Diyarbakır. As a result of the study, two parasitoids and two predators were obtained. These are: Cheiloneurus claviger Thomson, 1876; Metaphycus sp. (Hymenoptera: Encyrtidae: Encyrtinae) and Brumus (Exochomus) quadripustulatus (Linnaeus, 1758), Chilocorus bipustulatus (L.) (Coleoptera: Coccinellidae). B. (Exochomus) quadripustulatus and C. bipustulatus are the first records on K. hermonensis as predators in Turkey. K. hermonensis: Cheiloneurus claviger and Metaphycus sp. are the first records on K. hermonensis as parasitoids in Turkey.

***Key words**: Kermes hermonensis, Cheiloneurus claviger, Metaphycus sp., Brumus (Exochomus) quadripustulatus, Chilocorus bipustulatus, Turkey.*

INTRODUCTION

Kermesidae family (*Kermesidae: Hemiptera*) with 91 species in 9 genera (1 fossil species in 1 fossil genus) generally specialized on the plants belonging to *Fagaceae*. Family of *Kermes* Boitard genus is the richest species in the world as well as in Palaearctic region with 33 species (Ben-Dov et al., 2013; Spodek, Ben-Dov, 2014). All species were recorded on *Quercus* spp. Although in general for all scale insect, almost all of the description of *Kermes* species based on adult female stages, first instar stages were used for the systematic studies as well (Bodenheimer, 1953; Balachowsky, 1950, 1953; Borchsenius, 1960; Pellizzari et al., 2012; Spodek, Ben-Dov, 2014).

Ten species have been recorded up to now belonging to genus *Kermes* and *Nidularia* Targioni-Tozzetti in Turkey (Ülgenturk et al. 2013). Bodenheimer himself described three *Kermes* species in Turkey between 1951 and 1953, but unfortunately either the type material and dry materials are not in good conditions and they need more attention indeed. Although *K. bekirii* Bodenheimer, *K. muhlisi* Bodenheimer, *K. sadrii* Bodenheimer and *K. safinazae* Özkök were described from Turkey, there are not complementary studies on the *Kermesidae* species in Turkey. The other members of the family *Nidularia balackhowskii* were found recently on *Quercus* spp. in many places. (Ülgentürk *et al*., 2013). *Kermes hermonensis* Spodek & Ben-Dov was described as a new species in Turkey by Kaydan et al. (2014).

Scale insect family species *Kermesidae* (*Hemiptera: Coccoidea*) are restricted to the northern hemisphere and they are distributed throughout the Nearctic, Oriental and Palaearctic regions (Ben-Dov et al., 2015). The family contains about one hundred valid species in ten genera and the majority of species of the family are known to develop exclusively on *Quercus* species (*Fagaceae*) (Ben-Dov et al., 2015). Females and males develop mainly on twigs, branches and in bark crevices, while some species develop on leaves (Sternlicht, 1969; Bullington, Kosztarab, 1985; Hu, 1986; Podsiadlo, 2005).

Most *Kermesidae* species are not known that they cause any visible damage to their host trees. However there are reports of infestations of some species that have led to branch dieback, flagging, reduced growth rates and occasionally tree death. These occurrences are mainly in urban areas (Kozár, 1974; Hamon, 1977; Solomon et al., 1980; Viggiani, 1991;

Pellizzari et al., 2012; Podsiadlo, 2012). *Kermesidae* species belong to two genera named *Nidularia* Targioni-Tozzetti and *Kermes* Boitard in the Mediterranean and European regions. Species of *Kermes* (*Hemiptera: Kermesidae*) are specialist sap-feeders on species of *Quercus* and they can be economically important at high population densities.

On the other hand, these insects can be important for honey bees in honey production. Among the most important natural enemies of *Kermes* species are encyrtids within the genus *Psilophrys* (Japoshvili, 2005; Japoshvili, Noyes, 2006a). However, there are some *Blastothrix* species that also parasitize *Kermes* spp. (Trjapitzin, 1989; Japoshvili, Karaca, 2003). Undoubtedly, these parasitoids have an important effect on scale about the population of the species.

The *Encyrtidae* constitute the majority of parasitoids attacking to the psyllid insects. Members of the family are important in biological control. More than 400 encyrtid species have been used or are used today for suppression of various crop pests (Japoshvili, Noyes, 2006b). There are more than 1270 described species of encyrtids in the Palaearctic Region (Yasnosh, Japoshvili, 1999; Japoshvili, 2005-2007a, b; Japoshvili, Karaca, 2003; Japoshvili, Noyes 2005-2006b).

The *Coccinellidae* are generally considered as an useful insects, because many species of it feed on aphids which are pests in gardens, agricultural fields, orchards, and similar places. Colonies of such plant-eating pests lay hundreds of eggs and then the larvae commences feeding immediately. However, some species do have unwelcome effects; among these, the most prominent are the subfamily *Epilachninae,* which are plant eaters. Thirteen genera contain 66 species that are placed here into this large trophic group that has scale insects as its prey. Members of the superfamily *Coccoidea* (the scale insects); this superfamily includes various related families, notably *Coccidae* (soft scales), *Diaspididae* (armored scales), *Pseudococcidae* (mealybugs), *Dactylopiidae* (cochineal scales), *Kermesidae* (gall-like scales), *Eriococcidae* (felt scales), *Cerococcidae* (ornate pit scales), and Asterolecaniidae (pit scales) (Anonymous 2016a).

The aim of this study was to determine the natural enemies of the harmful *Kermes hermonensis* on *Quercus infectoria* trees in Diyarbakır.

MATERIALS AND METHODS

Soft scale insect samples were collected from the province of Diyarbakır in the Southeastern Part of Turkey in 2013. Specimens were taken from both wild and cultivated plants during irregular surveys carried out in the spring and summer seasons of the one-year study. Each sample was put into a plastic bag and taken to the laboratory for examination.

Representative specimens were sent to various taxonomic specialists for confirmation of identification. Host identification (*Kermes hermonensis*) was made by Dr. Malkie Spodek (Department of Entomology, Agricultural Research Organization The Volcani Center, P.O. Box 6, Bet Dagan, 50250 ISRAEL), the coccinellids identification was made by Prof. Dr. Nedim Uygun (Çukurova University, Faculty of Agriculture, Department of Plant Protection, 01330 Adana, Turkey) and the parasitoids identification was made by Prof. Dr. George Joposhvili (Institute of Entomology agricultural University of Georgia-Georgia).

Samples were collected from ornamental plants from Diyarbakır in Turkey. Each sample was placed into a plastic bag and taken to the laboratory for examination.

RESULTS AND DISCUSSIONS

As a result of this study, two parasitoids species *Cheiloneurus claviger* Thomson, 1876, *Metaphycus* sp. (*Hymenoptera: Encyrtidae: Encyrtinae*) and two predators species *Brumus (Exochomus) quadripustulatus* (Linnaeus, 1758), *Chilocorus bipustulatus* (Linnaeus, 1758). (*Coleoptera: Coccinellidae*) were obtained.

Kermes hermonensis Spodek, Ben-Dov (*Hemiptera: Sternorrhyncha: Kermesidae*)

Distribution in World: Israel (Spodek, Ben-Dov, 2014),

Distribution in Turkey: Diyarbakır (Kaydan et al., 2014).

Host plant: *Quercus species* (*Fagaceae*) (Ben-Dov et al. 2015), *Quercus infectoria* Oliv. (*Fagaceae*) (Kaydan et al., 2014).

Material examined: Diyarbakır (38° 09' 41° 12' 54'E at altitude of about 663 m.).

Cheiloneurus claviger Thomson, 1876 (*Hymenoptera: Encyrtidae: Encyrtinae*)

Recorded hosts: *Acanthopulvinaria orientalis* (Nasonov) (*Coccidae: Acanthopulvinaria*) (Japoshvili, Çelik, 2010; Myartseva, 1984); *Ceroplastes ceriferus* (Fabricius) (*Hemiptera, Coccidae*) (Japoshvili, Çelik, 2010; Xu, Huang, 2004); *Ceroplastes japonicus* Green (*Hemiptera: Coccoidea: Coccidae*) (Japoshvili, Çelik, 2010; Japoshvili, Noyes, 2005; Japoshvili, 2000); *Chloropulvinaria aurantii* (Cockerell) (*Hemiptera: Coccidae*) (Xu, Huang, 2004); *Coccus hesperidum* L. (*Hemiptera: Coccoidea: Coccidae*) (Japoshvili, Çelik, 2010); *Kermes hermonensis* Spodek, Ben-Dov (*Hemiptera: Sternorrhyncha: Kermesidae*) (Japoshvili et al., 2015); *Kermes vermilio* Planchon (*Hemiptera: Sternorrhyncha: Kermesidae*) (Japoshvili, Çelik, 2010; Marotta et al., 1999)

New record host in Turkey: In the present study *Kermes hermonensis* was recorded as a new host of *Cheiloneurus claviger* in Turkey.

Distribution: Armenia, Austria, Azerbaijan, Bulgaria, Croatia, Czech Republic, Czechoslovakia, Egypt, Europe, France, Georgia, Germany, Greece, Hungary, Iran, Israel, Italy, Japan, Kazakhstan, Moldova, Montenegro, Netherlands, Palaearctic, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Tadzhikistan, Transcaucasus, Turkey, Turkmenistan, Ukraine, United Kingdom, USSR, Uzbekistan, Yugoslavia (Federal Republic) (Anonymous, 2016b).

Material examined: 2♀♀ Locality: Diyarbakır (Diyarbakır 38° 09' 41° 12' 54'E at altitude of about 663 m.).

Metaphycus sp. (Hymenoptera: Encyrtidae: Encyrtinae)

Target Pests: Soft brown scale, black scale and citricola scale.

Crops suitable: Citrus, olives, passion fruit, figs, custard apples and a wide range of ornamentals including gardenia, oleander, ferns and palms.

New record host in Turkey. In the present study *Kermes hermonensis* was recorded as a new host of *Metaphycus* sp. for Turkey.

Material examined: 2♀♀ Locality: Diyarbakır (Diyarbakır 38° 09'41° 12' 54'E at altitude of about 663 m.).

Brumus (Exochomus) quadripustulatus (Linnaeus, 1758) (*Coleoptera: Coccinellidae*)

Recorded hosts. The pine ladybird a polyphagous predatory in both adult and larval stages preys aphids and scale insects (Uygun, 1981; Çelik, 1983; Bolu, 2002; Bolu, 2004; Bolu et al., 2007).

New record host in World. In the present study *Kermes hermonensis* was recorded as a new host of *Brumus (Exochomus) quadripustulatus* from Turkey for world.

Distribution in World: Albania, Austria, Balearic Is., Belarus, Belgium, Bosnia and Herzegovina, Britain I., Bulgaria, Corsica, Crete, Croatia, Cyprus, Czech Republic, Danish mainland, Estonia, European Turkey, Finland, French mainland, Germany, Greek mainland, Hungary, Italian mainland, Latvia, Lithuania, Luxembourg, Macedonia, Moldova, Norwegian mainland, Poland, Portuguese mainland, Romania, Russia Central, Russia North, Russia South, San Marino, Sardinia, Sicily, Slovakia, Slovenia, Spanish mainland, Sweden, Switzerland, Netherlands, Ukraine, Yugoslavia (Anonymous, 2016b).

Distribution in Turkey: Balıkesir, Denizli, İzmir (Giray, 1970); Aegean Region (Soydanbay-Tunçyürek, 1976); Artvin, Rize (Bozan, Aslıtürk, 1975); İzmir (Öncüer, 1977); Eastern Mediterranean Region (Uygun, 1981); Ankara (Düzgüneş et al., 1982); Gaziantep (Çelik, 1983); Erzurum (Özbek, Çetin, 1991); Southeastern Anatolia Region (Bolu, Uygun, 2003; Bolu, 2002-2004; Bolu et al., 2007); Adana, Niğde (Ulusoy et al., 1999); Diyarbakır, Elazığ, Mardin (Bolu, 2005).

Material examined: 10 adult ladybirds was obtained in total.

Locality: Diyarbakır (Diyarbakır 38° 09' 41° 12' 54' E at altitude of about 663 m.).

Chilocorus bipustulatus (L.) (*Coleoptera: Coccinellidae*)

Recorded hosts: Heather ladybirds feed on aphids and scale insects, small insects mainly belonging to the family of *Coccidae* and

Diaspididae (Uygun, 1981; Bolu, 2005; Bolu et al., 2007).

New record host in world. In the present study *Kermes hermonensis* was recorded as a new host of *Chilocorus bipustulatus* (L.) from Turkey for world.

Distribution in World: Albania, Austria, Azores, Balearic Is., Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Corsica, Crete, Croatia, Cyprus, Czech Republic, Danish mainland, Estonia, European Turkey, Finland, French mainland, Germany, Greek mainland, Hungary, Ireland, Italian mainland, Latvia, Lithuania, Luxembourg, Macedonia, Madeira, Malta, Norwegian mainland, Poland, Portuguese mainland, Romania, Russia Central, Russia North, Sardinia, Sicily, Slovakia, Slovenia, Spanish mainland, Sweden, Switzerland, The Netherlands, Ukraine, Yugoslavia (Anonymous, 2016b).

Distribution in Turkey: Aegean Region (Soydanbay-Tunçyürek, 1976); Artvin, Rize (Bozan, Aslıtürk, 1975); İzmir (Öncüer, 1977); Aydın, Denizli, İzmir (Uygun, 1981); Adana, Niğde (Ulusoy et al., 1999); İzmir, Manisa (Tezcan, Uygun, 2003); Southeastern Anatolia Region (Bolu, Uygun, 2003; Bolu et al., 2007); Diyarbakır, Elazığ, Mardin (Bolu, 2005).

Material examined. Total obtained was 1 adult ladybirds. Locality: Diyarbakır (Diyarbakır 38^{o} 09' 41^{o} 12' 54' E at altitude of about 663 m.).

This study showed that there are many hitherto unrecorded parasitoids and predators of *Kermes hermonensis* in Turkey. More studies should be conducted on the parasitoid fauna of *Kermes hermonensis*, including studies on their biology.

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BIOLOGICAL EFFICACY OF SOME SOIL HERBICIDES AT MAIZE (*Zea mays* L.)

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Abstract

Maize (Zea mays L.) is main grain, forage and strategical field crop in Bulgaria. One of the main negative factors for maize growing is the weeds. The aim of our study conducted in 2016 and 2017 is to evaluate the biological efficacy of some soil herbicides at maize hybrid P 1114. The experiment was stated on the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 4 replications, and the efficacy was recorded by the 10 score visual scale of EWRS. The herbicides Merlin® Duo (37.5 g/l isoxaflutole + 375 g/l terbutilazin), Adengo® 465 SC (225 g/l isoxaflutole + 90 g/l thiecarbazone-methyl + 150 g/l cyprosulfamide - antidote), Lumax® 538 SK (37.5 g/l mesotrione + 375 g/l s-metolachlor + 125 g/l terbutilazine) were examined. The herbicides were applied after sowing before germination of the crop (BBCH 00). The highest herbicide efficacy and the highest yields (11.86 t ha^{-1}) were obtained for the treatment with Merlin® Duo at rate of 2000 ml ha^{-1}. All evaluated herbicides were selective for the grown maize hybrid.

Key words: *maize, weeds, herbicides, efficacy.*

INTRODUCTION

Maize is the most common forage crop in Bulgaria that is grown for grain and silage (Yankov et al., 2013). According to data from the Ministry of Agriculture Food and Forestry in 2016 grain maize has harvested area of 406,942 ha with average yields of 5,470 kg ha^{-1} (www mzh.government.bg).

The weeds are one of the main yield limiting factors. They have high concurrence with the crop for water, light, space and nutrients (Tonev et al., 2007; Tonev, 2000). The maize grain yield can decrease from 24% to 96.7% (Mukherjee, Puspajit Debnath, 2013; Oerke, Dehne, 2004; Khan et al., 2003; Tonev et al., 2007; Zhalnov, Raikov, 1996). In Bulgaris economically most important weeds at this crop *Amaranthus retroflexus* L., *Datura stramonium* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Chenopodium album*, *Abutilon theophrasti* L., *Sinapis arvensis* L., *Echinochloa crus-gali* L., *Setaria glauca* L., *Sorghum halepense* L., *Convolvulus arvensis* L, *Cinodon dactilon* L. and *Cirsium arvense* L. (Hristova et al., 2012; Kalinova et al., 2012; Tonev et al., 2010). Studies conducted in Slovakia showed that the most distributed weeds in maize fields are *Chenopodium album* L., *Amaranthus* spp., *Echinichloa crus-galli* (L.) P. Beauv, *Datura stramonium* (L.), *Fallopia convolvulus* (L.) A. Lôve, *Persicaria* spp., *Cirsium arvense* (L.) Scop, *Elytrigia repens* (L.) P. Beauv, *Avena fatua* (L.) and *Abutilon theophrasti* Medik. (Týrand Vereš, 2012). Smatana et al. (2015) reported that in the maize fields of the country the weeds *Atriplex* spp. and *Setaria viridis* (L.) P. Beauv. are found. In India the most aggressive weeds are *Polygonum* spp. (*P. pensylvanicum, P. persicaria, P. orientale*), *Stellaria media, Stellaria aquatica, Oldelandia diffusa, Oldenlandia umbellate, Physalis minima, Solanum nigrum*. In Belgaum district of Karnataka, India, the most distributed weeds are *Cynodon dactylon, Dinebra retroflexa, Echinochloa colonum, Eleusine indica, Cyperus rotundus, Parthenium hysterophorus, Commelina benghalensis, Portulaca oleracea, Cynotis cuculata, Phyllanthus niruri* and *Amaranthus viridis* (Mukherjee, Debnath, 2013; Haji et al., 2012). One of the main weed control methods is the herbicide application (Janak et al., 2016; Umesha et al., 2015; Noor Muhammd et al., 2012; Skrzypczak et al., 2011; Pannacci and Covarelli, 2009; Tonev, 1986). Against the annual grass and broadleaf weeds very high eficacy is found after the application of Gardoprim Plus Gold 500 SK -

4000 ml ha^{-1} (99%), Lumax 538 SK - 4000 ml ha^{-1} (97%), Wing - 4000 ml ha^{-1} (97%) and Merlinflex - 420ml ha^{-1} (94,6%) (Dimitrova et al., 2013). Pannacci (2016) established that the application offoramsulfuron had 95% efficacy against *Amaranthus retroflexus* L., *Setaria viridis* (L.) Beauv., *Sinapis arvensis* L. and *Solanum nigrum* L. Quddus et al. (2011) recorded that formasulfuron + isoxadifen-ethyl + Urea sucsessfully controleed *Cyperus rotundus* and *Achyranthus aspera* - 87% and 75%, respectively.
The aim of our study is to evaluate the efficacy of some soil herbicides at maize.

MATERIALS AND METHODS

The field experiment is carried out in 2016 - 2017 in the field of training and experimental base of the department of Agriculture and herbology. The trial was conducted by the randomized block design in 4 replications. The size of the experimental plot was 28 m². The maize hybrid P1114 (590 FAO from the latehybrid group) was grown in the experiment. Predecessor of maize during the experimental years was winter wheat. After predecessor's harvest, deep ploughing followed to disking tillage operations was performed. Fertilization with 500 kg ha^{-1}NPK (15:15:15) was done before sowing of maize and dressing with 300 kg ha^{-1} NH_4NO_3 during vegetation.
The reporting of the weeds was performed prior to treatment, on the 14th, 28th and 56th days after treatments. The efficiency against weeds was reported by the 10-score scale of EWRS. The results were compared with untreated control. The selectivity of the herbicides was reported by 9-score phytotoxicity scale of EWRS (0 - no damage, and 9 - complete crop destruction).
Variants of the trial were as follows: 1) Untreated control; 2) Merlin® Duo (37.5 g/l isoxaflutole + 375 g/l terbuthylazine) - 0.75 l ha^{-1}; 3) Merlin® Duo-1.00 l ha^{-1}; 4) Merlin® Duo - 1.25 l ha^{-1}; 5) Merlin® Duo - 1.50 l ha^{-1}; 6) Merlin® Duo - 2.00 l ha^{-1}; 7) Adengo® 465 SC (225 g/l isoxaflutole + 90 g/l thiencarbazone-methyl + 150 g/l cyprosulfamide-antidote) - 0.44 l ha^{-1}; 8) Lumax® 538 SC (37.5 g/l mesotrione + 375 g/l s-metolachlor + 125 g/l terbuthylazine) - 4.00 l ha^{-1}.

RESULTS AND DISCUSSIONS

The experimental field was infested with *Setaria viridis* (L.) P.Beauv., *Echinochloa crus-galli* (L.) Beauv., *Sorghum halepense* (L.) Pers. from seeds and rhizomes, *Chenopodium album* L., *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medik., *Datura stramonium* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Cynodon dactylon* (L.) Pers., *Convolvulus arvensis* L.
For control of *Sorghum halepense* L., *Convolvulus arvensis* L., *Echinochloa crus-gali* L., *Chenopodium album* L., *Amaranthus retroflexus* L. and *Abutilon theophrasti* L. in maize the combination of Stomp 33 EC + Mistral 4 SC could be applied (Kalinova et al., 2000). It is important to note that the use of pendimethalin has a lower risk of groundwater contamination than other herbicides such as alachlor (Brahushi et al., 2011).
The efficacy of the studied herbicides on the 14th day after treatment in 2016 and 2017 is presented on table 1. All annual broadleaf and grass weeds, except *X. strumarium*, were well controlled by Merlin® Duo in all the studied rates. Excellent efficacy against the weeds was also performed by Lumax® 538 SC and Adengo® 465 SC. Against the *S. halepense* from rhizomes, none of the tested products were able to control the weed, although on the 14th day after treatments, efficacy rates of 10 to 40% were reported. We did not expect any efficacy against the *C. dactylon* from the studied herbicides, irrespective of their application rate. Nevertheless, after the application of Adengo® 465 SC, on the 14th day after treatments light bleaching after the germination of the weed was observed. The symptoms disappeared very quickly and the weed completely restored. Against *C. arvensis* unsatisfactory efficacy of the examined herbicides was reported. The low efficacy of Merlin Duo (for treatments with rates of 1.25, 1.50 and 2.00 l ha^{-1}) and Adengo® 465 SC were expressed in a slight retention of weed growth and a decrease of the chlorophyll content in the leaves.
On the 28th day after treatments the efficacy against all annual weeds was kept or decreased from 5 to 15%, in comparison with the evaluation on the 14th day (Table 2). To a

greater extent, herbicidal efficacy decreases for the perennial weeds - *S. helepense, C. dactylon* and *C. arvesnis*. According to Kierzek et al. (2012) the best control of mixed weed infestation in maize is achieved after soil application of s-*metolachlor* + *terbuthylazine* + *mesotrione*, followd by foliar application of *nicosulfuron* + adjuvant Atpolan Bio 80 SL.

In the maize fields Tonev et al. (2016) establish high efficacy against annual gass and broadleaf weeds, as well as *Sorghum halepense* L., *Convolvulus arvensis* L, and *Cirsium arvense* L. after application of Flurostar® 200 EC + Nishin® 4 ODat rates of 700 ml/ha + 1300 ml/ha. If there is high infestation with *Chenopodium album* L. tank mixture of Mustang® 306.25 SC+ Nishin® 4 ODat rates of 600 ml/ha + 1300 ml/ha (Tonev et al., 2016).

For both years of the study, on the 56th day after treatment with Merlin® Duo at rates of 0.75 l ha^{-1} and 1.00 l ha^{-1} and Adengo® 465 SC, due to a strong secondary weed infestation with *S. viridis* and *E. crus-gali* the efficacy decreases and reaches 65-80% (Table 3). For the herbicides Merlin® Duo applied at doses of 1.25 to 2.00 l ha^{-1} and Lumax® 538 S Cit was found to have very good results against these two weeds (56 days after herbicide application, 85% to 95% efficacy). All herbicides, except for the lowest dose of herbicide Merlin® Duo (0.75 l ha^{-1}), completely control (95-100%) the weed *S. halepense* developed from seeds. Against C. album, Merlin® Duo applied at doses of 1.25 l ha^{-1} to 2.0 l ha^{-1} had excellent efficacy - 95% to 100%. Efficacy is not satisfactory at the lowest tested rates of Merlin® Duo and from the herbicides Lumax® and Adengo® 465 SC (from 70% to 85%) against this weed. Independently of the herbicide and examined rates, in all variants, the herbicidal efficacy against the *A. retroflexus* was from 90% to 100%. From all annual broadleaf weeds, *X. strumarium* was the most resistant to evaluated herbicides and rates. In none of the variants, efficacy was satisfactory. For Merlin® Duo at all evaluated rates, the efficacy reported on the 56th day after treatment ranged from 55% to 85%. For the variants treated with Adengo® 465 SC and Lumax® 538 SC, the efficacy was 80% for both years of the experiment. These low results are most likely due to the fact that *X. strumarium* germinates unevenly in time and from different soil depths. This is also the reason for the late secondary infestation. From the herbicides Merlin Duo (at rates of 1.00, 1.25, 1.50 and 2.00 l ha^{-1}), Adengo® 465 SC and Lumax® 538 SC against *A. theophrasti*on the 56th day after the treatment 90% to 100 % efficacy was recorded. Against *D. stramonium* excellent efficacy from all herbicides and rates in the study was reported. All studied herbicidesexcept for the lowest rate of Merlin® (0.75 l ha^{-1}) excellently control *S. nigrum*. Against *P. oleracea*, excellent results were obtained with the highest evaluated rates of Merlin® Duo (2.00 l ha^{-1}) and Lumax®538 SC at a rate of 4.00 l ha^{-1}. The lower the Merlin® Duo rate was, the lower the efficacy on the 56th day after treatment was recorded (75% to 95%). From the product Adengo® 465 SC, on the third last efficacy reporting date, due to high secondary weed infestation, the efficacy gradually decreased to 85%. None of the herbicides in the trial were able to control *S. halepense* developed from rhizomes, *C. dactylon* and *C. arvensis*. On the 56th day after the treatments, the efficacy of all products against these weeds was 0%.

No visible signs of phytotoxicity were reported for any of the treatments.

The weeds decrease the yields and the quality of maize grain (Masqood et al., 1999). The results of the comparative analysis of the indicator yield per hectare showed that during the two years of the experiment, significant differences in the benefit of the individual treated variants compared to the untreated control were demonstrated (Table 4).

From the analysed data by Duncan's multiple range test it was found that for variant 6 (Merlin® Duo at rate of 2.00 l ha^{-1}) the highest maize grain yield was achieved - 11.85 t ha^{-1}average for the period. The lowest maize grain seed yield among the treated variants was obtained at variant 2 (Merlin® Duo at rate of 0.75 l ha^{-1}) - 7.84 t ha^{-1} average for the two experimental years. The yield from the untreated control (6.97 t ha^{-1}) was 34% lower than the yield of variant 6.

Table 1. Efficacy of the studied herbicides on the14th day after treatment (%)

Variants / Weeds	2016								2017							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	-	90	95	95	95	95	90	100	-	85	90	90	90	90	85	100
S. viridis	-	85	95	95	100	100	90	100	-	80	90	90	95	95	85	95
E. crus-galli	-	95	100	100	100	100	100	100	-	100	100	100	100	100	100	100
S. halepense (s)	-	85	95	100	100	100	85	100	-	80	90	95	95	95	80	95
C. album	-	100	100	100	100	100	100	100	-	100	100	100	100	100	100	100
A. retroflexus	-	70	80	80	90	90	90	90	-	65	75	75	85	85	90	90
X. strumariu	-	90	100	100	100	100	100	100	-	90	95	100	100	100	10	100
A. theophrasti	-	90	95	95	100	100	95	95	-	85	90	95	95	100	95	95
D. stramonium	-	90	100	100	100	100	100	100	-	90	95	100	100	100	100	100
S. nigrum	-	95	100	100	100	100	100	100	-	85	90	95	95	100	95	95
P. oleracea	-	10	30	40	40	40	40	30	-	10	25	35	40	40	40	35
S. halepense (r)	-	0	0	0	0	0	15	0	-	0	0	0	0	0	10	0
C. dactilon	-	0	5	10	15	20	20	0	-	0	0	5	10	15	20	0

Table 2. Efficacy of the studied herbicides on the 28th day after treatment (%)

Variants / Weeds	2016								2017							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
S. viridis	-	80	90	95	95	95	80	100	-	70	80	85	85	85	75	95
E. crus-galli	-	80	85	95	95	100	80	95	-	75	80	90	90	90	75	90
S. halepense (s)	-	95	100	100	100	100	100	100	-	90	95	100	100	100	100	100
C. album	-	85	90	100	100	100	80	90	-	80	85	95	95	100	75	85
A. retroflexus	-	100	100	100	100	100	100	100	-	90	95	100	100	100	100	100
X. strumariu	-	65	80	80	80	85	80	85	-	60	75	75	80	80	75	80
A. theophrasti	-	85	100	100	100	100	100	100	-	80	90	95	100	100	100	100
D. stramonium	-	90	90	95	100	100	90	90	-	85	90	95	100	100	85	85
S. nigrum	-	90	100	100	100	100	100	100	-	85	90	95	100	100	100	100
P. oleracea	-	90	95	100	100	100	90	100	-	85	90	95	100	100	90	95
S. halepense (r)	-	0	5	15	15	15	20	15	-	0	0	10	10	10	15	10
C. dactilon	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
C. arvensis	-	0	0	0	0	5	5	0	-	0	0	0	0	0	0	0

Table 3. Efficacy of the studied herbicides on the 56th day after treatment (%)

Variants / Weeds	2016								2017							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
S. viridis	-	70	80	90	90	90	75	90	-	65	75	85	90	90	70	90
E. crus-galli	-	70	80	90	95	95	75	90	-	65	75	85	90	95	75	90
S. halepense (s)	-	90	100	100	100	100	100	100	-	85	95	95	100	100	100	100
C. album	-	80	90	100	100	100	75	85	-	75	85	95	95	100	70	80
A. retroflexus	-	100	100	100	100	100	100	100	-	90	90	95	100	100	100	100
X. strumariu	-	60	75	75	80	85	80	80	-	55	70	75	80	80	80	80
A. theophrasti	-	85	100	100	100	100	100	100	-	80	90	95	100	100	100	100
D. stramonium	-	85	90	95	95	100	90	90	-	80	85	90	90	95	90	90
S. nigrum	-	90	100	100	100	100	100	100	-	85	100	100	100	100	100	100
P. oleracea	-	80	90	95	95	100	85	100	-	75	85	90	95	100	85	95
S. halepense (r)	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
C. dactilon	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
C. arvensis	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0

Table 4. Maize grain seed yield, t ha^{-1}

Treatments	Rates l ha^{-1}	2016		2017		Average	
		yield	Duncan	yield	Duncan	yield	Duncan
1. Untreated control	-	7.03	a	6.90	a	6.97	a
2. Merlin®Duo	0.75	7.90*	b	7.78*	b	7.84*	b
3. Merlin®Duo	1.00	8.10*	b	8.01*	b	8.05*	b
4. Merlin®Duo	1.25	8.89*	c	8.88*	c	8.89*	c
5. Merlin®Duo	1.50	9.50*	d	9.45*	d	9.48*	d
6. Merlin®Duo	2.00	11.90*	f	11.81*	f	11.85*	f
7. Adengo® 465 SC	0.44	11.01*	de	10.99*	de	11.00*	de
8. Lumax® 538 SC	4.00	11.06*	e	11.05*	e	11.05*	e

All variants with a star have significant difference with the untreated control. The values in a column, followed by different letters (a, b, c etc.), differ significantly in P <0.05.

CONCLUSIONS

The herbicide product Lumax® 358 SC at rate of 4.00 l ha^{-1} is superior in efficiency to Merlin® Duo at rates of 0.75 and 1.00 l ha^{-1} and Adengo 465 СК at rate of 0.44 l ha^{-1} against *Setaria viridis* L.

The highest efficacy against *Echinochloa crus-galli* L. and *Datura stramonium* L. for Merlin® Duo at rate of 2.00 l ha^{-1} was recorded.

In all variants, with the exception of the lowest tested Merlin® Duo rate, 90% to 100% efficacy against *Sorghum halepense* L. from seeds, *Abuthilon theophrasti* L. and *Solanum nigrum* L. was found.

The highest efficacy against *Chenopodium album* L.was observed after the treatment of Merlin® Duo at rates of 1.25, 1.5 and 2.00l ha^{-1}.

The most resistant annual broadleaf weed in the experiment was *Xanthium strumarium* L.

Visible signs of phytotoxicity were not observed for any of the treatments in the study. The highest maize grain yield was achieved for the treatment of Merlin® Duo at rate of 2.00 l ha^{-1}.

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PERFORMANCE OF MAIZE VARIETIES (*Zea mays* L.) WITH DIFFERENT RATES OF NITROGEN FERTILIZER AND COWDUNG IN MUBI, NORTHERN GUINEA SAVANNA, NIGERIA

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Abstract

The aftermath of insurgency, climate change and poor soil fertility, necessitated the search for crops that can perform better within a short duration under the vagaries of environment. Thus, field experiments were conducted in 2014 and 2015 rainy reasons at the FAO/TCP farm, Adamawa State University Mubi, Nigeria to study the Performance of maize varieties (Zea mays L.*) with different rates of Nitrogen fertilizer and cowdung. Treatments consisted of maize varieties [(Quality Protein Maize (QPM) and Extra Early White (EEW)], Nitrogen rates (0, 60 and 120 kg N ha^{-1}) and cowdung rates (0, 1 and 2 t ha^{-1}). In an experiment laid out in a split plot design, the varieties were assigned to the main plots and nitrogen with cowdung in a factorial combination assigned to the subplots and replicated three times. Data were collected on plant height, leaf area index, plant dried weight, days to 50% maturity and grain yield per hectare and analyzed using SAS system for windows. Result showed that EEW had the highest plants height, leaf area index and plant dried weight. Nitrogen at 120 kg ha^{-1} produced highest plant dry weight and grain yield. Similarly, cowdung at 1 t ha^{-1} gave highest plant height, leaf area index and early days to 50 % maturity. There were interactions between treatments on parameters measured. Base on the results, varieties were observed to perform very well, with EEW manifesting higher characteristics. Thus, Nitrogen at 120 kg ha^{-1} should be use in the cultivation and be complimented with 1 t ha^{-1} cowdung.*

Key words: *cowdung, maize varieties, nitrogen fertilizer, performance.*

INTRODUCTION

Maize (*Zea mays* L) is the most important cereal crop in the farming systems of the savanna zone of Nigeria (Tarfa et al., 2003). Maize is also widely believed to have the greatest potential among food crops for attaining technological breakthrough that will improve food production (Kamara and Sanginga, 2001). Of upmost importance is the protein component of quality protein maize which contains double amount of nutrients found in other varieties (Kamara, Sanginga, 2001). Because of the climate change, poor soil fertility and scarcity of inorganic fertilizer as well as its contribution to environmental consequences research focused has been shifted towards the combined application of organic and inorganic fertilizers as a way to arrest the anomaly in sub-Saharan Africa (Van Lauwe et al., 2001). The utilization of cattle manure as a soil amendment has been in existence with the Nigerian poor resource farmers (Haris, Yusuf, 2001, Iwuofor et al., 2002). However, information that is lacking is the management practices and rate of application for optimum crop production, more especially, to compliment inorganic fertilizers.

Thus, this research aimed at studying the effects of organic and inorganic fertilizers, their complimentary role on improved varieties of maize in Mubi and similar environments.

MATERIALS AND METHODS

Field experiments were conducted in 2014 and 2015 rainy reasons at the FAO/Tree Crops Plantation farm, Faculty of Agriculture, Adamawa State University Mubi, Nigeria (Latitude 10^0 15' N, and Longitude 13^0 16' E at an altitude of 696 m above sea level) to study the Performance of maize varieties (*Zea mays* L.) with different rates of Nitrogen fertilizer and cowdung. Treatments consisted of maize varieties [(Quality Protein Maize (QPM) and Extra Early White (EEW)], nitrogen rates (0, 60 and 120 kg N ha^{-1}) and cowdung rates (0, 1 and 2 t ha^{-1}). These treatments were arranged in an experiment laid out in a split plot design; with varieties assigned to the main plots and nitrogen with cowdung in a factorial combination assigned to the subplots and was replicated three times. Land for the experiment was ploughed and harrowed using tractor. Soil Samples were randomly collected at a depth of 0-15 cm and 15-30 cm and their physco-chemical properties determined. Total land area used was 945.5 m^2 with gross plot size of 13.5 m^2 and net plot size of 4.5 m^2, path ways of 0.5 m between plots and 1 m between replications were also created. Sowing for 2014 and 2015 raining season were done on 9th July, 2014 and 3rd July, 2015, respectively. Two seeds were sown per hole at a spacing of 0.75 m x 0.25 m and were later thinned to one plant per stand at 2 weeks after sowing (WAS). Nitrogen fertilizer rates were applied in two split doses; first dose from NPK (15-15-15) was applied together with cowdung at land preparation. The NPK (15-15-15) also supplied the recommended 26 kg ha^{-1} P and 50 kg ha^{-1} K. The second dose of N was applied at 5WAS using Urea. Weeds in the fields were controlled by applying pre – emergence herbicides on the sowing date, Primextra (290 g/liter S - metolachlor and 370 g/litre atrazine) at 4 L ha^{-1} were applied. Spraying of the herbicides were done using 20 L knapsack sprayer. This were then supplemented by hand hoe at 3 and 9 WAS to keep the plots free from weeds. Data were collected at 3 and 6 WAS and at harvest on plant height, leaf area index (LAI) and Plant dried weight, days to 50% maturity and grain yield ha^{-1}. Data generated were subjected to analysis of variance using SAS system for windows (SAS v8 2000). Means showing significant F - test were separated using DMRT at 5% level of probability.

RESULTS AND DISCUSSION

Result of the physico - chemical properties of the experimental sites in the seasons indicated that the soil textural class was sandy - loam, soil pH (H_2O), organic carbon (g kg^{-1}), organic matter (g kg^{-1}), Total N (g kg^{-1}), available P (mg kg^{-1}) and CEC (cmol kg^{-1}) in 2014 at 0 - 15 cm were 6.4, 3.5, 6.2, 1.0 and 5.3, respectively and at 15 - 30 cm were 6.2, 1.1, 2.2, 1.5 and 5.2, respectively. Similarly, in 2015 at 0 - 15 cm were 6.0, 10.4, 17.1, 2.2 and 6.1, respectively. While at 15 - 30 cm were 6.3, 9.1, 15.3, 2.2 and 6.2, respectively. The variation in the physico - chemical properties in the seasons may be due to the residual soil nutrient which made that of 2015 rainy season higher than that of the 2014 rainy season. The effect of variety, nitrogen and cowdung on plant height of maize in 2014 and 2015 raining seasons and combined (Table 1) shows highly significant ($P \leq 0.01$) differences between varieties; at 3 WAS in 2014 raining season and combined, QPM produced taller plants, 21.97 cm and 19.21 cm, respectively. However, plant heights were taller in EEW in the seasons and combined at harvest (190.77 cm in the combined seasons). Similarly, in Table 1 no significant ($P > 0.05$) effect of nitrogen on plant height recorded at 3 WAS. Highly significant effect was recorded at 6 WAS and at harvest. Application of 120 kg N ha^{-1} produced taller plants of 92.72 cm at 6 WAS and 193.01 cm at harvest in the combined seasons. Shorter plants were recorded with 0 kg N ha^{-1}. Also in Table 1 no significant effect of cowdung on plant height of maize was recorded except in 2015 raining seasons (17.14 cm) at 3 WAS and in 2014 raining season (95.00 cm) and combined (88.80 cm) at 6 WAS where 1 ton ha^{-1} cowdung had taller plants. There were no interactions between variety with nitrogen except in 2014 raining season and combined at 3 WAS; QPM with 120 kg N ha^{-1} had taller plants (23.46 cm and 22.93 cm, respectively) (Table 2). No interaction between varieties with cowdung except in 2014 raining seasons at 3 WAS. QPM with 2 ton ha^{-1} cowdung had the tallest plant (34.15 cm) (Table 2). No interaction between nitrogen with cowdung except in 2014 raining seasons and combined at 6 WAS and in 2014 raining season at harvest (Table 1). At 6 WAS in 2014 raining season 120 kg N ha^{-1} with 0 t ha^{-1} cowdung (103.17 cm) and 120 kg N ha^{-1} with 1 t ha^{-1} cowdung (101.60 cm) were statistically similar and had taller plants (Table 2). The interaction between variety, nitrogen with cowdung was significant only in the combined seasons at harvest; EEW, 120 kg N ha^{-1} with 1 t ha^{-1} cowdung gave the tallest plants (Table 2). The significant differences between varieties might be due to genetic characteristics. Some varieties are dwarf and others tall, some maize varieties with stand environmental stress more than others.

Table 1. Effect of variety, nitrogen and cowdung on plant height (cm) of maize (*Zea mays* L.)

Treatment	3 WAS			6 WAS			At harvest		
	2014	2015	CMD	2014	2015	CMD	2014	2015	CMD
Varieties (V)									
Extra early white	20.58_b	16.25	18.41_b	91.36	79.74	85.55	193.63_a	187.91_a	190.77_a
Quality Protein Maize	21.97_a	16.45	19.21_a	90.84	80.48	85.68	176.66_b	175.38_b	176.02_b
Level of significance	**	NS	**	NS	NS	NS	**	**	**
SE ±	2.486	0.755	1.299	2.669	5.608	3.106	2.901	8.959	4.709
Nitrogen (N) kg ha^{-1}									
0	13.16	16.07	14.62	81.76_c	$67.8b_b$	74.79_b	177.12_c	164.77_b	170.95_b
60	20.36	16.58	18.47	92.89_b	86.58_a	89.74_a	187.39_b	189.83_a	188.61_a
120	24.31	16.40	20.36	99.51_a	85.92_a	92.72_a	195.68_a	190.33_a	183.01_a
Level of significance	NS	NS	NS	**	**	**	**	**	**
SE ±	1.982	0.427	1.014	2.317	1.969	1.520	2.325	3.595	2.141
Cowdung (C) t ha^{-1}									
0	16.96	15.45_b	16.18	83.63_b	76.38	80.01_b	181.54	182.85	182.19
1	19.90	17.14_a	18.52	95.00_a	82.60	88.80_a	186.36	179.41	182.89
2	20.82	16.45_b	18.64	94.72_a	81.35	88.04_a	187.55	182.67	185.11
Level of significance	NS	**	NS	**	NS	**	NS	NS	NS
SE ±	1.982	0.427	1.014	2.317	1.969	1.520	2.325	3.595	2.141
Interactions									
V x N	*	NS	**	NS	NS	NS	NS	NS	NS
V x C	**	NS	NS	NS	NS	NS	NS	NS	NS
N x C	NS	NS	NS	**	NS	**	**	NS	NS
V x N x C	NS	NS	NS	NS	NS	NS	NS	NS	**

Means in the same column followed by the same letter are not significant at 5% using DMRT. CMD = combined
* = P ≤ 0.05, ** = P ≤ 0.01, NS = P > 0.05

Table 2. Interactions of variety, nitrogen and cowdung on plant height (cm) of maize (*Zea mays* L.)

Treatment	At 3 WAS in 2014 rainy season		At 3 WAS in the Combined seasons	
Variety	EEW	QPM	EEW	QPM
Nitrogen kg ha^{-1}				
0	20.29c	21.02b	18.25c	20.97b
60	20.28c	22.43a	18.21c	21.73b
120	21.16b	23.46a	18.78c	22.93a
SE ±	2.80		1.43	
Cowdung t ha^{-1}				
0	19.82d	23.99b		
1	20.42a	29.72ab		
2	21.49c	34.15a		
SE ±	2.80			

	At 6 WAS in 2014 rainy season			At 6 WAS in the combined seasons			At harvest in 2014 rainy season		
Nitrogen kg ha^{-1}	0	60	120	0	60	120	0	60	120
Cowdung t ha^{-1}									
0	56.90c	90.83ab	103.17a	61.93e	81.74cd	80.71d	154.33e	193.12b	196.67a
1	95.10a	88.30ab	101.60a	87.04c	88.61c	92.36b	182.60c	179.10d	197.37a
2	93.27a	97.13a	98.77a	91.04b	96.06a	91.04b	179.23d	190.4bc	193.0b
SE ±		4.01			2.63			4.03	

Combined seasons at harvest

Variety	EES			QPM		
Nitrogen kg ha^{-1}	0	60	120	0	60	120
Cowdung t ha^{-1}						
0	154.72e	206.30a	198.97a	165.22c	182.44b	185.51b
1	181.95b	191.34ab	203.38a	165.08c	173.97c	181.56b
2	185.49b	195.71a	199.05a	158.21d	182.65b	189.53b
SE ±			5.24			

EEW = extra early white; QPM = quality protein maize

Table 3. Effect of variety, nitrogen and cowdung on leaf area index of maize (*Zea mays* L.)

Treatment	3 WAS			6 WAS			At Harvest		
Variety (V)	2014	2015	CMD	2014	2015	CMD	2014	2015	CMD
Extra early white	0.08	0.05	0.06	0.32	0.29	0.31	0.40a	0.34	0.37a
Quality protein maize	0.08	0.04	0.07	0.30	0.28	0.29	0.36b	0.32	0.34b
Level of significance	NS	NS	NS	NS	NS	NS	**	NS	**
SE±	0.0062	0.0024	0.0033	0.0094	0.013	0.008	0.0042	0.029	0.0146
Nitrogen (N) kg ha^{-1}									
0	0.07	0.05	0.06	0.25b	0.23b	0.27b	0.37a	0.28	0.33b
60	0.07	0.05	0.06	0.34a	0.31a	0.32a	0.39a	0.36a	0.38a
120	0.08	0.05	0.07	0.36a	032a	0.34a	0.37b	0.36a	0.37a
Level of significance	NS	NS	NS	**	**	**	**	**	**
SE ±	0.004	0.0033	0.0024	0.0150	0.099	0.009	0.0089	0.092	0.064
Cowdung (C) t ha^{-1}									
0	0.07	0.05	0.06b	0.29	0.28	0.29	0.37	0.33	0.35b
1	0.08	0.05	0.07a	0.32a	0.29a	0.31a	0.39	0.35	0.37a
2	0.08	0.05	0.07a	0.33	0.28	0.31	0.39	0.32	0.36a
Level of significance	NS	NS	**	NS	NS	NS	NS	NS	**
SE ±	0.036	0.033	0.0024	0.0150	0.0099	0.009	0.089	0.092	0.064
Interaction									
V x N	NS	NS	NS	NS	NS	NS	NS	NS	NS
V x C	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x C	NS	NS	NS	**	NS	NS	**	NS	NS
V x N x C	NS	NS	NS	NS	NS	NS	NS	NS	**

Means in the same column followed by the same letter are not significant at 5% using DMRT. * = $P \leq 0.05$, ** = $P \leq 0.01$, NS = $P > 0.05$, CMD = combined

This result agreed with that of Azeez and Adetunji (2003) that, improved crop varieties exhibitgenetic characteristics and/or influence of the environment. And that of Yahaya (2008) and Olakanle (2009) that maize varieties varies in their performance. Also Jama et al. (2000) reported an increased in the growth of maize due to cow manure. The effect of cowdung may be due to its ability to improve water holding capacity, soil aeration, soil structure, nutrient retention and microbial activity. The significant interactions of some of the parameter might be due to varietal response to nutrient. The effect of variety, nitrogen and cowdung on LAI of maize in 2014 and 2015 raining seasons and combined (Table 3) shows no significant differences between varieties on LAI at 3 and 6 WAS and at harvest in 2015 rainy season.

Highly significant effect was recorded at harvest in 2014 raining season and combined, EEW had the highest LAI (0.40 and 0.37, respectively) (Table 3). No significant effect of nitrogen at 3 WAS. Highly significant effect was recorded at 6 WAS and at harvest. 120 kg N ha^{-1} had the highest LAI at 6 WAS and at harvest 60 kg N ha^{-1} took over (Table 3). Except in the combined seasons at 3 WAS and at harvest where cowdung at 1 t ha^{-1} had higher LAI no significant effect of cowdung recorded (Table 3). No interactions recorded except in 2014 rainy season at 6 WAS and at harvest (Table 3). Also in Table 3 no interaction between variety, nitrogen with cowdung except in 2014 raining season at 3 WAS and combine at harvest. In Table 4 the interaction that had higher LAI in 2014 raining season at 6WAS was 120 kg N with 1 t ha^{-1} cowdung (0.37) and at harvest was 60 kg N ha^{-1} with 2 t ha^{-1} cowdung (0.43). The significant differences between varieties might be due to genetic characteristics which could have affected plant height and number of leaves per plant, which might have in turn affected LAI. Environmental factors might have also contributed to the variation in LAI. Earlier, Aziz et al. (2014) reported that, nitrogen increase leaf area of maize. Increase in LAI can also be due to the application of cowdung. Table 5 shows the effect of nitrogen and cowdung on plant dried weight of maize in the seasons. No significant differences between varieties recorded except in 2015 rainy season at 6 WAS and in 2014 rainy season at harvest, EEW produced higher plant dried weights of 335.21g and 905.93g, respectively. Similarly, highly significant effect of nitrogen was recorded in the seasons and combined except in 2014 rainy season and the combined at 6 WAS (Table 5); 120 kg had the highest effect producing 805.66 g in the combined seasons at harvest. Also in Table 5, no significant effect of cowdung on plant dried weight in the seasons, except in 2014 rainy season at 3 WAS and the combined seasons at harvest; 2 t ha^{-1} cowdung produced maximum plant dried weight of 706.32 g in the combined seasons at harvest. Furthermore, there were no interactions except in the combined seasons at harvest (Table 5).

Table 4. Interactions of variety, nitrogen and cowdung on leaf area index of maize (*Zea mays* L.)

	At 6 WAS in 2014 rainy season			At harvest in 2014 rainy season		
Nitrogen kg ha^{-1}	0	60	120	0	60	120
Cowdung t ha^{-1}				0.31d	0.36c	0.37bc
0	0.16d	0.34b	0.37a	0.39b	0.38b	0.39b
1	0.29cd	0.32c	0.36ab	0.41a	0.43a	0.37bc
				SE ± 0.01		
2	0.31c	0.35ab	0.34b			
SE ±	0.026			0.01		
	At harvest in the combined seasons					
Variety	EEW			QPM		
Nitrogen kg ha^{-1}	0	60	120	0	60	120
Cowdung t ha^{-1}						
0	0.2926cd	0.3985a	0.421a	0.2840d	0.3440c	0.3647bc
1	0.3635bc	0.3958a	0.4045a	0.3188c	0.3733b	0.3728b
2	0.3520c	0.3493c	0.3695b	0.2842d	0.3763b	0.3597c
SE ±	0.016					

EEW = extra early white; QPM = quality protein maize.

Table 5. Effect of variety, nitrogen and cowdung on plant dried weight (g) of maize (*Zea mays* L.)

Treatment	3 WAS			6 WAS			12 WAS		
Varieties (V)	2014	2015	CMD	2014	2015	CMD	2014	2015	CMB
Extra Early white	36.19	69.69	52.94	197.71	335.21a	266.46	905.93a	487.07	696.50
Quality protein maize	40.33	73.19	56.77	185.04	297.08b	241.06	877.78b	435.54	656.66
Level of significance	NS	NS	NS	NS	**	NS	**	NS	NS
SE ±	1.867	2.995	1.765	3.211	4.441	2.741	10.965	22.096	12.333
Nitrogen (N) kg ha^{-1}									
0	32.82b	49.52c	41.17c	180.81	228.80b	204.81	721.11b	292.71b	506.91b
60	40.61a	68.09b	54.35b	198.24	257.32a	277.78	900.00a	534.35a	717.18a
120	41.36a	96.72a	69.04a	195.07	362.31a	278.69a	1055.44a	566.87a	805.66a
Level of significance	**	**	**	Ns	**	NS	**	**	**
SE ±	2.298	5.246	2.864	11.295	12.125	8.285	62.067	22.484	33.008
Cowdung (C) t ha^{-1}									
0	31.72b	68.37	50.05	177.48	314.07	245.78	816.22	446.18	631.2b
1	38.81a	75.41	57.11	187.21	306.67	246.94	952.22	431.77	691.99a
2	44.20a	70.54	57.37	209.38	327.69	268.54	106.67	505.97	706.32a
Level of significance	**	NS	NS	NS	NS	NS	NS	NS	*
SE ±	2.298	5.246	2.864	11.295	12.125	8.285	62.067	22.484	33.008
Interaction									
V x N	NS	NS	NS	NS	NS	NS	NS	NS	NS
V x C	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x C	NS	NS	NS	NS	NS	NS	NS	NS	NS
V x C x N	NS	NS	NS	NS	NS	NS	NS	NS	**

Means in the same column followed by the same letter are not significant at 5% using DMRT.
* = $P \leq 0.05$, ** = $P \leq 0.01$, NS = $P > 0.05$, CMD = combined

Table 6. Interactions of variety, nitrogen and cowdung on plant dried weight of maize in the combined seasons

Variety	EEW			QPM		
Nitrogen kg ha^{-1}	0	60	120	0	60	120
Cowdung t ha^{-1}						
0	316.750g	820.75a	864.55a	393.15f	550.22d	843.12a
1	687.483c	807.10a	688.33c	513.65d	622.40c	833.02a
2	661.25c	655.27c	767.00b	469.17e	847.32a	837.92a
SE ±	80.85					

EEW = extra early white; QPM = quality protein maize

In Table 6, QPM with 60 kg N ha^{-1} and 2 t ha^{-1} cowdung had the highest plant dried weight (847.32 g). The significant differences between varieties, the effect of nitrogen and cowdung on plant height and leaf area index might have given the plants ability to accumulate more assimilates and thus, more dry matter per plant. The effect of variety, nitrogen and cowdung on days to 50% maturity of maize in the seasons is presented in Table 7. Highly significant differences existed between varieties on days to 50% maturity in 2014 rainy season and combined, QPM took longer days to 50% maturity; 87.07 and 89.65 days, respectively. Similarly, in Table 7 highly significant effect of nitrogen was recorded in the seasons 0 kg N ha^{-1} had longer days to 50% maturity. There was a highly significant effect of cowdung on days to 50% maturity in 2014 rainy season and combined, 0 ton ha^{-1}took longer days to 50% maturity (Table 7). No interactions recorded (Table 7). The significant differences between varieties on days to 50 % maturity may be due to varietal differences; early maturing and late maturing. Similarly, nitrogen is known to promote cell division, enlargement and overall plant growth and development. Where fertilizer is lacking maize undergoes abnormal growth.

Table 7. Effect of variety, nitrogen and cowdung on days to 50% maturity and grain yield of maize (*Zea mays* L.)

	Days to 50% maturity			Grain yield (kg ha^{-1})		
Treatment	2014	2015	Combined	2014	2015	Combined
Varieties (V)						
Extra Early White	79.11b	92.78	85.5b	3960.9	4240.8	4100.9
Quality Protein Maize	87.07$_a$	92.22	89.65$_a$	3706.8	4533.1	4119.9
Level of significance	**	NS	**	NS	NS	NS
SE ±	0.302	0.164	0.172	146.289	174.498	113.853
Nitrogen (N) kg ha^{-1}						
0	85.17$_a$	93.50$_a$	89.34$_a$	3454.6b	2662.5c	3058.6c
60	82.67$_b$	92.28$_b$	87.48$_b$	3946.9a	4902.6b	4424.8b
120	79.94$_c$	91.72$_b$	85.83$_c$	4100.0a	5658.3a	4879.2a
Level of significance	**	**	**	**	**	**
SE ±	0.424	0.314	0.264	155.994	223.453	135.885
Cowdung (C) t ha^{-1}						
0	84.22$_a$	92.55	89.33$_a$	3773.5	4366.0	4069.8
1	81.33$_b$	92.28	87.47$_b$	4084.7	4164.8	4123.8
2	82.22$_c$	92.66	87.44$_b$	3643.2	4609.6	4126.4
Level of significance	**	NS	**	NS	NS	NS
SE ±	0.424	0.314	0.264	155.994	223.453	135.885
Interaction						
V x N	NS	NS	NS	**	NS	NS
V x C	NS	NS	NS	NS	NS	NS
N x C	NS	NS	NS	NS	NS	NS
V x N x C	NS	NS	NS	NS	NS	NS

Means in the same column followed by the same letter are not significant at 5% using DMRT.
* = P $\leq$ 0.05, ** = P $\leq$ 0.01, NS = P > 0.05

The effect of variety, nitrogen and cowdung on grain yield of maize in the seasons (Table 7) shows no significant differences between varieties on grain yield. Highly significant effect of nitrogen on grain yield was recorded in the seasons. Application of 120 kg N ha^{-1} had highest grain yield (4878.20 kg ha^{-1}) in the combined seasons (Table 7).

No interaction between varieties with nitrogen on gain yield, except in 2014 rainy season where EEW with 120 kg N ha^{-1} had the highest grain yield of 4264.20 kg ha^{-1} (Table 8).

No other interactions on grain yield. As more dry matter accumulated by plant, harvest index (grain yield) increased. Yield varies with variety; some varieties are high yielding than others (Odeleye, Odeleye, 2001).

Table 8. Interactions of variety, nitrogen and cowdung on grain yield (kg ha^{-1}) of maize (*Zea mays* L.) in 2014 rainy season

Variety	Extra early white	Quality protein maize
Nitrogen		
0	3880.25b	3028.96d
60	4264.20a	3827.16c
120	3935.80b	4066.67a
SE ±	220.61	

CONCLUSIONS

Maize varieties (Quality protein maize and Extra early white) were found to respond positively to fertilizer application with nitrogen at 120 kg ha^{-1} and 1 t ha^{-1} cowdung produced optimum yield. Thus, should be use in the cultivation.

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THE EFFECTS OF TILLAGE METHODS AND PLANT DENSITY ON GROWTH, DEVELOPMENT AND YIELD OF SOYBEAN [*Glycine max* (L.) Merrill] GROWN UNDER MAIN AND SECOND CROPPING SYSTEM: II. GROWTH-DEVELOPMENT COMPONENT

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Abstract

The aim of this study was to compare tillage methods and plant density on growth, development and yield of soybean [Glycine max (L.) Merrill] grown under main and second cropping systems. The field experiments were carried out at the experimental area of Faculty of Agriculture, Dicle University during 2013 and 2014. The experiments were conducted as split-split plot design based on randomized complete blocks with two sowing dates (normal and late) as the main plot, three tillage methods (no-tillage, reduced and conventional) as sub-plot, and three between row spacing (35, 55 and 70 cm) sub-sub-plot factor. The experiments were performed in three replications and soybean cultivar Nova (MG III) was used. According to the two-year average results of the study, tillage methods had significant effects on dry weight and leaf area index in the R5 phase of the tillage treatment and reduced soil tillage by soil application, lower dry weight and leaf area index (LAI) value than conventional tillage method. Leaf growth rate (LGR) and leaf area ratio (LAR) were found significant between early planting (0.13 cm^2/cm^2/ day, 0.15 cm^2/g) and late planting (0.09 cm^2/cm^2/day, 0.12 cm^2/g).

***Key words**: soybean, sowing time, tillage, plant density, yield, growth.*

INTRODUCTION

Due to the limited availability of arable land in the world, the nutritional deficiencies associated with the growing population are required to meet by the increase either in yield or in the unit area. For this reason, producers need alternative agricultural practices that can provide the highest yield potential with lower production costs. By means of this, while high yielding and and high quality varieties are developed using breeding studies, determination of effect of agronomic studies such as irrigation, fertilization, sowing time, tillage and sowing density plant growth and development intensively continue. Determination of the optimal number of plants and the optimal tillage methods from agricultural practices has been the subject of prior research in recent years (Peterson, Higley, 2001).

When main crop soybean cultivation is compared with second crop soybean the potential benefits of soybean can be counted as follows: intensive use of resources, reduction of soil erosion, reduction of production cost, and increase of income level of producers (Sanford et al., 1986).

Tillage methods in crop production affect plant growth and development. As a matter of fact, Sürek (2004) stated that protected soil tillage practices can delay or reduce the severity of drought stress in the second crop of soya agriculture. Besides, preplant wastes are one of the important components of reduced soil tillage method. They just not add required nutrients to the soil (Erenstein, 2003), but also help soil temperature to be balanced via reducing evaporation (Greb, 1966; Wilhelm et al., 1989) and consequently, affects crop yield (Biamah, 2005).

For most soils, plant residues increase the infiltration of water in the root zone (Bruce et al., 1987; Dick et al., 1987), reducing water surface runoff and soil loss, thereby providing favorable conditions for soil treatment and thus increasing product yield. When reduced or no tilling methods can be used as alternative methods to conventional soil treatment, since planting time are predated, second crop can expand the planting fields.

The growth and development of a plant in the agricultural ecosystem is affected by agricultural practices such as number of rows and number of plants. Different row spacing and plant density affects plant lighting, photosynthesis rate and consequently plant productivity. Narrow row spacing in grain plants results in increased light uptake during the first developmental period of the plant and may lead to higher seed yield compared to standard row spacing. Moreover, the ability of cultivated plants to compete with weeds in agricultural ecosystems depends, in part, on the plant growth rate. Plants that are capable of forming canopy from the first developmental period, such as soybean, are able to suppress the weed population more than other cultivated plants. Leaf area index, canopy formation rate and plant height significantly affect the competitiveness and tolerance of cultivated plants against weeds (Peterson, Higley, 2001).

Although our country, especially the Mediterranean, Aegean and Southeastern Anatolian Regions, have suitable ecological conditions for soybean production, unfortunately the plantation area and the production amount have remained very low. Therefore, in this study, it was aimed at determination of effects of different tillage methods on soybean growth and development in case of cultivation of main crop and second crop soybean using proper row spacing.

MATERIALS AND METHODS

The study was carried out at experimental area of Field Crops Department, Faculty of Agriculture, Dicle University, Diyarbakir located in South East Anatolian Region of Turkey in 2013 and 2014. The region has a warm climate in summer, and the mean annual rainfall is around 450 mm, most of which fall in a major cropping season which extends from November to June (Anonymous, 1990). Experimental soil has a heavy built (fine textured), it is poor in terms of organic matter and phosphorus with medium lime and moderate alkaline reaction and high cation exchange capacity no salt (Anonymous, 1995). The treatments were replicated three times in split-split plot based on randomized complete block design with sowing time (early and late) in the main plots, tillage systems (no-tillage, reduced tillage and conventional tillage) in the sub-plots and plant density of 35 x 5 cm, 55 x 5 cm and 70 x 5 cm with 571,400, 363,600 and 285,700 plants ha^{-1} in the sub-sub-plots. Conventional tillage (plough + disc harrow), reduced tillage (cultivator) and no-tillage (without any tillage) treatments were involved before sowing. On the basis of soil analysis, the crop was fertilized with 100 kg N and 100 kg P_2O_5 ha^{-1} applied as basal dose in the form of 20-20-0 fertilizer prior to sowing. In addition, top dressing nitrogen was provided at the time of full flowering stage at the rate of 100 kg ha^{-1} as ammonium nitrate (33% N) for all plots. Weeds were controlled by both Trifluralin (2.5 l ha^{-1}) as pre plant and by hand as needed. The field was uniformly irrigated at 10-days intervals until harvest period using overhead sprinklers. Soybean cultivar Nova (MG III) was sown as early sowing on May and late sowing on June. At R8 (Fehr, Caviness, 1977), all plots were harvested from two central rows in mid-September and in mid-October (for early and late sowing, respectively) and threshed for seed yield (kg ha^{-1}). In both years, the seeds from each plot were taken after harvest for determining oil and protein content of seeds.

Data was subjected to an analysis of variance (ANOVA) using a statistical software package (JMP version 5.0.1a). Least significant difference (Tukey's HSD test) was used to compare treatment means at P=0.05.

Plant Dry Weight (g plant^{-1})

In two different developmental periods (flowering-R1 and seedling-R5) as a mean of 5 plants in each plot; It was dried and weighed at 80°C until it reached constant weight and determined in grams.

Leaf Area İndex (cm^2 cm^{-2})

It was calculated according to the following formula, developed by Radford (1967), recommended by the Board (2000), using the WINFOLIA leaf area program as an average of 5 plants in each of the two different growth stages (flowering-R1 and seeding-R5):

$$LAI = \frac{\text{Total plant leaf area (cm}^2\text{)}}{\text{Total area covered by the plant (cm}^2\text{)}}$$

Plant Growth Rate (g/m^2/day)

It was calculated according to the following formula, Board (2000) and developed by Radford (1967):

$$PGR = \frac{W_2 - W_1}{T_2 - T_1}$$

W_1: $T_{1\,(time)}$ Dry weight of plants in T_1 (g plant^{-1});
W_2: $T_{2(time)}$ Dry weight of plants in T_2 (g plant^{-1});

T_1: 1. When dry matter is detected during development (day);
T_2: 2. When dry matter is detected during development (day).

Leaf Growth Rate (cm²/m²/day)
It was calculated according to the following formula, Board (2000) and developed by Radford (1967):

$$LGR = \frac{LA\dot{I}_2 - LA\dot{I}_1}{T_2 - T_1}$$

$LA\dot{I}_1$: $t_{1(time)}$ Leaf Area Index ($cm^2\ cm^{-2}$);
$LA\dot{I}_2$: $t_{2(time)}$ Leaf Area Index ($cm^2\ cm^{-2}$);
T_1: 1. When the leaf area index is determined during development (day);
T_2: 2. When the leaf area index is determined during development (day).

Leaf Area Rate (cm²/g)
It was calculated according to the following formula, Board (2000) and developed by Radford (1967):

$$LAR = \frac{(LA_2-LA_1)\ (\log_e W_2 - \log_e W_1)]}{[(\log_e LA_2 - \log_e LA_1)\ (W_2-W_1)]}$$

LA: Leaf Area (cm^2);
W: Plant Dry Weight (g).

Relative Growth Rate (g/g/day)
According to the following formula proposed by Gardner et al. (1985):

$$RGR = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

W_1: $t_{1(time)}$ Plant Dry Weight ($g\ plant^{-1}$);
W_2: $t_{2(time)}$ Plant Dry Weight ($g\ plant^{-1}$);
T_1: 1. When dry metal is detected during development (day);
T_2: 2. When dry metal is detected during development (day).

Net Assimilation Rate (g m^{-2} day^{-1})
Gardner et al. (1985) proposed the following formula:

$$NAO = PGR\ X\ \frac{\log_e L_2 - \log_e L_1}{T_2 - T_1}$$

PGR: Plant Growth Rate
L_1: $T_{1(time)}$ Leaf Area (m^2);
L_2: $T_{2(time)}$ Leaf Area (m^2).

RESULTS AND DISCUSIONS

According to the results of the experiment the two-year average values showing the effects of tillage methods and plant density on the yield and quality characteristics of soybean grown as the main and double-crop soybeans are given in the Table 2.

1. Plant dry weight at the first flowering period (R1) (g/plant)
The effect of planting time on plant dry weight was found significant, according to the effect obtained on the dry weight of the plant in flowering period (R1). In terms of the two-year average, while the plant dry weight was 16.02 g in early planting, when planting delay, a significant decrease in plant dry weight was observed (13.28 g). No significant difference between soil treatments was obtained (Table 1). In terms of the two-year average, it varied between 14.34-14.98 g (Al-Darby, Lowery, 1987); the amount of dry matter per plant is lower in no tillage than conventional or reduce tillage method. Similar findings were also obtained by researcher Janovicek (1991), indicating that the dry matter accumulation in the soilless system is lower than in the plow and the autumn. While there was a significant difference between the practices in terms of plant density, as the plant density increased with respect to the two-year average values, a decrease in plant dry weight was observed in the first flowering period. The highest value was obtained from a plant density of 70 x 5 cm (16.31 g/plant).

2. The plant dry weight in the seedling period (R5) (g plant^{-1})
The plant dry weight was found 32.38 g plant^{-1} in early planting, while the plant dry weight was reduced to 28.91 g plant^{-1} when the planting date was delayed, according to the obtained value for the effect on plant dry weight at the seeding period. The effect of the tillage method on the plant dry weight was found significant. The plant dry weight varied between 29.73-31.38 g plant^{-1} and the highest plant dry weight was obtained from conventional tillage treatment (31.38 g/plant).
In this regard, Yusuf et al. (1999) found that dry matter weights of total plant, stem, leaf and fruit of plants grown in the conventional tillage method were about 15-20% higher in the early

stages of development than those without soil treatment, whereas plants were grown in the R5-R6 stage. As a result of the reduction of this difference in reach, the seed yield, oil and protein ratio can be compensated. Similar findings were also obtained by Janovicek (1991), indicating that the dry matter accumulation in the soilless system is lower than in the plow and the autumn. The effect of plant density on the dry weight of the plant during seedling period was significant.

The plant dry weight varied between 28.83-32.76 g/plant, and as the plant density decreased, the plant dry weight gain was increased and the highest dry weight was obtained from the plant density of 70 x 5 cm (32.76 g/plant). Rahman et al. (2013) found different values in their study.

3. Leaf area index in the first flowering period (R1) (cm^2 cm^{-2})

There was not found effect on the leaf area index of sowing time, it was 2.29 cm^2 cm^{-2} in early sowing and 1.79 cm^2 cm^{-2} in late sowing. The leaf area index is defined as the green leaf area per unit area and is closely related to the seed yield and should be in the range of 3.5-4.0 in order to achieve the light uptake of 95% required for optimum seed yield (Board and Harville, 1992).

Canopy is an important factor determining the yield potential in the lineage. There is a significant relationship between total dry matter accumulation and plant growth rate and seed yield, and it is noted that these characteristics are strongly related to the plant canopy (De Bruin and Pedersen, 2009). Our findings were similar to those of Hu (2013) and Muhammad et al. (2009) and contrary to the results of Pedersen and Lauer (2004).

The effect of tillage method on leaf area index was not observed and ranged from 1.96-2.12 cm^2/cm^2. However, Pedersen and Lauer (2004) found that leaf area index values obtained from no tillage treatment were higher than those from conventional tillage method.

It was determined that plant density are significantly effective on the leaf area index. While the highest leaf area index value was obtained from the plant density of 35 x 5 cm (2.48 cm^2/cm^2), no difference was observed between the plant density of 35 x 5 cm and 70 x 5 cm. As can be seen from this, the leaf area index value increases as the plant density increases. In soybean, grown at low plant population density, due to less light intake during the flowering period reduction of leaf area and consequently decrease in plant growth rate took place (Andrade, 1995).

4. Leaf area index in seed growth period (R5) (cm^2 cm^{-2})

The effect of sowing time on the leaf area index was significant in the seeding period and was 5.02 cm^2 cm^{-2} in early sowing and 3.75 cm^2 cm^{-2} in late sowing. Kandil et al. (2013), similar results were obtained, and the effect of sowing time on the leaf area index was found to be significant. In addition, Pedersen and Lauer (2004) found that leaf area index decreased with the delay of planting. The effect of soil treatment on leaf area index was found significant. Although there is no difference between the effects on the leaf area index and no-tillage method (4.09 cm^2 cm^{-2}) and reduced tillage method (4.35 cm^2 cm^{-2}), the higher leaf area index was obtained (4.72 cm^2 cm^{-2}) in the conventional tillage method.

Pedersen and Lauer (2004) found that leaf area index value obtained from no-tillage method was higher than conventional tillage method.

Significant differences were observed in the plant density in terms of the leaf area index. The highest leaf area index value (5.19 cm^2 cm^{-2}) was obtained from the 35 x 5 cm plant density, while no difference was observed between the 55 x 5 cm plant density and the 70 x 5 cm plant density (3.88 cm^2 cm^{-2} and 4.09 cm^2 cm^{-2}, respectively).

In the study conducted by Rahman and Hossain (2011), the plant density indicated that the residual leaf area index value was increased, and as a result, my work did not produce similar results.

5. Plant Growth Rate (g m^{-2} day^{-1})

The effect on the growth rate of sowing time was found no significant and it was obtained (as 8.18 g m^{-2} day^{-1} in early planting and 7.18 g m^{-2} day^{-1} in late planting) Muhammad et al. (2009) have reported that plant growth rate is regressed when planting date is late, and the results are similar to our findings. However, Pedersen and Lauer (2004) found that the results of our study were inconsistent with our findings, indicating that plant growth rate was higher in early sowing.

The plant growth rate varied between 7.57-8.52 g m^{-2} day^{-1} and the plant growth rate value obtained from conventional tillage method was found to be the highest (8.52 g m^{-2} day^{-1}), while the effect of soil treatment on plant growth rate was not significant.

As a result of Pedersen and Lauer (2004)'s study, we obtained different findings from our study and stated that the value of plant growth rate in the conventional tillage method is lower than the value of the plant growth rate obtained in the no-tillage method.

The effects of plant density on plant growth rate were considered negligible. Plant growth rate values ranged from 7.86 to 8.22 g $m^{-2}$$day^{-1}$. While Egli and Bruening (2000) indicated that plants at significant plant populations experienced significant decreases in plant growth rate as a result of shading of each other, Rahman and Hossain (2011) and Cox and Cherney (2008) reported that plant density increased by an increase in plant growth rate.

6. Leaf growth rate (cm^2 cm^{-2} day^{-1})

The effect of sowing time on leaf growth rate was found to be significant, according to the effect obtained on leaf growth rate. Leaf growth rate was 0.09 cm^2 cm^{-2} day^{-1} in late sowing time application and 0.13 cm^2/cm^2/day in early sowing.

The effect of soil treatment on leaf growth rate was insignificant and leaf growth rate varied between 0.10-0.12 cm^2 cm^{-2} day^{-1}. However, in Pedersen and Lauer (2004), the leaf growth rate of conventional tillage application was lower than the leaf growth rate obtained without soil treatment and they had different results from our study. The highest growth rate was found to be 35x5 plant density (0.13 cm^2 cm^{-2} day^{-1}), whereas no significant difference was observed between 55 x 5 cm and 70 x 5 cm plant density (0.10 and 0.11 cm^2 cm^{-2} day^{-1}).

7. Leaf area rate (cm^2 g^{-1})

The effect of planting time on the leaf area ratio was found significant. When early planting was 0.15 cm^2 g^{-1}, leaf area ratio decreased to 0.12 cm^2 $g^{-1}$when planting date was delayed. The effects of the tillage method on leaf area ratio were insignificant compared to the two-year average. Leaf area ratio values varied between 0.13-0.15 cm^2 g^{-1}).

The effect of plant density on leaf area ratio was found to be significant and there was no significant difference between plant density of 55 x 5 cm (0.12 cm^2/g) and 70 x 5 cm (0.13 cm^2/g) and the highest leaf area ratio was obtained from 35 x 5 cm plant density (0.16 cm^2/g).

8. Relative growth rate (g g^{-1} day^{-1})

When the relative growth rate of early planting was 0.031 g g^{-1} day^{-1} and the planting date was delayed, this ratio was 0.038 g/g/day. The effect of the soil treatment on the relative growth rate has been reached as a result. The effect of plant density on the relative growth rate was found to be significant, with an increase in the relative growth rate (0.035 g g^{-1} day^{-1}) as the plant density increased.

According to this data, the net assimilation rate is decreasing as the plant development stage progresses, and it is estimated that this decrease is due to the fact that the plants are not shaded each other due to the increase of the leaf area index (Addo-Quaye et al., 2011).

9. Net assimilation rate (g m^{-2} day^{-1})

Indeed, Watson (1958) notes that there is a very strong inverse relationship between net assimilation rate and leaf area index. The effects of sowing time on the net assimilation rate were no-significant. The net assimilation rate increased with the delay of the sowing time and the highest values were obtained from the cultivations carried out on June 5 (Kandil et al., 2013).

The effects on the net assimilation rate of the tillage methods were nosignificant compared to the two-year average and the net assimilation rate varied between 2.77-3.12 g m^{-2} day^{-1}).

The effect of plant density on net assimilation rate was found to be significant. There was no significant difference between application of 55 x 5 cm plant density (3.22 g m^{-2} day^{-1}) and 70 x 5 cm plant density (3.27 g m^{-2} day^{-1}) and net assimilation rate was higher than application of 35 x 5 cm plant density (2.27 g m^{-2} day^{-1}). Similar results were obtained from studies conducted by Carpenter and Board (1997), indicating that plants grown at low plant frequencies had a higher rate of light utilization and higher photosynthetic rate than those grown at higher plant frequencies.

CONCLUSIONS

Data collected in the average results of two study years indicate that early sowing time were found significant on Plant dry weight [(PDW (R1) and (R5)], leaf area ındex [LAI (R5)], leaf growth rate (LGR) and leaf area rate (LAR) than late sowing time. Tillage methods had significant effects on dry weight and leaf area index in the R5 phase of the tillage treatment and reduced soil tillage by soil application, lower dry weight and leaf area index (LAI) value than conventional tillage method. The highest Plant dry weight (PDW) and net assimilation rate (NAR) were obtained in the lowest plant density otherwise the highest leaf area ındex (LAI), leaf growth rate (LGR), leaf area rate (LAR) and relative growth rate (RGR) were found on 35x5 cm row spacing.

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Table 1. Analysis of variance (mean square) for Plant dry weight at the first flowering period (R1) (g plant^{-1}). The plant dry weight in the seedling period (R5) (g plant^{-1}), Leaf area index in the first flowering period (R1) (cm^2 cm^{-2}), Leaf area index in seed growth period (R5) (cm^2 cm^{-2}), Plant Growth Rate (g m^{-2} day^{-1}), Leaf area rate (cm^2 g^{-1}), Relative growth rate (g g^{-1} day^{-1}) and Net assimilation rate (g m^{-2} day^{-1}) at different tillage systems and plant density of soybean grown under main and double-cropping systems

Practices	DF	PDW (R1) (g plant^{-1})	PDW (R5) (g plant^{-1})	LAI (R1) (cm^2 cm^{-2})	LAI (R5) (cm^2 cm^{-2})	PGR (g m^{-2} day^{-1})	LGR (cm^2 cm^{-2} day^{-1})	LAR (cm^2 g^{-1})	RGR (g g^{-1} day^{-1})	NAR (g m^{-2} day^{-1})
Sowing Time (S)	1	202.81*	326.21*	6.91	43.65*	3.65	0.03*	0.02*	0.0004	8.12
Tillage (T)	2	3.81	24.13	0.58	3.57*	8.43	0.005	0.002	0.0001	1.17
Plant Spacing (PS)	2	97.70**	141.57	5.09*	17.76**	1.39	0.009**	0.02**	0.0002*	11.47*
S x T	2	30.16**	42.81**	0.58	4.09	5.67*	0.007*	0.01*	0.0002*	0.72
S x PS	2	9.65	3.30	0.07	0.06	1.23	0.0006	0.001	0.0004	0.49
T x PS	4	6.54	12.01	0.78*	1.78	4.95*	0.004	0.009*	0.00001	1.96*
S x T x PS	4	10.39*	11.73	0.49	1.93	1.89	0.003	0.009*	0.00027	1.04

PDW: Plant dry weight; R1: First flowering period; R5: Seed growth period; PGR: Plant Growth Rate; LGR: Leaf Growth Rate; LAR: Leaf Area Rate; RGR: Relative Growth Rate; NAR: Net Assimilation Rate.

Table 2. Effect of tillage and plant density on Plant dry weight at the first flowering period (R1) (g plant^{-1}). The plant dry weight in the seedling period (R5) (g plant^{-1}), Leaf area index in the first flowering period (R1) (cm^2 cm^{-2}), Leaf area index in seed growth period (R5) (cm^2 cm^{-2}), Plant Growth Rate (g m^{-2} day^{-1}), Leaf area rate (cm^2 g^{-1}), Relative growth rate (g g^{-1} day^{-1}) and Net assimilation rate (g m^{-2} day^{-1}) at different tillage systems and plant density of soybean grown under main and double-cropping systems

Treatments	PDW (R1) (g plant^{-1})	PDW (R5) (g plant^{-1})	LAI (R1) (cm^2 cm^{-2})	LAI (R5) (cm^2 cm^{-2})	PGR (g m^{-2} day^{-1})	LGR (cm^2 cm^{-2} day^{-1})	LAR (cm^2 g^{-1})	RGR (g g^{-1} day^{-1})	NAR (g m^{-2} day^{-1})
Sowing Time									
Early	16.02 A	32.38 A	2.29	5.02A	8.18	0.13 A	0.15 A	0.031	2.65
Late	13.28 B	28.91 B	1.79	3.75 B	7.81	0.09 B	0.12 B	0.038	3.19
LSD (5%)	1.47	1.96	ns	0.77	ns	0.01	0.02	ns	ns
Tillage									
No-Tillage	14.98	30.79 AB	2.12	4.09 B	7.90	0.10	0.13	0.03	3.12
Reduced Tillage	14.62	29.77 B	2.05	4.35 B	7.57	0.10	0.14	0.03	2.77
Conventional Tillage	14.34	31.38 A	1.96	4.72 A	8.52	0.12	0.15	0.03	2.87
LSD (5%)	ns	1.29	ns	0.31	ns	ns	ns	ns	ns
Plant Density									
35x5 cm	13.01 C	28.83 B	2.48 A	5.19 A	7.90	0.13 A	0.16 A	0.035 A	2.27 B
55x5 cm	14.62 B	30.35 B	1.80 B	3.88 B	7.86	0.10 B	0.12 B	0.035 AB	3.22 A
70x5 cm	16.31 A	32.76 A	1.81 B	4.09 B	8.22	0.11 B	0.13 B	0.030 B	3.27 A
LSD (5%)	1.17	1.80	0.35	0.44	ns	0.01	0.01	0.003	0.55
Average	14.64	30.64	2.04	4.38	7.99	0.13	0.13	0.03	2.92

Columns marked with different letters are significantly different at $P \leq 0.05$; ns: no-significant; PDW: Plant Dry Weight; R1: First Flowering Period; R5: Seed Growth Period; PGR: Plant Growth Rate; LGR: Leaf Growth Rate; LAR: Leaf Area Rate; RGR: Relative Growth Rate; NAR: Net Assimilation Rate

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THE CHANGE OF SHEAR FORCE AND ENERGY OF COTTON STALK DEPEND ON KNIFE TYPE AND SHEAR ANGLE

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Abstract

The shear force and energy values of biological materials are very important data for suitable design of a cutting and pruning machines and related equipment. The objective of this study was to determine shearing force and shearing energy of cotton (Gossypium hirsutum L.) stalk at different shoots diameter as a function of knife type and knife edge angle. Dependent variables were maximum cutting force and cutting energy. The samples were obtained from the cotton experimental field at vegetation season for each plot. A universal test machine was used to measure the cutting force and the energy. The cutting energy was calculated by measuring the surface area under the cutting force-deformation curve. As a result, the main effect of the knife edge angle on the cutting force and energy were found significant. The best and minimal results were determined at serrated 2 knife types to be 69.61 N and 25.61 N cm, respectively, followed by the serrated 1 and flat knife. The highest values were observed at flat knife type. Nevertheless, the cutting force and cutting energy increased with an increase in the knife edge angle from 50° to 90°. The maximum values were obtained at 90° both cutting force and cutting energy. At this angle, while the maximum cutting force and cutting energy were determined to be 93.18 N and 31.60 N cm, respectively. The main minimum values were obtained at 50° angle. Cutting force and energy values of cotton stalk were found highly correlated with the stalk diameter. Cutting force and energy increased with increase diameter of stalk. The maximum cutting force and cutting energy were obtained at 29.20 mm^2 cross-sectional area as 102.30 N and 41.97 Ncm, respectively, while the minimum values of cutting force and cutting energy were obtained at 13.84 mm^2 cross-sectional area as 47.28 N and 16.76 N cm, respectively.

***Key words**: cotton, stalk, shearing force, cutting properties, design.*

INTRODUCTION

Cotton is a major raw material for textile sector in worldwide and produced several countries. One of the important countries in terms of the magnitude of total cotton production is Turkeys and it is Europe's largest textile manufacturer and ranks seventh in the world cotton production. In Turkey, Cotton is cultivated primarily in the Aegean Region, Çukurova Basin and Southeast Anatolia Region. With GAP (Southeastern Anatolian Project) irrigation project in Turkey, the irrigated farmland and cotton production in Southeast Anatolia region has developed rapidly since 2000 year. That is, cotton production area was shift Aegean and Çukurova region to Southeastern Anatolia region and two decades and nowadays, more than half of the national cotton production is produces in Southeastern Anatolia region (Sessiz et al. 2009). The increase in cotton production has increased provided the development of the cotton industry (Sessiz, Esgici, 2015a). Therefore, this has a strategic importance for the region. GAP covered in Diyarbakır, Şanlıurfa, Mardin and Batman as agricultural areas with more as well as the producers of the region opportunities for irrigation on the GAP in the provinces of through boreholes have opened their own facilities, irrigated farmland has increased significantly. With water, a significant increase in the area of cotton production has occurred. This increase, today more than half of Turkey's cotton production is covered by the South-Eastern Anatolia. GAP region produces 61.5 % of Turkey's total cotton production area in the 2015 (TUIK, 2015). This has led to the development of industries based on cotton in the region. This production ratio in region is important for region's development, human resources development and rural development. Therefore, increasing cotton production and yield, reducing of cotton losses and protection

of fiber quality are very important for sustainability of the production in Diyarbakır province.
Cotton production requires large-scale mechanization, from the operation of soil tillage to harvesting stage. In addition to conventional operations, cotton topping is another cultural practice that should be done during the vegetation period (Aydin, Arslan, 2018). Therefore, the production cost is quite high depending on cultural application during the production season. Especially, the cotton production costs are considerably high in harvesting and cutting of plant topping section. It has been reported by cotton producers that the cutting of cotton plant topping operation is directly related to the yield and reduces bollworm infestations without negatively affecting cotton yields. The similar results were reported by Obasi and Msaakpa (2005), Yang et al. (2008), Renou et al. (2011) and Aydin and Arslan (2018). According to these researchers, the cutting of top section of cotton plants increases the yield and quality of cotton and improves the earliness, limiting the vegetative growth of the plant and improving the development of generative organs of the cotton plants. To reduce of input operation costs, we need use of mechanical equipment.
To design a new harvester, the first step is to measure the cutting force and cutting energy. Several studies have been carried out to determine these parameters for cotton stems considering various cutting knife edge angles. In general, the cutting knife of a harvesting machine cuts the plant material and separates it into different parts by external force. Knife edge angle, knife approach angle, shear angle, and knife rake angle are the most important knife angles that can directly influence the cutting force and energy (Ghahraei et al., 2011). Why we conducted this study, because the cotton is one of important product for both our country and several countries in the world. However, cotton topping is done by worker during the vegetation period. To reduce production cost and increase yield, we have to use suitable cutting machine. So, firstly, we must determine cutting properties of cotton stalk.
The objectives of this study were to determine the optimum the knife type and knife edge angles that use the lowest cutting force and energy to be used in the design and to fabricate of the cotton stalk cutting machine and then the test it in the field conditions. For this purpose, cutting tests were carried out at 5 cm intervals from the top of the plant and cutting force and cutting energy were determined during the vegetation period.

MATERIALS AND METHODS

The field experiment was conducted at a commercial farm in 2017 during the growing season at the Bismil district, Diyarbakır province (latitude 37°53′N and longitude 40°16′E, 680 m altitude), Southeast part of Turkey, where cotton production is intensively done. The BA-440 cotton cultivar was planted on 12 April in 2017 by a pneumatic planter. A randomized complete block design with five replications was used in this study. Experimental field consisted of 18 plots with each measuring 15 m x 5 m with an inter row spacing of 0.7 m distance. The same agricultural practices were applied in all the plots during the growing season. The some mean values of these measurements are given in Table 1.

Table 1. The some properties of cotton plant

Properties	Mean values
Plant height, cm	92.00
Boll number on the plant, number	16.70
Number of branch on one the plant	14.00
Average yield (kg/da)	596.80

To determine the cutting tests of cotton stalks, the cotton plants were, randomly selected five different locations on the experimental field, uprooted in December 2017 from soil surface and then transported to the laboratory for cutting tests. Then, the leaves were removed from the plants in the laboratory (Figure 1). Prior to the tests, the stalks were cutted into four different groups according to diameters (Figure 1) and heights, namely at 0-10 cm (first internode, IN1), 11-15 cm (second internode, IN2), 16-20 cm (third internode, IN3) and 21-20 cm (fourth internode, IN4) from the top of cotton plants toward bottom. The cutting properties include the cutting force and cutting energy was determined along the stem from first internode to fourth internode.

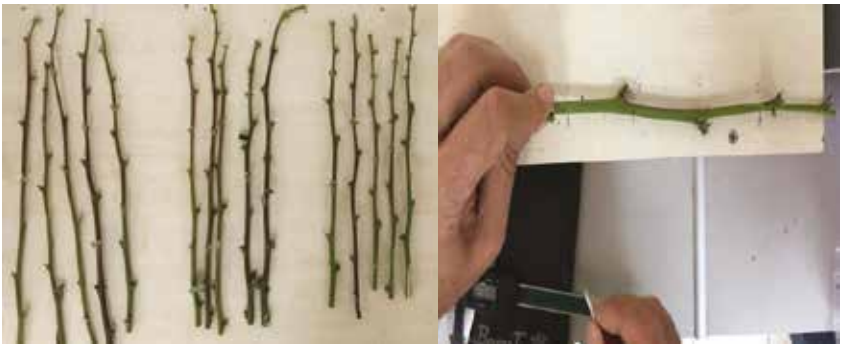

Figure 1. The cotton stalk and samples

The diameter of the cotton stalk (stem) decreases from the roots toward the shoots at the top of plants. Therefore, the average diameter of the stalk has changed between 4.00 mm-8.00 mm. According to height, the average diameters were considered as 4.2, 4.8, 5.4 and 6.10 mm in this study. The ranges of diameter of the stem were converted to cross-section area in mm^2 (13.84, 18.06, 22.89 and 29.20 mm^2). The stem diameters were measured before the test using a caliper with five replications at about the cutting point near the node section of an each test specimen. Testing was completed as rapidly as possible in order to reduce the effects of drying. The moisture content of each sample was determined according to ASABE standard method (ASABE Standards, 2008) by oven-drying 50 g of each sample at 105°C for 24 h before the cutting tests. The average moisture content was determined as 50.20% during the cutting tests.

The Universal Testing Machine (Lloyd LRX Plus) was used to determine the cutting force and the cutting energy of cotton stalks (Figure 2). Cutting experiments were carried out with three various knife types (Figure 2), two of them are serrated type, Serrated 1 (knife-edge thick), Serrated 2 (knife-edge thin), and Flat (knife-edge flat) with five knife edge angles (50°, 60°, 70°, 80° and 90°) and four different stalk diameters (4.20 mm, 4.8 mm, 5.40 mm and 6.10 mm). To determine the cutting force, cutting energy, and displacement, the knife was held and fixed to the crosshead of an universal testing machine (Figure 2). The maximum cutting speed of the machine, 3 mm s^{-1}, was used for all tests.

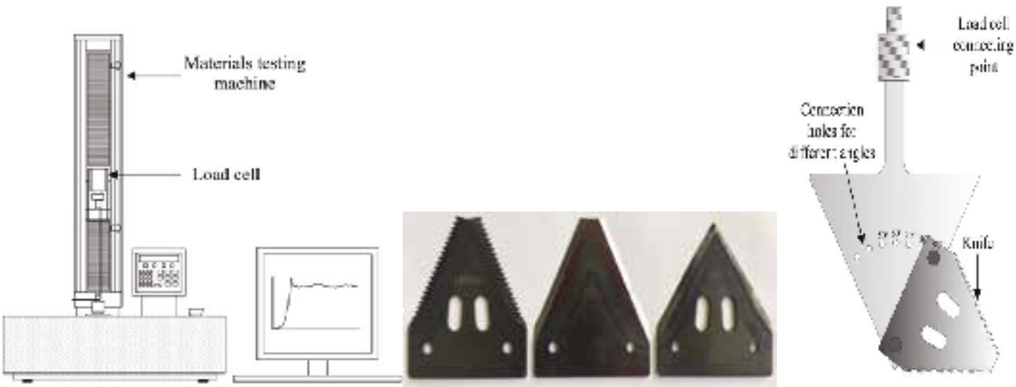

Figure 2. Materials testing machine and different cutting knives with edge angles.

The cutting force and cutting energy were determined as the maximum force and the maximum energy depend on type of knife, angle of knife-edge and stalk diameters. The cutting energy was calculated by measuring the surface area under the cutting force-deformation curve (Mohsenin., 1986; Persson, 1987; Yore et al., 2002; Kocabiyik, Kayisoglu, 2004; Chen et al., 2004; Taghijarah et al.,2011; Ghahraei et al., 2011; Sessiz et al., 2013; Sessiz et al., 2015b; Ozdemir et al., 2015; Nowakowski, 2016; Öztürk et al., 2017) with the force and displacement data by using a NEXYGEN computer program. A computer data acquisition system recorded all the force-displacement curves during the cutting process by testing machine. A typical force-deformation is given in Figure 3. As you seen in Figure 3, the first peak corresponds to the yield point (offset yield) at which stalk damage was initiated. The second peak (upper yield) corresponds to maximum force (Figure 3).

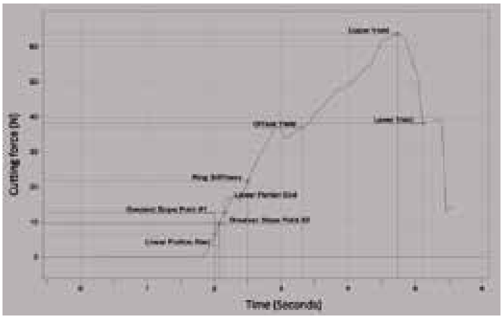

Figure 3. Typical force-deformation curve for cutting of cotton stalk

An analysis of variance (ANOVA) of the three factor randomized complete block design with five replications was performed to detect significant differences in the observations due to the effect of knife type, knife edge angle and stalk diameter of each factor using the MSTATC software. Means were compared at the 1% level of significance using Duncan's multiple range tests to identify the specific differences among treatments means.

RESULTS AND DISCUSSIONS

The effect of Knife type

The effect of knife type on cutting force and cutting energy are given in Table 2. Duncan test showed that there were significant differences among the three knife types ($p<0.01$) on cutting force and cutting energy. The results of the analysis of variance and Duncan's test also showed that the main effect of knife type, blade angle and cross-section area (stem diameter), and their interactions were found significant ($p<0.01$). The lowest and best results were determined at serrated 2 knife type as 69.61 N and 25.61 N cm, respectively, followed by the serrated 1 and flat knife. The highest cutting force values were observed at flat knife type as 78.31 N. However, there were not found significant differences between serrated 1 knife type and flat knife according to cutting energy. According these results, flat-edge knife type is not suitable, when compared to serrated knife types and we can recommend that the serrated type knife for a new design of cutting machine and pruning for cotton shoots topping.

Table 2. Analysis of variance of the cutting force and cutting energy with respect to knife type*

Knife type	Cutting force (N)	Energy (N cm)
Serrated type 1 (knife-edge thick)	73.30[b]	29.03[a]
Serrated type 2 (knife-edge thin)	69.61[c]	25.61[b]
Flat type (knife-edge flat)	78.31[a]	29.51[a]
Mean	73.74	28.05
LSD	2.326	1.485

*Means followed by the same letter in each column are not significantly different by Duncan's multiple range tests at the 1% level.

The Effect of Knife Edge Angle

The results of the analysis of variance of the cutting force and energy depending on different knife edge angle are shown in table 3. The main effect of the knife edge angle on the cutting force and energy were found significant. Moreover, reducing the knife edge angle led to a decrease in the cutting force and cutting energy (Ghahraei et al., 2011). The cutting force and cutting energy increased with an increase in the knife edge angle from 50° to 90°. Table 3 shows the results of the comparison among means of the cutting force and cutting energy. Also, according to results of variance analysis, the effect of interactions were found significant ($p<0.01$) on cutting force and cutting energy. There were found significant differences among from 50° to 70°. However, there was no significant difference among means for 70°, 80°, and 90° at the probability level of 1% and 5%. Nevertheless, cutting force and cutting energy gradually increased with increase knife-edge angle. A maximum cutting force and cutting energy of 93.8 N and 31.60 N cm were obtained with 90° edge angles, respectively. The main values were obtained at 50° angle as 59.38 N and 26.32 N cm, respectively. The similar results were observed by Kronbergs et al. (2011), they were noticed that the significant material deformation for the cutting knives with bevel angle 90º during the experiments. They argued that cutting energy depends on the material deformation process and friction forces. This situation is causes significant cutting energy increasing. According they results, the suitable knives bevel angle is change between 25° and 45°.The decrease of cutting force and cutting energy depend on knife edge angle allows proper design of the cutting unit and cutting machine for cotton stalk of top section and predicting the power requirements (Nowakowski, 2016; Ozdemir et al., 2015; Esgici et al., 2017). Prasad and Gupta (1975) reported that the optimum knife bevel angle value for cutting of corn stalk was 23°. According to Suryanto et al. (2009), the knife edge angle has an significant effect on the cutting force and energy. Dowgiallo (2005) also reported that besides the cutting edge, knife edge sharpness and knife speed are effect on cutting properties. Based on our results, a preferred knife edge angle of 50° for cutting the top of cotton stalk is recommended. We will consider these results for a new design and construct a prototype cutting machine for cotton stalk of topping in future.

Table 3. Analysis of variance of the cutting force and cutting energy with respect to knife edge angle*

Knife cutting angle (°)	Cutting force (N)	Energy (N cm)
90	93.18[a]	31.60[a]
80	78.05[b]	28.65[b]
70	72.78[c]	26.72[c]
60	65.30[d]	26.92[c]
50	59.38[e]	26.32[c]
Mean	73.73	28.04
LSD	3.003	1.329

*Means followed by the same letter in each column are not significantly different by Duncan's multiple range tests at the 1% level.

The relationship between the cross-sectional area with the cutting force and cutting energy are shown in table 4. The results of variance analysis showed that the independent parameters and their interactions has significant effect on cutting force and cutting energy. The significant differences were found between all of cross-sectional areas at a 1% probability level. There is high correlation between cutting energy and diameter. The cutting force and cutting energy increased with increase cross-sectional area. The maximum cutting force and cutting energy were obtained at 29.20 mm^2 cross-sectional area as 102.30 N and 41.97 N cm, respectively, while the minimum values of cutting force and cutting energy were obtained at 13.84 mm^2 cross-sectional area as 47.28 N and 16.76 N cm, respectively (Table 4). This information is very valuable for selecting a suitable equipment design for reduces energy requirement and consumption. Because, the selection of suitable cutting apparatuses and equipment are plays an important role in economizing on cutting force and energy requirement (Sessiz et al., 2013). This effect is in agreement with a previous study on maize stalks, in which both the cutting energy and maximum cutting force were directly proportional to cross-sectional area (Prasad, Gupta, 1975; Sessiz, 2005). The effect of stem diameter on the maximum cutting force and cutting energy is consistent with Chen et al. (2004), who reported that both the cutting energy and maximum cutting force are directly proportional to the cross-sectional area of hemp stalk. The results have shown that the cutting strength and cutting energy related to plant physical and mechanical properties (Igathinathane et al., 2010). Similar results were reported by Yore et al. (2002) for rice straw, by Kronsberg et al. (2011) for hemp stalk, by Alizadeh et al. (2011) for rice stem, and by Ghahraei et al. (2011) for kenaf stems, by Sessiz et al. (2013) for olive sucker, by Ozdemir et al. (2015) for grape sucker, by Sessiz et al. (2015b) for cane of some different grape variety, Öztürk et al. (2017) for soybean stem. These results also are in agreement with Aydin and Arslan (2018) who determined shearing force and energy for shoot of cotton plant at different height of plant. Proper equipment design to accomplish the cutting will maintain the quality of the harvested product while minimizing the force and energy needed to accomplish the task (Srivastava et al., 2006). Based on the results from this study, for cutting of cotton stalk, lower cotton shoots diameter, lower knife edge angle and serrated knife type can be recommended to minimize the shearing force and shearing energy requirements.

Table 4. The relationship between cutting properties and cross-sectional area of cotton stalk*

Cross-sectional area (mm^2)	Cutting force (N)	Energy (N cm)
13.84	47.28[d]	16.76[d]
18.06	64.69[c]	23.15[c]
22.89	80.73[b]	30.32[b]
29.20	102.30[a]	41.97[a]
Mean	73.75	28.05
LSD	5.201	1.329

*Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at the 1% level.

CONCLUSIONS

The results of the analysis of variance and Duncan's tests also showed that the main effect of knife type, blade angle and cross-section area (stem diameter), and their interactions were found significant on cutting force and cutting energy ($p<0.01$). The lowest and best results were determined at serrated 2 knife type as 69.61 N and 25.61 N cm, respectively, followed by the serrated 1 and flat knife. The highest values were observed at flat knife type. The cutting force and cutting energy increased with an increase in the knife edge angle from 50° to 90°. A maximum cutting force and cutting energy of 93.8 N and 31.60 N cm were obtained with 90° edge angles, respectively. The maximum cutting force and cutting energy were obtained at 29.20 mm^2 cross-sectional area as 102.30 N and 41.97 N cm, respectively, while the minimum values of cutting force and cutting energy were obtained at 13.84 mm^2 cross-sectional area as 47.28 N and 16.76 N cm, respectively. This information is very valuable for selecting a suitable equipment design for reduces energy requirement and consumption. Based on our results, a preferred knife edge angle of 50° for cutting the top of cotton stalk is recommended. We will consider these results for a new design and construct a prototype cutting machine for cotton stalk of topping.

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COMPARATIVE STUDY REGARDING THE INFLUENCE OF HERBICIDES ON THE YIELD OF SUNFLOWER CROPS, THE CROPS BEING OBTAINED WITH CONVENTIONAL, CLEARFIELD AND EXPRES SUN TECHNOLOGIES IN THE FIELD CONDITIONS OF MOARA DOMNEASCA

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Abstract

The sunflower is the most important cultivated plant in Romania for production of addible oil by seeds processing. The cultivated areas have increased a lot recently, ranging between 0.7-1 milions hectares. With the increase of the cultivated areas in the past years, the structure of the hybrids has changed very much. The hybrids cultivated by CONVENTIONAL technology decreased at the expense of hybrids resistant to Imazamox herbicide, type IMI, the cultivation technology being named CLEARFIELD and resistant to herbicide Tribenuron-methyl, the cultivation technology being named EXPRESSUN, named by the commercial brands of the herbicides. We may say with no fear of making mistakes that the cultivated area with conventional hybrids is very small, compared to the areas cultivated with the two hybrids resistant to the two herbicides mentioned. This situation leads to the fact that the weeds and their control became one of the most difficult to manage problems in sunflower culture. This is the reason for which we have initiated three years ago a study at the Experimental Didactic Centre from Moara Domneasca for the period 2015-2017, in order to reveal whether there are any differences among the three types of cultures as regards the obtained yields, what is the effect on weed control level and specially if the application of these herbicides in different periods has any influence on post emerging crops (remaining effect) in crop rotation process.

***Key words**: hybrids resistant to herbicide, Clearfield, Express Sun, Conventional, remaining effect of herbicides.*

INTRODUCTION

One of the most important technological links in sunflower culture is weed control (Şarpe, 1987; Budoi, Penescu, 1996; Popescu et al., 1990; Penescu, Ciontu, 2001; Alonso et al., 1998; Brighenti et al., 2012). The emergence of sunflower hybrids resistant to certain types of herbicides has made this problem of weed control very easy for farmers. The unprecedented expansion of parasitic weeds on the *Orobanche cumana* (broomsrape) root made the surface cultivated sunflower hybrids obtained through the conventional method (without *Orobanche* resistance) to be much more reduced. The links known to be essential in limiting the growth of this weed, such as rotation and crop rotation, were reduced from 6 to 4 years, and in the case of new hybrid cultures resistant to this parasitic weed, even less than 4 years, sometimes 2-3 years. A large number of researchers have studied the efficacy of a large number of simple or combined herbicides in combating weeds in classical sunflower culture technology, giving the farmers at that time the opportunity to have a clean, productive and economical culture (Şarpe et al., 1983; Popescu et al., 1990; Ribeiro, Raiher, 2013; Ciontu et al., 2003). The level of the contamination with weeds, but especially its structure, the large number of weed generations per year, have determined the breeders to obtain hybrids resistant to certain types of herbicides. In our research we aim at studying the influx of three sunflower culture systems, Conventional, Clearfield and Express

Sun, respectively, on the level of weed infestation, its structure, production and the effect on post-emergent crops.

MATERIALS AND METHODS

The experience was carried out in the Experimental Field of the Faculty of Agriculture at Moara Domneasca between 2015 and 2017, on a preluvosoil type of soil with a pH of 6.5, humus content of 2.5, clay content of 35%.

The following hybrids have been studied:

- in the *Conventional* technology, the hybrid *PERFORMER*, created at INCDA Fundulea;
- in the technology *Clearfield*, the *NEOMA* hybrid from Syngenta Company;
- in the technology *Express Sun*, the hybrid *PR64LE19* from Pioneer Company.

The method of setting the experience was that of the subdivided plots. The surface of the experimental plot was 25 m^2 (3.7 x 7.8 m), placed in 4 randomized repetitions.

The herbicides used in the three combat systems were applied at two stages:

- *Optimal stage* 4-6 leaves of the sunflower plants;
- *Late stage* 8-10 leaves of the sunflower plants.

In the *Conventional* technology, the herbicide Modown 4F (bifenox) was used in a dose of 2.0 l/ha and 2.0 + 2.0 l/ha.

The herbicides applied were those used in Romania respectively:

- In the *Clearfield* technology, the Pulsar 40 SG (imazamox) herbicide was used at a rate of 1.2 l/ha and 1.2+1.2 l/ha;
- In the *Express Sun* technology, the Express 50 WG (tribenuron-methyl) herbicide at the dose of 0.030 kg/ha and 0.030 + 0.030 kg/ha, respectively.

To combat the annual monocotyledonous weeds, the Dual Gold 960 EC (s-metholachlor) herbicide was used in all three technological variants at a rate of 1.5 l/ha applied pre-emergence, immediately after sowing. During the vegetation period (post-emergence), for the control of *Sorghum halepense* from rhyzoms, the product Killer Super 5 EC (quisalofop-p-ethyl) was used in the dose of 1.75-2.0 l/ha.

The following indicators were determined prior to harvesting:

- The number of weeds/m^2, their dry weight/m^2 and their structure by species;
- Plant density (plants/m^2);
- Sunflower heads diameter (cm);
- Sunflower head weight (g);
- Grain weight on sunflower head (g);
- TWK (g);
- MH (kg)- hectolitric masse;
- Yield (kg/ha);
- The residual effect of all herbicides on crops from the crop rotation.

In order to establish the statistical significance, the variance analysis method was used.

RESULTS AND DISCUSSIONS

Climate conditions during the research period

The climatic conditions (temperature and precipitation) were approximately normal in the three years of research (2015-2017), as can be seen in Figures 1 and 2, where we present the average of these values.

From the analysis of the climatic conditions, temperature and rainfalls, we can say that all three years of research have been almost normal climatic years, which has led to both weeds and sunflower culture to developed normally.

The deviations from the multi-annual norm of the place were more obvious in the case of the temperatures in August and September + 4.8°C, respectively 4.0°C, and in the case of rainfalls in July and August when a deficit of -44.6 mm was achieved, respectively 14.7 mm.

In these months, an excessive drought happens, but in case of rainfalls we had in March and April positive deviations of +94 mm, 94 mm respectively, above the multi-annual average of the place (Figures 1 and 2).

Weed control

The weed control, presented in Table 1, in the three years of research highlights the following:

- the dominant weed species were the annual dicotyledonous *Xanthium strumarium*: 10.0 pl./m^2, *Hibiscus trionum*: 5.0 pl./m^2, *Chenopodium album*: 4.0 pl./m^2, but the perennial ones also had a great presence: *Cirsium arvense* 4.0 pl./ m^2, *Convolvulus arvensis* 4.0 pl./ m^2 and *Sonchus arvense* 2.0 pl./m^2. We can say that the level of weed contamination was very high;

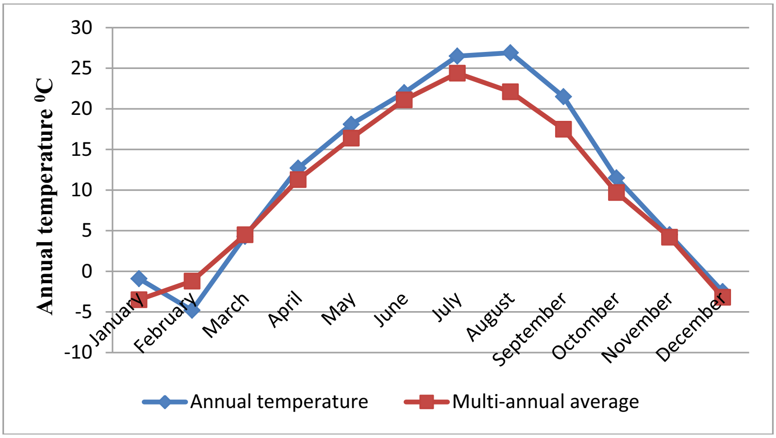


Figure 1. The evolution of average temperatures over the three-year study, compared to the multi-annual average from Moara Domnească (2015-2017 average)

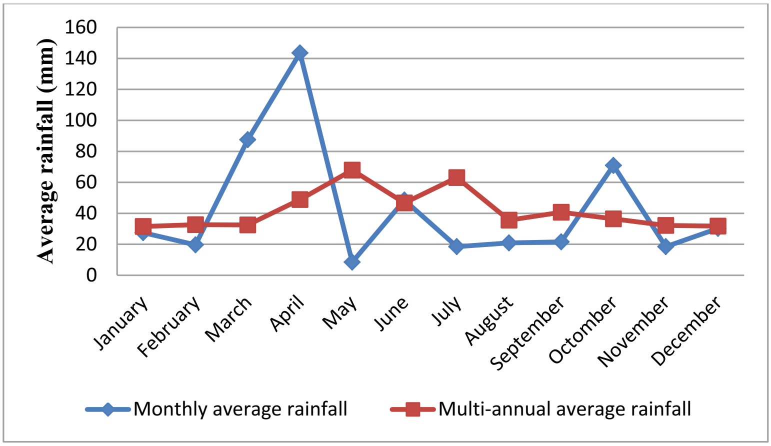


Figure 2. Evolution of the rainfalls, on average over the three years of study, compared to the multi-annual average from Moara Domnească (2015-2017 average)

- the best results in weed control were obtained in *Clearfield* and *Express Sun* technologies, approaching the values of Witness II (Standard II), in which three mechanical cultivations and three manual cultivations and untreated with herbicides were applied (Table 1). Poor weed control values were obtained in the conventional technology, ie the herbicide applied, Modown 4F (bifenox), failed to combat the entire weed spectrum experienced in the three years of experimentation due to the reduced control spectrum (Table 1).

The best values were obtained as follows: The Clearfield system of weed control in the Ist stage distinctly significantly positive, respectively very significantly positive at the dose of imazamox of 2.0 + 2.0 l/ha. And in the Express Sun System, distinctly significantly positive in variant with tribenuron-methyl 0.030 kg/ha, respectively very distinctly significant in the two applications version (0.030 + 0.030 kg/ha). Poor results were obtained in the Conventional version with the bifenox herbicide, the control results being close to the value of Witness I (untreated, unsown) with distinctly significantly negative values in both variants (Table 1). This aspect happened due to the reduced spectrum of Modown (bifenox), both in the variant with one treatment as well as in the one where two treatments were used repeatedly in both application stages.

Influence of the technological systems used on the productivity indicators

The results obtained during the three years of experimentation on the influence of the three weed control systems on the sunflower productivity elements are presented in Table 2 and highlight the following aspects:

- Very low values were obtained in all the indicators in the variants of Witness I (untreated with herbicides and unsown mechanically or manually), as well as in the variants where the Conventional system was used, the sunflower heads remained small (11.5 cm) for untreated Witness I, and in the variant where the Conventional technology was used, the diameter of the heads remained rather small 15.6 cm, compared to the value of the sown Witness II (24.1 cm);
- This is due to the large number of weeds that developed at free will, even if they were treated with bifenox 2.0 l/ha, resulting in small values of the diameter of the head, the weight of the grains on the head, the weight of the head and MMB (g). In these variants, the heads were small, with small seeds, many of them dried-up;
- Positive values of the productivity indicators obtained in the variants where Clearfield technology was used in which the diameter of the head exceeded 26 cm when applied only once and 27.2 cm when using 2 successive treatments;

Table 1. The weight of dry weeds (kg/ha) obtained before harvesting in the three experimental systems: *Conventional, Clearfield and Express Sun* (Average: 2015-2017), Moara Domeasca

System used	Experimental variants	Dose l,kg/ha	Weed quantity			
			Kg/ha	(%)	Difference kg/ha	Semnification
Stage I (4-6 leaves)						
Conventional	Untreated, unsown Witness	-	4800	Mt.I	4330	000
	Untreated Witness, mechanically and manually sown (3+3)	-	470	Mt.II	Mt.II	Mt.
	Bifenox 4 F	2.0	1200	255	730	000
	Bifenox 4 F + Bifenox 4 F	2.0 + 2.0	850	180	380	0
Clearfield	Untreated, unsown Witness	-	4912	Mt.I	4516	000
	Untreated, sown Witness (3+3)	-	701	Mt.II	Mt.II	Mt
	Imazamox	1.2	220	169	360	**
	Imazamox + Imazamox	1.2 + 1.2	170	176	531	***
Express Sun	Untreated, unsown Witness	-	4790	Mt.I	4465	000
	Untreated, sown Witness (3+3)	-	695	Mt.II	Mt.II	Mt.
	Tribenuron- methyl	0.030	216	169	479	**
	Tribenuron-methyl+ Tribenuron-methyl	0.030+0.030	104	186	591	***
Stage II (8-10 leaves)						
Conventional	Untreated, unsown Witness	-	4910	-	4456	000
	Untreated, sown Witness (3+3)	-	684	Mt.II	Mt.II	Mt
	Bifenox 4 F	2.0	1475	215	791	000
	Bifenox 4 F + Bifenox 4 F	2.0 + 2.0	1205	176	521	00
Clearfield	Untreated, unsown Witness	-	4875	-	4006	000
	Untreated, sown Witness (3+3)	-	876	Mt.II	Mt.II	MtII
	Imazamox	1.2	470	153	-406	*
	Imazamox + Imazamox	1.2 + 1.2	370	142	-506	**
Express Sun	Untreated, unsown Witness	-	4796	-	4398	000
	Untreated, sown Witness (3+3)	-	789	Mt.II	Mt.II	Mt.II
	Tribenuron - methyl	0.030	420	153	369	*
	Tribenuron - methyl+ Tribenuron - methyl	0.030+0.030	378	163	411	*

Dl 5% = 350 kg/ha
Dl 1% = 475 kg/ha
Dl 0.1% = 530 kg/ha

Table 2. Influence of the type of system used to control weeds on the productivity indicators of cultivated sunflower hybrids (Average: 2015-2017), Moara Domneasca

The system used	Experimental variants	Dose l,kg/ha	Density of plants /m^2	Diameter of calatidius (cm)	Weight of calatidius (g)	Grain weight / calatidius (g)	TWK (g)	MH (kg/Hl)
Stage I (4-6 leaves)								
Conventional	Untreated, unsown Witness	Mt.I*	5.3	11.5	82.4	28	27.1	28
	Untreated, sown Witness (3+3)	Mt.II**	5.4	24.6	146.2	45.6	43.0	38
	Bifenox 4 F	2.0	5.6	14.3	143.5	44.1	48.1	40
	Bifenox 4 F + Bifenox 4 F	2.0 + 2.0	5.5	15.6	144.2	44.1	48.2	45
Clearfield	Untreated, unsown Witness	Mt.I	5.5	10.2	86.5	26.0	30.2	30
	Untreated, sown Witness (3+3)	MtII	5.7	24.5	147.5	49.1	56.1	43
	Imazamox	1.2	5.8	26.0	151.2	49.7	55.1	45
	Imazamox + Imazamox	1.2 + 1.2	5.6	27.2	152.4	50.1	43.0	44
Express Sun	Untreated, unsown Witness	Mt.I	5.5	11.2	75.4	25.7	30.1	29
	Untreated, sown Witness (3+3)	Mt.II	5.5	25.1	134.5	49.8	42.5	44
	Tribenuron-methyl	0.030	5.6	26.5	147.5	50.6	54.2	45
	Tribenuron-methyl+ Tribenuron-methyl	0.030+0.030	5.7	26.1	151.2	50.8	57.1	43
Stage II (8-10 leaves)								
Conventional	Untreated, unsown Witness	-	5.4	10.5	74.2	24.1	28.5	24
	Untreated, sown Witness (3+3)	-	5.3	24.7	136.5	48.5	43.2	32
	Bifenox 4 F	2.0	5.4	17.5	128.4	36.2	40.2	35
	Bifenox 4 F + Bifenox 4 F	2.0 + 2.0	5.6	16.4	130.5	39.5	40.1	37
Clearfield	Untreated, unsown Witness	-	5.3	10.4	76.2	24.8	27.9	25
	Untreated, sown Witness (3+3)	-	5.8	25.4	128.5	41.5	43.2	34
	Imazamox	1.2	5.6	26.7	130.4	40.2	40.7	38
	Imazamox + Imazamox	1.2 + 1.2	5.4	28.3	132.5	46.3	41.5	37
Express Sun	Untreated, unsown Witness	-	5.7	10.6	69.5	23.7	29.1	28
	Untreated, sown Witness (3+3)	-	6.0	24.9	127.4	40.5	40.3	34
	Tribenuron-methyl	0.030	5.7	25.9	131.2	40.2	42.7	35
	Tribenuron-methyl+ Tribenuron-methyl	0.030+0.030	5.6	25.4	130.4	39.6	41.9	35

*Mt.I= Witness untreated with herbicides and unsown mechanically or manually (or Standard I);
**Mt.II= Witness untreated with herbicides, mechanically (3) and manually sown (3) (or Standard II).

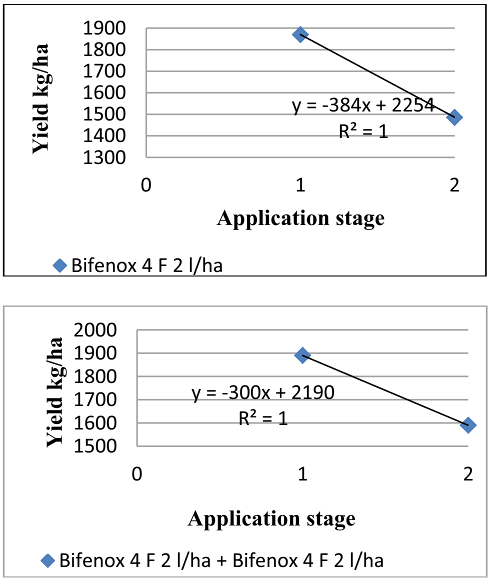
- Good results were also obtained in variants using *Express Sun* technology, values that exceeded the values of the diameter of calatidius in the case of Witness II mechanically and manually sown (3+3), values over 25.0 cm when the Express 50 WG herbicide (tribenuron-methyl) was applied only once and 26.5 cm when two successive treatments were applied. This is due to the almost complete destruction of annual and perennial dicotyledonous weeds, due to the systemic effect of these products, whereas in the case of the manually and mechanically sown standard, the perennial weeds (*Cirsium*, *Sorghum*, *Convolvulus*, *Polygonum*) have been sprouting until the harvest;
- The best values of the other productivity indicators (calatidius weight, grains weight, MMB) were obtained when the herbicides in *Clearfield and Express Sun* technologies were applied in Stage I (optimal stage). The more their application is delayed, the more the values of the productivity indicators worsen. In other words, in the case of sunflower culture it is recommended to use these products when the weeds are mostly grown and do not exceed 4-6 leaves or the height of 4-6 cm, and the sunflower plants are in the 4-6 leaves. The more the

application of herbicides is delayed, the values of the indicators change negatively due to the competitive effect of the weeds with the sunflower plants for the vegetation factors: water, light, nutrients.

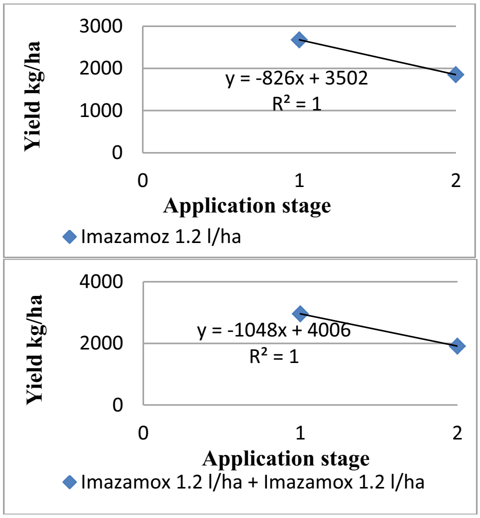
Influence of technological systems used to control weeds on sunflower production

The values of the productions obtained in the three technological systems used for weed control are presented in Table 3 and highlight the fact that the best results were achieved by the *Clearfield* and *Express Sun* technological systems when the herbicides were applied in the first stage (4-6 sunflower leaves). In Clearfield technology at 1.2 l/ha Pulsar 4 SC (imazamox) dose, the production was 2676 kg/ha, and when 2 treatments were applied, the production was 2958 kg/ha.

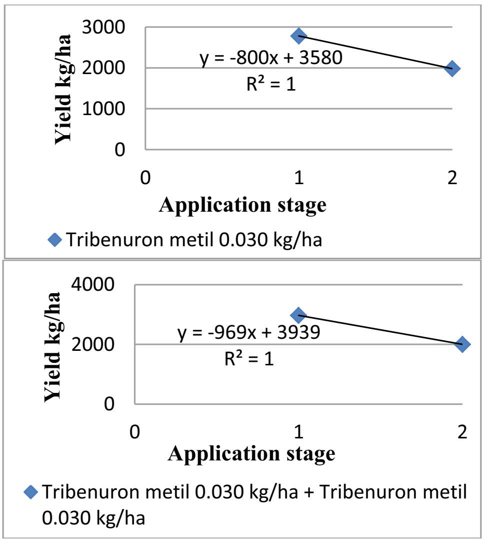
The late application of the herbicide and its application at the 8-10 leaves stage of the sunflower Stage II, 8-10 leaves, the production was reduced to 76% of the untreated Witness II value. In the variant with Conventional technology, it increased to 104% with *Clearfield* technology and 109% in the *Express Sun* technology variant, compared to the value of Witness II, manually and mechanically sown (3+3).



Figures 3, 4. The regression function for bifenox



Figures 5, 6. The regression function for imazamox



Figures 7, 8. The regression function for tribenuron-methyl

Unsatisfactory results were obtained in *Conventional Technology* when using the Modown 4 SC (bifenox) herbicide, which due to the reduced spectrum of control, made the production to remain way below the value of Witness II, sown and far from the productions obtained in the *Clearfield and Express Sun* technological variants. The regression functions (Figures 3, 4, 5, 6, 7, 8) between weed control technology and the application stage clearly shows that the best productions are obtained when these herbicides are applied in the Stage I (4-6 leaves of sunflower plants and 4-6 cm the height of the weeds).

Table 3. Influence of the type of system used to control weeds on sunflower yield (Average: 2015-2017), Moara Domneasca

System used	Experimental variants	Dose l,kg/ha	Yield			
			Kg/ha	%	Difference kg/ha	Semnifica -tion
Stage I (4-6 leaves)						
Conventional	Untreated, unsown Witness	-	1150	46	- 1325	000
	Untreated, sown Witness (3+3)	-	2475	100	Mt.II	Mt.II
	Bifenox 4 F	2.0	1870	75	- 605	000
	Bifenox 4 F + Bifenox 4 F	2.0 + 2.0	1890	76	- 585	000
Clearfield	Untreated, unsown Witness	-	1175	45	- 1395	000
	Untreated, sown Witness (3+3)	-	2570	100	Mt.II	Mt.II
	Imazamox	1.2	2796	109	226	*
	Imazamox + Imazamox	1.2 + 1.2	2958	115	388	**
Express Sun	Untreated, unsown Witness	-	1245	48	- 1315	000
	Untreated, sown Witness (3+3)	-	2560	100	Mt.II	Mt.II
	Tribenuron-metil	0.030	2780	109	220	*
	Tribenuron-methyl + Tribenuron-methyl	0.030+0.030	2970	116	410	**
Stage II (8-10 leaves)						
Conventional	Untreated, unsown Witness	-	1170	46	- 1320	000
	Untreated, sown Witness (3+3)	-	2490	100	Mt.II	Mt.II
	Bifenox 4 F	2.0	1486	60	- 1004	000
	Bifenox 4 F + Bifenox 4 F	2.0 + 2.0	1590	64	- 900	000
Clearfield	Untreated, unsown Witness	-	1148	46	- 1338	000
	Untreated, sown Witness (3+3)	-	2486	100	Mt.II	Mt.II
	Imazamox	1.2	2250	90	- 236	0
	Imazamox + Imazamox	1.2 + 1.2	2350	94	- 136	
Express Sun	Untreated, unsown Witness	-	1178	47	- 1320	000
	Untreated, sown Witness (3+3)	-	2498	100	Mt.II	Mt.II
	Tribenuron -methyl	0.030	2210	88	- 288	0
	Tribenuron -methyl+ Tribenuron- methyl	0.030+0.030	2201	888	- 297	0
Dl.5%=198kg/ha	Dl.1%=310 kg/ha		Dl.0.01%=415 kg.ha			

The residual effect of herbicides applied in the three sunflower weed control systems

Every autumn, after the sunflower, on the same site, after the preparation of the land for sowing (25-28 cm, 2 works with a disc harrow and a work with the combiner), autumn wheat was sown. In the *Conventional and Express Sun* technical variants, no phytotoxicity phenomena were observed, regarding the remaining effect of herbicides on wheat cultures. In other words, both the herbicide Modown and the herbicide Express, both for the single application and for the two successive treatments variants, in both application stages, no phytotoxic phenomena were observed in wheat plants either in the autumn immediately after sunrise, neither at the end of autumn nor spring. In variants where the *Clearfield technology* was used with the herbicide Pulsar 40SC (imazamox), in Stage I (4-6 sunflower leaves), a phytotoxic effect of 10-15% at a dose of 1.2 l/ha was noticed that is, 10-15% of the wheat plants were perished, the crops were hollow, the plants were yellow or white, and in the case of two successive treatments (1,2 + 1,2 l/ha), the phytotoxic effect was quite obvious, 35% at the one application dose and 40-45% more perished wheat plants when the products were applied in Stage II.

As such, when using Clearfield technology, after sunflower, it is recommended that no grainy cereals (wheat, barley, barley) to be sown in autumn, neither oats in spring. Due to the residual effect of the Pulsar 40 SC (imazamox) herbicide, especially on soils with little organic matter, pH less than 6.5 and high content of clay (over 35%).

CONCLUSIONS

The results obtained during the three years of experimentation 2015-2017, under the Experimental Field at Moara Domneasca of the

Faculty of Agriculture allow us to draw the following conclusions:

1. The best effect in weed control is achieved with *Clearfield and Express Sun technologies*. A weak effect in weed control has been achieved in *Conventional technology*, and it will be used only where it is possible to complete the weed control also with 1-2 mechanical hoes.

2. The best results on the productivity indicators were obtained in the *Clearfield and Express Sun* variants. Weak results were recorded in the *Conventional Technology* variant due to the reduced weed control spectrum of the Modown 4 F herbicide (bifenox).

3. Very good production results were obtained in the *Clearfield and Express Sun* technology variants when the herbicides were applied in Stage I (Optimal stage), 4 sunflower leaves and 4-6 cm weed height. Delaying the application of herbicides makes weeds grow very much, compete with sunflower plants and become very difficult to combat due to the highly developed root system and their size (in some cases over 15 cm high).

5. Following the *Clearfield technology*, it is imperative that the next plant be either leguminous or maize, given the residual effect of the imazamox herbicide.

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DETERMINATION OF THE EFFECTS OF DIFFERENT FERTILIZER APPLICATIONS ON SWEET CORN

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Abstract

Two varieties of sweet corn were compared; Gold Rush and Chocolate in this study. Three fertilization regimes were used: 1. Organic (1200 kg/ha); 2. Organic (600 kg/ha) and half urea (86.5 kg/ha); 3. Di amino phosphate (DAP) (50 kg/ha) and urea (173 kg/ha). Same irrigation amount was applied for all plots by using furrow irrigation method. NDVI was estimated by using RS technique to determine vegetation index. By using same technique canopy temperatures were estimated. Chocolate variety was highest in NDVI value (0.58) when Organic+Half Urea was applied. Plant height and leaf area were measured from each plot. Gold Rush variety produced the tallest plant with 119.4 cm at the Organic+Half Urea fertilizer application. The biggest leaf area (46.08 cm^2) was measured from Gold Rush variety with DAP+Urea fertilizer application. Sugar content also determined by using Rea fracto meter. The highest sugar content was 14% for the Gold Rush variety by applying Organic+Half Urea and DAP+Urea. When all findings were evaluated, Organic+Half Urea fertilizer application was found to give better results.

***Key words**: fertilizer, RS, NDVI, corn.*

INTRODUCTION

Remote sensing (RS) is the science of getting information about objects or areas remotely without contact (Anonymous, 2017), and it has been used very much in recent years for agricultural purposes (Tanriverdi, 2010; Tanrıverdi et al., 2017). One of the models in RS that have been used in this study is The Normalized Difference Vegetation Index (NDVI). NDVI is one of the most commonly utilized vegetation indexes and its' utility in satellite estimation and monitoring of global vegetation cover has been well indicated over the quarter of a century (Huete, Liu, 1994; Leprieur et al., 2000). NDVI is associated with some biophysical properties of canopy crops such as leaf area index (LAI), fractional vegetation cover, vegetation cover and biomass. Furthermore, the virtually essential pigment in converting of light energy to stored chemical has a direct effect on NDVI. RS of chlorophyll content in crop canopies can help ensure a low-cost alternative to crop or soil example (Scharf et al., 2002; Hatfield et al., 2008). Moreover, chlorophyll content is nearly related to N supply and also utilized to diagnose corn N status and predict corn grain output potential (Bullock, Anderson, 1998; Pandey et al., 2000; Vetsch, Randall, 2004). NDVI rises near-linearly with rising LAI and enters an asymptotic phase in which NDVI rises leisurely with rising LAI. Thermal infrared radiation IR plays a crucial role in determining the earth's climate and its sensitivity. It is, therefore, important to have an accurate IR radiation parameterization in atmospheric general circulation models (GCMs) used for studying climate change.

Sweet corn (*Zea mays* L. *saccharata*) is a variety of high sugar content of corn. Sweet corn is the result of natural mutation recessive genes that control the conversion of sugar to starch inside the endosperm of the nucleus of an atom. Corn is the second most important crop in the world after wheat and before rice. Also there is an increasing tendency for commercial production of sweet corn (Arun Kumar et al., 2007). In order to increase the productivity of the crop and enhancing the quality different types of fertilizer should be applied to the soil and in form of synthetic or organic fertilizers.

In Iraq, many farmers are still following traditional methods to agricultural works. That was the reason new technological tools (RS, SPAD 502 meter Minolta Japan, Thermal Infrared, GreenSeeker HandHeld Greenseeker, USA) were used in this study because of increase yield and quality of agricultural crop. With this way this study can be quid for farmers in Iraq.

Kanipanka and Zrigwez are agricultural regions in sweet corn breeding, two different locations famous for their soil and environmental suitability. This study was carried out to increase agricultural income in these two regions and to use different organic and chemical fertilizers to determine the effect of fertilizer varieties on plant production. In recent years, the demand for this crop has increased. For this reason, the main aim of this study is to investigate the correct fertilizer preferences by making experiments with various fertilizer applications with the help of collected data using RS to increase corn production.

MATERIALS AND METHODS

Field Expermental Site. The field experiment was carried out on 31 August 2016 in two research stations with different soil types (Table 1) of Sulaimaniya directorate of agriculture research; Kanipanka~40 km away from Sulaimanya, at 582 m above sea level with (35°22’25’’N, 45°43’25’’E) and Zirgwez Agricultural Station~30 km away from Sulaimanya, and located at 830 m above sea level with (35°22’50’’N, 45°27’50’’E). The two cultivars used in this project, Gold rush and chocolate were provided by the Sulaimaniya Directorate of Agriculture Research. To make the decision of fertilizer amount soil samples collected from the study area (Table 1).

Table 1. Soil chemical and physical value of both locations

Soil chemical and physical	Location	
	Kanipanka	Zirgwez
Soil texture	Salty clay	Salty clay
Ec Ds 25°	0.16	0.18
PH	0.85	7.76
N%	0.13	0.1
Available P(ppm)	7.44	7.75
Soluble K^+ Meq/L	0.051	0.051
Soluble Na^+ Meq/L	0.186	0.108
Soluble Ca^+ Mq/L	1.6	1.3
Soluble Mq^+/L	2.1	3.7
CL Meq/L	0.9	0.9
OM%	1.53	1.39
$CaCo_3$%	19.5	21.5
HCO_3 Meq/L	0.9	1.1
CO	0.6	0.9

To understand the climatically differences at the two location of the study area, air temperature were measured (Table 2).

Table 2. Average temperature of study both locations

Months	Location			
	Kanipanka		Zirgwez	
	maximum	minimum	maximum	minimum
August	45	21	43	26
September	39	16	37	17
October	30	8	31	14
November	25	2	21	6
December	11	-2	12	1

Experimental treatments and design. The experimental design was a split plot design with 3 fertilizers (Table 3) randomised on the main-plots and 2 genotypes randomised on sub-plots in three replicates. Each plot was 3 furrows with 2 m length and 0.75 m. The genotypes within the experiment were sown at the same plant population 66,000 plants/ha.

Table 3. Type and amount of fertilizers

Fertilizer type	Applied quantity
Organic	(1200 kg/ha)
Organic+ Urea	(600 kg/ha)+(86.5 kg/ha)
Di amino phosphate + Urea	(50 kg/ha)+ (173 kg/ha)

Field irrigation. After the end of planting all the seeds starts the irrigation process and the first day is the first day of the plant life.

Field Measurements

NDVI. NDVI was measured on three occasions; before Nitrogen application, pre and pose a study for each plot in three replicates using GreenSeeker Hand Held Green seeker, USA. This instrument measures the amount of NIR and red NIR reflected by the canopy.

Leaf chlorophyll content. These transmittance values are used by the device to derive a relative SPAD meter value that is proportional to the amount of chlorophyll in the sample. In this experiment, three measurements were taken from each leaf at the base, middle and top of the flag leaf for three plants in each plot.

Chlorophyll content measurement. These transmittance values are used by the device to derive a relative SPAD meter value that is proportional to the amount of chlorophyll in the sample. In this experiment, three measurements were taken from each leaf at the base, middle and top of the flag leaf for three plants in each plot.

Canopy temperature. This parameter was measured in two occasions; before second amount of N application and at tasselling using infrared thermometer AR320 Infrared Thermometer China manufacturer with infrared temperature.

Leaf area. The length of the leaves of three randomized plants was measured in each plot at slicking stage (Leaf area= length * 0.64).

RESULTS AND DISCUSSIONS

NDVI. Two measurements were recorded in before second N amount application, pre and pose tasseling for all plots (Table 4). The statistical analysis showed that the interaction of variety and fertilizers had significant differences in Zirgwez location before the second amount of N was applied. Chocolate variety was higher in NDVI value when (Organic+Half Urea) fertilizer was applied NDVI 0.58. However, the analysis of NDVI at Kanipanka location was non-significant for both cultivars at all three fertilizer types condition.

Table 4. Mean data for NDVI before second N amount application, pre and pose tasseling stage at both locations

Location	Kanipanka						Zirgwez					
Variety	Gold rush			Chocolate			Gold rush			Chocolate		
Fertilizer	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea
before N	0.39	0.46	0.42	0.48	0.38	0.39	0.33	0.44	0.37	0.35	0.38	0.45
at tasseling	0.46	0.54	0.53	0.53	0.51	0.54	0.46	0.46	0.36	0.54	0.58	0.56
after tasseling	0.43	0.46	0.45	0.42	0.44	0.49	0.41	0.36	0.4	0.42	0.40	0.39

Leaf chlorophyll content. Three measurements were taken from each leaf at the base, middle and top of the flag leaf for three plants in each plot (Table 5).

The cultivars were differed in leaf chlorophyll content at tasselling stage in Zirgwez location. The fertilizers had various effects on the two cultivars, the leaf chlorophyll content of Gold rush variety was higher than the Choocalate variety (43.72) (SPAD value 1 and 2) respecttively. At Kanipanka location, the fertilizers had affected the leaf chlorophyll content. However, this effect was the same for both cultivars.

Table 5. Mean data for leaf chlorophyll content before second N amount application, pre and pose tasseling stage at both locations

Location	Kanipanka						Zirgwez					
Variety	Chocolate			Gold rush			Chocolate			Gold rush		
Fertilizer	Organic	Organic + Half Urea	DAP+Urea	Organic	Organic+Half Urea	DAP+Urea	Organic	Organic+Half Urea	DAP+Urea	Organic	Organic+Half Urea	DAP+Urea
before N	30.23	23.77	38.04	40.12	30.13	36.51	35.2	34.91	37.53	33.48	36.92	32.1
at tasseling	23.77	28.37	41.13	33.36	35.79	41.54	26.06	34	37.33	32.63	34.71	43.72
after tasseling	28.23	33.88	43.63	35.02	36.78	46.03	29.74	34.44	37.79	33.97	41.53	43.37

Plant height. Plant height was measured from ground level to the tip of the tassel of the plant using a ruler on 3 randomly selected plants in each plot for all replicates (Table 6).

The cultivars showed variation in plant height Gold rush variety produced the tallest plant with 119 cm at Zirgwez location.

Canopy temperature. This parameter was measured in two occasions; when plant height reached 25 cm and at tasselling using infrared thermometer (Table 7). The value of canopy temperature was the highest at Kanipanka location for gold rush variety 19.09 when the parameter was measured at 25 cm plant height when DAP and Urea was applied.

When the parameter was measured at tasseling stage, the value of canopy temperature was the highest at Zirgwez location for the Chocolate variety in organic treated plots 20.43. However, when the treatments were compared statistically no significant variations were found.

Sugar content: This data was measured after harvesting from 3 cobs for each treatment Rea fracto meter device. The highest sugar content was 14% for the Gold rush variety that was treated with DAP + Urea and Organic + Half Urea (Table 8). However, no significant differences were found when the treatments were statically compared.

Table 6. Mean data for plant height at tasseling stage at both locations

Location	Kanipanka						Zirgwez					
Variety	Gold rush			Chocolate			Gold rush			Chocolate		
Fertilizer	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea
Plant height cm	99.3	122.8	110.8	92.8	92.8	94.8	104.47	119.40	114.87	81.97	86.17	85.61

Table 7. Mean data for canopy temperature when plant height reached 25 cm and at tasselling

Location	Kanipanka						Zirgwez					
Variety	Chocolate			Gold rush			Chocolate			Gold rush		
Fertilizer	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+Half Urea	DAP+ Urea	Organic	Organic+Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea
before tasseling	18.57	18.79	19.09	17.21	17.19	16.77	13.42	13.69	13.58	13.62	13.69	13.58
at tasseling	17.21	17.19	16.77	17.32	16.96	16.89	20.11	20.16	20.28	20.43	19.93	19.07

Table 8. Mean data for % sugar content of the corn

Location	Kanipanka						Zirgwez					
Variety	Gold rush			Chocolate			Gold rush			Chocolate		
Fertilizer	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea	Organic	Organic+ Half Urea	DAP+ Urea
N Leaf	8	9	8	7	7	7	8	8	7	6	7	7

CONCLUSIONS

The challenge in agriculture is to optimize the trade-off between quality and the environmental impacts associated with high fertilizer application in extensive production system. This study was set out to identify variation between a set of sweet corn for physiological traits associated with fertilizer use efficiency using RS technologies.

The results showed that NDVI as RS toll might be suitable to use as a method to determine N efficiency in sweet corn with respect of the environmental conditions.

Optical sensing showed cultivars variation in leaf chlorophyll content especially at tasseling stage indicates the potential of this parameter in assessing plant nutrient levels.

NDVI measuring technologies gives high opportunity in estimating plant nutrient levels.

There is an opportunity for the utilization of these parameters NDVI, chlorophyll content and canopy temperature in other projects.

It's useful to apply RS technologies in plant nutrient content projects especially for large areas.RS has chance in spatial analysis of crop nutrient requirements and fertilizer management.

By using GreenSeeker HandHeld for determine the NDVI is an easy, cheap and precise way.

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EFFECT OF HIGH DILUTIONS OF SODIUM CHLORIDE SOLUTIONS ON WHEAT GERMINATION - PRELIMINARY STUDY

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Abstract

The effect on germination and growth of high (homeopathic) dilutions on different plant species was studied since 1923, beginning with the experiments of Kolisko. Few other similar experiments with isopathic models are mentioned in the literature, illustrating the effects of homeopathic treatments on germination and growth of wheat, but they used mainly arsenic oxide (As_2O_3) as a stressor and then the same substance as therapy in a diluted form. Other studies were performed with sodium chloride as a stressor, but mainly on cowpea and beans. Our study used wheat in an experiment with sodium chloride as a stressor and various solutions of Natrium muriaticum (high dilutions of sodium chloride) as therapy. Different results were obtained regarding the number of germinated seeds and the length of the coleoptiles, showing that homeopathic dilutions of Natrium muriaticum have a different effect on the wheat germination and its growth, depending on the treatment dilution, opening a new field of research in the domain of abiotic stress in plants treated with high diluted medicines.

Key words: *wheat, germination, growth, abiotic stress, Natrium muriaticum.*

INTRODUCTION

High dilutions of medicinal substances have been used with success in the last two centuries, according to the principles of similarity, like cures like, in homeopathic medicine.

Homeopathic preparations are diluted and mechanically agitated (potentized) substances prescribed on the basis of the principle of similitude (like cures like), as stated in the textbooks of homeopathic medicine (Bungetzianu, Chirilă, 1983).

Three main types of scales are used, the decimal, the centennial and the 50-milesimal. The symbols used for these three scales are: D or X for decimal, C for centesimal and LM for 50-milesimal.

In the case of the decimal scale, dilutions are made in successive steps of 1/10 for each new dilution, in the centesimal scale, the proportion being 1/100 for each step and in the 50-milesimal dilution the proportion is 1 / 50000.

The dilution techniques are classified in two major categories, the Hahnemannian (symbol DH, CH) and the Korsakovian (K) techniques. Sometimes the Hahnemannian dilutions are simply named by letters D or C. For Hahnemannian technique, dilutions are done using a new vial at each new dilution step, and in the case of the Korsakovian method, using a single vial, which is emptied and refilled at each new dilution step, approximating that the liquid remaining on the vial walls would represent 1/100 of the initial volume contained therein, and would always be filled with diluents up to the starting level.

Homeopathic medicines usually have an initial tincture called mother tincture, which is most commonly obtained by maceration, in case of plants. The most widely used solvent is concentrated alcohol of 90 degrees to obtain a mother tincture, and for the subsequent dilutions alcohol of 45 degrees. For solids, deconcentration is initially carried out by trituration of the substance in a mortar with lactose powder. The proportion of mixtures will also be in the 1/10 or 1/100 scale (1 gram of useful substance is mixed with 9 grams of lactose for the decimal scale or 99 grams of

lactose for the centesimal scale). Homeopathic solutions may be used as such or in the form of lactose or sucrose granules impregnated with the initial solution.
Recent studies of electron microscopy HRTEM and FESEM show that all homeopathic dilutions, starting with centesimal 6 (CH 6) or 50-milesimal 1 / LM 1 dilution, contain nanoparticles with elements of the original substance, which place homeopathy in the domain of nanomedicine (Rajendran, 2015).
The effect on germination and growth of high (homeopathic) dilutions on different plant species was studied since 1923, beginning with the experiments of Kolisko.
A number of experiments on wheat are mentioned afterwards in literature, illustrating the effects of homeopathic treatments on germination and growth of wheat impaired by abiotic stress, as isopathic models, where a substance in high concentration is used as a stressor and then, as therapy, in a high homeopathic dilution.
The durum wheat (*Triticum durum* L.) seedling model stressed with arsenic trioxide and treated with the homeopathic preparation arsenic trioxide 45 X, is the most frequently investigated model in plants. This isopathic approach was introduced by an Italian team of researchers (Betti et al., 1997). Other studies were performed with sodium chloride as stressor, but mainly on other species of plants. Different researchers concluded that salinity has a negative impact from the initial stages of seed germination and seedling growth, it affects the physiological and biochemical processes in mature plants (Nawaz et al., 2012) and among abiotic stress factors, it is one of the most important in many arid and semiarid regions (Ibrahim, 2016).
In 2005, Tighe studied the effect of *Natrium muriaticum* 12 CH, 18 CH and 24 CH on Cress (*Lepidium sativum*) germination and growth, after the plant was stressed with a sodium chloride solution 1%, by watering in the solution for 16 hours. This type of experiment is known as the sodium chloride cress seedling model. After 96 hours of incubation, germination decreased with potency levels 12 CH and 24 CH, while the 18 CH potency had no significant effect. Seedling growth showed a trend towards inhibition also with 12 CH and 24 CH.
Natrium muriaticum in 6 CH and 30 CH dilutions, compared with NaCl 5% was also studied on *Phaseolus vulgaris* and it was concluded that *Natrium muriaticum* had a significative effect of stimulating the growth, especially in 6 CH dilution (Lensi et al., 2010).
Another model consisted of seeds of *Vigna unguiculata* pre-treated with *Natrium muriaticum* and then stressed with NaCl. In the pretreated group the germination was increased, when compared with the control group, stressed with the same substance (Mondal et al., 2012). Sukul et al., in 2012, showed that another remedy, Sepia 200 CH in 1:1000 dilution counteracts the effect of salt stress in cowpea seedlings. In 2016, a study performed by Mondal also on cowpea seeds pointed out that potentized *Natrum muriaticum* 200 CH can be used with positive results on plants grown on brackish soils.
In 2015, Delian and Lagunovsci-Luchian studied the effects of saline stress on germination and vigour of primed *Daucus carota* L. seeds and concluded that potassium phosphate and ascorbic acid can be used as an alternative priming treatment to obtain higher percentage of germination and early vigorous seedling growth under saline stress conditions.
Other interesting studies concerning priming and salinity are quoted by Delian et al., in 2017, and describe the germination rate of tomato seeds after priming with natrium chloride and gibberellic acid (GA), in a study performed by Nakaune et al. (2012) who report values of 4.9 and 4.6 times higher at 36 hours after sowing compared to hydro-primed seeds, with endogenous abscisic acid levels being similar after sowing. The results suggest that the effect of natrium chloride is produced by an increase of the GA4 content via GA biosynthetic genes activation, with increase in the expression of genes related to endosperm cap weakening.
Recently, in September 2017, Sarkar et al. demonstrated that *Natrum muriaticum* 30 CH and 200 CH, as well as the high concentration of sodium chloride solutions produce biological effects, initiating their action on specific binding sites of a protein, like bovine serum albumin (BSA). Repetition of the same dose of

the drug produces an increasing saturation of the binding sites of the protein.

Agrohomeopathy is a novelty in Romania both for plant protection, growth and plant immunity. Our study is the first experiment of this kind, exposing a variety of wheat to a moderately high salinity level and observing the effects of different treatments with *Natrium muriaticum* on germination and growth, comparatively with the control samples.

MATERIALS AND METHODS

The wheat Glossa variety (*Triticum aestivum* L.) was used in the experiment. The wheat is the most frequently used model in agrohomeopathic research, due to the easy possibility to observe its germination and growth in a relatively short time, in normal or controlled modified conditions.

The experiment took place in a natural environment (in the laboratory) and the growth chamber of USAMV Bucharest, for two different variants of study, at 23°C, 60% relative humidity each and a 12/12 h day/night regime in the growth chamber and 10/14 h day/night regime for the natural conditions, in November, 2017.

We used ten sterile Petri dishes with sterile sand, 10 seeds of wheat in each Petri dish. The seeds and the sand were wetted with 10 ml of a sodium chloride solution 4g/l and immediately afterwards we treated them with 10 different solutions, which were numbered from 1 to 10 (so that until the end of the experiment nobody knew which solution was used for the dishes). Each Petri dish was wetted with 5 ml of a different solution (*Natrium muriaticum* D6, D12, C5, C7, C9, C15, C30, C200 and two variants of spring water, one of them being mechanically agitated before the use, corresponding with the succusions made for the CH 7 potencies). The homeopathic dilutions of *Natrium muriaticum* were obtained from the pharmacy, being produced by Plantextrakt, Romania. The salt for the sodium chloride solution was taken from Naturalia, Bucharest (salt without Iodum).

One variant consisting in 10 dishes was placed in the growth chamber (Figure 1) and the other one in a natural environment (Figure 2). Each day afterwards we observed the rate of germination and the length of the shoots, on a period of 9 days.

Figure 1. Petri dishes in the growth chamber

Figure 2. Petri dishes in a natural environment

The correspondence between the numbers and the different solutions applied was written and kept for the evaluation of results, being the following:

1. *Natrium muriaticum* CH 30;
2. Spring water (Borsec) as control;
3. *Natrium muriaticum* CH 7;
4. *Natrium muriaticum* CH 9;
5. *Natrium muriaticum* CH 15;
6. *Natrium muriaticum* CH 5;
7. *Natrium muriaticum* CH 200;
8. *Natrium muriaticum* D12;
9. *Natrium muriaticum* D6;
10. Potentized (mechanically agitated) spring water CH 7 as additional control.

RESULTS AND DISCUSSIONS

Synthesis of previous agrohomeopathic experiments

Till present time, other researchers used mainly arsenic oxide (As_2O_3) as a stressor for the germination and growth of wheat and then the same substance as therapy in a diluted form (Table 1).

Table 1. Overview of bioassays of wheat with abiotic stress (after Jager et al., 2011)

Author	Stress	Treatment	Work variable
Auquiere et al., 1988	NaCl, CuCl, $K_2Cr_2O_7$	*DH* NaCl, *DH* CuCl, *DH* $K_2Cr_2O_7$	Shoot growth; fresh and dry weight of shoots, grains and roots
Kovac et al., 1991	As_2O_3 0.1%	*DH* As_2O_3, PC	Shoot growth
Betti et al., 1997	As_2O_3 0.1%	*DH* As_2O_3, PC	Germination
Brizzi et al., 2000	As_2O_3 0.1%	*DH* As_2O_3, PC	Germination
Brizzi et al., 2002	As_2O_3 0.1% 0.12%	*DH* As_2O_3, PC	Shoot growth
Binder et al., 2005	As_2O_3 0.1%	*DH* As_2O_3, PC	Shoot growth
Brizzi et al., 2005	As_2O_3 0.1% 0.16%	*DH* As_2O_3	Germination, shoot growth
Lahnstein et al., 2009	NaCl, CuCl, $K_2Cr_2O_7$	*DH* NaCl, *DH* CuCl, *DH* $K_2Cr_2O_7$	Shoot growth; fresh and dry weight of shoots, grains and roots

D, C = decimal, centesimal potency; H = Hahnemannian potency; PC = potentized control (as additional control).

The agrohomeopathic effect on wheat

The germination rate and the length of shoots responded differently to the treatments made with the different agrohomeopathic solutions applied, the highest growths in day 9 being observed for CH7 and CH15 as illustrated in Figure 3 and Figure 5.

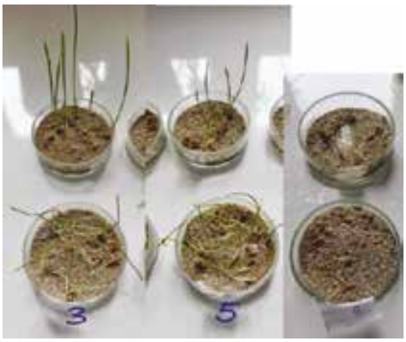

Figure 3. Length of wheat shoots treated with CH7, CH15 and D6 in growth chamber and natural environment

Examining the evolution of germination, the D6 dilutions leaded to no germinated wheat seed in the growth chamber and 70% germination in natural environment, still, all these with the lowest growth from all samples afterwards (Figure 4).

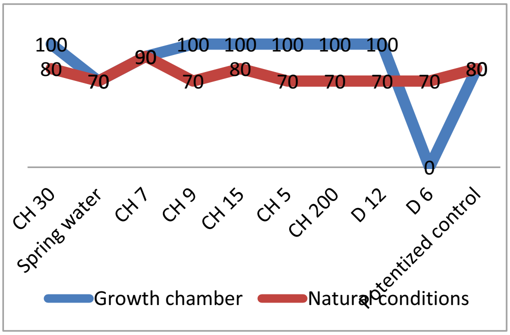

Figure 4. The germination percentage of wheat seeds in growth chamber and natural environment

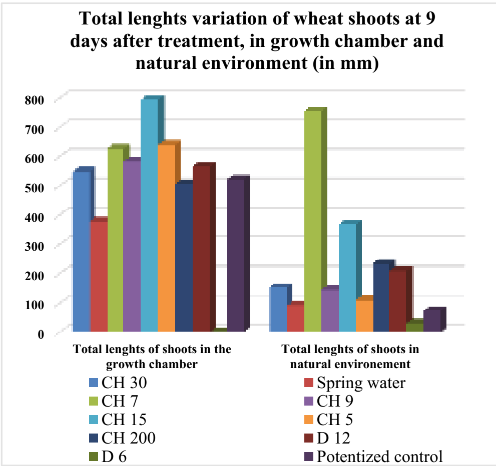

Figure 5. Length of wheat shoots in growth chamber and natural environment (in mm)

As it can be seen in the figure 4, the growth of seeds was also not the same in the different samples and in both variants we noticed an increase in the growth of shoots in samples 3 and 5 (CH 7 and CH 15). The worst results were noticed in the sample 9, in both variants, where *Natrium muriaticum* D6 was used. Seeds from control samples had a modest growth, comparatively with other samples, with the exception of sample 9, where we noticed the smallest growth in the natural environment or no growth present in the growing chamber. The sample with water CH 7 had a better tendency of germination and growth when compared with sample 2, where simple water was used, in our study, a fact which was also pointed out by Brizzi et al., in 2000 and 2011 (agitated/succused water has better effects on germination when compared with simple water), but is infirmed by other researchers (Baumgartner et al., 2008; Scherr et al., 2009). The D6 dilution is considered a low homeopathic dilution and it is possible that the low dilutions have inhibitory effects when compared with higher ones.

CONCLUSIONS

The study points out that homeopathic treatments have a certain effect on plants, confirming other studies from literature, depending on the dilution which was used. In our study, low dilutions like D 6 have an inhibitory effect on germination and growth, while higher dilutions stimulate germination and growth. Our present work has to be confirmed by other researches, but it can open a whole domain to be explored, when abiotic stress in plants is concerned. It is also important to take into consideration the possibility to intervene with homeopathic treatments in the case of brackish soils.

Another interesting domain concerns priming solutions tested and used as hydropriming, osmopriming, chemopriming etc. to overcome the action of stressors and we have in view in future to use dilutions of *Natrium muriaticum* as priming solutions to test the effects on germination and growth of wheat, as the next step of our researches.

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THE INFLUENCE OF THE INTERACTION OF SOME MINERAL FERTILIZERS ON THE ACCUMULATION OF SOME NUTRITIVE ELEMENTS IN WHEAT GRAINS

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Abstract

In the paper, it is presented the influence of the combined fertilization of phosphorus-potassium and phosphorus-nitrogen on the content of wheat grains in certain nutritive elements. The experience, in a three year stationary rotation (soybean-wheat-corn), was located on a baticalcaric argic chernozem and the research aimed at studying the influence of the combined application of different doses of nitrogen, phosphorus and potassium, at wheat culture. The wheat grains were determined in terms of their nitrogen, phosphorus, potassium, calcium, magnesium, copper, manganese and zinc content. The determinations were performed on the fertilized variants with P_0, P_{40}, P_{80}, P_{120}, on constant grounds K_0, K_{80}, respectively N_0, N_{100} and N_{200}. The application of progressive doses of phosphorus on constant levels of nitrogen and potassium, has led to the increase of nitrogen, phosphorus, potassium, magnesium and manganese content, but to the decrease in calcium, copper and zinc content in all analyzed samples. It is also noted that the application of the P_{120} dose did not result in increases for the content of the determined nutrients, and moreover, they often resulted in decreases in their content.

***Key words**: doses, fertilizers, nutritive elements, wheat grains.*

INTRODUCTION

Research on the efficacy of wheat mineral fertilizers at the national and international level has shown a strong influence on the yield and quality of wheat fertilizers.

In the soil-plant-fertilizer system there is a permanent circuit of nutrients. Wheat plants extract different amounts of N, P and K from the soil through both the active organs and the grains, and at harvesting the roots, a part of the plant mass, remain on and in the soil. Thus, a wheat crop whose yield is of 5-6 t / ha, extracts the following average amounts of nutrients from the soil: 100-140 kg/ha N, 50-60 kg/ha P_2O_5, 130-160 kg/ha K_2O, 19-24 kg/ha Ca, 12-24 kg/ha Mg, 10-21 kg/ha S, 0.2-0.4 kg/ha Zn, 0.5-0.6 kg/ha Mn, 0.6-3.5 kg/ha Fe, 0.08-2 kg/ha Cu, 0.006-0.2 kg/ha B and 0.004-0.01 kg/ha Mo (Hera Cr., Borlan, 1980).

To provide a high yield-forming effectiveness of fertilizers, one shall maintain the adequate ratios between the nutrients applied (Kostadinova, 2014; Hirzel, Matus, 2014).

The content of wheat grain in nutrients differs according to the cultivated variety, soil type, soil water supply, fertilizer doses and their application period. The efficiency of the chemical fertilizers applied to wheat on the protein content of the kernel, the improvement of the technological indices of flour processing (Oproiu, 1981; Popescu et al., 1981) was demonstrated.

The quantitative and qualitative yield increase at the level of current requirements is not possible without the use of fertilizers (Petcu et al., 2003).

In long-term experiments, fertilization can cause soil and concentration changes in available macro-elements, which in turn can also affect the micro-element concentration (Li et al., 2007).

For example, the application of phosphorus in humidity conditions greatly decreases the water solubility of micro-nutrients and implicitly their extraction from the soil (Bierman, Rosen, 1994).

Wheat grains contain almost all the nutrients indispensable to human existence, representing an important source of nutrients in human and animal nutrition, in the form of proteins, lipids, carbohydrates or mineral substances.

The content of magnesium, potassium, sodium and calcium as well as the quantitative ratios between them make it possible to determine the nutrition applicability of the plants grown for animal feed, as well as the nutritional value of the yield allocated to human consumption (Wojciech et al., 2017). An excessive uptake of specifications or anions limits the content of other, sometimes very important, macro- and microelements. High potassium contents deteriorate, whereas high calcium and magnesium contents improve the quality of animal feed (Murawska et al., 2013).

The presence of micro-elements in crop plants is not only a topical issue in agricultural technology, but also an essential quantitative indicator in food and feed standards (Fageria et al., 2008; Van Campen, Glahn, 1999). The deficiencies of some micro-elements in the soil also affect the quality of the yield, with repercussions even on the human body.

For example, zinc deficiency is the most widespread in the world (Fageria et al., 2002). Worldwide, about 50% of the soils are deficient in Zn, and a high proportion of Zn low-grain foods may be a major factor in the occurrence of Zn deficiency in humans (Welch, 1993).

Copper is a component of antioxidant enzymes, and its deficiency in diet can affect the proper functioning of the human and animal body antioxidant system (Hänsch, Mendel, 2009).

MATERIALS AND METHODS

The experiments in a three-year stationary rotation (soybean-wheat-corn) were carried out at the Research and Development Unit of Caracal on a baticalcaric - argic chernozem, following the parcel three-factor subdivision, with three repetitions. The slightly alkaline soil (pH = 7.7) in the arable bed is poorly supplied with nitrogen (0.130% total N), medium to well supplied with phosphorus (43.7 ppm P mobile) and well to very well supplied with potassium (233.8 ppm K).

The following items were tested in the wheat culture: factor A - phosphorus fertilization with four graduations: $a_1= P_0$, $a_2= P_{40,}$ $a_3= P_{80}$, $a_4= P_{120}$; factor B - potassium fertilization with two graduations $b_1= K_0$ și $b_2= K_{80}$; factor C - nitrogen fertilization with three graduations $c_1= N_0$, $c_2= N_{100}$, $c_3= N_{200}$.

Progressive doses of phosphorus were applied on constant nitrogen and potassium samples, also taking into account the initial soil content in the three macro-elements.

The influence of the interactions of fertilizer doses applied to the yield obtained during the three years of experimentation was examined, and at the end of the experimental period certain qualitative features of the yield were determined.

From the yield of the last year of experimentation, average samples were analyzed for the analyzed variants, from which determinations were made regarding the content of wheat grains in total nitrogen, phosphorus, potassium, calcium, magnesium, copper, manganese and zinc .

The Nt determination was performed using the Kjeldhal method (Kjeldhal Raypa wet/ acidification - Kjeldhal Pro-Nitro distiller and sodium hydroxide titration).

For the other nutrients analyzed (P, K, Ca, Mg, Cu, Mn and Zn), the mineralization of the samples was made by calcination at 450°C, and the mineral residue obtained was solubilized with 0.5 N hydrochloric acid. As far as the concentration of the macro- and micro-elements is concerned, the standard methods of determination were used.

Phosphorus was determined by the colorimetric method using the UV-VIS spectrophotometer, and K and Ca were determined by flame emission photometry using the Flame Photometer PFP 7.

The determination of the Mg, Cu, Mn and Zn content was achieved by atomic absorption spectrophotometry using the AA Spectrometer S Series.

RESULTS AND DISCUSSIONS

The autumn wheat culture responds positively to the application of mineral fertilizers, especially when the assortments and doses interact with primary macro-elements (NP or NPK) and the ratio between them is sufficiently balanced, more particularly between nitrogen and phosphorus (Borlan et al., 1994).

Long-term experiments play an essential role in understanding complex plant-soil-fertilizer interactions and provide data on fertilizer application, being a rich source of scientific information on the agronomic conditions over a long period of time (Lupu et al., 1993).

Nitrogen fertilization is one of the most effective yield-formation factors (Candráková et al., 2009; Jankovic et al., 2011). It demonstrates a comprehensive effect together with other crop management factors on the yield level, as well as on the quality characteristics of the grain (Liszewski, 2008; Valkama et al., 2013).

The right fertilization should be based on the balance of nutrients, considering their uptake from soil as well as from fertilizers (Staugaitis et al., 2014; Mandic et al., 2015).

Wheat is one of the world's most valued grain crops because wheat grains contain almost all the nutrients indispensable to human nutrition. Experimentally, 100 grams of grain contain on an average: 10.4 grams of protein, 61.7 grams of carbohydrate, 1.45 grams of lipids, 12.5 grams of fiber, 0.7 grams of B vitamins (B_1, B_2, B_3, B_6), 1.44 milligrams of vitamin E, 38 mg Ca, 493 mg P, 126 mg Mg, 3.21 mg Fe, 397 mg K, 2.63 mg Zn, 2 mg Na.

Together with the achievement of increased yield on the surface unit, there is widespread concern for obtaining adequate agri-food products for human and animal nutrition.

The yield quality of the last year of experimentation was evaluated by the content of macro- and micro-elements in wheat grains, being directly influenced by the doses of mineral fertilizers applied, as well as by their interaction.

Constantly using K_0 and K_{80}, depending on phosphorus fertilization, the wheat grain content was analyzed in primary and secondary macro-elements (N, P, K, Ca and Mg - Table 1), and in micro-elements (Cu , Mn and Zn - Table 2).

Phosphorus application in progressive doses, in the absence of potassium or using K_{80}, slightly increased the content of macro-elements in wheat grains, with the exception of calcium (0.035% Ca for P_0K_0 at 0.034% for $P_{40}K_0$/ $P_{80}K_0$/ $P_{120}K_0$). Compared to calcium, nitrogen, phosphorus, potassium, and magnesium were found to have slightly increased at the same time with the values of phosphorus doses. The P_{120} dose did not bring any significant changes compared to P_{80}.

Applying progressive phosphorus doses against constant potassium levels caused a slight decrease in the concentration of the analyzed micro-elements as a result of increasing doses, except for manganese (43 ppm Mn for P_0K_0 at 52 ppm Mn for $P_{120}K_0$ and 49 ppm Mn for P_0K_{80}/$P_{120}K_{80}$ at 53ppm Mn for $P_{40}K_{80}$).

Table 1. Content of macro-elements (%) of wheat grains depending on phosphorus and potassium fertilization

Doses	K_0					K_{80}				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
P_0	2.31	0.27	0.33	0.035	0.121	2.14	0.28	0.34	0.036	0.125
P_{40}	2.49	0.37	0.34	0.034	0.131	2.44	0.35	0.40	0.035	0.133
P_{80}	2.38	0.42	0.41	0.034	0.135	2.41	0.41	0.41	0.035	0.136
P_{120}	2.49	0.42	0.39	0.034	0.137	2.45	0.38	0.36	0.028	0.129

Table 2. Content of micro-elements (ppm) of wheat grains depending on phosphorus and potassium fertilization

Doses	K_0			K_{80}		
	Cu	Mn	Zn	Cu	Mn	Zn
P_0	6.18	43	37	5.81	49	33
P_{40}	6.46	49	27	3.87	53	33
P_{80}	3.74	51	31	3.35	51	33
P_{120}	4.11	52	29	3.67	49	31

Ensuring a K_{80} agro-base reduced the content of wheat grains in copper and zinc and increased the content of manganese for all levels of phosphorus fertilization, and even for unfertilized variants where it had not been applied.

Depending on the phosphorus fertilization, the wheat grain content was analyzed in primary and secondary macro-elements against constant background of N_0, N_{100} and N_{200} (N, P, K, Ca and Mg - Table 3), but also in micro-elements (Cu, Mn and Zn - Table 4).

The application of progressive doses of phosphorus at constant nitrogen values led to an increase in the content of macro-elements in wheat grains, even in the absence of nitrogen fertilizers. The exception was calcium, the content of which decreased with increasing phosphorus doses at all levels of nitrogen fertilization (0.032% Ca for $P_0N_0$0 at 0.030% Ca for $P_{80}N_0$; 0.038% Ca for P_0N_{100} at 0.028% Ca for $P_{120}N_{100}$; 0.037% Ca for P_0N_{200} at 0.035% Ca for $P_{120}N_{200}$).

Regarding the micro-element content, phosphorus fertilization at constant nitrogen values caused a decrease in Cu and Zn concentrations and an increase in Mn concentrations, as phosphorus doses increased regardless of the nitrogenous agro-base.

Table 3. Content of macro-elements (%) of wheat grains depending on phosphorus and nitrogen fertilization

Doses	N_0					N_{100}					N_{200}				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
P_0	1.76	0.30	0.35	0.032	0.129	2.32	0.28	0.35	0.038	0.124	2.6	0.25	0.31	0.037	0.117
P_{40}	1.89	0.36	0.37	0.032	0.135	2.52	0.31	0.36	0.034	0.130	2.98	0.41	0.38	0.037	0.132
P_{80}	1.79	0.40	0.40	0.030	0.132	2.42	0.40	0.40	0.037	0.134	2.99	0.46	0.43	0.037	0.142
P_{120}	1.96	0.37	0.40	0.031	0.134	2.60	0.40	0.38	0.028	0.132	2.87	0.43	0.35	0.035	0.133

Table 4. Content of micro-elements (ppm) of wheat grains depending on phosphorus and nitrogen fertilization

Doses	N_0			N_{100}			N_{200}		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
P_0	5.34	51	29	5.96	45	37	6.69	43	39
P_{40}	4.39	56	21	3.72	49	28	4.67	49	41
P_{80}	3.51	54	21	3.30	51	31	3.82	49	40
P_{120}	3.62	55	22	3.46	48	29	4.61	49	39

The copper content dropped from 5.34 ppm for P_0N_0 to 3.51 ppm for $P_{80}N_0$; from 5.96 ppm for P_0N_{100} to 3.30 ppm for $P_{80}N_{100}$ and from 6.69 ppm for P_0N_{200} to 3.82 ppm for $P_{80}N_{200}$.

Zinc had the same downward trend, along with increasing phosphorus doses, except for the N_{200} agrobase, where the values remained at about the same level (39-40-41 ppm). Manganese is the microelement whose concentration increased simultaneously with the applied phosphorus doses.

If we follow the variation of the concentration of the analyzed micro-elements for each level of phosphorus fertilization, depending on the nitrogen agrobase, it can be observed that zinc and copper are the micro-elements whose content is higher when N_{200} is secured and the Mn content is higher on unfertilized variants.

In all the samples in the analyzed variants the minimum and maximum nutrient content varied as follows:

- the total nitrogen content ranged between 1.76% for P_0N_0 and 2.99% for $P_{80}N_{200}$;;
- the phosphorus content was between 0.25% for P_0N_{200} and 0.46% for $P_{80}N_{200}$;
- the K content ranged between 0.31% for P_0N_{200} and 0.43% for $P_{80}N_{200}$;
- the Ca content was between 0.028% for $P_{120}K_{80}$ and 0.037% for $P_{80}N_{200}$;
- the Mg content ranged between 0.117% for P_0N_{200} and 0.142 for $P_{80}N_{200}$;
- the Cu content was between 3.30 ppm for $P_{80}N_{100}$ and 6.69 for P_0N_{200};
- the Mn content ranged between 43 ppm for P_0K_0 and 56 ppm at $P_{40}N_0$;
- the Zn content was between 21 ppm to $P_{80}N_0$ and 43 ppm for $P_{80}N_{200}$.

The application of fertilizers with phosphorus and nitrogen resulted in an increase of the total nitrogen content in wheat grains, the highest content of 2.99% being recorded on the $N_{200}P_{80}$ variant.

Regarding the wheat grains' phosphorus content, we can observe that on a constant background of nitrogen, it increases to the dose of P_{80}, after which, at the application of the maximum dose of P_{120}, there is a decrease of the phosphorus content both at N_0 and at N_{200}, as well as a restriction on the N_{100} agrofond.

On the background of N_0, N_{100} and N_{200}, the potassium content of the berries increases at the same time as the increase of phosphorus doses up to the P_{80} level, and the application of the P_{120} dose resulted in a restriction of the potassium content for N_0 and a decrease for the N_{100} and N_{120} variants.

On a constant background of nitrogen, there is generally a decrease in grains' calcium content along with the increase in the applied phosphorus doses.

On a constant background of nitrogen, an increase in the magnesium content for the grains may be attributed to the increase in the applied phosphorus doses, with a peak reached on the $P_{80}N_{200}$ variant.

On a constant background of nitrogen, the administration of progressive doses of phosphorus lead to a decrease in copper content for wheat grains.

In terms of manganese content, the application of different phosphorus doses on a constant nitrogen background led to an increase in nitrogen, while the application of different doses of nitrogen on a constant background of phosphorus resulted in a decrease in phosphorus.

On a constant background of N_0 and N_{100}, the progressive application of phosphorus doses led to a decrease in zinc content for wheat grains, but on the background of N_{200} it increased, with a maximum value of 43 ppm for the $P_{80}N_{200}$ variant.

CONCLUSIONS

Experimenting the combined application of phosphorus and potassium fertilizers and phosphorus and nitrogen fertilizers to wheat crops also allowed for a qualitative assessment of the yield obtained under such conditions and depending on the interaction between the examined factors.

Providing a constant nitrogen pool (N_{100} and N_{200}) on which progressive phosphorus doses were applied resulted in better nitrogen, phosphorus, potassium, magnesium and manganese grains. Calcium, copper and zinc decreased simultaneously with the increase of the phosphorus dose on the same nitrogen agrobase.

Against the K_{80} agrobase, the application of increasing phosphorus doses leads to an increase in nitrogen, phosphorus, potassium, magnesium and manganese concentrations, and a decrease in the calcium, copper and zinc content. The same trend is also evident for the agrobase K_0. The application of phosphorus fertilizers influences the accumulation in wheat grains, especially in macro-elements, with the exception of calcium.

Regardless of the provided agrobase, there are no significant differences in the concentrations of the analyzed elements between the doses of P_{80} and P_{120} so that the maximum phosphorus dose can be justifiedfrom an economic point of view.

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FACTORS AFFECTING ENERGY CONSUMPTION IN HAMMER MILLS

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Abstract

The use of hammer mills is widespread, since the size of granular or fibrous materials can be reduced, the simplicity of the structures and the fineness of the milled product can be easily controlled. However, in these mills, the grinding activity, which is an important indicator of energy utilization efficiency, is lower than in other mills. In this study, the effects of technological (physico-mechanical properties, fineness grade, particle size distribution etc.), mechanical (hammer annulus velocity, rotor dynamic characteristics, air flow in crusher unit etc.) and constructive (dimensions of crusher unit, hammer shape, the size of the gap between the unit walls and the hammer edges, the feeding technique of the crusher unit, the technique of unloading the product, the screen area, the impact plate etc.) have been investigated how such factors affect energy consumption in hammer mills.

Key words*: hammer mill, specific energy consumption.*

INTRODUCTION

The machinery used in breaking and grinding of granular products, in accordance with the breaking efficiency, is called mill. Nowadays, it is necessary to mechanically apply a force in grinding of grains and other forage plants (Güzel, 1999). The purpose of grinding is to increase the specific surface area of the material by reducing the size of the material. With the help of this process which is frequently required in the product processing technique, besides many benefits, the product is transformed from crude to finished product. On the other hand, an increase in the surface area occurs when the size of the product is reduced and the volume decreases. The benefits it provides are: the mix ability of the material is facilitated to obtain more homogeneous mixtures, the coating property of the material is increased, the growth period in the surface area of the material accelerates the drying period, the dissolution and absorption rates increase. Crushing and grinding in the direction of the stated aims should be carried out carefully in the production of mixed feeds, since it is an important process affecting the quality of the feed (Yıldız et al., 2008). In the production of feed, crushing and grinding operations can be carried out with disks, cylindrical rollers and hammer mills (Figure 1.)

Figure 1. General types of hammer mills

However, hammer mills are more widely used because of their high capacity of work, their low repair and maintenance costs and their ability to process mixtures of materials consisting of granules of different sizes (Yıldız, 2002).

MATERIALS AND METHODS

Hammer mills are rough grinding mills with hard and soft shredding operations. The hammer-shaped pushing elements, which are fixedly or movably connected about a shaft, are subjected to a high-speed crushing product. The granules are disintegrated as they pass through the sieve surrounding the mill body (Figure 2).

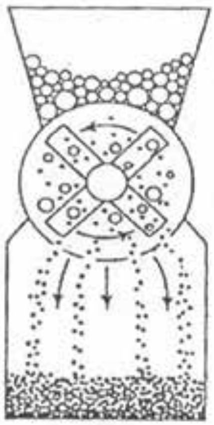

Figure 2. Schematic view of the material milled by the hammer mill

The shredding device consists of hammers that are freely connected at their ends on a rotating shaft (Güzel, 1999). Among these hammers, those with a rectangular shape and a thickness between 4.5 mm and 10 mm are more common. Although their construction is simple, they have a long lifespan. The hammerheads can be made wider to function as a pneumatic conveyer to transport the processed material to a certain distance. Hammers with notched edges are more effective in crushing-grinding. Crushing and grinding with hammer mills has an important effect not only on hammers but also on sieves that enclose crushing unit at the same time. The sieve surrounding the crushing unit is positioned according to the positions of the hammers, the numbers and the feeding situation of the product. Sieve hole shapes can be round, oblong and angular (Figure 3). The sieve hole diameter and shape is an important factor determining the processing precision and mill capacity. Sieves are made of steel sheet with a thickness of 2-3 mm. Sieves made of thick sheet are more durable and long lasting.

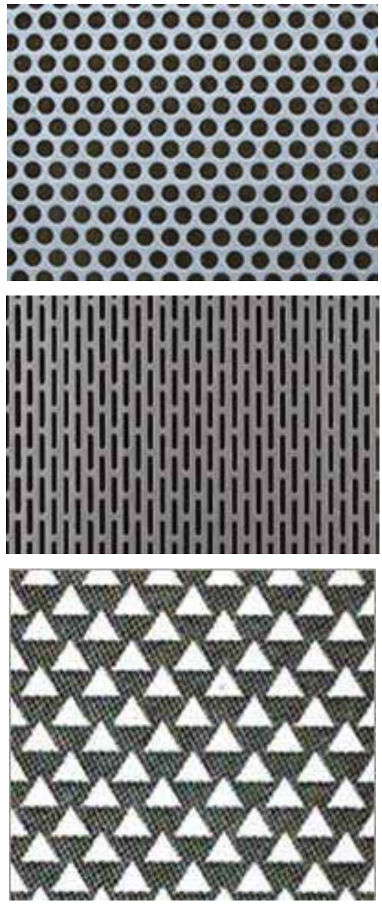

Figure 3. Some examples of sieve hole shapes

However, as the processed material becomes difficult to pass through the sieve holes, the dusting rate will increase (Yıldız, 2002).
The penetration of the granular material into the crushing unit is provided by the feeding gap. The feeding gap can be tangential or axial to the breaking unit (Yıldız et al., 2008).

RESULTS AND DISCUSSIONS

a. ***Effective factors on energy consumption***

Reducing the grain size in the processing of grain products for food and feed purposes is the most important and most energy-consuming process (Dziki, 2008).
The following two points need to be taken into account in terms of energy consumption; the first one is that the grain size should be selected accordingly and avoided unnecessary

excessively fine grinding. The second one is that the mills to be used during the grinding process must be sensitive to the selection of the constructive and operating parameters.

The grain size should be large enough to ensure that the feed is optimally assessed by the animal.

The materials that are made into small granules can be better digested in the organism. Because, as the granules become smaller, the area that digestive enzymes will act is broadened and the nutrients are digested in a shorter time.

However, it is not desirable to reduce the grain too much. Because of the over shrinking particles in the digestive system increases the rate of transit and the time they are affected with the relevant enzymes is shortened. This affects the digestion process negatively.

The performance of animals consuming mixed feeds of different grain sizes may vary. It is suggested that the broilers consume different grain sizes during different ages. Nir and Şenköylü (2000) suggest that broilers consume different grain size feeds at different ages. As a matter of fact, according to the age of the animals in the broiler feed, have been reported to vary between 900-1100 µm for 1-7 cut ages, 1100-1300 µm for 7-21 cut ages and 1300-1500 µm for> 21 cut ages respectively.

Goodband (2002) reported that the optimum grain size for wheat in pig feed was varied between 800-900 µm, and that there was no benefit of grain size below 1000 µm in the broiler feed.

Similarly, another study conducted by Dmitrewski (1982) found that average grain size should be above 3000 µm for cattle and above 1000 µm for pig and broiler chickens, and that excessively fine grind is harmful to the digestive tract, has been reported to increase consumption.

Grinding efficiency is; (mechanical properties of the milled product, fineness grade, particle size distribution etc.), mechanical (hammer circumferential speed, rotor dynamic characteristics, air flow in the crusher unit etc.) and constructive features (dimensions of the crusher unit, the size of the gap between the hammer edges, the feeding technique of the crusher unit, the method of emptying the milled product, the sieve area, the impact plate, etc.).

b. *The effect of technological features on energy consumption*

Factors such as fineness grade, shape, humidity, hardness, density, strength, porosity, abrasiveness and stickiness affect the energy consumption of the product grinded in hammer mills. Some studies have reported that the mechanical properties of the material contained in the technological factors are significantly influenced by the moisture content and the increase of the moisture content of the wheat increases the specific energy consumption (Glenn, Johnston 1992; Mabille et al., 2001; Islam, Matzen, 1988). Some studies have reported that the mechanical properties of the material contained in the technological factors are significantly influenced by the moisture content and the increase of the moisture content of the wheat increases the specific energy consumption (Glenn, Johnston, 1992; Mabille et al., 2001; Islam, Matzen, 1988). In general, it has been reported that, in grinding applications, the increase in fineness due to the reduction in particle size has also been associated with decreasing the grinding efficiency (Dmitrewski, 1982; Stamboliadis, 2007). In addition, in a study by Dziki (2008), it was reported that wheat grinding increased the grinding efficiency of hammer mills when used with crushing application, which can change the mechanical properties of the grains. In the same study, the specific energy consumption of the broken wheat varieties varied between 72.3-146.7 kj kg^{-1} for the Turnia variety and 67.0-114.4 kJ kg^{-1} for the Slade variety depending on the moisture content and the specific energy consumption after the crushing application was found to be in the range of 47.6-100.5 kJ kg^{-1} for the Turnia variety and 44.6-85.3 kJ kg^{-1} for the Slade type respectively. The specific energy required for the milling of barley and oats (elastic crust) is greater than the milling of wheat, rye and corn. On the other hand, due to the decrease in moisture content and the increase in grinding activity, the specific energy requirement seems to increase (Ayık, 1997).

c. *The effect of mechanical properties on energy consumption*

Mechanical properties of hammer mills; such as hammer circumferential velocity, hammer shape, feed rate and technique, type of crusher unit, size and shape of crusher unit, hammer-sieve range, grinding product evacuation technique, effective hole area / total sieve area ratio of used sieves, sieve surface properties and geometry, depends on the operational and constructive parameters of the crusher unit. It has been reported that the hammer circumferential velocity changes at an optimum range of 60-80 ms-1 in terms of grinding efficiency and that the specific energy consumption increases significantly at hammer peripheral speeds above these values, due to the increase in ventilation resistance in the crusher unit (Dmitrewski, 1982). It also suggested that hammer mill dimensions are important for energy consumption and that for high-capacity mills D (rotor diameter) / L (rotor width) = 1.5-1.7 and for low-capacity mills D / L = 4-7. In addition, it has been reported that the most useful hammer shape is that the thickness is between 1.5 and 10 mm, the indentations are flat rectangular plates, and the reduction in hammer thickness reduces the specific energy consumption by up to 15% (Dmitrewski, 1982).

d. *The effect of constructive properties on energy comsumption*

The sieves included in the constructive factors are the most important factor affecting the grinding efficiency of the hammer mills. The sieve hole diameter determines the fineness and grinding capacity of the material being milled. The increase in grinding capacity for a given fineness grade is a key factor in increasing the grinding efficiency (Fang et al., 1997; Koch, 1996). Therefore, in order to increase the grinding capacity, the ratio of "hole area / total sieve area" should be kept as high as the sieve strength permits (Figure 4).

Figure 4. Increase in effective sieve surface

These ratio values, also referred to as the sieve area utilization factor, vary between 8% and 35%, depending on the size of the sieve holes (Dmitrewski, 1982). In agricultural applications, round-hole sieves are generally used, while sieves with pocket-shaped (grater type) holes are used in industrial heavy-duty machines for feed production (Figure 5).

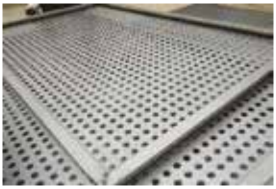

Figure 5. Round hole sieve and Pocket shapes holes

Due to the sharp edges, the porthole sieves increase the grinding capacity as well as increase the crushing effect. However, the negative aspect of these sieves is that they wear very quickly. The round-hole sieves can be drilled conically to increase the grinding capacity.

It has also been noted that placing steel bars 7 mm or 9 mm high on the sieve parallel to the rotor shaft results in an average reduction in energy consumption of up to 15% (Dmitrewski, 1982), by increasing both the grinding capacity and the disintegration effect.

In a study by Beyhan, in 2008, the values of specific energy consumption after grinding with round and oblong hole sieves, different hammer circumferential speed and sieve hole sizes were examined.

For round-hole and oblong-hole sieves, the specific energy consumption values are found as in Table 1.

As the distance between the hammer and the surface of the sieve increases, the grinding capacity decreases and the specific energy consumption increases; it has been reported that in the case of feeding tangentially to the circular wall of the crusher unit, the grinding capacity is increased and accordingly the specific energy consumption is reduced (Dmitrewski, 1982).

Table 1. Specific energy consumption values for sieve types

Sieve Type	Shd (mm)	Sec ($kWht^{-1}$)	Clarification
Rounded hole	2.5	9.38-10.36	Increase with a curvilinear change
	4.5	5.84-9.42	
	6.5	2.45-4.00	
	Hw (mm)		
Oblong hole	1.5	9.40-5.64	Decrease with a curvilinear change
	2.0	6.85-4.70	
	2.5	3.61-3.46	

Sec: Specific energy consumption values obtained after increasing the specific surface area given to the material; **Shd:** Sieve hole diameter; **Hw:** Hole width.

CONCLUSIONS

The grinding efficiency in hammer mills varies depending on the properties of the material to be grinded and the characteristics of the hammer:

- ✓ The material to be grinded; factors such as fineness grade, shape, moisture content, hardness, density, strength, porosity, abrasiveness and stickiness are effective in energy consumption;
- ✓ On the other hand, such as hammer circumferential velocity, hammer shape, feed rate and technique, type of crusher unit, size and shape of crusher unit, hammer-sieve range, grinding product discharge technique, hole area / total screen area ratio of used sieves, sieve surface and geometry, the construction of the crusher unit and the operating parameters significantly affect energy consumption.

Undoubtedly, the work on this subject should not only focus on determining the energy that should be given to the system but also concentrate on the applications that will bring down this energy the most. For example, in recent years, increasing the grinding efficiency by reducing the surface energies by modifying the surface properties of the grains by adding surface active materials to the grinding systems is one of the more popular research topics.

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EFFECT OF LOW TEMPERATURE ON DIAPAUSE EGGS OF *Dysdercus cingulatus* (*Hemiptera*: *Pyrrhocoridae*)

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Abstract

Dysdercus cingulatus (Red cotton bugs) is the most damaging pest of cotton in India and many parts of Asia which reduces yield of cotton. Observing the damage caused to crop the present study designed to know the insect's diapause in relation with abiotic factors. It deals with the diapause termination in eggs of D. cingulatus, when the eggs were incubated at 5ºC for number of days and then transferred to optimal temperature (25ºC), the percentage of hatching decreases as the day increase. Thus temperature play an important role in diapause induction and termination.

Key words*: diapause, diapause termination, Dysdercus cingulatus.*

INTRODUCTION

In nature abiotic factors such as temperature, light and humidity are important factor to limit the survival and development process of species. Insect can survive only in optimal temperature; light and rainfall, if any of these aspects are unfavourable, insects undergo an arrest state called diapause. Diapause is a delay in development due to adverse environmental condition. Diapause in arthropods is a dynamic process; the term diapause suggested by Andrewartha (1952) is a period of arrest in which development comes to a complete rest. Andrewartha (1952) coined the term "diapause development" to refer to the ongoing progression of events that occur during diapause and eventually results in termination.

According to (Du, Chen, 2011; Koštal, 2006) temperature is one of the significant environmental stimuli controlling the termination of diapause.

When exposed to extreme temperatures, most insect employ behavioural, physiological or genetic adaptation mechanisms to adjust their body temperature to which they can with- stand (McMillan et al., 2005; Overgaard, Sorenson, 2008; Nyamukondiwa, Terblanche, 2009; Karl et al., 2011).

The finding of (Heming, 2003) reveals that eggs are the primary stage of insects' life cycles, and they have an upper and a lower temperature limits that they can tolerate. The temperatures outside of the limits would retard or completely inhibit the insect's development or kill the insects.

When embryonic development of insect eggs is stressed by environmental factors, especially temperature, consequent development and reproduction could be affected. However, how the thermal environment experienced in early ontogeny affects biological characteristics of both sexes and thermal tolerance capacities in later development stages is not well-studied (Bowler, Terblanche, 2008).

As observed in onion maggot, completion of diapause occurred at a wide range of temperature (4-25ºC): The optimal temperature was approximately 16ºC (Ishihawa, 2000) and diapausing temperature of *Sorghum midge* were in the range from 20 to 30ºC, which were optimum for diapause termination and adult emergence, moisture acted to initiate diapause termination, but photoperiod had no significant effect on the termination of larval diapause (Baxendale, Teetes, 1983).

In recent study on *R. Irregulariter dentatus* the optimal temperature for development was around 15ºC. A relatively high temperature of 25ºC prevent from hatching within 210 days which showed that diapause development were slow or arrested (Yamaguchi, Nakamura, 2015). It was observed that on the cold termination of diapause in the eggs of the silkworm *Bombyx mori*, the physiological mechanism of termination process was attributed to the conformational change of a specific protein named Time-Interval-

Measuring-Enzyme (TIME), which is regulated by the time-holding peptide (PIN) (Ti et al., 2004).
The present study on laboratory experiments is designed to know the insect's diapause in relation with abiotic factors i.e. temperature which plays significant role in termination of diapause in the eggs of *Dysdercus cingulatus*.

MATERIALS AND METHODS

Dysdercus cingulatus population was established from approximately 20-25 individual bugs originally collected from the field of cotton in Aurangabad city (19°32'N / 75° 14' E). They were reared at 22± 3°C and L: D 10: 14 photoperiod in glass bottle with muslin cloth and rubber band tied at the bottle mouth.
The bugs were fed with water soaked cotton seeds, changing the feed on every alternate day. The adult male and female were separated and kept pairs in Petridish of 9 cm diameter and whatmann no.1 filter paper placed at the bottom.
After copulation and breeding, female lays eggs in batches of 90-140 approximately. Freshly laid eggs were collected with the help of painting brush.
Batches of 10 eggs each were made and exposed to different temperature to observe hatchability. Group of 10 eggs in each batch were made and incubated at low temperature i.e. 5ºC for diapause induction for 10 days, 20 days and 30 days under darkness L:D 0:24 respectively, and then returned to 25ºC under darkness L:D 0:24 the diapause termination was recorded.

RESULTS AND DISCUSSIONS

The effect of different temperature on hatchability was observed and found that at 25ºC 100% hatchability so it was taken as optimal temperature for hatchability shown in Table 1 and Figure 1.

Table 1: Effect of different temperature on percentage hatching of eggs

Sr. No.	No. of Eggs	Temperature (°C)	% of Hatching
1	10	5°C	0 ± 0.6
2	10	15°C	90 ± 1
3	10	25°C	100 ± 0
4	10	30°C	50 ± 1
5	10	35°C	16.6 ± 1
6	10	40°C	0 ± 0

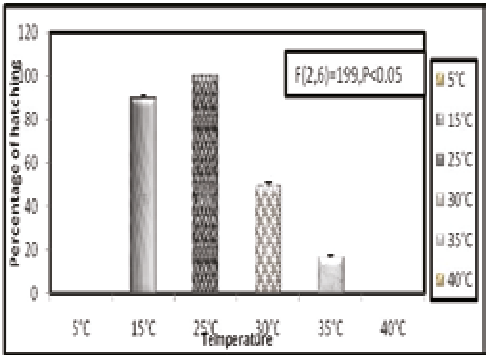

Figure 1. Effect of different temperature on the percentage of eggs hatching

Table 2 showing time taken for hatching of eggs and required for hatching of eggs maintained in laboratory (0:24 LL:DD photo period) at 5°C for 10, 20 and 30 days when transferred to 25°C.

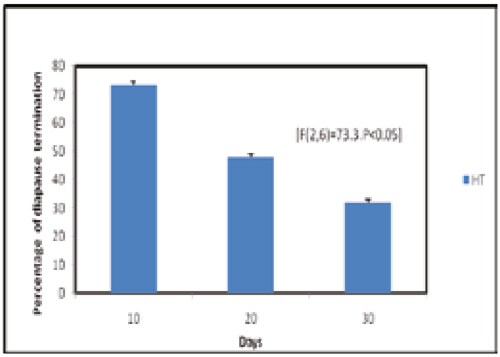

Figure 2. Effect of temperature i.e. 5ºC on diapause termination in eggs of *D. cingulatus*

Table 2. Time taken for hatching of eggs and required for hatching of eggs maintained in laboratory

Sr. No.	No. of days of incubation at 5ºC	Days required for termination at 25ºC	No. of eggs hatched at 25°C	% of Diapause termination	Mean value of day required for diapause termination
1	10	26.33± 3.05	18.33± 1.15	73.3 ± 1.1	26.3± 3.3
2	20	33.33± 2.08	12± 1	48 ± 1	33.3± 2.1
3	30	67.66± 3.05	8± 1	32 ± 1	67.6± 3.0

The effect of low temperature on percentage of hatching was shown in table 2 and figure 2, respectively. The result shows that eggs kept at 5ºC for 10 days were terminated after 26 ± 3.3 days with 73% of hatching. However after 20 days incubation the hatchability of eggs declined with increase in number of days to 33 ± 2.1, similarly after 30 days incubation eggs showed lowest hatchability i.e. 32% in 68 ± 3.0 days.

The effect of low temperature on percentage of hatching was shown in table no.2 and figure 2 respectively. The result shows that eggs kept at 5ºC for 10 days were terminated after 26 ± 3.3 days with 73% of hatching. However after 20 days incubation the hatchability of eggs declined with increase in number of days to 33 ± 2.1, similarly after 30 days incubation eggs showed lowest hatchability i.e. 32% in 68 ± 3.0 days.

As incubation period at low temperature increases, it's result shows decrease in percentage of hatching, which reveals that low temperature effects percentage hatching $F (2,6) = 73.3$, $P<0.05$. Low temperature often plays a role in the termination of winter diapause *Pyrrhocorris apterus* adult, when exposed to 5ºC it undergoes diapause and then transferred to 25ºC a less number terminate from diapause and start ovipositing (Hodek, 1968).

The relationship between temperature, incubation time and the hatching success were studied on *Austrophlebioides marchani* in laboratory conditions (Parnrong, Campbell, 1997). They observed that hatching time and hatching success are temperature dependent. Similar result were observed in temperate Australian mayfly eggs, which hatch between 9-25ºC, whereas European mayfly eggs hatched in an incubation temperature range between 3-25ºC (Giberson, Rosenberg, 1992) while in *Hexagenia rigida* eggs hatching occurs at 12-32ºC and if incubation is done at low temperature and high temperature above 36ºC it enters diapause (Friesen et al., 1979).

CONCLUSIONS

It is concluded from the results that the optimal temperature for egg hatching is 25ºC if the temperature is reduced to 5ºC or less than that eggs enter diapause. The induced diapause eggs get terminated when returned to optimal temperature but it increases duration for hatching. Hence abiotic factor like temperature is a one of the crucial environment factor that affects the biological processes and has major effect on survival of the insects.

To survive in the unfavourable temperature insect eggs induce diapause and terminate from diapause on favourable temperature. Insect greatly vary in their ability to survive low temperature and are considered highly successful animals that respond to seasonal changes by induction and termination of diapause.

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RESEARCH AT NIRDPSB BRASOV ABOUT *IN VITRO* BEHAVIOR OF POTATO PLANTLETS BELONGING TO NEW AMELIORATED LINES AT SRDP TARGU SECUIESC

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Abstract

Through in vitro propagation accomplish a rapid multiplication of biological material free of viruses and so it can be reduce the number of generations in the field. This research is directed on behavior of in vitro plantlets belonging to 14 lines ameliorated at Station of Research and Development for Potatoes Targu Secuiesc and studing of different parametes of these: average length of a plant, average number of leaves/plant, average length of a root, average weight of a plant. Another part includes microtuber production from these lines (and studding two parameters: number of microtubers obtained / plant and wight of microtubers) and highlighting the best lines.

***Key words**: potato, in vitro material, plantlets, microtubers, nutrive medium.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a vegetative propagated crop, is inclined to accumulation of infections by bacteria, fungi, viruses. Worldwide, viral diseases are considered as a limiting factor in seed potato production (Meybodi et al., 2011). Micropropagation allows the rapid multiplication of disease-free potato clones in a short time in a controlled environment. Using of micromultiplication techniques has opened new perspectives in for seed potato production and multiplication. These techniques allow a better control and maintenance of growth vigor and more efficient control of the phytosanitary state (Molet, Lavieville, 1990; Fourage, 1991; Senac, 1991, 1992, quoted by Morar, 1999). A huge amount of disease-free potato plantlets can be produced by the micropropagation method (Khurana et al., 2003). Microtubers are small *in vitro* tubers, which can be produced all over year on the complete plantlets or on plants organs (Ranalli, 1997). Over all each plantlet or explant can produce one microtuber with a 3-10 mm diameter and weight of 0.2-0.7 g (Struik, Lommen, 1990).

The aim of this work was to multiply potato plantles *in vitro*, especially to determine different parameters of plantlets and to produce microtubers. Microtubers production is one of the strategies under this perspective because of their small size and weight, which gives them tremendous advantages in terms of disease free, storage, transportation and mechanization Kanwal et al. (2006), in addition to the possibility of being stored for extended periods of time (McCrown, Joyce,1991).

MATERIALS AND METHODS

For research on the *in vitro* behavior of potato plants belonging to the lines studied

In vitro multiplication is an extensive method used to increase seed potato nuclear stock. This technique is widely used in many countries, is very flexible and offers a high rate of multi-plication.

The use of tissue culture technology in the rapid multiplication of disease-free planting material has facilitated the production of seed potatoes, being used as a standard methodology in the production of free potato viruses from a valuable stock but with infections. Virus eradication technology and the establishment of an *in vitro* collection of virus-free germplasm is an important prerequisite for the production of seed potatoes, having as its starting point the

culture of meristems. Plantlets free of virus, tested by the DAS ELISA technique, were used to analyze 4 parameters: average length of a plant; average number of leaves/plant; average length of a root; average weight of a plant.
The data were recorded 29 days after inoculation; the monofactorial experience, in which the analyzed factor was the genotype, comprised 14 variants, divided into 3 repetitions.
The minicuttings, containing a single node, were inoculated into culture vessels (test tubes) containing Murashige-Skoog (MS) medium. The axillary/apical bud develops rapidly, so a plant has regenerated in 4 weeks.
As a control, was established for each analyzed parameter the average values.

For research on potato microtubers production belonging to the lines studied

In the Vegetable Tissue Culture Laboratory of NIRDPSB Brasov in period September-October 2017. Inoculation of minicuttings was made to study two parameters: number of microtubers obtained/plantlet and average weight of a microtuber.
Potato uninodal segments (microcuttings) are inoculated into a solid base medium Murashige-Skoog (1962), specific to the growth and rooting phase. The microcuttings cultures are kept in the growth room under controlled conditions of light and temperature for 30 days. The temperature is 22 ± 1°C / day and 18 ± 1°C / night, with a photoperiod of 16 hours light and 8 hours dark.
The pH of the nutrient medium was adjusted to 5.7 with 0.1N NaOH or HCl before autoclaving at 121°C for 15 minutes, pressure 1.1 kg/cm^2.
After microcuttings formed plantlets well-developed, 80 ml liquid tuberization medium was introduced/recipient.
The liquid microtuberization medium contains the same substances from Murashige-Skoog medium but in a reduced amount by half. Instead have an increased energy source: sucrose is higher (80 g/l) (20 g/l was in phase of formation and rooting of plantlets) and also in this phase is added coumarin and kinetin.
Cultures of plantlets were incubated in the climatic chamber in the dark at a temperature of 20°C for about 12 weeks. After microtuberization period (Figure 1), in January 2018 the data were recorded.
Potato plantlets were extracted from the culture recipents and the harvested microtubers were washed to remove all traces of the medium to avoid further infections that might occur during their storage. Microtubers obtained were harvested (Figure 2), washed, treated with fungicide, counted and stored for refrigeration at temperatures of 4-5°C in the dark.
The experience was monofactorial. The studied factor being the genotype, which comprised 14 variants, divided into 3 repetitions. As a control, the average of the determined values was determined for each analyzed parameter.
The aim of the researches was *in vitro* identification of genotypes with valuable characteristics.

Figure 1. Microtubers obtained in culture recipients

Figure 2. Harvested microtubers

RESULTS AND DISCUSSIONS

For *in vitro* behavior of potato plants belonging to the lines studied

By comparing variants studied differences to control variant with DL of 5, 1 and 0.1%, it appears that a single line, TS 12-1489-1574, presented superiority in the growth of the plantlets'lenght, with a significant difference (statistically ensured) by 1.44 cm; TS 11-1475-1633 line has registered a slowdown in plant growth, deviating by -1.43 cm (a significant negative difference) from mean (control).

It is found that the other lines are quite constant in plant growth, indicating stability in their behavior towards environmental factors (*in vitro* specific growth climate).

TS 12-1489-1574 line will provide in the future a possible biologically advantageous material in sense that it will develop a plant with high height (Table 1).

Table 1. Influence of genotype on the mean length of a potato plantlets/line

Line	**Length of the plantlets**		**Diff. (cm)**	**Sign.**
	(cm)	**(%)**		
TS 12-1489-1574	10.27	116.27	1.44	*
TS 11-1480-1633	9.83	111.36	1.00	ns
TS 11-1468-1633	9.33	105.70	0.50	ns
TS 11-1467-1633	9.27	104.95	0.44	ns
TS 11-1486-1642	9.17	103.81	0.34	ns
TS 12-1502-1675	9.07	102.68	0.24	ns
TS 12-1497-1573	9.00	101.93	0.17	ns
Mean (Ct)	8.83	100.00	-	-
TS 11-1472-1633	8.77	99.28	-0.06	ns
TS 96-1207-169	8.50	96.26	-0.27	ns
TS 12-1488-1574	8.50	96.26	-0.33	ns
TS 09-1442-1525	8.50	96.26	-0.33	ns
TS 09-1441-1525	8.17	92.49	-0.66	ns
TS 12-1501-1582	7.83	88.71	-1.00	ns
TS 11-1475-1633	7.40	83.81	-1.43	o

DL 5% = 1.41 cm DL 1% = 1.90 cm DL 0.1%= 2.54 cm

Another element studied was the average leaf number on the plantlet (Table 2), to which it was again chosen as control mean values of number of leaves/plantlets, situated at 8.38 leaves. This average is found to be 5th place, following a number of 10 lines, with a smaller number of leaves of which 9 lines have insignificant differences and only one line (TS 12-1488-1574) a significant negative difference of -1.38 leaves. Positive results get the lines: TS 11-1472-1633, TS 12-1502-1675 , which provides valuable genetic material, with a large number of leaves of 11.00 and 10.67, respectively, contributing with statistically positive differences by 2.62 considered very significant and 2.29, respectively, distinctly significant; these two lines give us valuable information on the creation of superior genetic material which will form a well-developed plant, with a large number of leaves and thus with a large assimilation surface. A number of 11 lines obtain results closely to mean variants, with insignificant differences indicating and by this time the stability of genetic material created at Station of Research and Development for Potatoes Targu Secuiesc.

Mean length analysis (Table 3) of the roots formed by plantlets belonging of the 14 genotypes (lines) studied, compared to their mean positioned control in the middle of these variants, values of limit differences compared to DL of 5, 1 and 0.1 %, indicating the starting lines TS 09-1442-1525 and final TS 12-1489-1574 with a diametrically opposed behavior, which get differences from significantly higher (3.55 cm) to significantly lower (-3.18 cm). Among these, 12 lines obtain insignificant results of differences in root length. TS 09-1442-1525 line which obtained the longest root length, but also the 6 lines that follow to the level of the control, can be considered as valuable genotypes, viewed from the perspective of the formation of an elongated root, capable of extracting water from the soil and finally to fight against drought, therefore, to resist in and water stress conditions. Mean weight analysis (Table 4) of potato plantlets freshly taken gives us indications about ability of these genotypes to form well-developed plantlets, capable of growing and for later to develop vigorous and, of course, productive plants. It is distinguished TS 11-1480-1633 line which records the greatest weight of the plantlet (245.37 mg) with a significant positive difference by 70.29 mg to control (mean of values). Although the differences recorded by the other lines compared to control are insignificant they give us some indications over *in vitro* plantlets regeneration capacity, these values of weights being between 218.10 mg (TS 09-1442-1525) and 121.93 mg (TS 11-1486-1642).

Table 2. Results on average number of leaves/plant

Line	Average number of leaves		Diff.	Sign.
		(%)		
TS 11-1472-1633	11.00	131.26	2.62	***
TS 12-1502-1675	10.67	127.29	2.29	**
TS 11-1480-1633	8.67	103.42	0.29	ns
TS 09-1442-1525	8.67	103.42	0.29	ns
Mean (Ct)	8.38	100.00	-	-
TS 11-1467-1633	8.33	99.44	-0.05	ns
TS 11-1475-1633	8.33	99.44	-0.05	ns
TS 12-1489-1574	8.00	95.47	-0.38	ns
TS 96-1207-169	8.00	95.47	-0.38	ns
TS 11-1468-1633	8.00	95.47	-0.38	ns
TS 09-1441-1525	8.00	95.47	-0.38	ns
TS 11-1486-1642	7.67	91.49	-0.71	ns
TS 12-1501-1582	7.67	91.49	-0.71	ns
TS 12-1497-1573	7.33	87.51	-1.05	ns
TS 12-1488-1574	7.00	83.53	-1.38	o

DL 5% = 1.35 DL 1% = 1.82 DL 0.1% = 2.43

Table 3. Results on the average length of root/line

Line	Length of the plant		Diff. (cm)	Sign.
	(cm)	(%)		
TS 09-1442-1525	11.50	144.65	3.55	*
TS 11-1475-1633	10.00	125.79	2.05	ns
TS 11-1472-1633	9.17	115.30	1.22	ns
TS 12-1488-1574	9.00	113.21	1.05	ns
TS 12-1502-1675	8.67	109.01	0.72	ns
TS 12-1497-1573	8.33	104.82	0.38	ns
TS 11-1486-1642	8.17	102.73	0.22	ns
Mean (Ct)	7.95	100.00	-	-
TS 11-1468-1633	7.67	96.44	-0.28	ns
TS 11-1480-1633	7.33	92.24	-0.62	ns
TS 12-1501-1582	7.17	90.15	-0.78	ns
TS 11-1467-1633	7.00	88.05	-0.95	ns
TS 96-1207-169	6.33	79.66	-1.62	ns
TS 09-1441-1525	6.17	77.57	-1.78	ns
TS 12-1489-1574	4.77	59.96	-3.18	o

DL 5% = 3.16 cm DL 1% = 4.26 cm DL 0.1% = 5.69 cm

Table 4. Results on the average weight of a potato plant on the line

Line	Weight of the plant		Diff. (mg)	Sign.
	(mg)	(%)		
TS 11-1480-1633	245.37	140.15	70.29	*
TS 09-1442-1525	218.10	124.57	43.02	ns
TS 11-1475-1633	214.07	122.27	38.99	ns
TS 09-1441-1525	203.80	116.40	28.72	ns
TS 96-1207-169	201.53	115.11	26.45	ns
TS 12-1489-1574	176.83	101.00	1.75	ns
Mean (Ct)	175.08	100.00	-	-
TS 11-1468-1633	169.1	96.58	-5.98	ns
TS 12-1502-1675	159.37	91.03	-15.71	ns
TS 12-1497-1573	153.8	87.85	-21.28	ns
TS 12-1488-1574	153.23	87.52	-21.85	ns
TS 11-1467-1633	149.57	85.43	-25.51	ns
TS 11-1472-1633	143.93	82.21	-31.15	ns
TS 12-1501-1582	140.47	80.23	-34.61	ns
TS 11-1486-1642	121.93	69.64	-53.15	ns

DL 5% = 59.81 mg DL 1% = 80.71 mg DL 0.1% = 107.72 mg

For behavior of potato lines experimented in microtuberization

The response of varieties analyzed in terms of the number of microtubers (Table 5) shows the superiority of TS 11-1486-1642 genotype compared to the control mean (1.16 microtubers). Which shows an increase in the number of microtubers/plant (1.73). Which is very significant (+ 0.58 microtubers/pl), followed by the genotype TS 12-1489-1574 which recorded 1.57 microtubers/pl. and a distinctly significant difference (+0.41 microtubers/pl.). By comparing the experimental differences with limit differences obtained regarding the influence of the genotype (Table 6) on the average weight of a microtuber, it appears that line TS 11-1468-1633 (0.901 g) was superior to the mean of all values (considered control 0.643 g), showing a significantly positive difference in producing microtubers (+0.258 g), whereas TS 12-1502-1675 and TS 12-1488-1574 presented significant negative differences (-0.218 g and -0.224 g). By comparing the number of microtubers / plant and the average weight of a microtuber, it is noted that the TS 11-1486-1642 line produced the highest number of microtubers/pl. (1.73) with an average weight of 0.646 g. The TS 11-1468-1633 line is distinguished with a high average weight of microtuber (0.901 g), but with a number of them/pl. low (1.03).

Table 5. Influence of the genotype on the number of microtubers obtained/plant

Genotype	Number (microtub.)	%	Diff. (microtub.)	Sign.
TS 11-1486-1642	1.73	149.79	0.58	***
TS 12-1489-1574	1.57	124.80	0.41	**
TS 12-1501-1582	1.20	103.70	0.04	ns
TS 09-1442-1525	1.20	103.70	0.04	ns
Mean (Ct)	1.16	100.00	0.00	-
TS 11-1467-1633	1.13	97.94	-0.02	ns
TS 09-1441-1525	1.10	95.06	-0.06	ns
TS 11-1472-1633	1.07	92.18	-0.09	ns
TS 12-1488-1574	1.07	92.18	-0.09	ns
TS 11-1468-1633	1.03	89.30	-0.12	ns
TS 11-1475-1633	1.03	89.30	-0.12	ns
TS 96-1207-169	1.03	89.30	-0.12	ns
TS 11-1480-1633	1.03	89.30	-0.12	ns
TS 12-1497-1573	1.03	89.30	-0.12	ns
TS 12-1502-1675	0.97	83.54	-0.19	ns

DL 5% = 0.23 microtub. DL 1% = 0.31 microtub. DL 0.1%= 0.42 microtub.

Table 6. The influence of genotype on the weight of the microtubers obtained

Genotype	Weight (g)	%	Diff. (g)	Sign.
TS 11-1468-1633	0.901	140.11	0.258	*
TS 12-1501-1582	0.854	132.71	0.210	ns
TS 11-1480-1633	0.784	121.87	0.141	ns
TS 11-1472-1633	0.768	119.35	0.124	ns
TS 11-1467-1633	0.732	113.84	0.089	ns
TS 12-1489-1574	0.682	105.95	0.038	ns
TS 11-1486-1642	0.646	100.45	0.003	ns
Mean (Ct)	0.643	100.00	0.000	-
TS 09-1441-1525	0.624	97.02	-0.019	ns
TS 96-1207-169	0.599	93.16	-0.044	ns
TS 11-1475-1633	0.580	90.12	-0.064	ns
TS 12-1497-1573	0.524	81.51	-0.119	ns
TS 09-1442-1525	0.468	72.70	-0.176	ns
TS 12-1502-1675	0.425	66.05	-0.218	o
TS 12-1488-1574	0.419	65.17	-0.224	o

DL 5% = 0.215 g   DL 1% = 0.291 g   DL 0.1%= 0.388 g

CONCLUSIONS

The development of modern biotechnology techniques, has allowed to obtained scientific and practical results, concretized in efficient methods of rapid multiplication of new breeding creations and the production of seed material from the first biological links, of higher phytosanitary quality with profitable productions and competitive productions at national and international level.

In vitro results on plant height indicate that genotype TS 12-1489-1574 has achieved a preeminent value of 10.27 cm, with a significant positive difference, statistically assured +1.44 cm, compared to the mean of values (8.83 cm) obtained at the 14 lines. TS 12-1489-1574 will deliver in the future, a possible biologically advantageous material.

The mean number of leaf/plant oscillated between 11.00 (TS 11-1472-1633 line) and 7.00 (TS 12-1488-1574 line), being visible superior characteristics of TS 11-1472-1633 and TS 12-1502-1675 lines, which obtain statistically positive differences by 2.62 considered very significant and +2.29, respectively distinctly significant. They offer a valuable genetic material that will form a well-grown plants with a large number of leaves and so with a large assimilation surface. Statistical analysis made to determine the influence of genotype on mean length of roots, indicates the superiority of TS 09-1442-1525 line, which achieves a significant, positive difference (+3.55 cm). This line can be considered as a genotype capable of extracting soil water reserve and finally fight with effects of drought.

The mean weight of a freshly taken plantlet was inclined in favor of TS 11-1480-1633, which records the greatest weight of the plantlet (245.37 mg) with a significant positive difference by 70.29 mg from the mean (control).

TS 11-1486-1633 line was the most representative, being a productive line, which was distinguished by a number of 1.73 microtubers/plant and a difference very significant positive (+0.58 microtub.) compared to the control. This is followed by TS 12-1489-1574 which recorded a difference distinctly significant (0.41 microtub.), compared to the value of control.

Regarding the weight of microtuber line TS 11-1468-1633, produce big microtubers, with a significant positive difference (+0.258 g).

In the future we can focus on TS 11-1486-1633 and TS 12-1489-1574 lines if our purpose is obtaining a big number of potato tubers, with a weight (0.646 g and 0.682 g) which is over all mean values (control). If we want to obtain potato tuber with high caliber, we recommend TS 11-1468-1633 line.

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THE EFFECT OF NITROGEN FERTILIZER ON THE YIELD AND QUALITY IN THE SWEET MAIZE

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Abstract

The aim of the study was to determine the effect of nitrogen fertilizer application on fresh ear yield and quality properties in sweet maize in Isparta conditions during the years of 2012-2013. In this research, five different N doses (0, 50, 100, 150 and 200 kg ha^{-1}) were applied on the variety of Merit sweet corn. The study was carried out at Agricultural Research and Applied Center of Suleyman Demirel University in Turkey. This research was conducted in randomized block design with three replication. Plant height, first ear height, ear length, ear diameter, single fresh ear weight, fresh ear yield and crude protein ratio were determined. According to results of two years, nitrogen applications increased plant height, ear length, ear diameter, single fresh ear weight, fresh ear yield and crude protein ratio while first ear height was not affected by nitrogen doses. According to results of this research, 150 kg ha^{-1} nitrogen application is recommended for high fresh ear yield and quality in sweet maize in Isparta.

***Key words**: sweet maize, nitrogen doses, ear characteristic, fresh ear yield, crude protein ratio.*

INTRODUCTION

Cereals are the main crops and occupied wide range of important parts of the world. Approximately 55% of protein, 15% of fats, 70% of glosides and generally 50 to 55% of calories consumed by humans in the world are provided by the cereals (Normohammadi et al., 2001). The economic importance of corn is clear as it has spread its planting in new world thousands of years ago; because all of its parts such as grain, branches and leaves, even corncob and corn silk is used numerously in human nutrition (20-25%), fed livestock and poultry (70-75%) and pharmaceutical industry (5%) (Mirhadi, 2001).
Sweet corn (*Zea mays* L. var. *saccharata*) is the same botanical species as common corn, the main difference being that the endosperm in the grains of fresh sweet corn have a greater polysaccharide content at commercial maturity. Sweet maize, which provides consumption, is especially important in food industry. In recent years, the production for fresh consumption and Conversion industries as two valuable careers are considered. Since the significant increase in crop production is achieved, the average yields of crops are yet less than their yield potential. The yield potential with full-product cultivars, under ideal management conditions and with optimal physical and also chemical environment will be achieved. Providing appropriate amount of fertilisation required for plant growth through appropriate distribution methods is one way to increase the crop yield (Fathi, 1999).
Nitrogen is one of the most prevalent elements and it is a component of amino acids, proteins, nucleic acids, chlorophyll and many other metabolites essential for survival of the plant. Nitrogen determine setting and maintenance of the photosynthetic potential of the plant reproductive capacity. Numerous field experiments conducted throughout the world has shown that nitrogen is the most important growth-limiting factor. Nitrogen application is one of the important nutrient amendments made to the soil to improve growth and yield of many crop plants (Reddy, 2006). Deficiencies of nitrogen profoundly influence the morphology and physiology of plants. Plants under low levels of nitrogen develop an elevated root: shoot ratio with shortened lateral branches. Higher levels of NO_3 inhibit root

growth and leads to a decrease in the root: shoot ratio (Scheible et al., 1997; Zhang et al., 1999).

Under nitrogen deficiency, plants exhibits stunted growth and small leaves. In the beginning of nitrogen deficiency, the older leaves show chlorosis when compared to younger leaves because of high mobility of nitrogen through phloem. Nitrogen deficiency induces the chloroplast disintegration and loss of chlorophyll. Necrosis occurs at later stages and if nitrogen deficiency continues, it ultimately results in plant death (Alimohammadi et al., 2011).

The objective of this research was to determine the effects of different rates of nitrogen fertilizers on fresh ear yield and quality properties of sweet corn.

MATERIALS AND METHODS

The study was carried out at Agricultural Research and Applied Center of Suleyman Demirel University (37°45′N, 30° 33′E, altitude 1035 m) located in Turkey, between 2012 and 2013 years.

The major soil characteristics, based on the method described by Rowell (1996) were as follows: the soil texture was clay-loam (clay: 31.2%, silt: 45.1%, sand: 23.7%); organic matter was 1.1% by the Walkley-Black method; total salt was 0.3%; lime was 7%; sulphur was 12 mg kg^{-1}; extractable P by 0.5 N $NaHCO_3$ extraction was 3.3 mg kg^{-1}; exchangeable K by 1 N NH_4OAc was 119 mg kg^{-1}; pH was 7.1 in soil saturation extract. Soil type was a calcareous fulvisol.

Sowing was done by hand on 15 May in 2012-2013. The experiments were evaluated in a randomized complete block design with three replications. Seeding row spacing 75 cm and distance within rows of 20 cm were used. Plot sizes were 3 x 5 m = 15 m^2. Sweet corn fertilized at the rates of 0, 50, 100, 150 and 200 kg N ha^{-1}.

Triple super phosphate (TSP 46%) and ammonium nitrate (AN 33%) were used as fertilizers. Crop was drip-irrigated. Crop management was similar to those commonly applied to commercial crops in the area.

Sampling to determine the parameters analysed was carried out in most treatments when the ears reached commercial quality and size, corresponding to 90 days after sowing.

Plant height, first ear height, ear length, ear diameter, single fresh ear weight, fresh ear yield and crude protein ratio were determined.

The data were analyzed together using the Proc GLM (SAS 1998). Means were separated by LSD at the 5% level of significance.

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Table 1. Results of Analysis of Variance Traits Determined

	df	Plant height	First ear height	Ear length	Ear diameter	Single fresh ear weight	Fresh ear yield	Crude protein ratio
Year	1	**	ns	ns	ns	*	ns	ns
Block	4	ns	ns	ns	ns	ns	ns	ns
Nitrogen	4	**	ns	**	**	**	**	**
Year*N	4	ns	ns	ns	ns	ns	ns	ns
Error	16							

**Significant at 1 percent level, *Significant at 5 percent level, ns: non significant.

RESULTS AND DISCUSSIONS

The results of variance analysis showed that plant height, first ear height, ear length, ear diameter, single fresh ear weight, fresh ear yield and crude protein ratio values in sweet maize were influenced significantly by nitrogen treatments (Table 1).

In present study, increasing N fertilization increased plant height value. The highest plant height were obtained from 100, 150 and 200 kg ha^{-1} N rates (177.5, 181.6 and 177.7 cm), while the lowest plant height (154.4 cm) was obtained from control plot (Table 2). These results are in agreement with those reported by

Grazia et al., 2003, Gözübenli, 2010 and Alimohammadi et al., 2011.
The effects of nitrogen fertilization on first ear height of sweet corn was found statistically not significant. The first ear height value from 60.5 to 70.2 cm as was determined. Contrary to this study, some researchers increased the first ear height by increasing the nitrogen dose (Koçak, 1991; Gözübenli, 1997; Turgut, 2000; Kara, 2006).
Other important quality characteristic for sweet maize is the size of ear. As the nitrogen dose increased, the increase in the ear length and ear diameter were also determined. The highest ear length (22.7 cm) and ear diameter (47.6 mm) were obtained from 150 kg ha^{-1} N rates, while the lowest ear length (15.8 cm) and ear diameter (39.6 mm) were obtained from control plot. Similar results were reported by other researchers (Turgut, 2000; Altıparmak, 2001; Grazia et al., 2003; Saruhan, Şireli, 2005; Kara, 2006; Gözübenli, 2010; Alimohammadi et al., 2011).
On account of single fresh ear weight, highest values were obtained from 100, 150 and 200 kg ha^{-1} N rates (272.9, 285.4 and 276.5 g), while the lowest value (151.9 g) was obtained from control treatment (Table 2). On the other hand, nitrogen fertilisation increased fresh ear yield relative to control. The highest fresh ear yield (26.03 t ha^{-1}) was obtained from 150 kg ha^{-1} N rate, while the lowest value (8.13 t ha^{-1}) was obtained from the control plot. The results are quite in line with those of Koçak, 1991 and Altıparmak, 2001.
Lower nitrogen fertilization decreased the grain weight because it affects the number of endospermic cells and starch granules in the early post flowering period, as well as reduces the source of assimilates during the filling period (Uhart, Andrade, 1995).
Crude protein content of forage is one of the most important criteria for forage quality evaluation (Holechek et al., 1989; Vogel et al., 1993). Increasing N fertilization rates resulted in an increase in CP ratio of sweet maize (Table 2). The highest CP ratio were obtained from 100, 150 and 200 kg ha^{-1} N rates (12.11, 12.88 and 12.31%), while the lowest CP ratio (10.04%) was obtained from control plot (Table 2).
Similar results were reported by Koçak (1991) and Altıparmak (2001).

Table 2. The plant height, first ear height, ear length, ear diameter, single fresh ear weight, fresh ear yield and crude protein ratio values of sweet maize at different nitrogen doses at the mean of two years

Nitrogen fertilization (kg ha^{-1})	Plant height (cm)	First ear height (cm)	Ear length (cm)	Ear diameter (mm)	Single fresh ear weight (g)	Fresh ear yield (t ha^{-1})	Crude protein ratio (%)
0	154.4 c	60.5	15.8 d	39.6 d	151.9 c	8.13 d	10.04 b
50	163.5 b	61.6	20.8 c	43.5 c	235.9 b	19.63 c	10.92 b
100	177.5 a	68.3	21.8 ab	45.0 bc	272.9 a	23.08 b	12.11 a
150	181.6 a	70.2	22.7 a	47.6 a	285.4 a	26.03 a	12.88 a
200	177.7 a	67.6	21.4 bc	47.1 ab	276.5 a	24.59 ab	12.31 a

CONCLUSIONS

As a consequence of nitrogen dose increased, the increase in the plant height, ear length, ear diameter, single fresh ear weight, fresh ear yield and crude protein ratio while first ear height was not affected.
According to results of this research, 150 kg ha^{-1} nitrogen application is recommended for high fresh ear yield and quality in sweet maize in Isparta.

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THE BIOCHEMICAL METHANE POTENTIAL OF *Miscanthus giganteus* BIOMASS UNDER THE CONDITIONS OF MOLDOVA

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Abstract

Biomass production is one of the key sectors with significant potential for the production of renewable energy and diversification of rural economy in the Republic of Moldova. Obtaining biogas from biomass is one of the possibilities to produce renewable energy and to reduce greenhouse gas emissions. The decisive factors for the cultivation and exploitation of energy crops are the productivity, the chemical structure and the cost of production of phytomass. The genus Miscanthus in general, and Miscanthus giganteus in particular, has been selected and studied as one of the most promising energy crops in Europe, over the past two decades, because of an array of attributes including high photosynthetic efficiency, high stress tolerance, perennial growth, low nutrient requirements and high content of carbon. We investigated some biological peculiarities and the biochemical composition of Miscanthus giganteus biomass under different harvest regimes and on different dates: single mowing regime (June 16, August17, October 2) and double mowing regime (1st mowing on June and 2nd mowing on October). The samples were collected from the 3-year-old Miscanthus giganteus (cv. Titan) plants cultivated in the experimental land of the Botanical Garden (Institute). The results of our study, based on the near infrared spectroscopy (NIRS) technique, indicated that the biomass of Miscanthus giganteus harvested in June contained 1.63% nitrogen, 41.7% cellulose, 31.7% hemicelluloses, 4.9% acid detergent lignin and 7.4% ash; in August - 0.35% nitrogen, 50.1% cellulose, 32.0% hemicelluloses, 7.7% acid detergent lignin and 4.5% ash, but the biomass obtained by late single mowing in October - 0.59% nitrogen, 45.1% cellulose, 29.4% hemicelluloses, 6.6% acid detergent lignin and 6.6% ash, respectively. The chemical composition of the biomass of Miscanthus giganteus obtained during the 2nd mowing, did not differ essentially from the biomass obtained as a result of the 1st mowing in June, however, the amount of acid detergent lignin and hemicelluloses varied, reaching 5.7% and 28.0%, respectively. The biochemical methane potential of the biomass of Miscanthus giganteus, obtained during the 1st mowing in June, reached 314 L/kg, in August - 259 L/kg, of late single mowing regime in October - 277 L/kg and 2nd mowing in October - 293 L/kg, respectively.

Key words: *biochemical methane production potential, harvest dates, Miscanthus × giganteus biomass, near infrared spectroscopy.*

INTRODUCTION

Taking into account the global rise in the demand for energy and the concerns associated with serious negative consequences, such as the depletion of the resources of fossil fuels and climate change caused by the growing greenhouse gas emissions, the use of alternative sources of energy is considered advantageous and environmentally friendly. The European Union intends to achieve a 20% share of renewable energy in overall energy consumption until 2020, leading to an increased share in the forthcoming years. Biomass production is one of the key sectors with significant potential for renewable energy production and diversification of rural economy in the Republic of Moldova. Phytomass has significant potential for meeting the future energy need, besides, agricultural lands offer an alternative to the traditional agriculture, which is referred to as energy farming (Hăbăşescu, 2011; Ţîţei, 2015). The use of traditional species as energy crops risks conflict with food uses. Domesticating new species specifically for energy production may allow access to species that are better suited to energy production and avoid diversion of food species. The accelerated domestication of new species should be able to take advantage of the growing understanding of the process of domestication and the knowledge of biological peculiarities, productivity and chemical composition of plants.

Perennial grasses play an important role as an extensive CO_2 sink significantly increases the content of soil carbon, dry organic matter possesses many beneficial attributes as energy crops, and there has been increasing interest in their use for this purpose in the US and Europe since the mid-1980s. The C_4-plants, having a more effective photosynthetic pathway, possess such features as resistance to aridity, high photosynthetic yield and a high rate of CO_2 capture when compared with C_3 plants (Lewandowski et. al., 2003). Based on their physiological properties, they have great potential of biomass production, frequently higher than the productivity of trees. Promising perennial grasses, C_4 photosynthetic pathway, belong to the genus *Miscanthus* Andersson, which includes about 16-25 species, found mostly in East Asia. The *Miscanthus sinensis* Andersson*,* *Miscanthus sacchariflorus* (Maxim.) Franch*.*, *Miscanthus floridulus* Warb. ex K. Schum. and Lauterb*.*, *Miscanthus lutarioriparius* L. Liu ex S.L. Chen and Renvoize and *Miscanthus giganteus* Greef et Deu. are the most commonly known and used (Xi, Jezowski, 2004; Arnoult, Brancourt-Hulmel, 2015; Lewandowski et al., 2016; Weijde et al., 2016; Kiesel, Lewandowski, 2017; Tejera, Heaton, 2017; Borso et al., 2018) .

The natural hybrid between *Miscanthus sinensis* x *Miscanthus sacchariflorus,* sterile triploid plant, was developed in Japan, introduced as ornamental plant in Denmark in 1935 and distributed to some European countries towards the end of 1970s. But its exact taxonomic position had not been examined in detail for a long time, and the plant was called *Miscanthus sinensis giganteus*, until Greef and Deuter (1993) conducted the syntaxonomy and nominated it as *Miscanthus×giganteus* Greef et Deu., and included it in genus *Miscanthus*, section *Triarrhena*, family *Poaceae* (Xi, Jezowski, 2004).

Transport energy in the form of biofuels requires sustainable high biomass production in a form that facilitates conversion to high quality fuel. The suitability of plants as feedstock for industrial conversion to biofuels and biomaterials varies depending upon the type of plant biomass and the processes used. The second generation of biofuels under development is based on the conversion of the structural carbohydrates; the plant cell wall has received much attention in recent years. The biogas obtained from biomass is one of the possibilities to produce renewable energy and to reduce greenhouse gas emissions. Its quantity and quality depend on the substrate used in the anaerobic digestion process. Identification of cell wall characteristics desirable for biorefining applications is crucial for lignocellulosic biomass improvement (Klimiuk et al. 2010; Dandikas et al., 2014; Arnoult, Brancourt-Hulmel, 2015; Lewandowski et al., 2016; Weijde et al., 2016; Kiesel, Lewandowski, 2017)

Near infrared reflectance spectroscopy (NIR) has been used in agriculture research for years, as a robust method, low cost and doing non-destructive measurements with limited sample preparation, providing quantitative and qualitative information (Vidican et al., 2000; Harmanescu, 2012; Jin et al., 2017).

The aim of this research was to evaluate some biological peculiarities and the biochemical composition of *Miscanthus giganteus* biomass obtained under different harvest regimes and on different dates, as feedstock for biogas production, under the conditions of the Republic of Moldova.

MATERIALS AND METHODS

The cultivar *Titan* of *Miscanthus giganteus,* which was cultivated in the experimental plot of the Botanical Garden (Institute), latitude 46°58′25.7″ and longitude N28°52′57.8″E, served as subject of this study. The green mass of 3-year-old plants was harvested manually under different harvest regimes and on different dates: single mowing regime (June 16, August 17, October 2) and double mowing regime - 1st mowing (June 17) and 2nd mowing (October 2). The dry matter content was detected by drying samples up to constant weight at 105°C. The content of neutral detergent fibre, acid detergent fibre and acid detergent lignin was evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research-Development Institute for Grassland Brasov, Romania. The biochemical biogas potential (Yb) and methane potential (Ym) were calculated according to the equations of Dandikas et al., 2014, based on the chemical compounds - acid detergent lignin (ADL) and hemicellulose (HC) values:

biogas potential Yb=727+0.25 HC-3.93 ADL; methane potential Ym=371+0.13HC-2.00ADL.

RESULTS AND DISCUSSIONS

It is known that the growth and development rates of plants influence of the biomass accumulation, the leaf share and the dry matter content in harvested biomass.

The biological peculiarities of *Miscanthus giganteus* are described in Table 1. The third growing season for *Miscanthus giganteus* began on April 10. The cultivar *Titan* was characterized by faster growth. The plants developed shoots that reached a height of 157 cm in mid-June, 260 cm in mid-August and in the period when the panicle development started, the first days of October - 385 cm. Analysing the results of the study on the influence of the harvest time on the leaf: stem ratio, we found that stem mass increased from 10.16 to 52.69 g, but the leaf mass from 10.94 to 25.64 g, which caused a decrease in the leaf content in the harvested biomass from 53.50 to 32.73%.

We may mention that after the harvest, the plants of the cultivar *Titan* of *Miscanthus giganteus* were characterized by a moderate rate of revival and, in early October, the stems reached 193 cm. The content of dry matter was higher (38.90%), in comparison with the first mowing (20.46%).

The biochemical composition and the biochemical methane potential of *Miscanthus giganteus* biomass under different harvest regimes and on different dates is presented in Table 2. The obtained results showed that the concentrations of nitrogen, ash, the neutral detergent fiber fraction, the acid detergent fiber fraction and the concentrations of lignin and cellulose differed significantly depending on the harvesting period.

Table 1. Some biological peculiarities of cv. *Titan* of *Miscanthus giganteus*

Harvesting period	Plant height, cm	Stem		Leaf		Leaves content in biomass, %
		green mass, g	dry matter, g	green mass, g	dry matter, g	
16 June	157	60.18	10.16	42.83	10.94	53.50
17 August	260	65.95	25.53	43.42	16.36	39.05
2 October (1st mowing)	385	102.0	52.69	61.64	25.64	32.73
2 October (2nd mowing)	193	30.11	11.76	26.54	10.30	46.69

Table 2. Biochemical composition and biochemical methane potential of cv. *Titan* of *Miscanthus giganteus*

Indices	Harvesting period			
	16 June 1st mowing	17 August 1st mowing	2 October 1st mowing	2 October 2nd mowing
Dry matter, g/kg	204.6	389.6	464.5	389.0
Nitrogen, %	1.63	0.35	0.59	1.09
Ash,%	7.4	4.5	6.6	7.6
Acid detergent fibre, %	46.6	57.8	51.7	46.7
Neutral detergent fibre, %	72.3	89.8	81.1	74.7
Acid detergent lignin,%	4.9	7.7	6.6	5.7
Cellulose, %	41.7	50.1	45.1	41.0
Hemicellulose, %	31.7	32.0	29.4	28.0
Biochemical biogas potential, L/kg VS	614	505	541	573
Biochemical methane potential, L/kg VS	314	259	277	293

In the biomass harvested in mid-August, there was a significant decrease in the nitrogen content (0.35%) and an increase in cellulose (50.1%) and acid detergent lignin content (7.7%), because of the more unfavourable weather conditions caused by the amount and distribution of rainfall and the number of days with air temperature above 30°C. The hemicellulose content was approximately the same level as in the biomass harvested in mid-June, but higher than in the biomass obtained as a result of the late single mowing, in October. The chemical composition of the biomass of *Miscanthus giganteus* obtained during the 2nd mowing in October did not differ essentially from the biomass harvested in mid-June, however, the amount of acid detergent lignin and hemicelluloses varied, reaching 5.7% and 28.0%, respectively.

The differences in the chemical composition of the biomass affected the biochemical biogas and methane potential of the biomass of

Miscanthus giganteus. The biochemical gas forming potential varied from 505 to 614 L/kg VS. The calculated biochemical methane potential under single mowing regime ranged from 259 to 314 L/kg VS, and 2nd mowing in October - 293 L/kg, respectively.

The obtained values are in good accordance with Kiesel and Lewandowski (2017) who reported, the substrate-specific methane yield of *Miscanthus x giganteus* biomass decreased with later harvest dates and reached 247 L/kg VS in October, and the significantly highest SMY was measured in the both cuts of the double-cut regime. In contrast Klimiuk et al. 2010 observed lower yields, 100 L/kg VS in *Miscanthus giganteus* silages prepared in autumn.

CONCLUSIONS

The obtained results showed that leaf: stem ratio, dry matter content, concentrations of nitrogen, ash, neutral detergent fiber fraction, acid detergent fiber, lignin and cellulose differed significantly depending on the harvesting period, which have influenced the methane yield.

The biomass of *Miscanthus giganteus* harvested in June contained 53.50% leaves, 20.46% dry matter, 1.63% nitrogen, 41.7% cellulose, 31.7% hemicelluloses, 4.9% acid detergent lignin and 7.4% ash; in August-38.96%, 39.05%, 0.35%, 50.1%, 32.0%, 7.7% and 4.5%, but the biomass obtained by late single mowing in October -46.45%, 0.59% nitrogen, 45.1% cellulose, 29.4% hemicelluloses, 6.6% acid detergent lignin and 6.6% ash, respectively. The biochemical gas forming potential varied from 505 to 614 L/kg VS. The calculated biochemical methane potential under single mowing regime ranged from 259 to 314 L/kg VS, and 2nd mowing in October - 293 L/kg, respectively.

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INDUCING TRANSIENT GENE EXPRESSION IN *Nicotiana tabacum* PLANT BY AGROINFILTRATION METHOD

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Abstract

In this study I will demonstrate an Agrobacterium-mediated transient gene expression method, into Nicotiana tabacum leaves. By efficient infiltration of Agrobacterium cells carrying appropriate gene constructs into tabacum leaves, transient expression assays can be performed within several days without using expensive instruments or complicated procedures. By syringe infiltration, I will demonstrate a simple, efficient and scalable methodology to induce a target-gene into plant tissue. In addition, DNA, RNA and protein can be extracted from agroinfected leaves and used for reverse transcription-polymerase chain reaction (RT-PCR), polimerase chain reaction (PCR), Southern blot, Northern blot, Western blot, immunoprecipitation, and enzyme assays.

Key words*: Agrobacterium, gene, transfer.*

INTRODUCTION

Agrobacterium tumefaciens is a Gram-negative, non-sporing, which can cause crown gall disease (large tumour-like swellings that typically occur at the crown of the plant) of a wide range of dicotyledonous plants (Tzfira T., Citovsky V., 2008). According to Tzfira T. and Citovsky V., *Agrobacterium* is the only cellular organism that is naturally capable of transferring genetic material between the kingdoms of life and genetically transform, under laboratory conditions, a large number of plant species.

Transient gene expression systems is widely used for the functional characterization of genes and recombinant protein production in plant cells (Kapila et al., 1997). *Agrobacterium*, mediated transient gene expression (agroinfiltration), is based on infiltration of *Agrobacterium tumefaciens* culture, transformed to express the genes of interest, into intact plant leaves. The *Agrobacterium* assay, subsequently transfers a DNA segment, called transfer-DNA or T-DNA, into the target plant cells, thus inactivating the gene and mutation can then be analyzed. The T-DNA carries effector genes that allow transient gene expression (Hamilton, 1997; Vezina et al., 2009). This method has many advantages, including simple, rapid, and effective transformation compared with stable genetic transformations. Leaves of *Nicotiana tabacum* show reliable and high transformation efficiency for this specific method. In a syringe infiltration, an *Agrobacterium* suspension is infiltrated into the abaxial side of the leaves using a syringe *via* a needle hole, but without a needle. Using this method, the ability of various constructs can be simultaneously assessed on the same leaf. In such an assay, target gene is cloned into a T-DNA expression cassette driven by the CaMV 35S promoter, in a binary vector, into an *Agrobacterium tumefaciens* strain (Matoba et al., 2011; Whaley et al., 2011; Gleba et al., 2014; Zheng et al., 2012).

The binary vectors containing the T-DNA can carry inserts of up to over 100 kbp (Hamilton, 1997). T-DNA transferred to a plant cell will relocate to the nucleus, where its genes can be transcribed and expressed (Kapila et al., 1997). The majority of the plant cells in the infiltrated region express the transgene and the highest expression level, in 2-3 days after infiltration (Naomichi et al., 2016).

The main advantage of syringe infiltration is that different genes, either alone or in combination, can be expressed together in a single leaf (Liu et al., 2010). This assay has the potential to identify inserts, that affect the production of an autonomous replicating gene that can spread systemically, through a plant and subjected to several analyses as polimerase chain reaction (PCR), Southern blot, Northern blot, Western blot, immunoprecipitation and enzyme assays.

MATERIALS AND METHODS

Preparation of *Agrobacterium tumefaciens* competent cells

A single *Agrobacterium tumefaciens* strain LBA4404 colony was incubated in 5 ml LB overnight at 28°C. Two ml of the overnight culture was added to 50 ml of LB broth in a 250 ml flask and shacked vigorously (250 RPM) at 28°C until the culture grew to an OD_{600} of 0.5-1.0. The culture was then chilled on ice and the cells were collected by centrifugation at 2700 RPM for 5 minutes at 4°C. Then, the supernatant was discarded and the cells were resuspended in 1 ml of 20 mM $CaCl_2$ ice-cold solution. The mixture was kept on ice and 0.1 ml aliquots were prepared in pre-chilled Eppendorf test tubes. After being flash frozen by immediate immersion in liquid nitrogen, the *Agrobacterium* competent cells were kept at -80ºC for use.

Direct *Agrobacterium* transformation

Direct *Agrobacterium* transformation by freeze-thaw method was used to transform LBA4404 competent *Agrobacterium* cells strain carrying pART27-ERI-1 hairpin construct into new *Agrobacterium* competent cells strain C81C1. 6 *µg* of pART27 ERI-1 construct DNA were added to 100*µl* of the competent *Agrobacterium* and gently mixed. The mixture was first freezed in liquid nitrogen for 5 minutes. The cells were then thawed by incubating at 37°C water bath for 25 minutes. After adding 1 ml of LB medium, the mixture was incubated at 28°C for 3 hours with gentle shaking. The whole mixture of the transformed *Agrobacterium* suspension was spread on LB agar plate containing 100 mg/ml spectomycin and 50 mg/ml rifampicilin final concentration and incubated for 2 days at 28°C. Grown colonies were then picked up and inoculated in 5 ml of LB with the same concentration of the above mentioned antibiotics. Culture tubes were kept at 28°C over night under vigorous shaking. Small scale DNA preparation was performed as described written below in *Isolation of Plasmid DNA with Mini-Prep Method*. The collected plasmid was then back-transformed onto *E.coli.* DH5α strain as described, as follows, in *Transformation of E.coli competent cells (heat-shock method)*. This step was done in order to verify the presence of the pART27 ERI-1 construct in the selected transformed *Agrobacterium.*

Isolation of plasmid DNA with Mini-Prep method

The mini-prep method was used for small-scale preparations extracting DNA for restriction digests and to check the cloned inserts before attempting a higher scale extraction. The integrity of the plasmid DNA was checked by gel electrophoresis and ethidium bromide staining.

1.5 ml of overnight bacterial culture was transferred to an eppendorf tube and the bacterial cells were collected by centrifugation at 13200RPM for 2 min in a microcentrifuge. Bacterial pellet was resuspended in 100 *µl* of Solution I (25 mM Tris-HCl, pH 8, 10 mM EDTA, pH 8, and 50 mM glucose) and cells were vortex vigorously and incubated at room temperature for 5 min. 200 *µl* of fresh Solution II (0.2 N NaOH and 1% SDS) was added followed by a further incubation for 5 min at 4°C in order to lyses the bacterial cells and release the plasmids. After thorough mixing, 150 *µl* of Solution III (5 M potassium acetate, pH 5.5) were added in the tube, mixed and left for 5 min on ice to let the genomic DNA and cellular debris precipitate. The solution was centrifuged for 10 min at 13200RPM in a microcentrifuge and the supernatant was transferred to a new sterile tube. The mixture was then washed with neutral phenol and extracted with chloroform/isoamylalcohol (24:1). The nucleic acids were precipitated at room temperature for 2 min by the addition of 2 volumes of 100% ethanol. The DNA was pelleted after 10 min of centrifugation at 13200RPM and the pellet was washed with 70% ethanol. After air-dry the pellet was resuspended in 50 *µl* sterile deionised water and stored at -20ºC, the efficiency yield is 3-5 *µg* of DNA/ ml of bacterial culture.

Transformation of *E.coli* competent cells

Competent bacterial cells were transformed by a simple heat-shock procedure. An aliquot of competent cells was thawed on ice and ligation prep. was added, and the tube was gently shaked to mix the contents. The mixture was stored on ice for 20 min and then the tubes were transferred to a water bath at 42°C and left for exactly 90 seconds. After, the tubes were rapidly transferred to an ice bath and allowed to chill for 2 min. 400 *µl* of LB was added and cells were incubated at 37°C for one and a half hour. During this time the bacteria can recover and express the antibiotic resistance marker encoded by the plasmid. To maximize the efficiency of transformation, the cells could be gently agitated. The cells were concentrated by centrifugation for 1 min 13200 RPM and gently resuspended in about 30 *µl* LB and 30 *µl* X-gal (5-bromo-4-chloro-3-indolil-β-D-galactoside) which is converted by β-galactosidase into a blue compound. The transformed cells were spread over the surface of the LB plates containing the appropriate antibiotics (ampicillin) by use of a sterile bent glass rod grown overnight at 37°C. The recombinant cells were selected by their ability to grow on LB ampicillin plates, under a white/blue selection, where the white colonies were the recombinant ones.

RESULTS AND DISCUSSIONS

In this research direct *Agrobacterium (A. tumefaciens)* cells strain carrying the desired gene (pART27-ERI-1 hairpin construct) was transferred to plant cells.

Experimental part as well as pART27-ERI-1 hairpin constructruction, were performed in Plant Molecular Biology Laboratory of the Institute for Molecular Biology and Biotechnology (IMBB-FORTH, Greece), during my PhD research.

Greenhouse grown *Nicotiana tabacum* wild type was used for agrobacterium injection. Plants were grown in the greenhouse with air-conditioning system. 10-15 *µl* of the transformed *A. tumefaciens* contains the expression vector pART27 ERI-1 were incubated in 5 ml LB supplemented with 100*µg/ml* spectomycin and 100 *µg/ml* rifampicilin over night.

Figure 1. *Agrobacterium* injection. A sterile needle tip was used to wound the lower surface of the leaf prior *Agrobacterium* suspension to be infiltrated into the abaxial side of the leaves using a needleless syringe

Once the *A. tumefaciens* is inside the leaf, gene of interest will transform to a portion of the plant cells and the gene is then transiently expressed over the plant. Towards the preparation of *Agrobacterium* infiltration, the transformed cells were harvested at OD_{600} of 0.8-1.0 by centrifuging at 2800 RPM at 4°C. The pellet was resuspended with 5ml of MMA medium (MS salts, 10 mM MES pH 5-6), and 5 *µl* of 200 mM acetosyringone was added. The pellet was resuspended by gentle pipetting and incubated for more than one and a half hour at 28°C with gentle shaking. The cells were then collected by centrifuging at 2800 RPM at 4°C and washed twice with 5 ml cold 10 mM $MgCl_2$ .The pellet was then resuspended in 5 ml cold 10 mM $MgCl_2$ to give an absorbance at 600 nm of 0.2-0.3.

***Agrobacterium* injection**

Suspensions of preinfiltrated *Agrobaterium* in a blunt-tipped plastic syringe were forced into intact leaves still attached to the plant. A sterile needle tip was used to wound the lower surface of the leaf prior injection and by pressing the tip of the syringe against the surface of the leaf, an *Agrobacterium* suspension was forced against the wounded area of the leaf (Figure 1). After being agroinfiltrated, plants were kept in the lab at room temperature conditions. Any possible effect that can be observed from the phenotype of the plant will then be subsequent for plant DNA isolation. Confirmation of DNA integration can be done by performing polymerase chain (PCR) reaction or Northern Blot assay.

Nicotiana benthamiana and *Nicotiana tabacum* (tobacco) are preferably used as favourite model plants. Especially for a short life cycle, carries relatively large and easily infiltratable leaves that produce recombinant proteins at high levels and the leaf does not show necrosis upon infiltration with most *Agrobacterium* strains. In this experiment I used the green fluorescent protein gene (*GFP*)-transgenic *Nicotiana tabacum* plants, elaborated in Plant Molecular Biology Laboratory of the Institute for Molecular Biology and Biotechnology (IMBB-FORTH, Greece). The *GFP* is expressed temporary over 2-5 days, in the infiltrated area and *GFP* is visualized as bright green fluorescence under UV light. Following syringe infiltration, inducing ERI-1 gene, either alone or in combination, it is shown that can be expressed together in a single leaf (Figure 2).

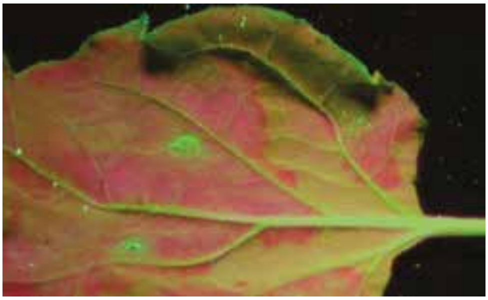

Figure 2. Inducing green fluorescent protein gene (*GFP*) and ERI-1 gene expression, in the infiltrated leaf area of transgenic *tabbaco* plant. *GFP* is visualized as bright green fluorescence under UV light

CONCLUSIONS

Most of the pART27 ERI-1 insert have been identified based on their ability to interfere with the local and systemic silencing of the green fluorescent protein gene (*GFP*) in GFP-transgenic *Nicotiana tabacum* plants in an agroinfiltration assay. The *GFP* is expressed temporary over 2-5 days, in the infiltrated area and *GFP* is visualized as bright green fluorescence under UV light. Following syringe infiltration, inducing ERI-1 gene, either alone or in combination, it is shown that can be expressed together in a single leaf. This assay has the potential to identify ERI-1 gene expression, that affect the production of an autonomous replicating gene that can spread systemically, through a plant and subjected to several analyses as polimerase chain reaction (PCR) for identification.

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THE ROLE OF BUFFER ZONES IN ENSURING THE COEXISTENCE OF GM AND NON-GM MAIZE

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Abstract

Cultivation of genetically modified (GM) maize MON 810 in the European Union, including Romania, requires specific management. The adventitious presence of genetically modified organisms in conventional crops, can affect their coexistence. Two field experiments with genetically modified maize (MON 810) and conventional maize (non-GM) were placed at A.R.D.S. Simnic- Craiova under the climatic conditions of the agricultural years 2011-2012 and 2012-2013. The rate of cross-pollination (highlighted by percentage of Xenia on the ear) was influenced by: the buffer zones, the isolation distance and the prevailing wind direction. The presence of buffer zones (ten row of Sudan Grass) has reduced the cross-fertilization rate by 40.5% in 2012 and by 20.0% in 2013. As a result, the use of buffer zones can be considered one of the important strategies for ensuring coexistence a the genetically modified maize with non-genetically modified maize, in the same area of culture.

Key words: *buffer zones, cross-pollination rate, GM and non-GM maize, percentage of xenia.*

INTRODUCTION

Biotechnology has major implications in agriculture, food production and processing, and medicine.

Genetically modified maize MON810 resistant to *Ostrinia nubilalis*, obtained by transgenesis is authorized for cultivation in the EU, including Romania.

According to the Ministry of Agriculture and Rural Development (MADR 2017), in 2007, the Romanian farmers cultivated 332.5 hectares of genetically modified maize, after which the surface increased to over 6,100 hectares in 2008, and then the surfaces continuously decreased yearly.

Since 2016, farmers have completely abandoned MON 810, probably because of the very complex rules on product traceability.

To ensure that the development of biotechnology and GMOs, in particular, is made safe, the EU has set up a legal framework comprising several legislative rules.

Coexistence refers to certain rules that provide farmers with the practical possibility to opt for conventional, organic or GM crops, in line with legal obligations for labeling and purity standards (EU, 2006).

Recommendation 2010/C. 200/01 on guidelines for the development of national coexistence measures to avoid the adventitious presence of GMOs in conventional and organic crops, was adopted on 13 July 2010 (MADR, 2010). According to this recommendation, each Member State, individually, to achieve as low adventitious presence of genetically modified organisms (below 0.9%) in conventional crops and other crops, must take into account their specific needs at regional and local level on the cultivation of GMOs.

In Romania, it was adopted Order no. 61/2012 which provides as the main coexistence measure for GM maize with conventional maize, ensuring minimum separation distances of 200 m from neighboring pollen sources (MADR, 2012).

Many field experiments have been carried out in recent years to collect technical data under real coexistence conditions (Marceau et al., 2013, Popescu et al., 2010, Popescu et al., 2011), however, this data is not always taken into account in current legislation.

The adventitious presence of GMOs in conventional maize crops is mainly due to cross-pollination (pollen-mediated gene flow).
Urechean and Bonea (2017), previously reported that the best ways to reduce the GM's adventitious presence in conventional maize crops (below 0.9%) are: delayed sowing (so that there is no coincidence in flowering); a minimum isolation distance of 20 m for consumption maize and 100 m for the lots obtaining hybrid seed maize, and the use of buffer zones.
In this work, our purpose is to evaluate the role of buffer zones in reducing the cross-pollination rate and in the adventitious presence of GMOs in conventional maize crops under real culture and coexistence conditions.

MATERIALS AND METHODS

Two field experiments with genetically modified maize and conventional maize were placed at Agricultural Research and Development Station (A.R.D.S.) Simnic in the climatic conditions of the agricultural years 2011-2012 and 2012-2013
Experience I with an area of 4560 sq meters, where the sweet maize - Deliciul verii (pollen receiver) was sowed between two plots of genetically modified maize - MON 810 (pollen donor) at a distance of 100 m (West and East - 2012 and North and South - in 2013).
Experience II with an area of 4740 sq meters, where the sweet maize - Deliciul verii was sown between two plots of genetically modified maize - MON 810 at a distance of 100 m from it (West and East - 2012 and North and South - in 2013), with buffer zones (10 rows of Sudan Grass) halfway through the isolation distance (Photos 1, 2, 3, 4).
From a climatic point of view, the agricultural year 2011-2012 was an extremely dry year with an excessive pedological drought accompanied by extreme drought and extreme heatduring the blooming period. The agricultural year 2012 - 2013 was, in general, a favourable year for maize crops
For the study of the cross-pollination rate between two types of maize with different colour of the grains, the percentages of xenia were calculated (Watanabe et al., 2006) (Photo 5).

Photo 1. Aspects from experimental fields: MON 810, 2012

Photo 2. Aspects from experimental fields: Deliciul verii, 2012

Photo 3. Aspects from experimental fields: MON 810, 2013

Photo 4. Aspects from experimental fields: Deliciul verii, 2013

Photo 5. Xenia (MON 810 x Deliciul verii)

From every experience, from conventional maize parcels (Deliciul verii) there have been taken 5 consecutive ears from the middle part of the every row and the average of xenia has been determined for them. All the results were expressed as the average per ten row ±Standard error (SE).

The coefficient of variance calculated after Saulescu N.A and Saulescu N.N. (1967).

RESULTS AND DISCUSSIONS

In 2012, in Experience I, when the two plots (West 100m and East 100m) were sown without buffer zones, the average percentage of xenia was the same 0.42% respectively, but higher values are found on R3 and R5 of the plot in the West and on the R7-R10 interval of the East plot (the first rows from the MON 810 plot) (Table 1).

The variability of the percentage of xenia was identical, with very high values in both plots (CV = 35.1%).

Because there were no strong winds during the flowering period, we can say that only the local air currents could have influenced the percentage differences from row to row within the same plot.

In Experience II, when the two plots (West 100 m and East 100 m) were sown with a buffer zone of 10 rows of Sudan Grass, the average percentage of xenia was significantly lower compared to Experience I (0.26% for W and 0.24% for E) (Table 1).

Table 1. Variation of the percentage of xenia in sweet maize (Deliciul verii) cultivated at 100 m distance away from the MON 810 (pollen donor) with or without buffer zones, in 2012

No. row	Experience I		Experience II	
	W 100 m	E 100 m	W 100 m	E 100 m
R1	0.4	0.4	0.4	0.2
R2	0.4	0.2	0.3	0.2
R3	0.8	0.2	0.3	0.2
R4	0.4	0.5	0.3	0.2
R5	0.5	0.3	0.3	0.2
R6	0.3	0.4	0.2	0.2
R7	0.4	0.6	0.2	0.3
R8	0.4	0.5	0.2	0.3
R9	0.3	0.5	0.2	0.3
R10	0.3	0.6	0.2	0.3
Average	0.42	0.42	0.26	0.24
Standard error (±SE)	0.05	0.05	0.02	0.02
Coefficient of variation (CV%)	35.1	35.1	26.9	21.5
Average experience	0.42		0.25	
% reduction	40.5			

W= West; E= East

Higher values of the % of xenia were recorded on the R1-R5 interval for the West plot and on the R7-R10 interval for the East plot, as expected with them being the rows closest the pollen donor (MON 810).

A large variability in the percentage of xenia was observed in both plots (CV = 26.9% and CV = 21.5%), but less than in Experience I.

Comparing the average of the two plots (West and East) in Experience I and Experience II (Figure 1), it is very clear that the presence of buffer zones had an essential role, almost halving the average of the percentage of xenia (- 40.5%).

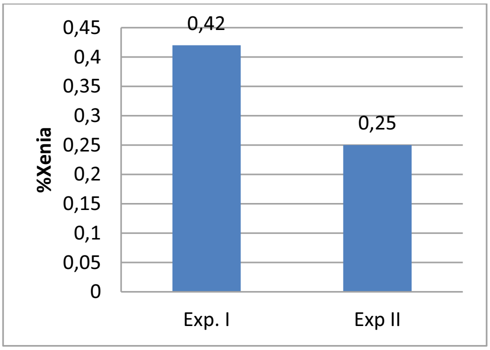

Figure 1. Reducing the percentage of xenia due to presence of buffer zones, 2012

Also, Messéan et al. (2009) reported that the use of buffer zones in combination with isolation distance results in a lower cross-pollination rate.

In 2013, in Experience I, in the two plots that were sown without buffer zones, the average percentage of xenia was very close, respectively 0.41% in South and 0.44% in North (Table 2).

Table 2. Variation of the % of xenia in sweet maize (pollen receptor) cultivated at 100 m distance away from the MON 810 (pollen donor) with or without buffer zones in 2013

No. row	Experience I		Experience II	
	S 100 m	N 100 m	S 100 m	N 100 m
R1	0.6	0.3	0.6	0.2
R2	0.5	0.4	0.5	0.4
R3	0.4	0.3	0.6	0.2
R4	0.4	0.2	0.2	0.5
R5	0.4	0.4	0.3	0.4
R6	0.4	0.5	0.2	0.3
R7	0.4	0.5	0.2	0.1
R8	0.4	0.6	0.2	0.2
R9	0.4	0.6	0.3	0.5
R10	0.2	0.6	0.3	0.6
Average	0.41	0.44	0.34	0.34
Standard error (±SE)	0.03	0.04	0.05	0.05
Coefficient of variation (CV%)	24.2	32.5	48.4	48.4
Average experience	0.43		0.34	
% reduction	20.0			

S = South; N = North

Higher values of the percentage of xenia were recorded on the first rows (R1 - R2) of the southern plot and on the last rows (R6 - R10) on the northern plot, thus on the nearest rows to the MON 810 plot.

The percentage of xenia being somewhat bigger in the North towards the middle of the plot, we can say that this year, only local air currents (which were from North to South) could have influenced a little the cross-pollination rate because the wind speed was small.

The coefficient of variation (CV%) is high for both plots, but something more homogeneous for the plot in the South.

In Experiment II, where the distance between the donor and the pollen receiver was 100 m in the South and North with a buffer zones of 10 rows of Sudan Grass in the middle of the isolation distance (50 m), the average percentage of xenia was lower compared to Experience I (0.34% both in the South and in the North - Table 2).

Higher values were recorded for the southern plot over the R1-R3 interval and in the northern plot for the R9-R10 interval which was expected, these being the first rows closest to the pollen donor (MON 810).

The average values, the standard deviation and the coefficient of variation were identical for both plots.

Comparing the Southern and North plots of Experience I and Experience II (Figure 2), we can say that the presence of protective curtains (10 rows of Sudan Grass) played an essential role, reducing the degree of contamination by 20.0% (in 2013).

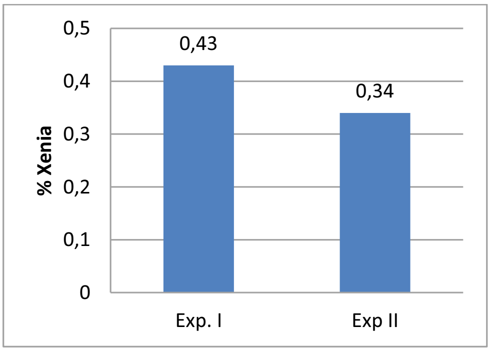

Figure 2. Reducing the percentage of xenia due to presence of buffer zones, 2013

Similar observations on the cross-pollination rate in maize for the distance of 100 m, respectively between 0.1 and 0.4% were reported by Messeguer et al. (2006), Pla et al. (2006) in Spain and by Baltazar et al. (2015) in Mexico.

Comparing the experimental years (2012, 2013), we can say that the predominant wind direction in the area of culture (during pollination period) as well as the intensity and frequency of the local air currents, significantly changes the average percentage of xenia.

Our results on the influence of the wind direction are consistent with those obtained by Weber et al. (2007), who observed that speed and direction of windmay vary between areas and years, so they can not be reliably embedded in strategies to avoid cross-pollination.

Other authors consider that the coincidence of flowering between donor and receiver plants as well as local wind conditions are other major factors of influence in plant coexistence (Hüsken et al., 2007; Warwick et al., 2009).

CONCLUSIONS

The rate of cross-pollination of conventional maize (Deliciul verii) with genetically modified maize (MON 810) was determined by: the presence or absence of buffer zones, the isolation distance and the predominant wind direction during the pollination period.

In 2012, at 100 m West and East from MON 810, the average of the percentage of xenia was 0.42% (Experience I) and 0.25% (Experience II) and in 2013 at 100m South and North distance from MON 810, the average of the percentage of xenia was 0.43% (Experience I) and 0.34% (Experience II)

The presence of buffer zones has led to a reduction in the cross-pollination rate by 40.5% in 2012 and by 20% in 2013.

As a result, the use of buffer zones can be considered as one of the important strategies for ensuring coexistence in the same area for GM maize and non-GM maize.

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COMPARISON BETWEEN PRISTINE PURE-BEECH STAND AND MIXED BEECH-OAK STAND USING AN UNMANNED AERIAL VEHICLE

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Abstract

Percentage Canopy Cover (PCC) has an important role in forest inventories and, as an ecological indicator, can also determine light conditions, habitats or microclimates. Canopy gaps, which are the result of natural causes or anthropogenic activities, have an important influence on the incoming light that controls how regeneration and the understory biota evolve. Unmanned aerial vehicle (UAV) remote-sensing has become an efficient, increasingly used alternative method, with the advantage of spatially-explicit mapping of the smallest canopy gaps. Following endeavors for 'near-natural' silviculture vertical analyses of tree crowns could provide substantial information for a consecutive management. In this paper, UAV-based measurements of canopy cover, tree crowns and canopy gaps are made in a pure-beech pristine forest and a managed old mixed beech with oak stand. Very high resolution images (3-4 cm/pixel) were acquired and assembled into orthomosaics. The canopy gaps and the upper tree crowns were delineated and the PCC, area (A), perimeter (P) and position of each tree crown were determined. For canopy gaps, calculations of: area (A), perimeter (P), perimeter/area (P/A), circularity (C) and gap shape complexity index (GSCI) were made. The results represent the mentioned structural determinations and the spatial distribution of trees and gaps in both study plots, results which are further compared and discussed.

***Key words**: UAV, PCC, canopy gaps, pristine forest.*

INTRODUCTION

Forest canopy cover, also known as the crown cover or Percentage Canopy Cover (PCC) (Pretzsch, 2009) represents the proportion of the ground covered by the projection of the tree crowns (Jennings et al., 1999) and is an important part of forest inventories. Also, canopy cover is useful as an ecological indicator that can show different habitats, microclimates or light conditions (Korhonen et al., 2006).

In forest canopies, gaps have an important role, especially for the regeneration and the understory biota diversity perspectives (Getzin et al, 2014). Canopy gaps are made by natural causes (windthrow, insects, disease etc.) or by humans in the thinning process and have an important influence of the incoming light that controls how the understory layer will evolve (Proulx, Parrott, 2008). The size, the shape complexity and the spatial distribution of the gaps are key elements in the establishment of the future formations (Koukoulas, Blackburn, 2004; Koukoulas, Blackburn, 2005).

Traditional ground-based methods are difficult to manage efficiently when measuring the ground projection of tree crowns and canopy gaps in plots with sizes of over one hectare (Proulx, Parrott, 2008). Furthermore, if there are desired determinations of the smallest dimensions of canopy gaps and tree crowns, then a very efficient method is mapping by drone remote-sensing (Getzin et al., 2014). And since the diversity of the understory species is related to the direct light radiation, it is important to also monitor the smallest gaps (Montgomery, Chazdon, 2002; Moora et al., 2007).

The main strength of using UAV remote sensing represents the very high resolution of the images, provided at low-cost, that can be used to accurately map gaps under one square meter, which can be further used in assessing

the understory biodiversity (Getzin et al., 2014).
In this work, measurements of canopy cover, tree crowns and canopy gaps are made with the use of an UAV in an old mixed beech with oak stand for further purposes as: improved forest management decisions; spatio-temporal dynamic of forest; biodiversity assessment; comparison with other unmanaged/managed forests etc.

MATERIALS AND METHODS

Study area

The study area is represented by 2 plots in different stands. First plot is located in the State Forest „Kranzberger Forst”, compartment Rehbuckel („Kleiner Spessart”), near Freising, Bavaria, Germany (Figure 1) with a total area of 7.0 ha. The plot is located in a stand dominated by European beech (*Fagus sylvatica* L.; age approx. 140 years) and common oak (*Quercus robur* L.; age around 170 years) on a loamy soil. The stand was managed for decades in a selection system for crop trees (mostly oak) but by the time beech gained space due to stronger competition. At present harvest of mature trees and release for the best individuals takes place. Natural regeneration of beech is wide spread.

Figure 1. Mixed beech and oak stand (red area) - „Kleiner Spessart” (Source: Google)

The second plot is located in „Izvoarele Nerei” Nature Reserve, Caraș-Severin County, SW Romania. The Reserve is included in the Semenic - Cheile Carașului National Park and proposed to be included in UNESCO Biosphere List, representing one of the largest pure beech virgin forest in Europe with an area of over 5000 ha. The stand (Figure 2) has a total area of 3.9 ha and is represented by pure beech trees with an average age of over 300 years.

Figure 2. Pure beech stand (red area) - „Izvoarele Nerei” Reserve (yellow line) (Source: Google)

Aerial images

Images of very high resolution were acquired with a rotary-wing UAV - DJI Phantom 3 Professional (Figure 3). The flights took place at 100 m altitude and were made during noon time (to avoid as much as possible the shading effect). The total flight area included a buffer area used in order to have good quality images in the vicinity of the stand borders. The details of the flights are presented in Table 1.

Table 1. Flight details

Plot	No. of photos (#)	Date of acquisition	Planned overlap of photos (%)	Flying altitude (m)	Ground resolution (cm/pixel)
Kleiner Spessart	437	October 2016	86	100	3
Izvoarele Nerei	286	June 2016	85	100	4

Figure 3. UAV equipment - DJI Phantom 3 Professional

Data processing and calculations

Based on the captured images, orthomosaics were assembled (Figure 4) using Agisoft Photoscan Professional software. The orthomosaics were reprojected into the national coordinate systems (ETRS1989_UTM32_M -

Germany; Stereographic 1970 - Romania). Corrections based on GPS ground control points followed by shifting and filtering of each orthomosaic were made.

The canopy gaps and the upper tree crowns were delineated throughout a manual digitizing process using ArcGIS software in order to create geospatial polygons. Based on resulted polygons, the following: canopy cover, area and perimeter for each tree crown and the positions of the trees (as being the centroid of the crown polygons) were calculated. Regarding the properties of the canopy gaps, determinations of: area (A), perimeter (P), perimeter/area (P/A), circularity ($C = 4\pi A/P^2$) and gap shape complexity index ($GSCI = P/\mathrm{sqrt}(4\pi A)$) were made. While the first four are basic measurements, the GSCI is an important index for forest gaps (Koukoulas and Blackburn, 2004) and has significant correlation with species richness (Getzin et al., 2012).

Furthermore, a pattern analysis regarding the spatial distribution of gaps and tree crowns was made.

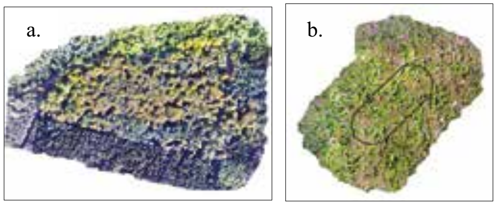

Figure 4. Orthomosaic of the study stand assembled with Agisoft Photoscan Professional software: a. „Kleiner Spessart" stand; b. „Izvoarele Nerei" stand

RESULTS AND DISCUSSIONS

Structural determinations

In „Kleiner Spessart" stand of mixed beech and oak, a canopy cover of 74.49% (Figure 5) from the total area of 7.0 ha was calculated, a total number of 201 canopy gaps and 675 trees from upper layer (Figure 5) were identified.

In „Izvoarele Nerei" pure beech stand, a canopy cover of 81.57% (Figure 6) from the total area of 3.9 ha was calculated, a total number of 248 canopy gaps and 704 trees from upper layer (Figure 6) were identified.

A summary of the determinations is presented in Table 2.

Table 2. Summary of measurements and determinations

Plot	Type	# per ha	A (m^2)			P (m)			P/A			C			GSCI		
			Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Kleiner Spessart	Gaps	29	1.8	89.5	1457.1	6.1	56.4	696.3	0.30	1.42	4.07	0.03	0.42	0.91	1.05	1.78	5.87
	Upper tree crowns	96	6.4	77.8	274.3	9.6	35.9	78.1	-	-	-	-	-	-	-	-	-
Izvoarele Nerei	Gaps	64	0.2	20.2	388.28	1.7	24.1	256.2	0.55	2.79	8.78	0.07	0.43	0.92	1.04	1.70	3.67
	Upper tree crowns	180	3.4	45.3	149.9	6.9	27.6	62.1	-	-	-	-	-	-	-	-	-

Table 3. Spatial analysis of plots

Plot	Pattern type		Z-score		Observed Mean Distance		Expected Mean Distance		P-value	
	Gaps	Crowns	Gaps	Crowns	Gaps	Crowns	Gaps	Crowns	Gaps	Crowns
Kleiner Spessart	dispersed	dispersed	2.81	13.56	11.65	7.58	10.55	5.96	0.004964	0.000000
Izvoarele Nerei	dispersed	dispersed	7.09	24.33	7.98	5.54	6.46	3.75	0.000000	0.000000

Figure 5. Upper tree crowns and the overall canopy cover of the stand „Kleiner Spessart”

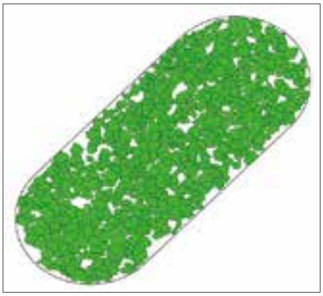

Figure 6. Upper tree crowns and the overall canopy cover of the stand „Izvoarele Nerei”

Spatial analysis

Regarding the spatial analysis, the average nearest neighbour algorithm reported a dispersed pattern for both plots (Table 3) when looking to canopy gaps (Figure 7) and upper layer tree crowns (Figure 8).

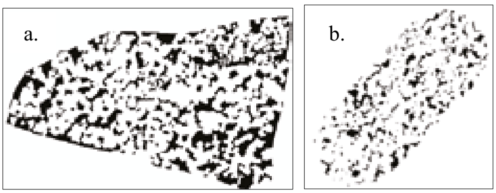

Figure 7. Spatial distribution of the delineated gaps: a. „Kleiner Spessart” plot; b. „Izvoarele Nerei” plot

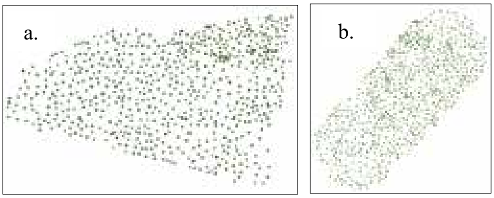

Figure 8. Spatial distribution of the highest trees: a. „Kleiner Spessart” plot; b. „Izvoarele Nerei” plot

CONCLUSIONS

Using UAVs to measure gaps and tree crowns, especially when they have small dimensions (e.g.: 0.2 m^2/gap in this study) can be very effective compared to terrestrial mapping which is a lot more time-consuming or to satellite images that have coarser resolutions (≥50 cm/pixel). In this study, measurements for the canopy cover, tree crowns and canopy gaps with an UAV were successfully accomplished.

Looking at the GSCI values (the value of 1.00 is the minimum one and represents 0% shape complexity - similar to a circle), canopy gaps in the „Kleiner Spessart” plot have values ranging from 5% to 487% in shape complexity and those in „Izvoarele Nere” plot have values ranging from 4% to 267% in shape complexity.

Considering that numbers above 1.732 for GSCI are classified as high complexity (Jenks and Caspall, 1971), 40% of the canopy gaps in the „Kleiner Spessart” plot are in this case. Related to the recent studies where GSCI is significant correlated with the species richness (Getzin et al., 2012) and could be used to assess biodiversity, some of the current outliers and the +78% shape complexity of the mean GSCI value should indicate that species richness level is above normal for the „Kleiner Spessart” plot. The forest management activities in this plot could be the cause of the regularly distributed patterns of canopy gaps and trees.

Further attempts could focus on an evaluation of GSCI to enhance forest management for biodiversity or to find references.

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MODAL ANALYSIS OF FIELD SPRAYER BOOM DESIGN FOR DIFFERENT MATERIALS

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Abstract

During spraying, vibrations of sprayer booms, which take place because of unevenness of soil and tractor velocity, affect the success of spraying. When vibrations increase, this situation cause disruption of spraying pattern and leaf coverage. Thus, the yield of agricultural product drops. Manufacturers desire durable field sprayer boom during the spraying operation. To do this, they manufacture various suspension systems to damp vibrations. Beside vibration, weight of the field sprayer boom is very important. Most manufacturers start to use aluminum due to its lightness and corrosion resistance reasons. In this study, for the same field sprayer boom design, materials of mild steel and aluminum are compared via modal analysis with finite element analysis in a CAD Program. According to results of modal analysis field sprayer boom which made of mild steel has 7.4 Hz natural frequency under its own weight that applied from own center of gravity. And also same field sprayer boom which made of aluminum has 8.15 Hz natural frequency under its own weight from own center of gravity. The costs, weights, and manufacturability of materials are also compared.

Key words*: modal analysis, field sprayer boom, material selection for field sprayer boom, manufacturability.*

INTRODUCTION

Field sprayer boom is one of most used and manufactured plant protection machines in Turkey. Day by day, these machines are developed by researchers, manufacturers, also farmers. During the application, due to tractor velocity and soil unevenness, field sprayer boom vibrations occur. To damp these vibrations; dampers, springs, piston-cylinder with cushioning are also used. However, in Turkey, most of manufacturers do not study on damping the vibrations, they only try to reduce boom weight. To do this some manufacturers prefer aluminum because of lightness and corrosion resistance against pesticides. However, vibration effects of aluminum also must be searched. Kennes et al. (1998) investigated a tractor mounted field sprayer with a spray arm width of 12 m by the finite element method. The vibrations from the field were observed at a frequency of 0.3 Hz and 3 Hz. Vibration amplitude was also 2 cm in width. Nielsen and Sorensen (1998) investigated the suspension of an active field sprayer boom in their work. They made active and passive suspensions for simulation and compared the results. The passive suspension system consists of a construction itself and a spring that acts as a damping damper. A hydraulic piston and a spring are also installed for the active suspension. According to results, field sprayer boom must include both active and passive suspension systems.

Borchert and Schmidt (2015) studied on the characterization of the horizontal axis motion of tractor mounted field sprayers in their work. After the mathematical model of the field sprayer boom was discovered, this mathematical model was analyzed in Matlab /Simulink program. They also operated the related field sprayer boom on a vibration machine at 0-3 Hz and measured the working amplitude. Then observed data was a standard deviation of 1.4% between the maximum amplitude and the actual amplitude given by the Matlab program.

Koç (2017) solved the structural analysis of a field sprayer with a field sprayer boom width of 21 m in his work with finite element method. For the same geometric design but different materials (aluminium and steel) were used.

According to fatigue analysis results, it is understood that aluminum material is better than mild steel.
Aim of this study, is to study modal analysis of a field sprayer boom for same geometric design but different in materials (mild steel St 37 vs. 6061 aluminum) by the aid of finite element method. By this way, for same geometrical design aluminum and steel field sprayer booms' vibration characteristics are compared. Beside this result, manufacturing costs of both materials are determined and compared with each other.

MATERIALS AND METHODS

Modal analysis is used to determine vibration characteristic of a body under a load. Modal Analysis can be applied both Mathematically and Experimentally also in 3D CAD programs. General vibration characteristic of a system can be defined as below:

$$m\ddot{x} + c\dot{x} + kx = F$$

One of mostly manufactured field sprayer boom's construction is determined. The field sprayer boom has no damping element on it. Therefore, mathematical defining of undamped free vibration system can be as below:

$$m\ddot{x} + kx = 0$$

To solve the differential equation, C and s as coefficient of differential equation and x is function of t (Rao, 2004).

$$x(t) = Ce^{st}$$

From here:

$Ce^{st}(ms^2 + k) = 0$ and $Ce^{st} \neq 0$

$$(ms^2 + k) = 0$$

$$s_{1,2} = \pm\sqrt{-\frac{k}{m}} = \pm i\omega_n \text{ (rad/s)}$$

$$\omega_n = 2\pi f_n$$

In modal analysis with finite element method, general mathematical defining of a field sprayer boom can be defined as above.

To apply modal analysis in CAD program, firstly one side of boom (for easily resolving the problem) with 16 m width field sprayer was measured geometrically. Then modelled and assembled in 3D CAD program Autodesk Inventor 2017 (Figure 1). In field sprayer boom 40*40*3 rectangular profiles are used (both in aluminum and mild steel). After modelling, both materials mild steel and aluminum 6061 were chosen for analysis.

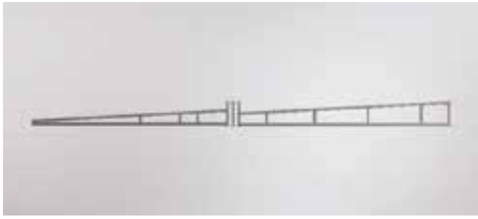

Figure 1. Modelling of field sprayer boom geometrically

Aluminum 6061 and mild steel is applied to geometric design, and then weights are calculated as shown in Table 1.

Table 1. Weights of different materials for same geometric design

Properties	Aluminum Boom	Mild Steel Boom
Weight (kg)	13,164	38,274
First Section Weight (kg)	5,672	16,492
Second Section Weight (kg)	7,492	21,782

After modelling and assembling field sprayer booms the Autodesk Inventor 2017, in stress analysis module. For analysis, modal analysis is chosen and then fixed points are marked which are joint of second section.
The weights (shown Table 1) are converted to loads and applied from each centre of gravity of each boom section.
In this study, only vertical forces are evaluated. Because horizontal inertial forces changes during a time period also field sprayer booms' construction are designed to resist vertical forces.
After meshing, simulating and solving are started and results are compared. Beside the modal analysis results, manufacturing costs are also calculated and compared. In region, manufacturing costs are as below.

Table 2. Costs of raw materials and manufacturing

Properties	Aluminum	Mild Steel
Raw material cost ($/kg)	3,75	0,625
Manufacturing cost ($/kg)	1,5	1,25

RESULTS AND DISCUSSIONS

Manufacturability Evaluation of Different Materials

According to results of weight and manufacturing costs, total costs of aluminum boom and mild steel boom are calculated as Table 3. Total manufacturing costs of aluminum is higher than steel. As known, thermal expansion coefficient of aluminum is higher than steel. Because of that reason, it is difficult welding of aluminum. Excessive heat can cause welding distortion whether cooler blocks is not used. Therefore, the difficulty of a process increase, cost of process of the manufacturing also increases.

Table 3. Costs of raw materials and manufacturing in region

Properties	Aluminum	Mild Steel
Raw material cost ($/kg)	3,75	0,625
Manufacturing cost ($/kg)	5,75	1,25
Total weight (kg)	13,164	38,274
Total cost ($/kg)	125,058	71,76375

Aluminum has better corrosion resistance than mild steel. Manufacturers use steel joints for aluminum field sprayer boom, because of hardness differences joints can enlarge holes of aluminum plates, which are located end of second section.

Modal Analysis Evaluation

In Figures 2 and 3, modal analysis results of mild steel and aluminum are shown.

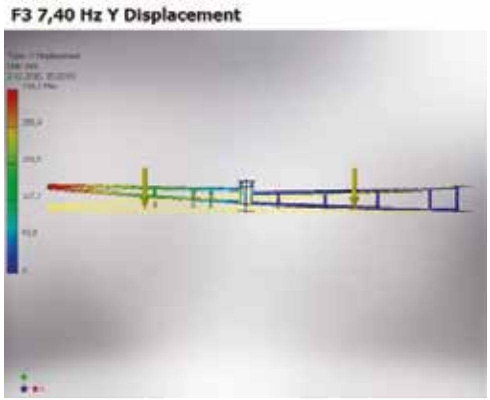

Figure 2. Modal Analysis Results of mild steel

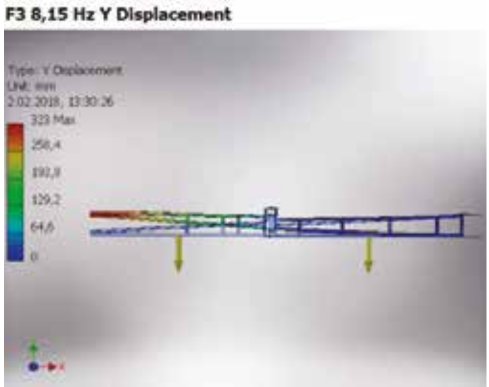

Figure 3. Modal Analysis Results of aluminum

According to results; mild steel has 7,40 Hz natural frequency under own load. Beside this, mild steel has 8,15 Hz natural frequency as shown in Table 4.

Table 4. Costs of raw materials and manufacturing

Properties	Aluminum	Mild Steel
Natural Frequency (Hz)	8,15	7,4
Total Weigh of Boom (kg)	13,164	38,274
Total Load (N)	129.138	375.467

Under own loads booms natural frequency differs. Although mild steel field sprayer is heavier than aluminum field sprayer boom, natural frequency of mild steel field sprayer boom is lower than natural frequency of alumium field sprayer boom which means under own load mild steel field sprayer vibrates less than aluminum field sprayer boom. Therefore spray pattern of pesticide that applied from mild steel field sprayer boom is better than aluminum field sprayer boom.

CONCLUSIONS

According to the results of the modal analysis of two different materials, it is understood that aluminum boom (8,15 Hz) can vibrate more than steel boom (7,4 Hz) under their own loads. Beside the vibration characteristics of these booms, manufacturing costs are also important. As in results, cost of steel boom is approximately half of the aluminum boom. However, it is not easy to weld aluminum boom because of high thermal expansion, heat transfer coefficient and low melting temperatures. For connecting two sections with each other, steel pins are used. Because of hardness difference (for steel pin and aluminum boom), it is not proper to use these materials together. Whether aluminum booms are preferred for manufacturing spraying booms, vibrating damping elements must be used to damp excess vibrations.

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EVALUATION OF TILLAGE SYSYEMS ON SOIL FUNGUS MICROFLORA UNDER WINTER WHEAT CULTIVATION

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Abstract

Soil fungus microflora promotes aggregation and has an important role in the transformation of relatively variable plant nutrient sources such as nitrogen, phosphorus, and sulfur and in making them useful for the plant. The effect of soil tillage treatments on soil fungus microflora is not fully known. This study was carried out in semi-arid climatic conditions in Diyarbakır in order to investigate the effectiveness of conventional tillage, reduced tillage and direct seeding practices on soil fungus microflora in the 2016-2017 winter wheat cultivation period. Soil samples taken from the rhizosphere of the wheat plant were transferred to nutrient agar and potato dextrose agar medium after dilution in order to determine the changes in the number of soil fungi in detail during the sowing time and flag leaf phenological period. As a result of the study, it was determined that different tillage practices do not make a statistical difference on the soil fungus microflora. However, unlike the soil samples taken in sowing period, it was determined that the number of fungi increased significantly in the parcels subjected to direct seeding during the flag leaf phenological period.

Key words: *soil fungus microflora, wheat, soil tillage, no-till.*

INTRODUCTION

Because microorganisms mediate many processes that support agricultural production, soil microbial activity is important for sustainable agriculture (Lupwayi et al., 1998). Fungi, bacteria and actinomycetes are the main soil microorganisms. Soil microorganisms and enzyme activity are important indicators of soil quality (Pajares et al., 2011). As is known, soil microorganisms and enzyme activity can activate potential soil constituents, which in turn helps increase product yield. Activities that act on soil physicochemical character, such as soil processing methods, directly affect microorganisms and enzyme activity in the soil (Ji et al., 2014). Soil fungus microflora promotes aggregation and has an important role in the transformation of relatively variable plant nutrient sources such as nitrogen, phosphorus, and sulfur and in making them useful for the plant (Shannon et al., 2002; Ghorbani et al., 2008). The soil organic matter promoted by fungal activity increases aggregate stability, cation exchange capacity, water retention capacity, water absorption rate and soil porosity (Nannipieri et al., 2003; Yin et al., 2010).

The effects on soil microorganisms of modern agriculture are quite complex. But understandding them is important for effective and sustainable management of agricultural ecosystems (Buckley, Schmidt 2001).

The widespread adoption of tillage methods in agricultural practice has the potential to increase soil microbial biomass and activity and the specific effects on fungal microorganisms are not fully known. Potential effects of soil tillage methods on increasing soil microbial biomass and activity and specific effects on fungal microorganisms are not fully known (Franzluebbers et al., 1995; Feng et al., 2003; Ng et al., 2012).

Soil degradation has now become an important environmental problem that limits agricultural sustainability throughout the world and reduces the production capacity of the land.

The soil degradation problem is the result of the deterioration of one or several of the physical, chemical and biological soil properties that constitute the soil quality parameters. The effects of different tillage methods on the physicochemical and biological properties of the soil are also quite different.

Among the tillage systems, conventional tillage is a tillage system in which most of the residue is buried in the soil, less than 15% of the residue is left on the soil surface after sowing, and heavy machine traffic is applied. Mouldboard plough is the main equipment of conventional tillage system. Conservation tillage method is a tillage system in which at least 30% of the soil surface is covered with plant residues after planting or in the critical erosion period (Köller, 2003).

In conventional systems, intensive and deep tilling of the soil results in numerous adverse effects on the physical, chemical and biological properties of the soil, and significant improvements in both environmental and soil quality parameters are obtained in conservative soil treatments and direct seeding systems. In this point, it is important to determine the level of soil cultivation and planting methods on the number of soil fungus in terms of high yield and healthy plant growth (Treonis et al., 2010; Wall et al., 2012; Zhang et al., 2015).

In the year 2016, approximately 1.2 million hectares of wheat are sown in the Southeastern Anatolia Region which corresponds to 15.79% of the total wheat cultivation area of Turkey and it is the third most important production area after Central and Western Anatolia (TÜİK, 2017a, b). In wheat cultivation, especially after the corn harvest, the conventional tillage practices are still widespread in the region.

In this study, it was aimed to determine the effect of conventional tillage, reduced tillage and direct seeding on the number of soil fungal microorganisms, which is a major effect during sowing and flag leaf penology in winter wheat growing.

MATERIALS AND METHODS

The study was carried out within the period of 2016-2017 wheat cultivation season at Dicle University Research Farm in Diyarbakır province located in Southerneast of Turkey (37°53'22'' latitude N, 40°16'38'' longitude E, 670 m above sea level). The study carried out on trial plots of 10 x 14 m, was planned in completely randomized parcel design with three replications. The study field has a clay (C) texture in 0-20 cm surface layer, consisting of 56.1% clay, 39.1% silt and 4.8% sand with pH of 7.3 and organic matter content of 1.0%. A semi-arid climate prevails in the study area. Three different tillage systems were applied in the study (Table 1) for wheat cultivation: conventional tillage (CT), reduced tillage (RT) and direct seeding (NT).

Table 1. The systems and the treatments

Systems	Treatments
Conventional Tillage (CT)	• Stubble chopper • Mouldboard plough • Disc harrow (2 times) • Scraper • Seeding
Reduced Tillage (RT)	• Cultivator • Disc harrow (2 times) • Scraper • Seeding
Direct Seeding (NT)	• Stubble cutting • Seeding

Commonly used bread wheat variety in the region was used as wheat seed. Irrigation of the wheat was carried out by sprinkler irrigation system. For the fertilizer requirement of wheat plants, 15-18 kg N da^{-1}, 8 kg P_2O_5 da^{-1} and 15-20 kg K_2O da^{-1} were given. No pesticides were used during the growing season. In the parcels that contains excessive amount of weeds, only the weeds were removed by hand and removed from the parcel.

Soil samples were obtained from two different periods; during the sowing and in the flag leaf phenological period of winter wheat. Accor-ding to the simple random sampling method in each parcel, 27 soil samples were taken for each period in three replicates.

Soil samples were collected from 5-20 cm depth of points determined according to simple random sampling method with the help of a shovel, and is placed in a polyethylene bag (Bora, Karaca, 1970; Saygılı et al., 2006). The obtained soil samples taken from rhizosphere layer were dried in room conditions and then passed through a 2 mm sieve to prepare for dilution technique. Before studying, a preliminary study performed to determine the amount of dilution suitable for determining the number of soil microbial populations in the dilution technique; 1/100000 and 1/1000000. In addition to Nutrient Agar (NA) medium used as a general feed for bacteria, Potato Dextrose Agar (PDA) medium was used for each sample in order to understand the effect of the medium in the study.

Before the analysis, Nutrient Agar (NA) and Potato Dextrose Agar (PDA) commercial formulations were prepared and autoclaved at 121°C for 20 minutes and sterilized under 1 atm pressure. Soil samples were dried and passed through a 2 mm sieve and soil particles under the sieve were used in the study. The soil sample obtained by sieving in this way was pounded into a sterile ceramic mortar and came to henna consistency. After weighing 10 g on a precision scale, was put in a 250 ml. volume of sterile Erlenmeyer. Sterile water was added to 10 g of soil sample in the sterile Erlenmeyer to complete the volume of 100 ml and it has been thoroughly mixed. The dilution rate here is 1/10. The soil-water suspension in the prepared Erlenmeyer was agitated for 5 minutes by means of a magnet stirrer. 1 ml of the soil-water suspension in the prepared Erlenmeyer is transferred to a test tube containing 9 ml. sterile water and mixed. 1ml of suspension in this test tube transferred to a test tube containing 9 ml sterile water and mixed again. This procedure was repeated in the same manner for 5 test tubes containing 9 ml of sterile water. The starting dilutions of the last two dilutions will be diluted to 1/100000 and 1/1000000.

100 µl (microliter) of the last two dilutions (1/100000 and 1/1000000) were pre-prepared with a micropipette using a sterile tip for each use, transferred to sterile petri dishes containing rested NA and PDA media, and spread through the sterile glass bag. Thus, for every soil sample, a total of 4 Petri dishes were inoculated to a Petri dish containing 1/100000 dilution 1 NA and 1 PDA media and to a Petri dish containing 1/10000 dilution 1 NA and 1 PDA media. The inoculated Petri dishes were wrapped with parafilm, incubated at 24±1°C, and counts of bacterial colonies were recorded every day for 10 days (Çınar, Biçici, 1991; Saygılı et al., 2006). All laboratory studies were carried out in a sterile sowing cabinet. In this respect, it is aimed to prevent contamination from air or other sources. The data obtained on the basis of ten days observations were recorded. The logarithmic transformation was performed to the obtained data because of a positive correlation between the variance of the groups and averages and statistical analysis was then performed by the SPSS program. The results were evaluated according to the statistical analysis of raw data.

RESULTS AND DISCUSSIONS

As a result of the study, the number of fungi was not statistically affected by soil tillage method, sampling time, medium and dilution. But the effect of sampling time*soil tillage interaction was found statistically significant ($p<0.05$).

On the other hand NT was significantly increased the number of fungi compared to CT and RT treatments (Figure 1). Frey et al. (1999) got the similar results that the fungal abundance was 10-60% higher in NT than in CT at all sites. Similar studies support the high number of fungi in conservation soil treatment applications (Minoshima et al., 2007; Van Capelle et al., 2012; Zhang et al., 2012).

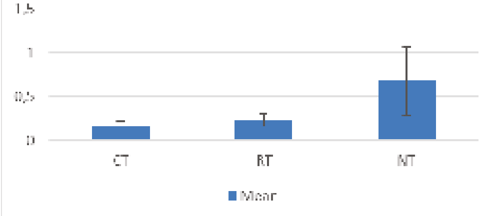

Figure 1. The means of fungi after tillage practises ($F=1.037$, $df=2,192$)

As is seen in Figure 2, there was a significant increase in the number of soil fungi from the time of sowing until the time of flag leaf in NT.

Among the methods, statistically different and high values were determined for the number of fungi in NT.

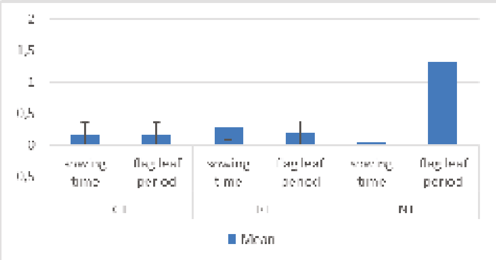

Figure 2. Effects of sampling time*soil tillage interaction on the number of fungi (F=4.249, df=2,192, P<0.05)

CONCLUSIONS

As we started to study, we expected the number of soil fungi to be statistically affected by all factors that we apply, but it didn't happen so. The number of soil fungi was significantly high in NT treatment than in RT and CT. But it is only statistically differed in sampling time*soil tillage interaction (p<0.05).

The abundance of soil fungi was related with soil moisture. Conservation tillage and direct seeding practices which keep soil moisture at ideal levels for fungal growing, leading to an increase in the number of fungi.

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TOTAL CONTENT OF SOME ANTIOXIDANTS IN TEN ROMANIAN POTATO GENOTYPES

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Abstract

Being a valuable source of important nutrients (starch, fibres, amino-acids, vitamins, minerals) potato tubers represent a complex matrix in the human diet, having potential health benefits. This vegetable has a significant contribution to the dietary intake of some antioxidants such vitamin C, carotenoids and phenolic compounds. The present study evaluated the total content of phenolic compounds (TPC) and of carotenoids (TCC) in skin and flesh of ten Romanian potato genotypes grown in Brasov, over two years. Significant differences of all parameters tested were observed across genotypes. Lower levels of TPC and TCC were found in the flesh than in the skin of the tubers. Blue genotype 'Blue Purple of Galanesti' had the highest values for all parameters excepting the carotenoids in flesh and skin which were higher in the variety 'Sevastia'. This study contents information regarding the content of valuable micronutrients of several Romanian potato cultivars with good nutritional quality.

***Key words**: antioxidants, potato, total carotenoids content, total phenolic content.*

INTRODUCTION

Many studies on potato composition revealed that there is much more in potatoes than starch. Indeed, the potato tubers contain important phytochemicals (as phenolic compounds, carotenoids, vitamin C) that may prevent the development of diabetes, cardiovascular diseases and certain types of cancers (Ezekiel et al., 2013).

Some of the reasons that led the choice of this subject (study polyphenol and carotenoids content of potato tubers) were: the special bioavailability of this plant, economic considerations (lower price compared to other sources of antioxidants), the content of anthocyanin and the presence of valuable polyphenols (chlorogenic acid, quercetin and kaempferol glycosylated with rutinose significant amounts of catechin, usually acylated glycosides of rutinose and glucose) (Andre et al., 2006; Ezekiel et al., 2013).

Regarding the carotenoids, violaxanthin, antheraxanthin, lutein and zeaxanthin are found in potatoes (Morris et al., 2004).

The variation of carotenoids content among potatoes species and among the varieties in the same species is wide, with total carotenoids content up to 60 times higher in *S. phureja* and *S. tuberosum* and up to a 20-fold difference within the same species (Morris et al., 2004; Burgos et al., 2009; Lu et al., 2001; Andre et al., 2006).

In particular, potato antioxidants have been shown to have favourable impacts on several measures of cardio-metabolic health, including lowering blood pressure, improving lipid profiles and decreasing markers of inflammation (Valcarcel et al., 2015; Wang et al., 1999).

Tubers with increased level of polyphenols or carotenoids could have an impact on human health, because these compounds posses antioxidant, antiglycemic, antiviral and anti-inflammatory activities (Duthie et al., 2000; Wang et al., 1999). This impact could be strong especially for people where potato is the most important food crop and therefore would be of interest to consumers and producers (Valcarcel et al., 2015).

The main objective of this work was to evaluate the amount of total polyphenols and carotenoids content of ten Romanian potato cultivars grown in Brasov over two years with different climatic conditions (Table 1).

MATERIALS AND METHODS

Biological material. The following potato genotypes were studied:

- BV 1791/1, BV 1871/4 - Romanian breeding lines from NIRDPSB Brasov;
- 'Christian', 'Roclas', 'Sevastia', 'Marvis', 'Castrum', 'Brasovia', 'Cosiana' (new Romanian varieties);
- 'Albastru Violet Galanesti' ('Blue Purple of Galanesti') (genotype with strong pigmentation in the flesh).

Seed tubers were planted in May in Brasov (coordinates lat. 45.6744234, long. 25.539622) in 2016 and 2015, with three replicates. Similar fertilizer chemical inputs were applied in both years. The climatic conditions of the experimental years are presented in table 1. Mature tubers were harvested 160 days after planting in Brasov in 2016 and 148 days in Brasov in 2015. After harvest, marketable tubers (medium size and free of damage and defects) were selected, washed, stored at 4°C until the sample preparation.

Table 1. Climatic conditions in the experimental years

Month	Year	Mean temperature (°C)	Rainfall (mm)
May	2015	13.2	82.6
	2016	12.4	100.4
June	2015	16.3	107.7
	2016	19.0	121.2
July	2015	17.9	95.9
	2016	19.7	28.8
August	2015	17.3	78.5
	2016	18.4	85.8
September	2015	13.5	54.7
	2016	15.0	38.0
October	2015	8.2	42.7
	2016	6.9	96.0
Average / Sum	**2015**	**14.4**	**462.1**
	2016	**15.2**	**470.2**

Sample preparation

Composite samples (4 to 10 tubers from each cultivar, depending of their size) were prepared by pooling tubers with a potato peeler. The tuber flesh was quartered from stem to bud and one of the quarters sliced. The tissues were freeze-dried (ScanVac CoolSafe 55-9 Pro Freeze Dryer, Denmark), ground to a fine powder (using a coffee grinder) and stored to -20°C until analysis.

Extraction. The extraction was carried out following the method described by Valcarcel et al. (2015). So, 0.2 g of freeze-dried potato skin or 0.6 g of flesh were weight into a 50 ml centrifuge tubes and 5 ml of ethanol solution 80% (v/v) in pure water were added. The tubes were shaken 5 min at room temperature and centrifuged 15 min at 10 000 rot/min. A part of supernatant was transferred to 1.5 ml tubes and stored at -20°C until analysis.

Total Phenolic Content (TPC) Analysis. The TPC was determined spectrophotometrically by Folin Ciocalteu method (Singleton et al., 1999) with several modifications (Digantha, Sastry, 2014). 20 µl of skin extracts and 50 µl of flesh extracts were mixed with 50µl, respectively 100µl pure water in a 96 well flat bottom assay plate (NUNC, Denmark). 50ml Folin Ciocalteu reagent were added and mixed for 1 min. After 5 min., 80 µl of a 20% solution (w/v) of Na_2CO_3 were added and mixed with a pipette; the microplates were shaken for 5 min. in the plate reader. After that, the plates were incubated at room temperature in the dark, agitating at 150 rpm on a MicroPlate Shaker (Biosan PST-60HL-4, Latvia) for 90 min. The absorbance of the samples was determined at 725 nm (Tecan SunRise, software Magellan). Gallic acid was used as standard and total phenolic content was expressed as milligrams GAE (Gallic acid equivalents) per gram of dry weight (DW) materials.

Total carotenoids content (TCC) analysis. Total carotenoids content was determined according to Burgos et al. (2009) without alkaline hydrolysis. Extraction of TCC from 0.5 g of powdered skin or 2 g of powdered flesh was sequentially carried out in triplicate with acetone, shaking in 50 ml tubes at 10 000 rot/min for 15 minutes. The supernatants were combined and 5 ml of petroleum ether and 20 ml of ultra-pure water added. The tubes were shaken vigorously by hand and centrifuged at 10 000 rot/min for 1 minute. The top organic phase was removed and washed with pure water, separating both phases as described above. The top organic phase was again removed and the absorbance of an aliquot was measured at 450 nm against petroleum ether using a UV VIS spectrophotometer Spectronic Genesys 5 (Milton Roy).

Statistical interpretation

Analysis of variance (ANOVA) and Duncan's multiple range test were used.

RESULTS AND DISCUSSIONS

Total polyphenol content

The experiments conducted in Brasov in 2015 and 2016 show that cv. 'Blue Purple of Galanesti' had the highest mean TPC value in skin and flesh tissue, in the years 2015 and 2016. The samples with the lowest TPC values were from the breeding line BV 1791/1 in both years for both tissues flesh and skin, respectively (Table 2).

The levels of TPC ranged from 0.59 to 3.38 and 2.68 to 10.79 mg GAE g^{-1} DW in the flesh and skin, respectively, with flesh and skin contents showing a significant difference between the cultivars (Table 2). The skin of the potatoes studied contained on average 4.62 and 4.12 times more phenolic compounds (TPC) than the flesh, in 2015 respectively in 2016 (Table 2). TPC in both tissue (skin and flesh) was positively correlated with the colour of the tissue, with a Pearson coefficient 0.813 ($p<0.01$) and 0.596 ($p<0.01$) for skin and flesh respectively.

The results obtained are comparable with those reported by other researchers, with values ranging from 1.56 to 12.9 and 0.54 to 3.59 mg GAE g^{-1} DW in the skin, respectively in the flesh (Valcarcel et al., 2015), from 1.4 to 2.4 mg GAE g^{-1} DW in the flesh (Cornacchia et al., 2011), from 0.92 to 12.37 mg GAE g^{-1} DW for whole tubers (Andre et al., 2006; Xu et al., 2009) from 1.00 to 4.3 mg GAE g^{-1} DW in the skin (Kahkonen et al., 1999; Makris et al., 2007).

Despite the fact that some vegetable sources have higher TPC than potatoes, in many countries the potatoes are consumed in higher quantities and so, potatoes make an important contribution to the phenolic compounds daily intake. A recent study in USA estimated that potatoes were the third highest contributor to the daily intake of phenolic compounds, after oranges and apples, with a daily intake consumption of 171 g day^{-1} (Chun et al., 2005). These properties of potatoes could be greater if the cultivars with high TPC level become popular for the people. Unfortunately, the cv. 'Blue Purple of Galanesti' (reported in this study with the higher TPC level in the flesh and skin) is not accepted with pleasure by the consumers because the tubers are small, elongated and with deep eyes. Maybe in the future, the potato breeders correct these quality parameters by developing new cultivars with functional food characteristics.

Total carotenoids content

The experiments conducted in Brasov show that cv 'Sevastia' had the highest mean TCC value in both skin and flesh. Genotypes with the lowest quantified values were BV 1791/1 in 2015 and 2016 for both tissues, flesh and skin respectively (Table 3).

The levels of TCC ranged from negligible quantities to 8.92 and 4.38 mg kg^{-1} DW in the skin and flesh, respectively, with flesh and skin contents showing a significant difference for both years (Table 3). On average, the skin of the potatoes analysed contained between two and three times more TCC than in the flesh (2.24 times in 2015, 3.24 times in 2016).

These data are in agreement with other studies: it has been reported (Breithaupt, Bamedi, 2002) that the total quantity of the four main carotenoids analysed in eight commercial potato varieties was between 0.38 and 1.75 mg kg^{-1}, which would be equivalent to 1.90-8.75mg kg^{-1} DW assuming 80% of water in the fresh samples. Other authors (Morris et al., 2004, Lu et al., 2001) found that for varieties 'Pentland Javelin' (white flesh) TCC was 1.60 mg kg^{-1} DW and for variety 'Yukon Gold' (yellow flesh) and 'Superior' (white flesh) were 1.11 and 0.64 mg kg^{-1} FW, respectively (equivalent to approximately 5.55 and 3.20 mg kg^{-1} DW). Excepting the cultivars 'Sevastia', 'Christian' and 'Roclas', the majority of the varieties included in this work had relatively low content of TCC in the flesh, with values below 0.24 and 1.14 mg kg^{-1} DW (Table 3). TCC in both tissue (skin and flesh) was very weak positively correlated with the colour of the tissue, with a Pearson coefficient 0.357

(p<0.01) and 0.196 (p<0.01) for skin and flesh, respectively.

Tubers grown in Brasov in 2016 had an average higher TCC and TPC content than those grown in Brasov in 2015. As reported and other researchers, potato peels are a great source of phenolic compounds because almost 50% of phenolic are located in the peel and adjoining tissues (Albishi et al., 2013; Al-Weshahy et al., 2009). The results presented in this study revealed higher TPC and TCC in skin compared with the flesh tissue. There is a lot of information in the literature about TPC in potato varieties grown in different controlled conditions. In this study, there were analysed several new Romanian varieties and some cultivars with strong colour in the flesh and skin tissue.

Table 2. Total polyphenol content (mg GAE/g DW) of potato samples (Brasov, 2015-2016)

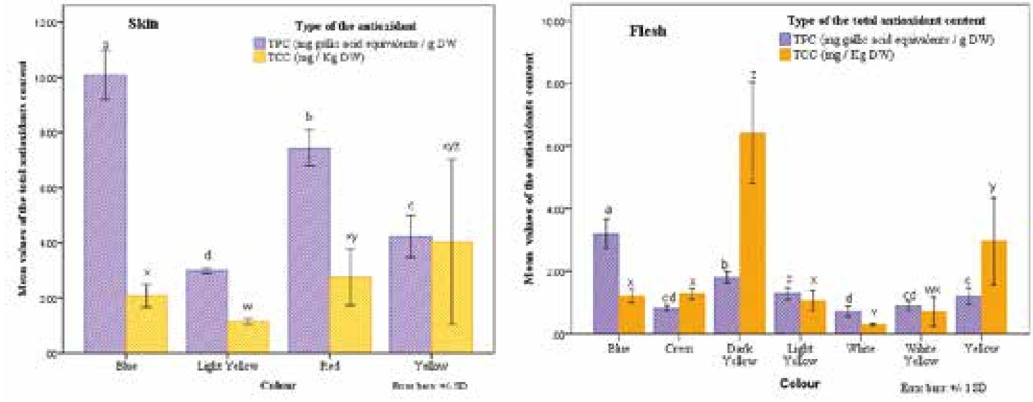
Cultivars/ genotypes	Flesh/ Skin colour	Year 2015		Year 2016	
		Flesh	Skin	Flesh	Skin
BV 1791/1	W/LY	0.59±0.012 *(g)**	2. 68±0.354 (*h*)	0.82±0.207 (*f*)	2.72±0.247 (*i*)
BV 1871/4	C/R	0.78±0.136 ef*)*	7.96±0.914 *(b)*	0.88±0.118 (e*f*)	8.32±0.604 *(b)*
AVG	B/B	3.03±0.208 (*a*)	9.39±1.262 *(a)*	3.38±0.400 *(a)*	10.79±1.114 (*a*)
Brasovia	WY/Y	0.92±0.057 *(d)*	3.35±0.130± *(f)*	1.04±0.158 *(e)*	3.90±0.307 (*gh)*
Castrum	LY/Y	1.15±0.113 (*c*)	4.01±0.264*(e)*	1.43± 0.246(*c*)	4.55±0.164 *(j)*
Christian	Y/R	1.25± 0.088(*c)*	6.54±0.614*(cd)*	1.53± 0.341(*c*)	6.93±0.108 (*d)*
Cosiana	WY/R	0.73±0.027 (*ef)*	7.16±0.828 *(bc)*	1.03±0.249 (*e*)	7.68±0.420 (c)
Marvis	WY/Y	0.75±0.017 (*ef)*	4.51±0.317 *(de)*	0.91±0.315 (ef*)*	4.66± 0.350*(f)*
Roclas	Y/Y	0.88±0.230 (*de)*	3.15± 0.167*(g)*	1.27±0.218 (d)	3.56±0.807(*g*)
Sevastia	DY/Y	1.66±0.150 *(b)*	4.99± 0.063(*d*)	1.95±0.142 (*b*)	5.57±0.226 (*e)*
Mean		1.164	5.383	1.424	5.868

*Means with different letters are significantly different at p<0.05 in each column. Values reported for the two Romanian trials are the mean of three filed replicates. Abbreviations: GAE-Gallic Acid Equivalents; DW-dry weight; BV 1791/1, BV 1871/4 breeding lines; AVG-Albastru Violet de Galanesti (Blue Purple of Galanesti); W-white; WY-white yellow; C-cream; LY-light yellow; Y-yellow; DY-dark yellow; R-red; B-blue; C-Cream.

Table 3. Total carotenoids content (mg /kg DW) of potato samples (Brasov, 2015-2016)

Cultivars/ genotypes	Flesh/ Skin colour	Year 2015		Year 2016	
		Flesh	Skin	Flesh	Skin
BV 1791/1	W/LY	0.24±0.048 *(h)**	1.24±0.424 *(f)*	0.32±0.033 *(g)*	1.53±0.025 *(g)*
BV 1871/4	C/R	1.18±0.634 *(d)*	2.08±0.632 *(d)*	1.42±0.392 *(de)*	2.98±0.392 *(e)*
AVG	B/B	1,14±0.325 (d)	2,43±0.286 *(c)*	1,31±0.273 *(de)*	nd
Brasovia	WY/Y	0,38±0.105 *(g)*	1,57±0.122 *(e)*	0,38±0.105 *(g)*	1,84±0.048*(ef)*
Castrum	LY/Y	0,82±0.208 *(e)*	1,46±0.162 *(e)*	1,30±0.049 *(de)*	2,68±0.035*(ef)*
Christian	Y/R	1,45±0.942 *(c)*	2,44±0.622 *(c)*	2,22±0.825 *(c)*	4,55±0.692 *(c)*
Cosiana	WY/R	0,69± 0.092*(ef)*	1,32±0.102 *(ef)*	1,68±0.412 *(d)*	3,53±0.528 *(d)*
Marvis	WY/Y	0,47±0.026 *(g)*	1,43±0.094 *(e)*	0,79±0.033 *(f)*	1,54±0.077 *(f)*
Roclas	Y/Y	3,22±0.725 *(b)*	5,10±0.944 *(b)*	3,42±0.046 *(b)*	8,04±0.831 *(b)*
Sevastia	DY/Y	4.84±0.492 *(a)*	7.83±0.863 *(a)*	4.78±0.082 *(a)*	8.92±0.723 (a)
Mean		1.443	4.685	1.762	3.957

*Means with different letters are significantly different at p<0.05 in each column. Values reported for the two Romanian trials are the mean of three filed replicates. Abbreviations: GAE-Gallic Acid Equivalents; DW-dry weight; BV 1791/1, BV 1871/4 breeding lines; AVG-Albastru Violet de Galanesti (Blue Purple of Galanesti); W-white; WY-white yellow; C-cream; LY-light yellow; Y-yellow; DY-dark yellow; R-red; B-blue; C-Cream; nd-non determined.



A. B.

Figure 1. Mean total polyphenols content (TPC) and total carotenoids content (TCC) according to the colour of the skin (A) and flesh (B) colour of the tubers. Bars represents mean values of samples collected in 2015 and 2016 with different skin and flesh colour. Error bars represent standard deviations. The letters different upper the bars indicate significant difference at $p<0.05$. Abbreviations: TPC-total polyphenols content; TCC-total carotenoids content; DW-dry weight; SD-standard deviation

Results regarding the influence of skin and flesh colour on TPC and TCC are presented in Figure 1. The tubers with blue skin had the highest TPC values. These values were significantly different from other colours, except red-skinned varieties (Figure 1). In the flesh tissue, blue potatoes had the highest TPC values, significant different from all the other colour variants. Blue and red colours are due to the presence of anthocyanins (Andre et al., 2008) and the higher TPC values obtained for this kind of tubers colour can be attributed to these pigments with high antioxidant potential. Strong correlations between TPC and antioxidant activity were reported in previous studies (Andre et al., 2006; Hu et al., 2012; Reyes et al., 2005; Valcarcel et al., 2015) which specified that both phenolic and flavonoid compounds are the main contributors to the antioxidant potential. Climate data for the growing season in Brasov (Table 1) show that average temperatures in 2016 were slightly higher than in 2015. This difference was accentuated in June, July and August and was accompanied also by increased rainfall. TCC and TPC content seems to be higher in early developing tubers (Morris et al., 2004) so these climatic conditions during the summer months could contributed to difference observed. Reddivari et al. (2007) reported that tubers planted in the location with higher average temperature and increased rainfall contained higher levels of carotenoids.

CONCLUSIONS

Ten Romanian potato genotypes with different colour of the skin and flesh tissue (grown in Brasov two years) were analysed for estimation their total polyphenols and carotenoids content. The results obtained were in the range of values reported in the literature.

Significant differences between the different cultivars tested were observed for TCC and TPC. Higher contents of antioxidants (polyphenols, carotenoids) were found in intense coloured fleshed tubers than in white counterparts, which should allow visual selection of varieties with enhanced levels of these compounds. So, the genotype ‘Blue Purple of Galanesti’ with blue skin and flesh had higher values of total polyphenols content and the cultivar ‘Sevastia’ (with a dark yellow colour of the flesh) had the highest values of total carotenoids content.

Potato consumption has a great importance in population’s food and this study offer preliminary information to researchers and producers on the level of some phytochemicals such carotenoids and polyphenols, antioxidants with functional properties.

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ESTIMATION OF THE LIQUID COVER OF SELECTED DICOTYLEDONOUS PLANTS IN VARIOUS PHASES OF DEVELOPMENT DURING SPRAYING

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Abstract

Chemical method of plant protection is an indispensable part of modern agriculture. Researchers emphasize the importance of properly spraying, but the characteristics of the sprayed plants are not taken into account during the experiment. A study was conducted in order to estimation of the liquid cover of selected dicotyledonous plants in various phases of development during spraying. The research was carried out in laboratory conditions at a specially prepared site used for examining the plants` surfaces. On the basis of the carried research, as well as its analysis, we can ascertain that taking into consideration the characteristic sprayed surface of plant makes it much easier to choose the right nozzle for spraying.

Key words*: spraying, degree coverage, estimation liquid cover.*

INTRODUCTION

In modern agriculture, the most important is to achieve the highest yields. Achievement this goal, it is necessary to perform chemical methods of plant protection. However, the spraying carried out improperly may contribute to a reduction in the effectiveness of plant protection products and may cause the need to repeat the treatment. Additionally, the spray liquid can be drifted to an area that was not the target of the treatment and cause environmental pollution (Gil et al., 2013; Reimer, Prokopy, 2012). The spraying procedure is the most effective when the spray liquid covers the largest area of the plant (Ferguson et al., 2016). Many researchers emphasized that the choose of appropriate technical and technological parameters, the use of appropriate nozzles, as well as the use of adjuvants and control of meteorological conditions in real time determine the correct course of the spraying operation (Derksen et al., 2014; Doruchowski et al., 2017). So far, the spray characteristic of plants has not been taken into account during the spraying. However, methods available for assessing plant characteristics, eg. LAI, MTA, fAPAR, phenological phase of the plant life cycle, do not define the characteristics and parameters of plants directly related to the spraying process (Feng et al., 2013; Savoy, Mackay, 2015). Therefore, the present research, carried out at the Institute of Agricultural Engineering of the University of Life Sciences in Wrocław, aimed estimation of the liquid cover age of selected dicotyledonous plants in various development phases.

MATERIALS AND METHODS

Research carried out in laboratory conditions, in the two stages. The first part of the research was concerned with determining the sprayed surface. The measurement stand is patent-protected. The scheme of measurement stand is shown in Figure 1. The main element of the measurement stand was the measuring chamber, in which photographs of the surface of horizontal and vertical projections were taken. The plant was placed on turntable. Photographs of horizontal surface were made over the plant. Photos of vertical surface were made before the plant. Photographs of vertical surfaces were made six times, after rotation of the plant on the turntable by 60°.The distance of the camera from the plant was the same, while the lens with a variable focal length was used. The measuring chamber has been equipped with additional lighting in the form of LED. The measuring chamber was fitted with vertical and horizontal meshes which the backgrounds of the pictures were taken. Area 1 of the square was 25 cm^2.

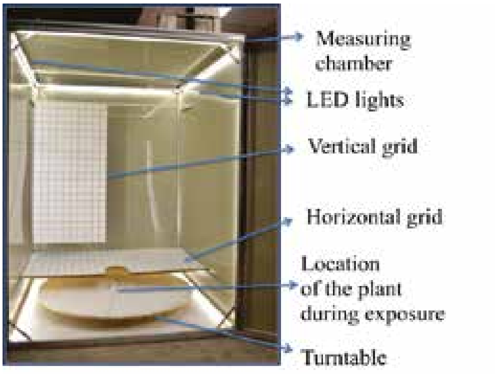

Figure 1. Measurement stand

Calculations of the surface of vertical and horizontal projections were carried out in the graphic program. After the graphical analysis of the photos, the position of the spray surfaces was calculated according to the following formula:

$$Wpo = \frac{projection\ vertical\ surfaces}{projection\ horizontal\ surfaces}\ (\text{-})$$

Five samples of sugar beets and potatoes in four development phases were used for the research.

In the second stage of the research, the degree of coverage of horizontal and vertical surfaces was determined using four nozzles single standard flat-fan (XR 11002), single air-induction flat-fan (CVI 11002), twin standard flat-fan (DG TJ60 11002), and twin air-induction flat-fan (CVI TWIN 11002). The scheme of the research position is presented in Figure 2. The primary element of the measurement stand was the nozzles' carrier. The nozzles' carrier functioned as a carrier itself. The driving speed was 2.2 m·s^{-1} and the liquid pressure was 200 k Pa.

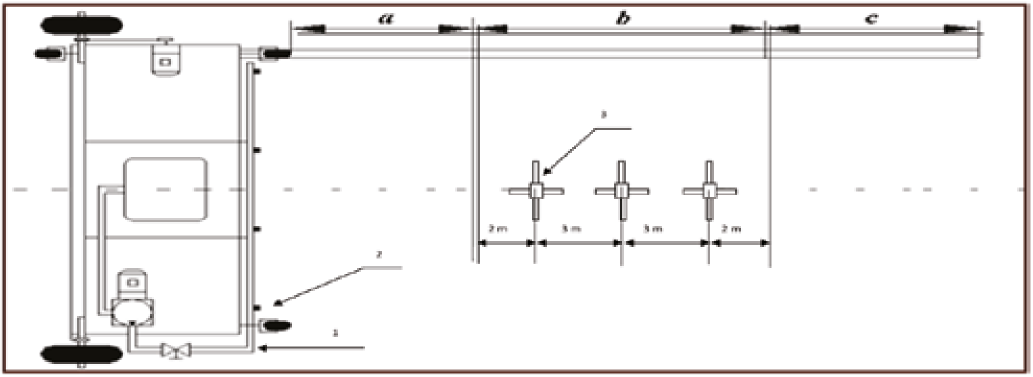

Figure 2. Schematic representation of the measurement stand: a - run line; b - a measurement line; c - ending line; 1 - nozzles carrier; 2 - nozzles; 3 - an artificial plant

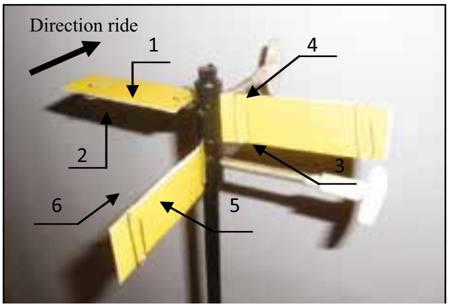

Figure 3. Photo of an artificial plant: 1 - upper level (Apog); 2 - lower level (Apod); 3 - vertical approach (Anj); 4 - vertical leaving (Aoj); 5 - vertical right (Apr); 6 - vertical left (Al)

The sprayed objects were artificial plants with attached WSP probes (Figure 3). The coverage degree of the examined objects was determined by the computer image analysis. Three representative fragments of the surface with 10 x 10 mm dimension were chosen on the probe's area, and then the program read the value according to the formula:

$P_{sp} = \frac{W_k}{W_p} * 100[\%]$ where:

P_{sp} - coverage degree [%];

W_k - surface covered with liquid [pixels];

W_p - 1 cm^2 surface [pixels].

In order to estimate the coverage of selected crop plants, total coverage was calculated on objects determined by product the sum of the surface of the horizontal and vertical projections of a given crop in a given development phase by the sum of the average coverage of the spray liquid horizontal and vertical objects.

$$P_c[cm^2] = \frac{[A_{hs}[cm^2] * P_{shs}[\%]] + [A_{vs}[cm^2] * P_{svs}[\%]]}{100}$$

where:
P_c - total coverage;
A_{hs} - area horizontal surfaces;
P_{shs} - degree coverage horizontal surfaces;
A_{vs} - area vertical surfaces;
P_{svs} - degree coverage vertical surfaces.

RESULTS AND DISSCUSSIONS

The spray characteristics of the sugar beet on the BBCH 14, 18, 31, 36 scale and potatoes on the BBCH 10, 30, 39, 67 scale are compiled in Table 1. Based on the analysis of the obtained data, it can be concluded that the sugar beets in the BBCH 14 and 36 development phase had a larger surface area of horizontal projections compared to vertical projections, opposite of the BBCH 31 scale, when the area of horizontal projections was the larger share area. A similar value of the surface of horizontal and vertical projections was characterized by sugar beets in phase 18 of the BBCH scale. On the other hand, potatoes were characterized by a larger share area of the surface of horizontal projections. With the growth of the plant, the area of the crops of the analyzed plant increased.

Table 1. Characteristic plant

Crop	Development phase	Area of vertical projections [cm^2]	Area of horizontal projections [cm^2]	W_{po} [-]
Sugar beet	BBCH 14	32.50	64.20	0.51
	BBCH 18	415.99	349.27	1.19
	BBCH 31	1470.34	612.90	2.40
	BBCH 36	2499.68	3467.60	0.72
Potato	BBCH 10	68.70	110.60	0.62
	BBCH 30	167.58	313.18	0.54
	BBCH 39	370.08	492.44	0.75
	BBCH 67	2100.86	2157.80	0.97

Results of the research on coverage degree of vertical and horizontal surfaces with the used selected nozzles presented on Figure 4. The highest value coverage vertical surfaces noticed for twin standard flat-fan DF 12002, while the highest value coverage of horizontal surfaces obtained for single standard flat-fan XR 11002. Taking into consideration air injection nozzles, it should be noted that cover horizontal and vertical surfaces was the higher values when used single flat-fan CVI 11002. However, the difference in coverage degree equals only 0.31% for vertical surfaces.

The results of the estimation of sugar beet cover during spraying are shown in Figures 5-8. Higher values of sugar beet coverage level on the BBCH 14, 18 and 36 scale would be obtained with a single flat-fan nozzle. However, when spraying sugar beets in the 31 BBCH scale, it would be more beneficial to use the DF 12002 double flat fan nozzles.
The results of the estimation of potatoes cover during spraying are shown in Figures 9-12. Higher values of the degree of coverage of potatoes in the analyzed scales would be obtained in the case of a single flat-fan nozzle.

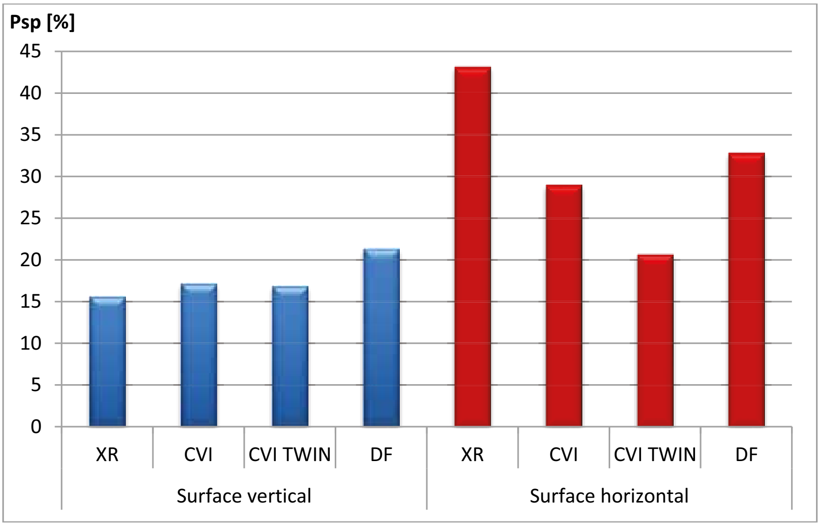

Figure 4. Coverage degree of horizontal and vertical surfaces for selected type nozzles

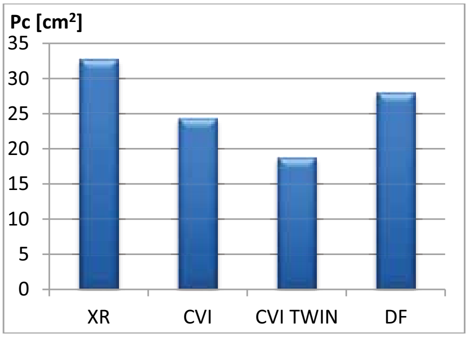

Figure 5. Estimation of the liquid cover beet sugar on the 14 BBCH scale

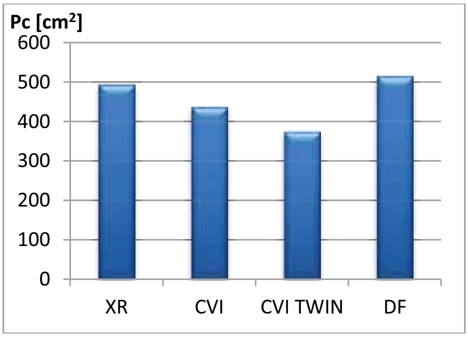

Figure 7. Estimation of the liquid cover beet sugar on the 31 BBCH scale

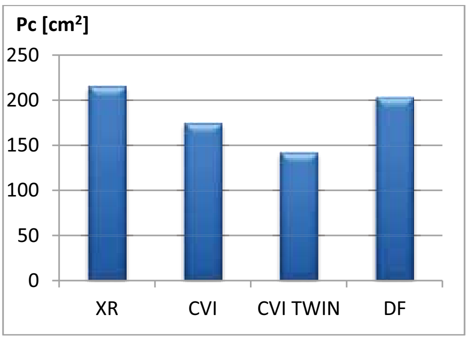

Figure 6. Estimation of the liquid cover beet sugar on the 18 BBCH scale

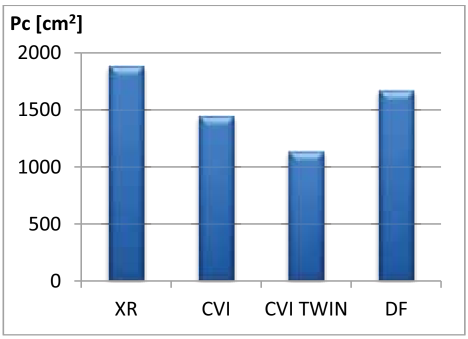

Figure 8. Estimation of the liquid cover beet sugar on the 36 BBCH scale

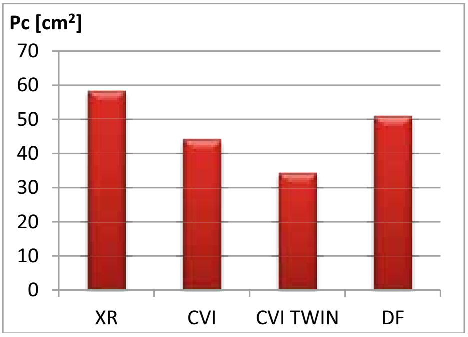

Figure 9. Estimation of the liquid cover potatoes on the 10 BBCH scale

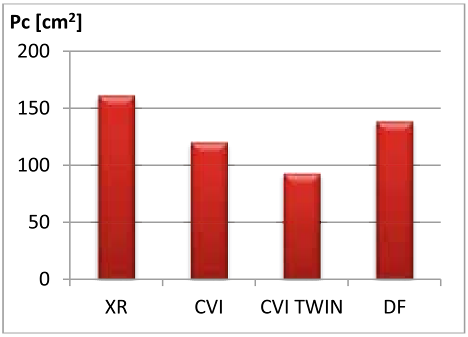

Figure 10. Estimation of the liquid cover potatoes on the 30 BBCH scale

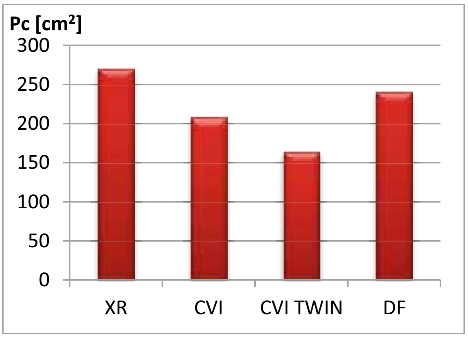

Figure 11. Estimation of the liquid cover potatoes on the 39 BBCH scale

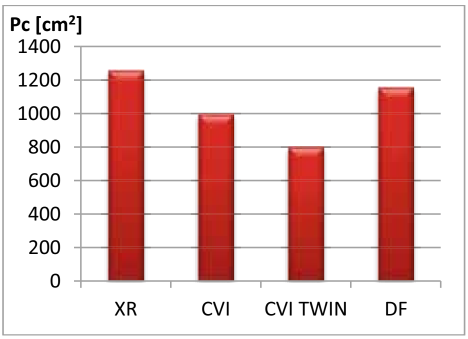

Figure 12. Estimation of the liquid cover potatoes on the 67 BBCH scale

CONCLUSIONS

1. The characteristic sprayed surface of plant and Wpo, that characterizes the sprayed plant and its developmental stage, makes it much easier to choose the right nozzle for spraying.
2. Analysis of the obtained results showed that the coverage depends on the type of nozzle used. Higher values of coverage of horizontal objects were noted using single-flat-fan nozzles. While higher values of coverage of vertical objects were obtained using double flat-fan nozzles.

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DIVERSITY, DISTRIBUTION AND ECOLOGY OF THE FOREST NATURAL HABITATS IN THE BRATOVOEȘTI FOREST, DOLJ COUNTY

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Abstract

The investigated area is located in Oltenia Plain, in the Valley Jiu at an altitude of 40-60 m.s.m. By the phyto-climatic point of view the Bratovoești forest is located in the forest Steppe of Oltenia. The Bratovoesti Forest is part of the protected area of community interest ROSCI 0045 Coridorul Jiului, and occupies an area of 904.51 ha. The Bratovoești forest is located on loess that were formed deep soils with good growth profile: alluvial-soils and luvisols. Also, some areas near shore Jiu, also on sandy rocks (sand and gravel) they have formed shallow soils poor in minerals (entic alluvisoils). In the researched area there are the following Natura 2000 forest habitats: 91E0-Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae), 91F0-Riparian mixed forests of Quercus robur, Ulmus laevis and Ulmus minor, Fraxinus excelsior or Fraxinus angustifolia along the great rivers (Ulmenion minoris), 92A0-Salix alba and Populus alba galleries. Of the rare and vulnerable species found in this forests habitats we can mention: Vallisneria spiralis, Utricularia minor, Nymphaea alba, Azolla filiculoides, Typha minima, Orchis purpurea, Epipactis palustris, Galanthus nivalis, Angelica sylvestris, Crocus flavus, Festuca gigantea. The biodiversity of the forest natural habitats from this area is endangered because the human impact is very high, although this area it is included in the important protected areas from Romania.*

Key words: *forest habitats, plant communities, Bratovoești.*

INTRODUCTION

The Bratovoiești Forest is part of the protected area of community interest ROSCI0045 Coridorul Jiului, and occupies an area of 904.51 ha. The investigated area is located on loess that were formed deep soils with good growth profile: alluvial-soils and luvisols. Of the rare and vulnerable species found in this forests habitats we can mention: *Orchis purpurea, Epipactis palustris, Galanthus nivalis, Angelica sylvestris, Crocus flavus, Festuca gigantea*.

The biodiversity of the forest natural habitats from this area is endangered because the human impact is very high, although this area it is included in the important protected areas from Romania.

The territory occupied by the Bratovoești forest is located in the north of the Moessice platform which embraces the Romanian Plain and is separated from the Precarpathian Depression through an intermediate area, which occupies the largest part of this territory. From a stationary point of view, we are particularly interested in the upper layer of the lithological formations that basically influence the genesis and physico-chemical properties of the soil.

In the researched territory, which is practically in the Jiu meadow, more developed aluviosoils have been formed on the higher lands that have not been flooded by the Jiu with a forest vegetation such as the “meadow oak-grass”, and on the lower places from the Jiu meadow less evolved soils - entic aluviosoils, eutric and gley aluviosoil, forest stands of PLA, PLN, SA and PLEA.

On the depressions in the Jiu meadow there were formed hydrisoils - gleisoils, stagnosoils where grows the black alder, the swamp cypress and less the willow.

Regarding the climatic data, the following can be distinguished:

- according to the climatic zoning (Geographical Monograph of R.P.R.), the studied territory is situated in the continental climate, the plain territory, the forest district, the central subdivision of the Romanian Plain (II.A.p.2.), with rainfall of about 500 mm/year and with an amplitude of temperature above 25°C;

- after Kopen, the territory under study is part of the C.f.a.x. sub-province, with the temperature of the warmest month of 22°C and the maximum precipitations at the beginning of the summer.

MATERIALS AND METHODS

The field data collection was done in accordance with geobotanical guidelines and rules, field trips being made, detailed descriptions and analyzes of phytocoenosis, and the types of plant communities in the forest that underlie the forest habitat edification. For the determination and description of the types of vegetal communities, there have been made surveys with a sample area of 400-1000 m^2. To identify the habitats we used the Romanian Manual for interpretation of EU habitats and Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, Annex I (Habitats Directive) and for the classification of the vegetal associations, we have used synthesis papers by Rodwell et al. (2002).

RESULTS AND DISCUSSIONS

In the researched area there are the following Natura 2000 forest habitats: 91E0*-Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*), 91F0 - Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia* along the great rivers (*Ulmenion minoris*), 92A0 - *Salix alba* and *Populus alba* galleries.

91E0*-Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)

Chorology: Jiu Meadow, Georoc Meadow, developed on alluvial soil, reaching a coverage of trees 60-90% and coverage of herbaceous plants 30-60%.

Typical plant communities of this habitat identified in Brotovoesti Forest: *Thelypterido palustris-Alnetum glutinosae* Klika, 1940*;* *Salicetum fragilis* Passarge, 1957; *Salicetum albae* Issler, 1924.

In the Bratovoesti Forest this plant communities have a rich phytodiversity: *Angelica sylvestris, Aegopodium podagraria, Ornithogalum pyrenaicum, Urtica dioica, Glechoma hirsuta, Allium ursinum, Fraxinus pallisae, Thelipteris palustris, Petasites albus, Ranunculus ficaria, Carex remota, C. sylvatica, C. riparia, C. vulpina, Eleocharis palustris, Scirpus sylvaticus, Mentha aquatica, M. longifolia, Arum orientale, Melica nutans, Cardamine impatiens, Moehringia trinervia, Agrostis stolonifera, Bidens tripartita, Persicaria (Polygonum) hydropiper, Lycopus europaeus, Caltha palustris* ssp. *cornuta, Festuca gigantea, Brachypodium sylvaticum, Impatiens noli tangere, Leucojum aestivum, Lysimachia nummularia, Galium palustre, Vincetoxicum hirundinaria, Geranium phaeum, Viola odorata, Thalictrum simplex, T. minus, Cucubalus baccifer.*

In the floristic composition of the ptytocoenoses of this natural habitat meet many undesirable species: *Glechoma hederacea, G. hirsuta, Galium aparine, Urtica dioica, Alliaria petiolata*. Some species grow very much invading the seedlings and other species of herbaceous layer and undergrowth. The invasive species threaten the conservation status of this habitat*: Phytolaca americana, Erigeron anuus, Ailanthus altissima, Gleditsia triacanthos, Acer negundo.*

91F0 - Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia* along the great rivers (*Ulmenion minoris*)

This natural habitat are poorly represented in the Bratovoesti Forest.

Chorology: Jiu meadow and Georoc meadow, developed on alluvial soil and luvisols.

Typical plant communities of this habitat identified: *Fraxino danubialis-Ulmetum* Soó, 1936 corr. 1963*; Quercetum roboris pedunculiflorae* Simon, 1960 (syn.: *Fraxino angustifoliae - Quercetum pedunculiflorae* (Chifu et al., 1998, 2004); *Fraxino pallisae-Quercetum pedunculiflorae* (Popescu et al., 1979; Oprea, 1997).

The floristic composition of the phytocoenoses of these plant communities is made up of a series of species of great value (e.g. orchid species).

The most important species of the phytocoenoses which edified this habitat are: *Quercus robur, Ulmus minor, Ulmus glabra,* *Fraxinus excelsior, F. angustifolia, F. pallisae, Populus nigra, P. tremula, P. alba, Salix alba, Acer tatarticum, A. campestre*, *Alnus glutinosa, Prunus padus, Humulus lupulus, Tamus communis, Hedera helix, Corydalis solida, Gagea lutea, Cornus sanguinea, Sambucus nigra, Frangula alnus, Ligustrum vulgare, Rubus caeius, Euonymus europaeus, E. verrucosus, Clematis vitalba, Aeopodium podagraria, Galuium aparine, Carex michelii, C. tomentosa, C. acutiformis, Iris pseudacorus, Glechoma hederacea, Leucojum aestivum.* In the phytocoenoses of this plant communities we must acording particular attention to species: *Vitis vinifera* ssp. *sylvestris*, *Convallaria majalis.* In this habitat are found channels and ponds where these species thrive: *Typha latifolia, Elodea canadensis, Eleocharis palustris, Phalaris arundinacea, Bolboschoenus maritimus, Lemna trisulca, L. minor, Ceratophyllum demersum, Azolla filiculoides, Caltha palustris* ssp. *cornuta, Spirodela polyrhiza, Vallisneria spiralis, Nuphar luteum, Utricularia minor, Nymphaea alba.*

92A0 - *Salix alba* and *Populus alba* galleries

This natural habitat are well-represented in the Jiu meadow.

Typical plant community: *Salici-Populetum* Meijer-Drees, 1936.

In the floristic composition of the ptytocoenoses of this natural habitat meet numerous mesophyle, meso-hygrophyle and hygrophyle species. These phytocoeonoses have a special composition, being characterised by the dominance of the species: *Salix alba, Populus alba, P. nigra, Fraxinus angustifolia, Cornus sanguinea, Crataegus monogyna, Rosa canina, Euonymus europaeus, Sambucus nigra, Prunus spinosa, Rubus caesius, Vitis vinifera* ssp. *sylvestris, Clematis vitalba, Humulus lupulus, Agrostis stolonifera, Althaea officinalis, Cicuta virosa, Lycopus europaeus, Lysimachia nummularia, L. vulgaris, Physalis alkekengi, Ranunculus repens, Scutellaria galericulata, Solanum dulcamara, Asparagus tenuifolius, Myosoton aquaticum, Poa trivialis, Eupatorium cannabinum, Bidens tripartita, Lythrum salicaria, Myosotis scorpioides, Equisetum arvense, Stachys palustris, Agropyron repens, Urtica dioica, Glechoma hederacea, Angelica sylvestris, Mentha aquatica, Rumex obtusifolius, Juncus buffonius, Cyperus fuscus, Typha latifolia.* The plant communities developed on alluvial soil reaching a coverage of trees 60-80% and coverage of herbaceous plants 20-60%. In the floristic composition of the ptytocoenoses of this natural habitat meet many undesirable species: *Rubus caesius* and *Amorpha fruticosa.*

Types of forests included in these natural habitats from Bratovoești Forest

Natural forest type and its productivity: 041.4. Medium productivity meadow ash (m); 632.4. Meadow oak-grass of average productivity (m); 632.5. Meadow highroad from the plain of average production (m); 041.1. Meadow ash (s); 632.1. Meadow oak-grass (s); 911.5. Low-productivity white poplar coppice from the meadows of the interior watersheds (i); 911.2. White poplar coppice of medium productivity (m); 921.5. Black poplar coppice of medium productivity (m); 931.2. Coppice mixed by PLA and PLN of average productivity (m); 951.7. Willow coppice from the meadows of the interior watersheds (m); 961.3. Poplar and willow coppice from interior watersheds (m); 931.1. Mixed PLA and PLN coppice of superior productivity (s); 971.1. Alder trees on gleaned soil (s) of high productivity; 972.1. Black alder coppice (s)

Classification guideline: 8.5.1.1. Forestry plain, grassland meadow Pm, brown groundwatery damp gley or semigley, edaphic medium-large; 8.5.1.2. Forestry-meadow grassland plain, Ps, brown groundwatery damp, gley or semigley, edaphic large; 8.5.2.2. Forestry floor, aluvial freative neutral, III; 8.5.2.3. Forestry plain, poplar grassland meadow Pm, humifer moderated alluvial, profoundly groundwatery damp, very rarely shortly flooded; 8.5.2.4. Forestry plain poplar grassland meadow Ps, aluvial intense humifer, groundwatery, frequently and rarely shortly flooded; 8.5.4.2. Forestry plain, deeply depressioned by the aldre grove Ps, turbogley and typical turbos.

Description of the types of stations: resorts in non-floodable meadow sectors or rarely and shortly flooded meadow areas of medium productivity for oak-grass or meadow grassland, situated on flat and small depressions; the lithologic substrate is made up of clay-sandy alluviums up to clay-argil ones, sometimes layered, sometimes stratified; soils: eutric aluviosoil, mollic, verticular, gley, gley-eutric with high trophicity, good aeration and moderate consistency; old, high stations in the Jiu meadow or its tributaries in the forest, with eutrophic specific, with eutric aluviosoil, mollic, vertic, gley, gley-euteric alluviosol; the lithologic substrate is made up of clayey and partly sandy alluviums; the subarbus and grassy floor: *Rubus* sp., *Viola* sp., *Poaceae* etc. hygrophyle species; superior productivity stations for oak and meadow glassland; stations located in the Jiu meadow area in the plain region, on high-lying terrain or very rarely and shortly flooded flat land; the lithological substrate consists of sandy or sandy-coarse alluviums; soils: eutric aluviosoil, entic; white poplar low productivity stations; stations located in the Jiu meadow or its tributaries in the forest plain, on high-lying terrains, abandoned river beds, more or less clumped, rarely flooded, with more or less evolved aluviosoils, stratified and carbonate formed on the substrate of sandy alluviums with light texture and moisture-dry humidity summer regime and damp in spring; middle productivity stations for black and white poplar; stations with high productivity with mesophit to ectrophic ecologic specificity, situated in the middle meadow of the Jiu River and its tributaries, in the forest plain, on aluviosol, medium evolved to evolved, stratified or with transition to zonal soils formed on sand substrate with light texture towards middle; eutrophic-specific higher productivity stations situated in the Jiu meadow in the forest plain on gleisoils and stagnosoils, very poorly salinated on deposits of fine alluviums and clay with heavy texture and humid moisture regime, damp to wet because of the groundwater that reaches to the surface, the land becoming marshy.

Conservation status and human impact

In the investigated area this habitats is characterized by the following data on the conservation status and human impact, future threats presented in Table 1.

Table 1. Habitats of European interests in the studied area from Bratovoiesti Forest

No.	Natural habitats	Natura 2000 code	Palearctic Hab. code	Conservation status	Human impact current pressures and; future threats
1.	Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)	91E0*	CLAS. PAL.: 44.3, 44.2 and 44.13	favorable up to unfavorably	E01.01 - continuous urbanization; G05.07 - missing or wrongly directed conservation measures; D.06- other forms of transportation and communication; K02 - biocenotic evolution, succession; I01 - invasive non-native species; B07- forestry activities not referred to above; D01- roads, paths and railroads; F04.02 - collection (fungi, lichen, berries etc.); B01.02 - artificial planting on open ground (non-native trees); B02.06 - thinning of tree layer.
2.	Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia* along the great rivers (*Ulmenion minoris*)	91F0	CLAS. PAL.: 44.4	favorable up to unfavorably	E01.01-continuous urbanization; G05.07- missing or wrongly directed conservation measures; D.06- other forms of transportation and communication; B01.02 - artificial planting on open ground (non-native trees); B04- use of biocides, hormones and chemicals (forestry); B07- forestry activities not referred to above; D01- roads, paths and railroads; F04.02 - collection (fungi, lichen, berries etc.); G01-outdoor sports and leisure activities, recreational activities;K02-biocenotic evolution, succession.
3.	*Salix alba* and *Populus alba* galleries	92A0	CLAS. PAL.: 44.141, 44.162 and 44.6	from favorable up to unfavorably-inappropriate	E01.01- continuous urbanization; G05.07- missing or wrongly directed conservation measures; D.06- other forms of transportation and communication; G05 - other human intrusions and disturbances; H04 - air pollution, air-borne pollutants; I01 - invasive non-native species; B07- forestry activities not referred to above; D01- roads, paths and railroads; F04.02 - collection (fungi, lichen, berries etc.).

CONCLUSIONS

Three types of forest natural habitats of Bratovoești Forest have been identified *in the Oltenia Plain, of the Valley Jiu.*

These natural habitats are represented by plant communities rich in mesophyle, meso-hygrophyle and hygrophyle species.

Of the rare and vulnerable species we can mention: *Vallisneria spiralis, Utricularia minor, Nymphaea alba, Azolla filiculoides, Typha minima, Orchis purpurea, Epipactis palustris, Galanthus nivalis, Angelica sylvestris, Crocus flavus, Festuca gigantea.* The phytodiversity of the forest natural habitats from Bratovoești Forest is endangered because the human impact is very high, although this area it is included in the important protected area from Romania. In the floristic composition of the ptytocoenoses of this natural habitat meet many undesirable species: *Rubus caesius*, *Amorpha fruticosa, Phytolaca americana, Erigeron anuus, Ailanthus altissima, Gleditsia triacanthos, Acer negundo.*

Limitational environmental factors and determinants: prolonged drought risks, lowering groundwater level; lowering the groundwater level; the coarse texture of the soil; lowering the level of the groundwater. Management measures imposed by ecological factors and risk: treatments - progressive cuts, conservation cuts, cuttings, grove cuts.

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PHYTOCHEMICAL RESEARCH ON AERIAL PARTS OF *Raphanus raphanistrum* subsp. *landra* (Moretti ex DC.) Bonnier & Layens

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Abstract

Raphanus raphanistrum subsp. landra is a wild annual plant from Brassicaceae family. Aerial parts of the plant can be used as food: the leaves can be used raw or cooked, flowers and young seedpods can be used raw in various dishes. In our country, it is considered a weed and it is not paying attention to its edible and pharmaceutical uses. The aim of this study is to evaluate the antioxidant, total polyphenols and flavonoid content of the ethanol extract of aerial parts (flowers, silique, and leaves) from this species. The plants were collected from ruderal areas. Ethanolic extracts from the flowers, silique, and leaves were analysed spectrophotometric. These preliminary studies on the phytochemical characteristics of Raphanus raphanistrum subsp. landra bring new and important information about nutraceutical importance and the possibility to be used in pharmaceutical industry.

Key words*: Raphanus raphanistrum subsp. landra, polyphenols, flavonoids, antioxidant capacity.*

INTRODUCTION

Plants for spontaneous flora play a very important role in the discovery of active principles that can lead to the development of drugs for the treatment of many diseases. Today it is a very great interest to use natural antioxidants in food and in pharmaceutical ingredients, that can replace successfully the synthetic ones (Soare et al., 2015; Taghvaei and Jafari, 2015; Eça et al., 2014).

The phenolic compounds from plants, vegetables, fruits and cereals like polyphenols and flavonoids (Cercel et al., 2017; Bălan et al., 2014), have the proprieties to reduce oxidative stress, and in this way they can be used in prevention and treatment in many diseses including cancer, neurological, cardiovascular, diabetes, hepatitic, immune deficiency diseses, and they also can be used in antiaging therapy (Yaseen et al., 2017; Nichita et al., 2016).

The phenolic compounds also influence anthocyanins stability during food processing and storage. Thus, the addition of phenolic compounds can be a valuable tool for improvement of nutritive value of functional food products (Alexe and Dima, 2014; Kopjar et al., 2009).

This study was carried out to investigate the antioxidant, total polyphenols and flavonoid content of the ethanol extract of aerial parts (flowers, silique, and leaves) from *Raphanus raphanistrum* subsp. *landra* with posibilities to use in food and pharmaceutical industry.

Raphanus raphanistrum subsp. *landra* is a spontaneos weed plant that can grow both in winter and in spring crops, ruderal areas, from steppe to forests (Georgescu et al., 2016; Ionescu and Ionescu, 2014). It is believed that it is the ancestor of cultivated radish (*Raphanus sativa*) (Nishio and Kitashiba, 2017).

This plant has edible uses, young leaves, flowers and seeds can be used in salads, deep-dish, raw or in powder form as spice (Maldini et al., 2017; Conforti et al., 2012).

The aerial parts of the plant are used in traditional medicine for their purifing, antiinflammatory effects, anti-rheumatic and hypoglicemic activity and for the treatment of various ailments such as gastrointestinal diseases (Conforti et al., 2008).

Marelli et al. (2015), showed that ethanolic extract of *Raphanus raphanistrum* subsp.

landra exerted antioxidant and antiproliferative proprieties and contain the highest amount of sterols and highest content of linolenic acid (9.4%) of the studied species. Jdei et al. (2017) showed that ethanolic extract of *R. raphanistrum* subsp. ***landra,*** according to the phenolic compositions have antioxidant, antibacterial, and anti-tyrosinase activities. Küçükboyaci et al. (2012) demostrated that aquous extract of this plant, have a potential source of antioxidant and minerals of natural origin. Anti-inflammatory and antioxidant activity has been also demonstrated by Conforti et al. (2011) and El and Karakaya (2004).

In our country *R. raphanistrum* subsp. l*andra* it is consider a weed and there are not studies regarding the nutraceutical potential of this plant.

MATERIALS AND METHODS

Plant material

The plants were collected from ruderal area in October 2017 at flowering stage (Figure 1). The plants were authenticated at horticulture botany department of U.A.S.V.M of Bucharest. Plants were separated into leaves, flowers and fruits.

Figure 1. *Raphanus raphanistrum* subsp. *landra* at flowering stage

Preparation of plant extracts

The ethanolic extracts were prepared after Romanian pharmacopoeia method.

1 g of the aerial plant fresh material was weighed accurately on analytical balance and extracted by 100 ml of ethanol 50% (v/v) for 30 minutes at boiling temperature in water bath. A whole content of flask was quantitatively percolated through paper filter into a calibrated flask and filled up to 100 ml with ethanol 50% (v/v).

Determination of total phenolic content

Total phenolic contents were determined spectrophotometrically using a modified method after Mitic et al. (2014), Tukun et al. (2014), Rakcejeva et al. (2012) and Abdelhady et al. (2011) using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents per g fresh material (mg GAE/g FW). To each sample 200 µl of sample extracts, were added 1200 µl distilled water and 300 µl Folin-Ciocalteu reagent and was mixed thoroughly. After 5 min, 1500 µl of 2% Na_2CO_3 was added. After 15 min. of incubation at room temperature in the dark, the absorbances were measured against the reagent blank at 750 nm and compared to a gallic acid calibration curve (R^2 = 0.996). The same procedure was repeated for all standard gallic acid solutions at different concentrations (0, 50, 100, 150, 250, 500 mg/L). The experiment was caried out in triplicate.

Determination of total flavonoid content

The $AlCl_3$ modified assay after Asănică et al. (2016) and Agbo et al. (2015) was used for quantification of the total flavonoid content of the ethanolic plant extracts. 300 µl of the sample extract or standard solutions of rutin (0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.035 mg/ml) was mixed with 1200 µl distilled water and 90 µl of 5% $NaNO_2$ was added. After 5 min, 90 µl of 10% $AlCl_3.6H_2O$ solution was added. After another 5 minutes 600 µl NaOH 1M was added, followed by 720 µl distilled water. The mixture was vigorously shaken.

The absorbance of the standards

The absorbance of the standards and samples extracts were measured against a blank at 510 nm with a UV-spectrophotometer. Total flavonoid content was expressed as mg rutin equivalents/g fresh weight (mg RE/g FW). The determination of total flavonoid content was done in triplicate.

Radical scavenging activity (RSA) assay

The measurement of the DPPH radical scavenging activity was performed according to a modified methodology described by Ibraheim et al. (2015), Garcia et al. (2012) and Brand-Williams et al. (1995) as follows : 0.5 ml hydro alcoholic plant extract was mixed with 3 ml Ethanol ≥99.8% absolute grade and 0.3 ml of ethanol solution of 0.1 mM DPPH. After 100 min of reaction, the absorbance of the samples was measured at 517 nm. The blank is represented by the mixture of 3.3 ml ethanol and 0.5 ml sample extract. The control solution is the mixture between 3.5 ml ethanol and 0.3 ml sample extract.

The scavenging activity was determined using the following formula:

$$AA\% = 100 - \frac{(\text{Abs sample} - \text{Abs blank}) \text{x} 100}{\text{Abs control}}$$

All the solvents used in all the experiments were of analytical grade. All the samples were analyzed in triplicates. All the absorbances were measured using Specord 210 Plus UV/VIS spectrophotometer

Chlorophyll a, b, total carotenoids content

The method was adapted after Asanică et al. (2017), Burducea et al. (2016), Pandia et al. (2013), Lichtenthaler and Wellburn (1983) and Arnon (1949) as follows: 1g of the samples (flowers, leaves, silique) were ground with 80% acetone (v/v). The extract was filtered with a vacuum pump until the residue becomes colorless and completed to volume to 50 ml. (Figure 3). The absorbance of the samples extracts was read at 663, 646 and 470 nm against the blank (acetone). The following formulas were used to calculate the chlorophyll a, b, and total carotenoids content of the samples:

Ca µg/ml = 12.21A663-2.81A646

Cb µg/ml = 20.13A646-5.03A663

$$\text{Cx+c } \mu g/ml = \frac{1000A470 - 3.27Ca - 104Cb}{229}$$

All the solvents used in the experiment were of analytical grade. All the samples were analyzed in duplicates. All the absorbances were measured using Specord 210 Plus UV/VIS spectrophotometer.

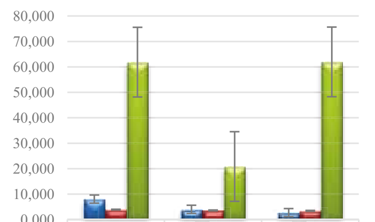
Dry matter content

The *Raphanus raphanistrum* var. *landra* parts (flowers, leaves and silique) were dry at 105°C in drying oven until they reach constant weight (AOAC, 2000).

RESULTS AND DISCUSSIONS

The results for total phenolic, total flavonoid content and radical scavenging activity presented in Figure 2, showed that the leaves have the highest total flavonoid content: 8.06 ± 0.028 mg RE/g FW and total phenolic content: 3.94 mg ± 0.003 GAE/g FW. The lowest content in TFC was recorded by the siliqvae: 2.73 ± 0.003 mg RE/g FW and the flowers had the lowest content in TPC: 3.61 ± 0.001 mg GAE/g FW and radical scavenging activity 20.85 ± 0.002%. The highest radical scavenging activity had the siliquae with 61.95% ± 0.001.

The total phenolic content was similar with Jovancevic et al. (2011) showed in the wild bilberrys (*Vaccinium myrtillus*) that ranged from 3.92-5.24 mg GAE/g FW.



	Leaves	Flowers	Siliqvae
TFC mg RE/g FW	8,067	4,022	2,737
TPC mg GAE/g FW	3,942	3,607	3,467
AA %	61,866	20,856	61,956

Figure 2. TFC, TPC and AA from aerial parts of *R. raphanistrum* subsp. *landra*

There was find also a positive correlation between total flavonoid, total phenols content, radical scavenging activity of all the aerial parts from *Raphanus raphanistrum* subsp. *landra* (Table 1).

Also, it was found a positive correlation between leaves, flowers and siliquae regarding the total flavonoid, total phenols content and radical scavenging activity (Table 2).

In the Figure 3 the highest values regarding the chlorophyll a, b, total carotenoids, total chlorophyll content and chlorophyll a/b ratio were recorded by *R. raphanistrum* subsp. *landra* leaves. The flowers had the lowest values.

Table 1. Correlation between TFC, TPC and AA from aerial parts of *R. raphanistrum* subsp. *landra*

	TFC mg RE/g FW	*TPC mg GAE/g FW*	*AA %*
TFC mg RE/g FW	1		
TPC mg GAE/g FW	0.998	1	
AA %	0.285	0.228	1

Table 2. Correlation between leaves, flowers and silique of *R. raphanistrum* subsp. *landra*

	Leaves	Flowers	Siliqvae
Leaves	1		
Flowers	0.999	1	
Siliqvae	0.997	0.999	1

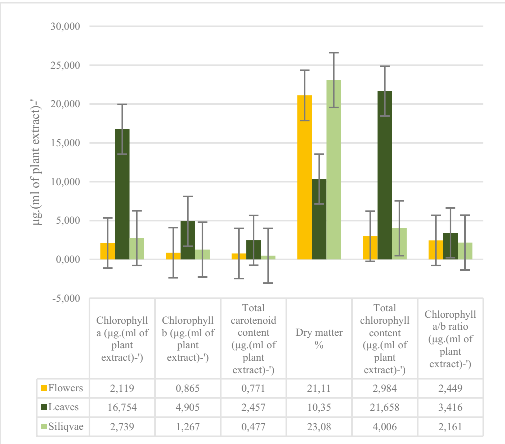


Figure 3. Chlorophyll a, b, total carotenoid and dry matter content of aerial parts from *R. raphanistrum* subsp. *landra*

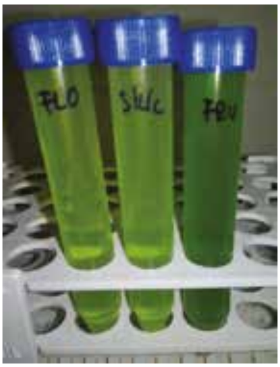


Figure 4. Chlorophyll acetone extract from flowers, siliquae and leaves of *R. raphanistrum* subsp. *landra*

Table 3 shows a positive correlation between chlorophyll a, chlorophyll b, total carotenoids of the flowers, leaves and siliquae.
Correlation to determine the relationship between the variables were calculated using MS Excel software.

Table 3. Correlation between chlorophyll a, chlorophyll b and total carotenoids of aerial parts from *R. raphanistrum* var. *landra*

	Chlorophyll a (µg.(ml of plant extract)-')	Chlorophyll b (µg.(ml of plant extract)-')	Total carotenoid content (µg.(ml of plant extract)-')
Chlorophyll a (µg.(ml of plant extract)-')	1		
Chlorophyll b (µg.(ml of plant extract)-')	0.998	1	
Total carotenoid content (µg.(ml of plant extract)-')	0.985	0.974	1

CONCLUSIONS

This preliminary work reveals that *Raphanus raphanistrum* susp. *landra* harvested in October can be a very interesting source of antioxidants principles similar to wild bilberries with great potential use in food and pharmaceutical industry. Future studies are needed on chemical composition and nutritional value of this plant.

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WHICH SHRUB SPECIES SHOULD BE USED FOR THE ESTABLISHMENT OF FIELD SHELTERBELTS IN ROMANIA?

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Abstract

In the current context of increasing air temperatures and decreasing rainfall, the field shelterbelts play an important role in the management of agricultural lands in Romania. The aim of this study was to highlight the most preferred shrub species used for establishment of the filed shelterbelts in Romania. Ten of the most common shrubs, namely Cornelian cherry, common dogwood, European smoketree, common hawthorn, European spindle, wild privet, blackthorn, dog rose, black elder and common lilac, were selected and ten criteria were taken into consideration. An Analytic Hierarchy Process (AHP) was performed in order to assess the performance of the selected alternatives (i.e. shrub species) by means of pairwise comparisons. Expert Choice Desktop software was used for the analysis. Based on the results of the analysed scenarios, recommendations were made.

Key words: *AHP, field shelterbelt, shrub species.*

INTRODUCTION

In the current context of climate change recorded in Romania and concretized through increasing both air temperatures and number of summer days and decreasing rainfall amount in all seasons, except autumn (Dumitrescu et al., 2014), the field shelterbelts play and will continue to play an important role in the management of agricultural lands, especially in the areas with a low share of forest vegetation.

In Romania, the first field shelterbelts were established in 1880 by Stănculescu in Ialomița County (Southern Romania) on his private land (Catrina, 2007). Since the second part of the last century, these multi-purpose plantations started also to be regarded as a significant source of wild fruits that have an important economic contribution for the forestry sector in Romania (Constandache et al., 2016).

According to the official definition (Law no. 46/2008 Forest Code), the forest shelterbelts represent formations with forest vegetation, located at a certain distance from one to another or close to an objective in order to protect it against the effects of harmful factors and/or for the land improvement from climatic, economic and aesthetic perspectives. Law no. 289/2002 divides the forest shelterbelts into five types, field shelterbelts being among the most important in terms of integrated environmental management. The main roles of the field shelterbelts consist in protecting and assuring favourable micro-environmental conditions for the agricultural crops (Vasilescu, 2008).

Thanks to several other benefits, such as soil and water conservation, carbon sequestration, wildlife habitat, source of timber and non-timber products, aesthetics, etc., the field shelterbelts were the subject of several national programmes. Even if only a few steps were done in the last two-three decades in terms of establishing new field shelterbelts, in the future the area of forest shelterbelts at national level is expected to increase. The central authority responsible for forestry in Romania (*i.e.* Ministry of Waters and Forests) did another important step by including in the draft of the National Forest Strategy the budget needed for planting a significant area of these types of forest cultures (MAP, 2017).

Due to its high diversity in terms of shrub and tree species, in Romania more than fifty woody species are commonly used in afforestation of the degraded lands and in establishment of the protective shelterbelts (Enescu, 2015).

The aim of this study was to highlight the most preferred shrub species used for establishment of the filed shelterbelts in Romania.

MATERIALS AND METHODS

Ten of the most common shrubs, namely Cornelian cherry (*Cornus mas* L.), common dogwood (*Cornus sanguinea* L.), European smoketree (*Cotinus coggygria* Scop.), common hawthorn (*Crataegus monogyna* Jacq.), European spindle (*Euonymus europaeus* L.), wild privet (*Ligustrum vulgare* L.), blackthorn (*Prunus spinosa* L.), dog rose (*Rosa canina* L.), black elder (*Sambucus nigra* L.) and common lilac (*Syringa vulgaris* L.) were taken into consideration in this study.

An Analytic Hierarchy Process (AHP) was performed in order to assess the performance of the selected alternatives (*i.e.* shrub species) by means of pairwise comparisons. Within AHP, the decision problem (*i.e.* the goal of this study) was decomposed into a hierarchy sub-problems (*i.e.* the criteria used), each of which can be independently analysed.

A scale ranging from 1 to 10 was used for each criterion, namely: criterion 1 - *growth rate* (from 1 - very slow growing rate to 10 - very fast growing rate), criterion 2 - *vegetative propagation* (from 1 - no vegetative propagation to 10 - very intense vegetative propagation), criterion 3 - *seed dimensions* (from 1 - the smallest seeds to 10 - the biggest seeds), criterion 4 - *height* (from 1 - the smallest to 10 - the highest), criterion 5 - *crown density* (from 1 - rare crown to 10 - very dense crown), criterion 6 - *root system* (from 1 - very less developed in depth and sidewise to 10 - very developed in depth and sidewise), criterion 7 - *demand for light* (from 1 - very shade tolerant to 10 - very high demand for light), criterion 8 - *soil requirements* (from 1 - extremely low requirements to 10 - very high requirements), criterion 9 - *temperature requirements* (from 1 - resistant to low temperatures to 10 - resistant to high temperatures) and criterion 10 - *ornamental value* (from 1 - very low value to 10 - very high value).

For each criterion Expert Choice Desktop software (version 11.5.1683) was used for the analysis.

Two scenarios were taken into consideration, namely scenario 1 (all criteria received equal importance) and scenario 2 (special attention - 40% of the overall variation - was given to the criterion *growth rate*).

RESULTS AND DISCUSSIONS

Based on the information available in the specialized manuals and studies, a detailed description of the ten selected shrub species was made in accordance with the ten selected criteria. The description took into consideration only the information related with the ten selected criteria.

Cornelian cherry has a very slow growing rate; it regenerates in a vegetative way; its seeds are 12-15 mm; it can reach 6-8 m in height; its crown is rare; it has a strong root system; it is a light-demanding species; it can grow on light sandy to heavy clay soils, with a pH ranging from slightly acid to very alkaline; it is a thermophilous species, but it can survive up to -30°C; it has a high ornamental value - its yellow flowers appear before the leaves and its leaves are reddish during autumn (Clinovschi, 2005; Constandache et al., 2006; Şofletea and Curtu 2008; Constandache et al., 2012; Da Ronch et al., 2016).

Common dogwood can be propagated in a vegetative way; its seeds are 5-8 mm long; it can reach 3-6 m in height; its root system is superficial; it is a light demanding species, but it can tolerate also the shade; it prefers consistently moist, well-drained soils, but it is also able to grow in a wide range of soils, from dry to humid with different pH levels; it is adapted to a high range of temperatures; it has a high ornamental value thanks to its reddish shoots (those that are situated in full light) and its reddish leaves during autumn (Clinovschi, 2005; Constandache et al., 2006; Şofletea and Curtu, 2008; Constandache et al., 2012; Popescu et al., 2016).

European smoketree has a rare crown; its seeds are small (3-4 mm); it can reach 1-3 (5) m in height; it grows in semi-shade conditions, on superficial soils, including the ones with moderate amount of carbon; it is a thermophilous species and it is resistant to drought and frost (-30ºC); it is very appreciated from the ornamental point of view due to its very rich inflorescence and its yellow to purple leaves during the fall (Clinovschi, 2005; Constandache et al., 2006; Şofletea and Curtu, 2008).

Common hawthorn has a slow growing rate; it can reach up to 8-10 m in height; its globulous seeds are 7-10 (14) mm in diameter and they are ussualy bird-dispersed; its root is deeply developed in depth, with numerous lateral branches; it could be either a light-demanding or a semi-shade species; it is able to grow on several types of soils and it can tolerate a large amplitude of temperatures (Forman and Baudy, 1984; Clinovschi, 2005; Şofletea and Curtu, 2008; Constandache et al., 2012).

European spindle can be vegetative propagated; it can reach 6 m in height; its seeds are 10-15 mm in diameter; it has a strong root system; it can grow in full or partial shade; it usually grows on well-drained, preferably alkaline soils; it is a thermophilous and a drought resistant species and it is appreciated for its coloured fruits (Clinovschi, 2005; Şofletea and Curtu, 2008; Constandache et al., 2012; Popescu et al., 2016).

Wild privet can be propagated in both vegetative and generative ways; its bird-dispersed fruit is a small black berry, 6-8 (10) mm in diameter; its root system is superficial; it is a shade tolerant species; it is able to tolerate a wide spectrum of soil conditions; it is resistant to drought; it has an ornamental value, specially thanks to its white flowers (Forman, Baudy, 1984; Clinovschi, 2005; Constandache et al., 2006; Şofletea, Curtu, 2008; Enescu et al., 2015).

Blackthorn has a slow growing rate; it is reproducing both in vegetative and generative ways; its seeds are 10 to 15 mm in diameter; it can reach a height of 2-3 (5) m; its roots are deeply developed in depth, with numerous lateral branches; it is a light-demanding species; it can grow on several types of soils; it is a xerophyte species, that is resistant to drought; thanks to its white flowers, it is appreciated from an ornamental point of view (Clinovschi, 2005; Şofletea and Curtu, 2008; Constandache et al., 2012; Popescu and Caudullo, 2016).

Dog rose can reach 2-3 m in height; its fruits are 15-20 mm in diameter; it is a light-demanding species; it has low requirements to soil conditions; it is able to tolerate a wide spectrum of soil conditions; it has an ornamental value especially thanks to its red fruits (Clinovschi, 2005; Şofletea and Curtu, 2008; Constandache et al., 2012; Soare et al., 2015).

Black elder usually reaches 4 to 5 m in height; it is able to reproduce both in vegetative and generative ways; its seeds are 6-8 mm in diameter; its root system is deeply developed in depth, with numerous lateral branches long up to 8 meters; it is a semi-shade species; it is a very exigent species, preferring rich in humus and nitrogen and deep soils; it is not drought tolerant (Clinovschi, 2005; Şofletea and Curtu, 2008; Constandache et al., 2012; Enescu et al., 2016).

Common lilac has a fast growing rate; it can be propagated both in vegetative and generative ways; its seeds are 10-15 mm long; it can reach a height of 3-4 (7) m; its crown is branched from the base; it is a light-demanding species; it prefers the soils with high amount in calcium; it is a thermophilous species, resistant to drought and frost; it is very appreciated thanks to its very diverse colored flowers (Clinovschi, 2005; Şofletea, Curtu, 2008; Constandache et al., 2012; Horț et al., 2013).

The AHP alternative ranking for the ten criteria, based on the information available in the specialized manuals and on the author's opinion, is given in Table 1.

Table 1. AHP alternative ranking

Criterion	*Cornus mas*	*Cornus sanguinea*	*Cotinus coggygria*	*Crataegus monogyna*	*Euonymus europaeus*	*Ligustrum vulgare*	*Prunus spinosa*	*Rosa canina*	*Sambucus nigra*	*Syringa vulgaris*
1. Growth rate	1	7	6	2	4	8	3	5	9	10
2. Vegetative propagation	4	9	2	1	5	10	8	3	5	7
3. Seed dimensions	9	2	1	5	8	4	8	10	3	8
4. Height	8	6	3	9	7	4	2	1	10	5
5. Crown density	2	5	1	10	3	4	8	9	6	7
6. Root system	7	1	6	9	3	2	8	4	10	5
7. Demand for light	7	6	4	5	1	2	10	9	3	8
8. Soil requirements	7	6	5	4	8	1	3	2	10	9
9. Temperature requirements	1	3	10	7	4	5	9	8	2	6
10. Ornamental value	7	5	10	1	6	4	3	2	8	9

In scenario 1 (all criteria received an equal share), the most preferred shrub species were: *Syringa vulgaris*, *Sambucus nigra* and *Prunus spinosa*, respectively (Figure 1). This means that these three shrub species should be used with priority in establishing new field shelterbelts to the detriment of the other seven, when the ten criteria taken into consideration are equally important. In the case of common lilac, its placing in the top could be explained by the fact that it is a fast growing species, that is able to reproduce both in vegetative and generative ways (thanks to its medium-size seeds, which can be disseminated at great distances), with medium requirements in terms of temperatures and with a high ornamental value. Its rare crown, very low developed root system and medium to high requirements in terms of soil conditions represent its drawbacks.

In the case when special attention - 40% of the overall variation - was given to the criterion *growth rate*, wild privet appeared in top three (Figure 2).

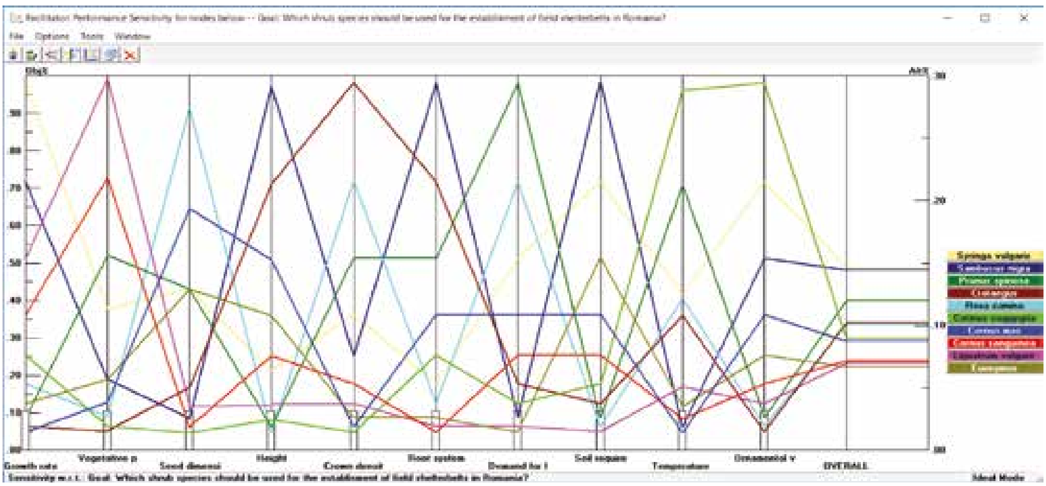

Figure 1. The ranking of the ten shrub species in the first scenario

The results of this second scenario could be useful in the case when someone wants to establish in a very short time a field shelterbelt composed by fast-growing species that are able to reproduce both in vegetative and generative ways (thanks to their small seeds), but in this situation the height of the shelterbelt, its density or its combined root system are not of great interest.

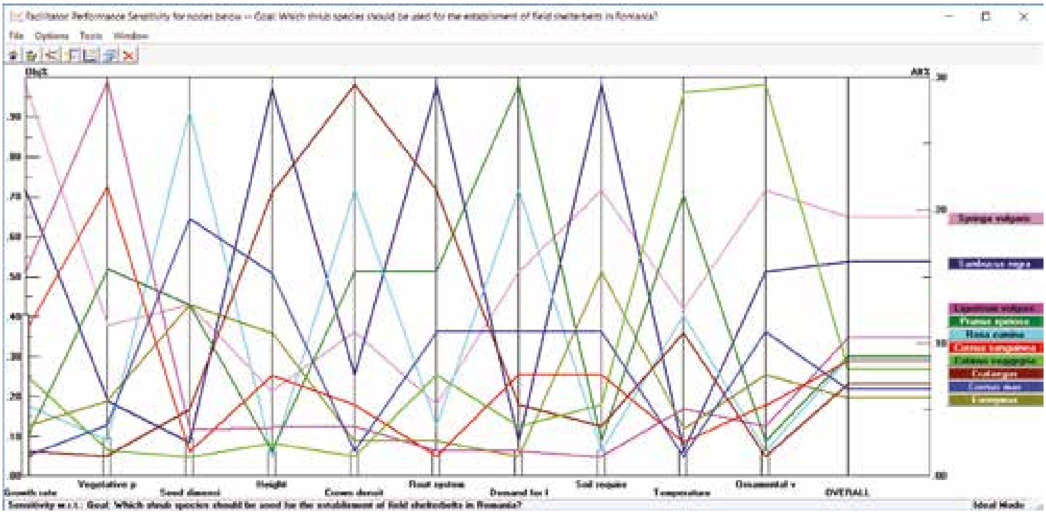

Figure 2. The ranking of the ten shrub species in the second scenario

CONCLUSIONS

The results of this study could be regarded as an attempt to take a decision regarding the shrub species that could be used in several scenarios, with special focus on biological characteristics, morphological traits, ecological requirements or other aspects.

By using the combination of the principles of AHP and a very easy to use software, such as Expert Choice Desktop, solving a multi-decision problem becomes very easy.

In the perspective of increasing the area of forest shelterbelts at national level, we believe that this multi-decision analysis approach will become of great interest both for the land owners but also for the foresters or other designers of different categories of shelterbelts, especially thanks to the fact that it is a very easy-to-use tool.

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MORPHOLOGICAL ASPECTS ABOUT GERMINATION OF *Vulpia myuros* (L.) C.C. Gmel. CARYOPSIS

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Abstract

Caryopsis harvested in 2014 from a population of Vulpia myuros, invasive in a rape seed crop, were tested for germination over 3 years and a half after harvesting (2015-2018). The germination tests were done on paper moistened with distillate water in Petri dishes, in several variants with Vulpia caryopsis and seeds of different species - Triticum durum, Brassica napus, Helianthus annuus. Viability of the seeds of the Vulpia population, stored under laboratory conditions, was preserved during all this time. The germination of the Vulpia caryopsis was simulated in the presence of germinated seeds of the other species. Germinated caryopsis of Vulpia myuros showed a hairy coleorhiza which allowed high adherence of the seedling to the soil surfaces.

Key words*: Vulpia myuros, caryopsis, germination, hairy coleorhiza.*

INTRODUCTION

An early and/or faster germination, broader germination requirements, and exploitation of germination niches are some of the traits, described by Gioria and Pysěk (2016) that explain the success of species becoming invasive in non-native habitats. To *Vulpia myuros* (annual fescue, rattail fescue), for example, the higher rate of germination in darkness or light has represented an advantage in conquering new spaces, unlike its less invasive congener, *V. bromoides* (Gioria and Pysek, 2016). According to Richardson's definition (Richardson et al., 2000), *V. myuros* can be considered an invasive species only in non-native natural areas. But, for agricultural crops it has acquired the character of a highly competitive weed, associated with minimal soil disturbance (no-till cropping systems) (Ball et al., 2007); its incidence is rising rapidly, such that in some regions it has emerged as a new weed (Georgescu et al., 2016).

Integrated weed management (IWM) strategies include measures based on seed and germination information under field conditions, such as seed production and their viability, dormancy and vernalization requirements, specific demands for seed germination and plant growth (Ball et al., 2008). Results of various experiments on seed and seed germination, conducted in the laboratory or in the field, registered the behaviour of rattail fescue both in the field crops and in grass plots: *V. myuros* is a rapidly germinating species whose seeds have persisted for more than a year in the soil (Peco et al., 2003), but no more than 2-3 year (Ball et al., 2008); it can germinate in both darkness and light, but the light has doubled the temperature range in which this species is able to germinate; when the seeds have been buried under the surface depth, the plants occurrence has been delayed (Dillon and Forcella, 1984). With a high seed production and a 2-3-month dormancy period, *V. myuros* is able to survive in summer and germinate in autumn when soil moisture is favorable (Dowling, 1996); in a California field experiment, increasing seeding density did not affect the density of the *V. myuros* plants, instead it has reduced the density of native perennial herbs and weeds (Brown and Rice, 2000).

Testing allelopathic potential on several plants, Kato-Noguchi et al. (2010) have showed that extracts of *V. myuros* inhibited the growth of the roots and shoots in different species; seed germination and growth of the wheat coleoptile and radicle were inhibited by the aqueous

extracts from annual fescue (Min et al., 1993); it is worth noting that compounds from aqueous extracts of some wheat genotypes showed allelopathic activity against other grass species, the ryegrass (Petcu et al., 2017).
Our aim in this study is to highlight the seed germination observations of *Vulpia myuros*, stored under laboratory conditions, over 3 years after harvesting; there are also descriptions for the morphology of the caryopsis of rattail fescue, before and after germination.

MATERIALS AND METHODS

Caryopsis harvested in 2014 from a population of *Vulpia myuros*, weed in a rapeseed crop (Georgescu et al., 2016), were stored in paper bags under laboratory conditions. The germination tests were done on paper moistened with distillated water in Petri dishes, in the following variants: i) 2015 - October: *V. myuros;* ii) 2016 - July: *V. myuros;* iii) 2017 - December: *V. myuros*, *Triticum durum*, *Brassica napus* and, respectively, *Heilanthus annuuus*, sown together; the germination was verified separately for each species, under the same conditions; iv) 2018 - January: *V. myuros*, *T. durum* and *B. napus* sown at a 3-days interval, on the same Petri dish.
For each variant 10 caryopsis or seeds/Petri dish were used, in 3 repetitions (replicates), for observing the germination status. All the grains used have had 100% of germination.
The aspects of all types of germinations were observed and the results have had recorded from the first day after sowing, using the S8APO stereomicroscope. Images of the micro-morphology of the caryopsis before and after germination were obtained at SEM FEI Inspect S 50. The experiments were carried out in the Research Center for Studies of Food Quality and Agricultural Products from UASVM Bucharest, Laboratory of microscopy and plant anatomy.

RESULTS AND DISCUSSIONS

Caryopsis morphology before and after germination. The fusiform caryopsis of *V. myuros* is merged to lemma and palea. Lemma, with basal rounded glabrous callus and distal straight awn, up to 15 mm long, is 5-nervate and scabrous (Stace, 2010; Häfliger and Scholz, 1981) (Figures 1, 2, 3).

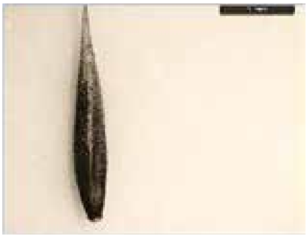

Figure 1. *Vulpia myuros* caryopsis (stereomicroscope)

Figure 2. Lemma basal callus (stereomicroscope)

Figure 3. Lemma basal callus (SEM)

Caryopsis germination. Hairy coleorhiza can be observed on germinate caryopsis (Figures 4, 5). This peculiar characteristic encountered to some *Poaceae* species, like *Lolium perenne*, *Festuca arundinacea* or *Oryza sativa*, allows a high adherence of the germinate caryopsis to the soil surface. (Morita et al., 1990, 1997; Debaene-Gill et al., 1994).

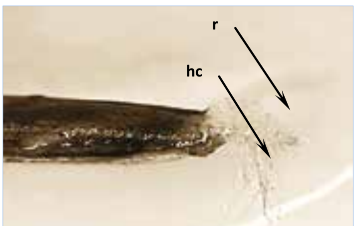

Figure 4. Hairy coleorhiza (hc) and radicle (r) of *V.myuros* germinate caryopsis

Figure 5. Hairy coleorhiza (SEM)

In October 2015 and July 2016, in conditions of light, germination and growing of the seedling were produced over 6 days (Figure 6). All the caryopsis had germinated (100%).

Figure 6. *V.myuros* germinate caryopsis in July, 2016

In December, *V. myuros* caryopsis germinated in dark conditions (coated Petri dishes). 24 hours after sowing in all variants the germination process was initiated both for *V. myuros* and the other species - *T. durum*, *B. napus* and *H. annuus*. In experiments with *V. myuros* only, caryopsis were in the early germination stages, a short hairy coleorhiza and the radicle being observed.

Figure 7. Germinate caryopsis of *V. myuros* of *T. durum* experiment. Coleoptile is present

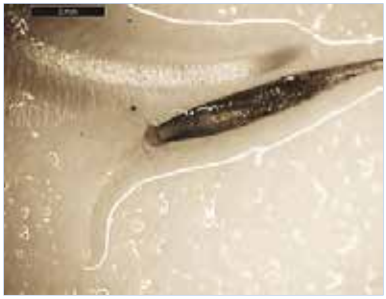

Figure 8. Germinate caryopsis of *V. myuros*. Above, the radicle of rape seed

In experiments with *T. durum*, caryopsis of the *V. myuros* were in an advanced germination stage: radicle has formed root-hairs and coleoptile was obvious in over 50 % of plants. Coleorhiza hairs were longer than those of the *V. myuros* only experiments (Figure 7). Seeds of *B. napus* stimulated, also the *V. myuros* caryopsis germination, but the number of seedlings with the coleoptile was reduced in comparison with the *T. durum* experiment (Figure 8).

Figure 9. Seedlings of rapeseed and *V.myuros* germinated caryopsis

Figure 10. *V.myuros* germinated caryopsis (detail)

Same situation was registered in *V. myuros* and *H. annuus* germination experiments. The germination of *V. myuros* caryopsis was produced at the same rate in the January experiments, even though these were sown after the seeds of *B. napus* or caryopsis of *T. durum* were germinated (Figures 9, 10).

CONCLUSIONS

Viability of the seeds of the *V. myuros* population, stored under laboratory conditions, was preserved during 3, 5 years.

The presence of seeds of other species, despite the time of sowing, accelerate the germination of the *V. myuros* caryopsis.

Germinated caryopsis of *V. myuros* showed a hairy coleorhiza which allowed high adherence of the seedling to the soil surfaces.

Future germination experiments with this populations of *V. myuros* in the fields are suggested.

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MELLIFEROUS POTENTIAL OF SILVER LINDEN TREES (*Tilia tomentosa* Moench.) GROWING IN THE FORESTS FROM SOUTH ROMANIA

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Abstract

Apart the linden trees are important as source of wood and have medicinal and ornamental uses, they are also important from the melliferous point of view, being a good source of food for melliferous insects as well as an important honey source for beekeepers. Among the different linden species, the silver linden is appreciated to have a good melliferous potential. Taking into account these aspects, the aim of this study was to investigate the melliferous potential of the silver linden trees (Tilia tomentosa Moench.) growing in the forests from South Romania. In this respect, a study was carried out in the years 2009, 2010 and 2011 in two forests located in South Romania. The following parameters were analyzed: 1) the average sugar yield of the silver linden flowers, which is given by the quantity of nectar secreted by the flowers and the nectar concentration in sugar; 2) the number of flowers per silver linden tree and per hectare; 3) the lifespan of the silver linden flowers. Our study performed in the two forests from South Romania showed the good melliferous potential of the silver linden trees, which was in average of 318 kg.ha^{-1} of honey, respectively 0.76 kg of honey per linden tree. However, it must be taken into account the influence on this good melliferous potential of the silver linden trees determined by the climatic conditions of the year and the number of flowers the linden trees can produce in the respectively year, as well as the number of linden trees from the forest.

Key words: *nectar secretion, nectar concentration in sugar, flowers, honey, silver linden.*

INTRODUCTION

Tilia genus (linden, lime) from *Tiliaceae* family includes about thirty species widespread in cool temperate regions from Europe, Asia and America, out of which only four occur naturally in Europe, i.e. small-leaved linden (*Tilia cordata* Mill.), large-leaved linden (*Tilia platyphyllos* Scop.), silver linden (*Tilia tomentosa* Moench.), and Caucasian linden (*Tilia dasystyla* Stev.) (Radoglou et al., 2009).
In Romania, linden is presented through three species, respectively *T. cordata*, *T. platyphyllos* and *T. tomentosa* (Ivanov et al., 2014), which form large natural stands, usually mixed with other hardwoods. Also, these species are frequently planted in parks in the cities and along roadsides because of their ornamental value. Their valuable features include the regular shape of their crown, the early development of leaf buds, and abundant flowering (Weryszko-Chmielewska and Sadowska, 2010). However, in Romania, the most important species from the point of view of beekeeping is *T. tomentosa*, which grows in both plains and hills (Sănduleac and Lăzărescu, 1960; Ion et al., 2008).
Linden trees are generally considered to be among the best nectariferous species (Waś et al., 2011). They are considered to be a good source of food for melliferous insects as well as an important honey source for beekeepers. The specific floral fragrance invites insects to feed with the abundant nectar that the yellowish-green blossoms produce.
The melliferous value is given by the long blooming period (about 12 days, even 16 days according to species) as well as to the good nectar secretion. Moreover, the different linden species have diverse blooming dates which extend their melliferous importance for honey production. Thus, in Poland the blooming

duration of the *T. cordata* and *T. platyphyllos* species was of 16 days, while the blooming duration of the *T. tomentosa* species was of 12 days, the total duration of all species being about six weeks (Weryszko-Chmielewska and Sadowska, 2010). Also in Poland, Dąbrowska et al. (2016) reported that the blooming period of all the analysed linden taxa lasted 7 weeks, on average, from June 7 to July 24.

The melliferous value of the linden trees is given also by their flowering period at a time when alternative floral resources are scarce. Therefore, not incidentally, the linden blossom is considered to be the "Queen of Nectar".

Linden honey is among the most known and popular honeys, together with acacia, rapeseed, and multifloral honey. A honey is called "linden honey" when it contains at least 30% of *Tilia* pollen (Bonod et al., 2003). Linden honey is defined by an intense specific flavour.

In Romania, it is estimated that over the years the value of the honey obtained from linden trees has been greater than the value of their timber cut during the same period.

Research indicates that honey has functional properties in human health promotion which depend largely on the floral source of the honey (Savatovića et al., 2011). Linden honey as well as acacia and multifloral honey have a high content in polyphenolic compounds and contain an important amount of C vitamin, which is necessary for human body (Purcărea and Chiş, 2011). In a study performed by Savatović et al. (2011) on antioxidant activity of three different Serbian floral honeys (acacia, linden and "Homoljski med"), it resulted that linden honey had the highest content of total phenolic and flavonoid compounds. This means that linden honey has a beneficial effect on human health.

Linden trees are valued not only for their high honey production, but also due to the medicinal proprieties of their flowers. Linden flowers (*Tiliae flos*) have been used in phytotherapy, as they have antispasmodic, sudorific, expectorant, diuretic and sedative effects (Rădulescu and Oprea, 2008). In Romania, the linden flowers are usually collected by foresters as a byproduct of the forests.

Apart the numerous usage of linden as universal and renewable raw material (wood, bark, fibre), as well as usage as medicinal, melliferous and ornamental species, the linden tree is presented as a Romanian cultural and literary symbol in various situations (Ţenche-Constantinescu et al., 2015).

The aim of this study was to investigate the melliferous potential of the silver linden trees (*Tilia tomentosa* Moench.) growing in the forests from South Romania.

MATERIALS AND METHODS

The study was carried out in years 2009, 2010 and 2011 in two forests located in South Romania, respectively one closed to Malu Spart village - Giurgiu county (44°26' North latitude and 25°40' East longitude) and one closed to Ciocăneşti village - Dâmboviţa county (44°36' North latitude and 25°52' East longitude).

In order to calculate the melliferous potential of the linden trees (also called "honey potential"), the following parameters were analysed: 1) the average sugar yield of the silver linden flowers; 2) the number of flowers per silver linden tree and per hectare; 3) the lifespan of the silver linden flowers.

Based on a sugar content of 80% for honey (CODEX STAN 12-19811), the melliferous potential was calculated as follow:

Melliferous potential (MP) = sugar/flower x no of flowers x blooming duration x 0.8^{-1} x 10^{-6}

where:

- MP is expressed in kg/ha;
- sugar/flower is expressed in mg of sugar/flower and it is calculated by the following formula:

  sugar/flower = (n x c)/100

where: n = nectar secreted by a flower during 24 hours (mg/flower);
c =nectar concentration in sugar (%);

- no of flowers is calculated at hectare based on number of trees per hectare and number of flowers per tree;
- blooming duration represents the number of days that a single flower is blooming.

The average sugar yield of a single flower was calculated based on two parameters, respectively the nectar secretion (the quantity of nectar produced by a flower, which is expressed in mg) and the nectar concentration in sugar (expressed in percent). Observations

regarding these two parameters were performed in 2009 and 2010 in both studied forests.
Before nectar determinations, it was intended to localize the nectary glands. In this respect, fresh open flowers were collected, and examined in laboratory under a binocular loupe coupled with a digital camera.
The nectar secretion (mg per flower) was determined in randomly chosen, unvisited flowers by insects (isolated flowers) at different stages of development. In this respect, there were selected five silver linden trees per forest and per year, and five tertiary branches full with inflorescences were isolated in tulle isolators (in total, 25 branches per forest and per year). The selected trees and branches were among those selected by foresters for collecting the linden flowers for medicinal purposes. The isolation operation is necessary to exclude the potential visits of the insects in order to gather nectar. After 24 hours from the isolation, the respectively branches were cut from trees and the nectar was immediately sampled from 10 flowers per each isolated branch using microcapillary method (see Ion et al., 2012). The nectar determinations were performed in the first half of the day. Immediately after extractions from flowers, for each nectar sampling, there was determined the nectar concentration in sugar using a hand refractometer.
The average number of inflorescences per tree was established in both forests in 2011 during the peak of flowering of silver linden trees. This activity was coupled with the activity of foresters to collect the linden inflorescences for medicinal purpose, and which involves cutting of some tertiary branches from marked trees. For our purpose, we have selected five silver linden trees from those marked by the foresters in each forest. In choosing the trees, there was payed attention their crowns not to be under the shallow of the crowns of other trees. The objective was the analysed trees to have flowers uniformly spread in their crown.
For each analysed tree, there were counted the number of secondary branches that grow off of scaffold branches, as well as the number of tertiary branches that grow off of secondary branches.
The counting of the inflorescences was performed on five tertiary branches per each selected tree (in total, five selected tree and five tertiary branches per tree, respectively 25 tertiary branches per forest), but only on the branches resulted from the cutting operations for collecting the inflorescences for medicinal purpose (Figures 1 and 2). The assessments of the total number of inflorescences per each analysed linden tree was done starting from the average number of inflorescences on tertiary branches and the average number of tertiary branches on a secondary branch, and the average number of the secondary branches on scaffold branches.

Figure 1. The counting of silver linden inflorescences in the forest

Figure 2. The silver linden inflorescences after counting

The average number of flowers per inflorescence was determined by randomly analysing a number of 10 inflorescences per each tertiary branch used for counting the number of inflorescences. So, the determination of the average number of flowers per inflorescence was coupled with the determination of the number of inflorescences. For each of the analysed inflorescences, the flowers were counted and registered. The average number of flowers per inflorescence multiplied by the average number of inflorescences per tree gives the average number of flowers per linden tree.

The number of flowers per hectare was calculated by multiplying the average number of flowers per linden tree by the average number of linden trees per hectare. The number of linden trees per hectare was estimated starting from the number of trees counted in each forest on an area of one thousand square metre (0.1 ha).

The lifespan of the linden flowers (expressed in days) was determined in 2009, 2010 and 2011. In this respect, there was determined how long a single flower in blooming lasts. The blooming duration of a flower was considered to be the number of days from the bud opening to the first signs of senescence on tagged flowers. The beginning of blooming was defined as the moment when the perianth was sufficiently open to permit the entry of an insect and the end of blooming when the perianth began to wilt.

In view to establish the lifespan of the silver linden flowers, the flower buds were randomly marked with tags at opening, respectively 10 flower buds per tree and 10 trees per forest in the two forests and the three years of study. The marked flowers were monitored until flower senescence. In this respect, twice a day, respectively in the morning and in the evening, the flowers were checked to see their flowering progress.

RESULTS AND DISCUSSIONS

1. The nectar secretion and the nectar concentration in sugar

The nectar glands are found on the upper surface of the sepals of the linden flowers; nectar drops accumulate in the depression of the sepals, being visible by naked eye (Figure 3).

The nectar secretion was in average of 1.8 mg/flower/24 hours and the nectar concentration in sugar was in average of 51.9% (Table 1). The nectar secretion varied from 0.2 to 4.2 mg/flower/24 hours and the nectar concentration in sugar varied from 42 to 57.5%, according to the forest location and climatic conditions of the year. These findings on nectar secretion and nectar concentration in sugar are according to those reported during the time in Romania either for *Tilia tomentosa* (Table 2) or for *Tilia* spp. (Table 3).

It has to be said that the nectar secreted by the linden flowers are directly exposed to the air, which means the nectar (in fact, the water from nectar) readily evaporates. This characteristic makes the nectar available to the insects to be strongly dependent on weather conditions during flowering, respectively on air humidity and temperature.

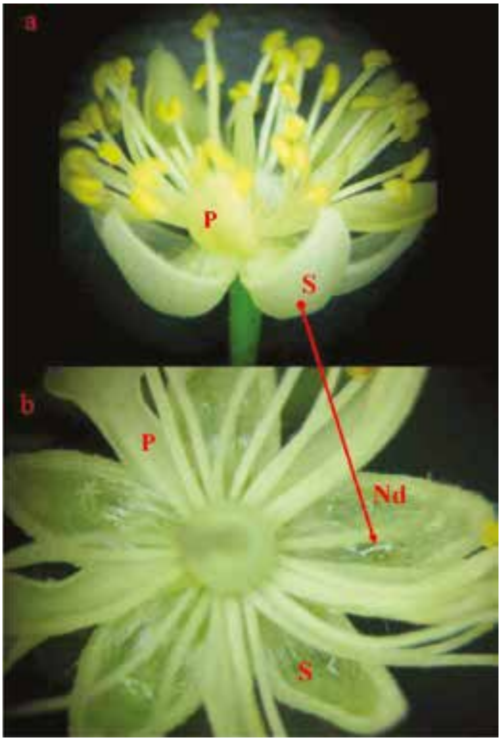

Figure 3. The flower at *Tilia* spp. with boat-shaped sepals (S), petals (P) and nectar drops in the upper side of the sepals (Nd): (a) flower seen from lateral; (b) flower seen from above

Table 1. Nectar secretion and nectar concentration in sugar of the silver linden flowers

Forest location	Year	Nectar secretion (mg nectar/flower in 24 hours)			Nectar concentration in sugar (%)		
		Min.	Average	Max.	Min.	Average	Max.
Malu Spart	2009	0.2	0.4	0.7	52.0	54.0	55.5
	2010	0.9	2.0	3.4	51.0	53.5	55.0
Ciocăneşti	2009	0.8	2.7	4.2	42.0	49.0	56.5
	2010	0.9	2.0	4.0	43.5	51.0	57.5
Average values		-	1.8	-	-	51.9	-

Table 2. Nectar secretion and nectar concentration in sugar of the silver linden flowers (*Tilia tomentosa*) - values reported in Romania

Forest location	Year	Nectar secretion (mg nectar/ flower/ 24 hours)	Nectar concentration in sugar (%)	Authors who reported the data
Giurgiu (South of Romania	1957	0.48	-	Sănduleac, 1965
	1958	0.40	48.5	
	1959	0.62	58.2	
Tulcea (South-East of Romania)	1958	0.65	61.0	
	1959	0.25	52.0	
	1960	1.20	-	Baculinschi, 1961
Average values		0.60	54.9	-

Table 3. Nectar secretion of the linden flowers (*Tilia* spp.) - values reported in Romania by Sănduleac (1965)

Forest location	Period	Nectar secretion (mg nectar/ flower/ 24 hours)
Moldova (East of Romania)	1958-1959	2.6 - 3.5
Banat (West of Romania)	1959-1960	2.4 - 3.3
Tulcea (South-East of Romania)	1958-1960	2.1 - 3.4
Giurgiu (South of Romania)	1957-1959	2.2 - 3.4

We have noted that the nectar concentration in sugar recorded the next day after a rainy day was very low, which means that a good water status of the linden trees dilutes the nectar concentration in sugar. However, a good water status of the linden trees was associated with a good nectar secretion, the nectar being indeed less concentrated in sugar.
It has to be underlined that day to day variation in nectar secretion was somewhat greater than daily variation in nectar concentration in sugar.
The daily yields of sugar of the silver linden flowers were between 0.2 and 1.3 mg per flower, with an average of 0.9 mg sugar per flower (Table 4).

Table 4. The daily yields of sugar of the silver linden flowers

Forest location	Year	Average sugar yield per flower (mg sugar/flower/24 hours)
Malu Spart	2009	0.2
	2010	1.1
Ciocăneşti	2009	1.3
	2010	1.0
Average value		0.9

2. The flower lifespan
Our results indicated that one flower lasted in bloom in average for five days, with limits of variations from 4 to 8 days. Weather conditions had significant direct effects on blooming duration of a single flower. Our observations indicated that the flowers blooming under low moisture conditions last up to 6 days. The highest longevity up to 8 days was seen for flowers blooming under wet conditions.

3. The number of flowers per linden tree
The analysed linden trees were large trees, with a basic structure formed by scaffold branches that start directly from the trunk and many lateral branches that are formed on the scaffold branches. It has to be noted that on each scaffold branches there are some secondary branches that grow off of them, and on each secondary branch there are some tertiary branches.
The number of inflorescences on each tertiary branch was in average of 71, with limits of variations between 42 to 106 inflorescences.
In average, on each inflorescence there were found a number of 9 flowers.
Based on the total number of tertiary branches estimated on linden tree, the average number of inflorescences per linden tree was found to be of 15,082, respectively 135,738 the average number of flowers per linden tree. Based on these data, and knowing the number of trees from studied forests, we calculated the number of flowers per unit of surfaces for each studied forest (Table 5).
We have noted that the most productive branches in term of inflorescences were the ones located in the upper part of the crown, especially those grown at an angle of 60 to 75 degrees from the vertical trunk. These branches

have the tendency to grow less vigorously and to produce more flowers than those growing in an upright position.

Table 5. Average number of silver linden flowers per ha

Forest location	Number of linden trees/ha	Average number of flowers per linden tree	Average number of flowers per ha
Malu Spart	400	135,738	54,295,200
Ciocăneşti	425		57,688,650

The linden trees that flowered abundantly and almost annually were those exposed completely to the light, respectively those linden trees that were not affected during the day by the shadow of the crowns of other trees. The linden trees under the complete shadow all over the day flowered very little or not at all. The linden trees under low shadow flowered more than the trees under intense shallow.

The parts of the same crown that was exposed to the light flowered more, producing more inflorescences than the ones under the shadow. The number of flowers in the crown was according to the intensity of shadow the crown was exposed to. Thus, there were crowns with parts under intense shadow that did not flowered at all.

4. The melliferous potential

The melliferous potential calculated for the two studied forests with silver linden trees are, in average, of 318 $kg.ha^{-1}$ of honey, with limits of variations from 68 to 469 $kg.ha^{-1}$ (Table 6).

The melliferous potential of an individual silver linden tree is in average of 0.76 kg of honey, with limits of variations from 0.17 to 1.1 kg of honey (Table 7).

Table 6. Melliferous potential of the silver linden trees from the studied forests - kg honey/ha

Forest location	Year	Average sugar yield per flower (mg sugar/flower/24 hours)	Average number of flowers per ha	Blooming duration of a single flower (days)	Melliferous potential of the linden trees (kg honey/ha)
Malu Spart	2009	0.2	54,295,200	5	68
	2010	1.1			373
Ciocăneşti	2009	1.3	57,688,650		469
	2010	1.0			360

Table 7. Melliferous potential of the silver linden trees from the studied forests - kg honey/linden tree

Forest location	Year	Average sugar yield per flower (mg sugar/flower/24 hours)	Average number of flowers per linden tree	Blooming duration of a single flower (days)	Melliferous potential of the linden trees (kg honey/linden tree)
Malu Spart	2009	0.2	135,738	5	0.17
	2010	1.1			0.93
Ciocăneşti	2009	1.3			1.10
	2010	1.0			0.85
Average					0.76

In our study we focused on the melliferous potential of the silver linden trees, but it has to be said that in the same forests usually several linden species grow naturally in a mix. So, speaking of the melliferous potential of the linden trees from a forest, it is quite difficult to estimate their melliferous potential since it has to be established the number of linden trees in the forest, but also the percentage of the linden species which has different melliferous potentials. Also, it has to be taken into consideration the several factors that influence the melliferous potential. Thus, besides that the melliferous potential is depending of the sugar yield produced by the flowers, respectively the nectar secretion and the nectar concentration in sugar which are influenced at their turn by climatic factors, there is of great importance also the floral abundance. For individual trees, the number of inflorescences, respectively the number of flowers (floral abundance) is influenced by the tree-specific factors that affect the ability of each tree to access the growing factors, such as tree age and position in the canopy. In fact, it has to be paid attention that all these factors influence the melliferous potential of the linden trees and determine variations in time and space of its values.

CONCLUSIONS

Our study performed in two forests from South Romania showed the good melliferous potential of the silver linden trees, which was in average of 318 $kg.ha^{-1}$ of honey, respectively 0.76 kg of honey per linden tree. However, it must be taken into account the influence on this good melliferous potential of the silver linden trees determined by several factors among which are counting the climatic conditions of the year and the number of flowers the linden trees can produce in the respectively year, as well as the number of linden trees from the forest.

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INVESTIGATION OF DIFFERENT TILLAGE AND SEEDING METHODS IN SAFFLOWER (*Carthamus tinctorius* L.) CULTIVATION

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Abstract

The aim of this study is to determine the effects of different tillage and seeding method on some soil physical properties and some plant growth parameters in safflower (Carthamus tinctorius L.). In the study, conventional tillage (CT), reduced tillage (RT) and direct seeding (DS) method were used.
According to results, the highest bulk density at 0-20 cm soil depth was found in CT method, followed by RT and DS method respectively. The highest porosity at 0-20 cm soil depth was determined in the DS method followed by RT and CT methods respectively. Soil penetration resistances for 0-25 soil depth were found to be 1.50, 1.96 and 2.37 MPa for CT, RT and DS methods, respectively. Weed growth for CT, RT and DS methods were found to be 26.3%, 31.3% and 42.5% respectively. Grain yields for CT, RT and DS treatments were 1340, 1160 and 1070 kg ha^{-1} were found, respectively. The highest seedling emergence rate was obtained at CT treatment and the lowest at DS treatment. The least seedling emergence time was obtained at the DS treatment and the longest at the CT treatment.

***Key words**: tillage systems, safflower, direct seeding, reduced tillage.*

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) seed is an important source of oil also its flowers are an important source of spice. Safflower flowers can be in red, orange, yellow and rarely white colors (Erbas and Baydar, 2017). Safflower oil in the semi-drying oil group is also evaluated in the construction of paints, varnishes, polishes and soaps (Ogut and Oguz, 2006). In our country, as of 2016, there are 39,500 ha plantation area, 58,000 tonnes total production and 147 kg da^{-1} seed yield (Anonymous, 2017). Safflower plant can be grown in different climates, as well as the use of all the equipment used in wheat-barley farming in the cultivation of this plant is an important advantage (Coskun, 2014). Although there are studies on different tillage methods for many plants, no studies have been done for safflower plants. In a study conducted by Kucukalbay and Akbolat (2015) on different tillage methods in chickpea cultivation, the highest yield was found in the conventional tillage, the highest weed growth was in direct seeding, the lowest penetration resistance was in the conventional tillage method, and the highest grain yield was obtained in the direct seeding method. Barut et al. (2010), in a four-year study of traditional, reduced and direct sowing methods in wheat farming; the highest penetration resistance were found in the reduced tillage and direct seeding methods, and the highest soil bulk density was found in the direct seeding method. Martinez et al. (2008) reported that for many years, in the study of conventional tillage and no-till method in wheat production, while the no-till method increased the stability of aggregate positively for many years, it affected other physical properties of soil negatively. Bayhan (2016) evaluated the different tillage and direct seeding methods of sunflower cultivation in terms of energy use efficiency. According to the results, the lowest energy output / input ratio is determined in reduced tillage method while the highest energy output / input ratio is determined in direct seeding method. Bayhan (2015) reported that in a study using disc harrow, rotary tiller, disc harrow + combined harrow and direct seeding methods as the

[1]The data of this study were taken from the master's thesis titled "Investigations of tillage and seeding methods at safflower cultivation".

tillage methods, the highest crop yield was obtained in the rotary tiller and the lowest crop yield was found in the method using disc harrow. Lopez-Garrido et al. (2014), in a study of conventional, reduced and non-till methods for sunflower cultivation, grain yield was lower in the no-till system, while penetration resistance was found to be higher than the other methods. Kasap and Dursun (2013) have tried five different tillage methods in their study to determine the different tillage methods used in the cultivation of chickpea and their effects on yield and yield component. According to the results obtained, the highest grain yield (260.6 kg da^{-1}) was obtained by the conventional tillage method whereas the least grain yield was obtained by direct seeding method (80 kg da^{-1}). Kaya et al. (2010) compared the yield and economic aspects of conventional tillage and direct seeding methods in dry climates of Chickpea-wheat planting. It has been reported that direct seeding methods on grain yields are ineffective and conventional tillage method is more profitable economically in chickpea cultivation. According to the results of many studies on soil tillage and direct seeding methods, the maximum weed growth was determined by direct seeding method while the least weed growth was determined by conventional tillage method (Cardina et al., 2002; Khattak, Khan 2005; Çakır et al. 2006; Celik, Altıkat, 2006; Jan et al., 2010).

The aim of this research is to determine the effect of soil tillage methods on soil physical properties, grain yield and plant growth parameters of safflower in the province of Eskişehir.

MATERIALS AND METHODS

A field experiment was conducted in a farm belong to a farmer during the growing season of 2016 at the Seyitgazi district of Eskisehir province of Turkey. According to Eskişehir province of Seyitgazi district of meteorological data; minimum, average and maximum air temperatures are -2.6°C, 10.6°C and 35.5°C respectively. The average annual precipitation of the Seyitgazi district is 365.0 mm. The content of soil organic matter of test area is 2.66% and pH is 6.32. It has also acidic and clay loam soil texture type.

As a seed in the experiment, Dincer variety safflower seeds were used. Dinçer variety, 90 to 110 cm in height, flower colour is orange, grain colour is white and has a medium thorn structure. In the trial, a four-wheel drive tractor with 90 HP power was used to seedbed preparation. The methods and equipment used in the experiment are given below in Table 1.

Table 1. Details of the tillage and sowing methods used in the experiment

Treatment	Details
CT (Conventional tillage)	Tillage with moldboard plow in the autumn with a depth of 30 cm + tillage with the cultivator at 15 cm depth in the spring (1 time) + scrapper + seeding with seed drill.
RT (Reduced tillage)	Tillage with cultivator at depth of 15 cm in the spring + scrapper + seeding with seed drill.
DS (Direct seeding)	Seeding with direct seeding machine.

Soil sampling cylinders with a volume of 100 cm^3 were used to determine the soil bulk density and porosity.

The samples taken from trial plot were dried in oven at 105°C for 24 hours according to Blake and Hartge (1986). Eijkelkamp (Eijkelkamp Equipment, Model 06.15 Eijkelkamp, Giesbeck, The Netherlands) Penetrologger was used to determine the soil penetration resistance.

Penetrologger memory is 1500 measurements, force resolution 1 N, maximum penetration resistance 1000 N measurement depth 80 cm, depth resolution 1 cm.

The experiment was consisted of 9 plots, three treatments and three repetitions according to randomized plot design. The experiment was consisted of 9 plots, three treatments and three repetitions according to randomized plot design.

In the CT treatment, first tillage was performed in the autumn by a 30 cm depth with a mouldboard plow. In the spring, seedbed was prepared with the cultivator and the field was made ready for seeding. Cultivator was used for soil preparation in RT treatment and seed drill was used for seeding.

In DS treatment, any soil tillage was not carried out for seed bed preparation.

Total herbicides were used in all treatments to control weeds before seeding.

Weeds were measured 20 days after seeding to determine the effect of soil tillage and seeding methods on weed growth.

For this purpose, weed quantities were determined by counting weeds remaining in a 1 m 2 frame at three points randomly determined in each parcel (Demirkan and Ark, 1991).

The average germination time (day) and emergence rate (%) of safflower seed were determined according to Mohanty and Painuli (2004). Also plant height (cm), head diameter (cm), number of branch (piece), first branch height and number of head per plant values were determined as plant growth parameters.

The yield was determined by the yield cycle method. In this method, circles with an internal area of 1 m^2 were used.

When the safflower was matured, the remaining plants in the circle which were dropped at three different location of each plot, were harvested by hand.

Then these plants were threshed and the safflower yield in the decare (kg/decare) was determined.

Data were analysed using analysis of variance (ANOVA). Means separation among the treatments was determined by LSD test at 5% significance.

RESULTS AND DISCUSSIONS

This study was conducted during the summer season of 2016. In the study, the conventional and reduced tillage methods applied by the farmer in the region where safflower was grown and direct seeding methods which could be alternative to this method were selected.

There was no statistically significant difference in terms of soil moisture content between the treatments.

In other words, the effect of the treatments on the soil moisture content was not significant (p=0.05).

The soil bulk density and porosity according to the treatments is given in Figure 1.

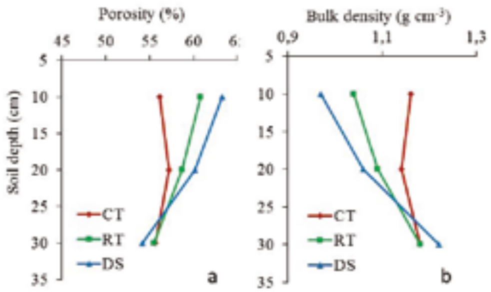

Figure 1. Soil porosity (a) and bulk density (b)

Soil porosity was decreased with depth increasing in all treatments. While the highest soil porosity was obtained in 0-10 cm soil depth at DS treatment (59.2%), the lowest soil porosity was obtained in 0-10 cm soil depth at CT treatment (56.3%). The difference between the treatments was found statistically significant in terms of soil porosity at 0-10 cm soil depth (p=0.05). While the treatments in 10-20 cm soil profile depth were different from each other, the differences between the treatments were not significant at 20-30 cm soil profile depth.

The highest soil bulk density was determined at CT treatment with a depth of 0-10 cm as 1.22 g cm^{-3} while the lowest soil bulk density was determined as 0.97 g cm^{-3} at a depth of 0-10 cm at DS treatment. The bulk density and porosity values obtained in the study are the opposite of the results obtained by Akbolat et al. (2009) and Kucukalbay and Akbolat (2015) for similar treatments. This may be because the first tillage in the CT and RT treatments have already been tilled at the autumn and the soil may be compacted in the time until the seeding. The soil penetration resistance results according to the treatments are given in Figure 2.

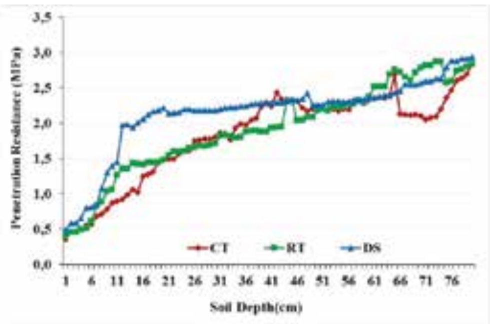

Figure 2. Soil penetration resistance results

The difference in the penetration resistance between the treatment was more evident in the soil depth of 0-25 cm. The penetration resistance difference in the profiles with a depth of 25 cm or more was declined gradually. The penetration resistance averages at 0-25 cm soil depth were determined as 1.50, 1.96 and 2.37 MPa for CT, RT and DS treatments, respecttively. The mean penetration resistances at 0-80 cm soil depth were 2.13, 2.28 and 2.48 MPa for CT, RT and DS treatments, respectively. As a result, the difference between treatments in terms of penetration resistance was found to be significant for 0-25 cm soil depth (p=0.05). Küçükbay and Akbolat (2014) reported that they found penetration resistances at soil depths of 0-25 cm for CT, RT and DS treatments as 1.50, 1.96 and 2.37 MPa, respectively.

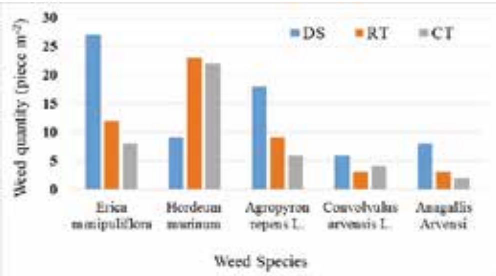

Figure 3. Weed species and amounts according to treatments

Our results are consistent with the findings of Kucukalbay and Akbolat (2015) for similar treatments.

In the study conducted to determine the effects of tillage and seeding methods on weed growth, the most weed growth was determined at DS treatment and the least weed growth at CT treatment. Among the weed species, the highest number of broom (*Erica manipuliflora*) was detected in the DS method while wild barley (*Hordeum murinum*) was detected in the RT and CT methods (Figure 3).

These varieties were followed by couch-grass (*Agropyron repens* L.), bindweed (*Convolvulus arvensis* L.) and scarlet pimpernel (*Anagallis arvensi*), respectively. According to the treatments, weed growth percentages for CT, RT and DS were 26.3%, 31.3%, 42.5% respectively. The difference between the treatments in terms of weed growth was found to be statistically significant (p=0.05). Ozpinar (2005) found to be the least weed growth in the CT method, Kucukalbay and Akbolat (2015) found to be the most weed growth in the RT method. Shresta et al (2002) found to be insignificant the effects of the soil tillage methods on weed growth. Plant growth parameters is given below Table 2.

Table 2. Average results for plant growth parameters and yields

Treatments	Seedling emergence rate (%)	Seedling emergence time (Day)	Plant height (cm)	Head diameter (cm)	Number of branch	First branch height (cm)	Grain yield (kg ha^{-1})	Head number per plant
CT	75[a]	22[a]	65.9	2.06	5.2	42.8	1340	10.53[a]
RT	49[b]	21[ab]	65.9	2.06	5.3	46.2	1163	8.86[b]
DS	51[b]	17[b]	66.5	2.21	5.3	43.1	1070	13.20[c]

Means that do not share a letter are significantly different.

The effect of treatment on seedling emergence time and seedling emergence rate according to Fisher Pairwise Comparison test was found to be statistically significant (p=0.05). The average plant emergence times of the treatments varied between 17-22 days. The earliest seedling emergence was found in DS treatment with 17 days, and the latest plant emergence was found in CT treatment with 22 days. Kucukalbay and Akbolat (2015) stated that seedling emergence time for CT, RT and DS treatment were found to be 22.4, 23.5 and 23.1 day in chickpea cultivation. They also reported that there was no difference between the methods in terms of seedling emergence time. Their results are compatible with our results. Soil tillage and seeding methods were found statistically significant on seedling emergence rate. While the highest seedling emergence rate was obtained at CT treatment with 75%, the lowest seedling emergence rate was obtained at RT method with 49% (Table 2).

The highest plant height was obtained at DS method with 66.5 cm and the lowest plant height at RT method with 65.9 cm. However, this difference was found statistically insignificant (p=0.05).

In the study conducted to determine the effect of tillage and seeding methods on yield of safflower, it was determined that the treatment was statistically insignificant on grain yield. The grain yields for CT, RT and DS treatments are obtained 1340 kg ha^{-1}, 1163, kg ha^{-1} and 1070 kg ha^{-1}, respectively.

While the lowest grain yield was obtained at DS treatment with 1070 kg ha^{-1}, the highest grain yield was obtained at CT treatment with 1340 hg da^{-1}. In a study conducted by Ozturk et al (2008), yield of safflower was found to be 1143 and 1140 kg ha^{-1} for irrigated and non-irrigated highland in 2002 growing season. These results are lower than the results we found for CT treatment that the method of commonly applied.

Head diameter development, which is the criterion for plant development, was found to be statistically insignificant among the treatments (p=0.05).

According to the treatments, mean head diameters were 2.06, 2.06 and 2.21 cm for CT, RT and DS, respectively. The highest head diameter was determined at DS treatment with 2.21 cm and the lowest head diameter with 2.06 cm at RT and DS treatments.

Tillage and seeding methods have been determined to be statistically significant on the number of head per plant.

The Tukey test was used to check the difference among the treatments. Head number per plant was determined as 10.5, 8.9 and 13.2 for CT, RT and DS treatments, respectively.

The effect of the methods used on the number of branches per plant was found statistically insignificant.

The average number of branches per plant varied between 5.33 and 5.20; The highest number of branches per plant was determined at RT treatment with 5.33, and the lowest number of branches was determined at CT treatment with 5.20.

The effect of the treatment on the first plant height was found statistically insignificant. The first branch heights of the plants for CT, RT and DS treatment were found to be 42.85, 46.20 and 43.11 cm, respectively.

CONCLUSIONS

There was no significant difference between the grain yields of treatments, even if the yield was relatively low in direct seeding treatment. The highest seedling emergence rate was obtained at CT treatment and the lowest at DS treatment. The least seedling emergence time was obtained at the DS treatment and the longest at the CT treatment. While the least number of heads per plant was determined at the RT treatment, the most heads per plant was determined at the DS treatment. while the most weed growth was obtained at DS treatment, the least weed growth was obtained at CT treatment. The highest penetration resistance (1.50 Mpa) was measured for 0-25 cm soil depth at DS treatment whereas the lowest penetration resistance (2.37 Mpa) was measured at CT treatment. While the bulk density in CT treatment was the highest, the bulk density in DS treatment was least.

It is a fact that the grain yield in the first years in reduced tillage method and direct seeding methods is lower than in the conventional tillage method. However, according to previous studies that increasing in soil productivity in the conservational tillage method will increase the yield in the next years. This research results present that, in terms of sustainability, safflower plant could be cultivated by applying conservational tillage methods as it is in other crops cultivation.

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NEW INSIGHTS INTO THE MULTIPLE PROTECTIVE FUNCTIONS OF DIATOMACEOUS EARTH DURING STORAGE OF AGRICULTURAL PRODUCTS

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Abstract

Testing and recommending means of fertilization as well as protective measures against the action of abiotic and biotic stressors during plant vegetation and post-harvesting, in a sustainable and environmentally friendly way, is one of the major challenges of researchers in the field, in close interdependence with the expectations of practitioners and last but not least of consumers. From this point of view, considering the chemical composition of diatomite, the use of diatomaceous earth may be a non-chemical alternative and / or complementary measure to the application of chemical fertilizers or plant-health treatments. The purpose of this review is to bring together recent, relevant data on the beneficial effects of diatomaceous earth use, in terms of insecticidal and fungistatic effects, also as mycotoxins adsorbent during storage of agricultural products.

Key words*: diatomite, insects, fungi, mycotoxins, storage, agricultural products.*

INTRODUCTION

During the storage of agricultural products, one of the main means of integrated pest management (IPM) is the reduction of chemical residues in food, by decreasing the doses used and the application of less toxic compounds. So, selected control strategies must be integrated for effective management of stored grain insects (Upadhyay and Ahmad, 2011).
Consequently, postharvest researches should be focused on reduction and elimination of synthetic pesticides, in close interdependence with the expectations of practitioners and last but not least of consumers. Accordingly, testing and recommending of protective measures against the action of biotic and abiotic stressors, during vegetation period, also post-harvesting, in a sustainable and environmentally friendly way, is one of the major challenges of researchers in the field (Rozman, 2015).
One of the very promising alternatives to traditional residual grain protectants (Kavallieratos et al., 2015) or chemical control of urban pests (Hosseini et al., 2014) is the use of diatomaceous earth (DE) or diatomite (Golob, 1997), a natural inert compound from geological deposits. It consists mainly of the fossilized skeletons of diatoms, which contain in principal silicon dioxide (87-94%) important amounts of alumina (Al_2O_3), also ferric oxide (Fe_2O_3) (Tsai et al., 2006; Kaufhold et al., 2008). The diatomaceous deposits result from an accumulation in oceans or fresh waters of the amorphous silica (opal, $SiO_2C_nH_2O$) cell walls of dead diatoms, which are microscopic single-cell aquatic plants (algae).
It has been widely reported that silicon (Si), a second most abundant element in soil, could reduce drought stress in plants (Rizwan et al., 2015). At the same time, it is known that silicon mediates the defense response to pests (see reviewed by Bakhat et al., 2018). In addition to improving soil characteristics (e.g. air penetration, water retention capacity, infiltration and so on), thanks to its physical properties (e.g. small particle size, high surface and high porosity) (http:// www.mineralszone. com/minerals/diatomite.html), DE has proven to be the efficacy of other uses as well, such as protection against stress agents during the storage of agricultural products. As recently it

was also mentioned by Lupu and Manole (2015), DE is one of the most effective mechanical insecticides, for controlling insects damaging cereal seeds.

The safety of such treatments for humans and animals health is fully justified, due to the fact that DE has no toxic effects on mammals (rat oral LD 50>5000 mg/kg of body weight) and do not leave toxic residues in the product. Otherwise, diatomaceous earths are classified by EPA (Environmental Protection Agency) in the category of GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA, 1995).

In view of these considerations, this compound was studied by many researchers: applied alone (Sahaf et al., 2007; Hosseini et al., 2014), in combination with different additives (Korunic and Riozman, 2010) or other treatments types (Korunic, 1997) such as essential oils (Yang et al., 2010; Popescu et al., 2016; Lupu et al. 2017), aromatic plants (Pacheco et al., 2016), microorganisms, such as entomopathogenic fungi (Lord, 2001; Michalaki et al., 2007) and last but not least, it was registered as a grains protectant in different countries (Korunic, 1997; Pinto Júnior et al., 2010). Between its successful characteristics there can be mentioned: its unique way of action, low insect resistance development, high persistence on grain, high level of adherence on grain, easy removal from the grain and low mammalian toxicity (Korunic, 2013).

In this context, the purpose of this review is to bring together recent, relevant data on the beneficial effects of diatomaceous earth use, in terms of insecticidal and fungistatic effects, also as mycotoxins adsorbent during storage of agricultural products.

INSECTICIDAL EFFECT OF DIATOMACEOUS EARTH

The insecticidal effects of DE depends on DE origin, commercial formulation, rates of application, exposure period, and last but not list, to the plant species treated, the insect species etc. (Wakil and Schmitt, 2015; Machekano et al., 2017; Perišić et al., 2018).

As Perišić et al. (2018) noticed, in the case of two DE originated from Serbia and one commercial formulation (Protect-It, Hedley Technologies Ltd. Canada) applied against *Rhyzopertha dominica* (F). (*Coleoptera, Bostrichidae*) which produces the lesser grain borer, it was noticed an adults increase mortality, when the exposure period was longer and the application rates were increased. Of course, the DE effects were somehow different, in relation with the plants species (wheat, barley, rye, oats and triticale grains) and not negative influences were registered as regard as some grains features (moisture, proteins and ashes content).

Several DEs based on natural deposits are now commercially available, and have proved very effective against stored grain pests. The area of Eastern Europe is considered "rich" in diatomaceous earth deposits, due to the existence of large areas with siliceous-based deposition. The DEs of these areas have been used in the past for several applications, including their use as insecticides, and some of them are now the main ingredients in commercially available formulations. For instance, an amorphous silica DE from the Former Yugoslavic Republic of Macedonia (FUROM) is the main ingredient for the DE formulation Protect-It (Hedley Technologies, Canada), which is one of the most commonly used DEs as insecticides worldwide (Athanassiou et al., 2011).

As Çetin and Taş (2012) states that Turkey is also considered to be a country where there are rich deposits of natural diatomite, with a reserve of about 125 million tons and the largest one (106 million tons) is at Hırka (Kayseri). Even under these circumstances DE-based products are not commercially available locally, to combat insects during grain storage, but Scanning Electronic Microscopy (SEM) preliminary studies on different DE proveniences have been carried out by Sağlam et al. (2017). They emphasized the existence of differences between Turkish DEs and commercial DEs, as regard as shape and size of DE bodies. The authors' conclusion was that there are necessary further studies with a view to better understand the interrelationship between the physical characteristics (e.g. shape and size) and the biological efficacy of DEs against stored product insect pests.

DE from six areas in Romania were analyzed from chemical and mineralogical point of view, in order to select the most effective one for development of ecological products intended to control insect pests in grain storehouses. The characterization was done using X-ray fluorescence, wet-chemical analysis, Fourier - Transform Infrared Spectroscopy (FTIR) and X-ray powder diffraction. The target occurrences were those at Adamclisi, Borodu Mare, Chiuzbaia, Padina Mare, Tăşad and Pătârlagele, ranging in age from Oligocene to Upper Pliocene. The mineralogy of all deposits was predominated by the presence of quartz, amorphous silica, feldspars and clay minerals, while the mineralogy of the diatomite rock from Tăşad was characterized by the presence of aragonite (up to 55 wt.%), due to contamination by late sediments, issued from a hot-spring activity. Romania samples of DE had satisfactory results as regard as the insecticidal effects; beginning with 100 ppm and good economically results starting with 300 ppm (Dumitraș et al., 2015; Dumitraș et al., 2017).

The insecticidal efficacy of the DE (obtained from three Romanian sources: Pătîrlagele, Urloaia and Adamclisi), applied at four doses (100, 300, 500 and 900 ppm), against granary weevil, *Sitophilus granaries* L. (*Coleoptera: Curculionidae*), with two essential oils was evaluated in an experimental model, in laboratory tests (Manole et al., 2015). The results showed that mortality induced by DE was at the levels between 83.33% and 100% in all variants, after 21 d, compared with untreated control and a standard product Silicosec®. It has also been demonstrated that the insecticidal efficacy was highly influenced by exposure time, dose and essential oil type.

According to Pacheco et al. (2016), as compared with some potential repellent action of aromatic plants or control variants, DE application emphasized statistical difference as regard as the number of dead insects of *Zabrotes subfasciatus* (Boheman) (*Coleoptera: Chrysomelidae: Bruchinae*) in common beans, in 210 and 240 storage days. On the other hand, it is important to mention that during 180 storage days, the aromatic tested plants did not affected the physiological quality of seeds, while after 90 days, DE increased abnormality symptoms in common-bean seedlings.

Using of some combined treatments, such as a mixture between a biological agent (*Beauveria bassiana*) (3 x 10^{10} conidia per kg), DE (150 ppm) and a neonicotinoid (Imidacloprid) (5 ppm) have been efficient against three beetle species (*R. dominica*), *Tribolium castaneum* Herbst. (*Coleoptera: Tenebrionidae*), *Cryptolestes ferrugineus* Stephens (*Coleoptera: Laemophloeidae*), and the *Psocoptera* species (*Liposcelis paeta* Pearman) (*Psocoptera: Liposcelididae*), the major stored wheat insect pest species under farm conditions of Pakistan (Wakil and Schmitt, 2015).The authors suggested that the obtained results have a practical importance, particularly for local farmers.

DE applied with a naturally derived insecticide (Spinosad), as DE Insecto had effective protective influence to the larvae the Indian meal moth, *Plodia interpunctella* (Hübner), assuring a mortality up to 86-97% of first instars of the insect, at 500 and 1000 ppm (Subramanyam et al., 1998). When Spinosad was applied alone at 1 ppm, the larvae mortality registered was 97.6-99.6% (Fang et al., 2002). In the case of maize grain, Machekano et al. (2017) noticed that a combination of DE with Spinosad and DE with deltameyhrin emphasized the same eficacity against *Prostephanus truncates* (Horn), *Sitophilus zeamays* (Motschulsky) and *T. castaneum,* as the commercial combinations of organophosphates and synthetic pyrethroids used in current commercial grain protectants: fenitrothion 1.0% w/w (10000 ppm) + deltamethrin 0.13% w/w (130 ppm). The authors also mentioned that such treatments are safer and possibly cheaper effective alternative, to protect seeds against pests attack.

Besides DE protective effects, it is very important to use lower effective doses (Kavallieratos et al., 2015). So, combining DE with low levels of other insecticidal compounds such as: DEBBM (a mixture of two natural compounds: bitterbarkomycin (BBM) and DE), DEA (a mixture of abamectin and DE) and DESgBAIT (a mixture of DE, silica gel Sipernat 50 S and food grade bait) was interesting and promising in the case of maize and wheat grains protection against *R. dominica* and *Sitophilus oryzae* (L.) (*Coleoptera: Curculionidae*) and one external feeder, *Tribolium confusum* Jacquelin du Val (*Coleoptera: Tenebrionidae*).

Successful results were also obtained for wheat, by Athanassiou et al. (2009), in laboratory conditions, when DE was applied in combination with bitterbarkomycin (BBM), an extract obtained from the roots of *Celastrus angulatus*, against adults of the maize weevil, *S. zeamais* the red flour beetle, *T. castaneum* and the rusty grain beetle, *Cryptolestes ferrugineus* (Steph.) (*Coleoptera: Cucujidae*). Application of BBM or DEBBM assured a higher mortality of *S. zeamais*, as compared with DE alone. Moreover, for *T. castaneum*, with few exceptions, by DEBBM application the mortality was significantly higher, as compared with that caused by DE or BBM. As for instance, after 14 days of exposure, mortality was 90% on wheat treated with 100 ppm of DEBBM. *C. ferrugineus* has been shown to be the most susceptible species, in which case, only after 5 days of exposure even at the lowest DEBBM dose, the recorded mortality was 90%.

In addition, so name botanical insecticides such as different plant materials, plant extracts, essential oils (Yang et al., 2010) or some natural products of plants (Isman et al., 2010) proved to be effective when are alone applied (Sahaf et al., 2007) or when added to other treatments combination, effects can be synergistic or antagonistic (Ziaee et al., 2014).

Often, applying only essential oils can result in very weak insecticide efficacy, because these are volatile rapidly, but associated with DE, even when *Carum copticum* (L.) was assured in a sub-lethal concentration, there was an increase of insects' locomotor activity. This higher insects' mobility results in more pronounced contact of the insect exoskeleton (cuticle) with DE, what it means implicitly a more lipids absorbtion (Lord, 2001), pronounced dehydration, more abrasion and ultimately death of the insect (Ebeling, 1971).

Moreover, as it was mention above, the DE particle size is defining for treatment to be successful as Ziaee et al. (2014) demonstrated. The synergistic effect of essential oil and DE on *T. confusum* was noticed when DE particle size was less than 37 μm, while in the case of larger particles (149 μm) there was not registered such effect. The author's explanation was that smaller particles assure a larger surface area relating to volume. In such conditions, DE has an improving capacity to maintain oils in their small pores, and their release will be very slow. A strong additive efficacy of garlic (*Allium sativum* L.) essential oil applied with DE against adult rice weevils *S.oryzae* and redflour beetles, *T. castaneum* was obtained by laboratory bioassays (Yang et al., 2010). In such combination, there was registered a longer lasting period of essential oil, but only a small increase in persistence. Thus, authors recommended extensive research to optimize the application rates of the mixture treatment, to improve toxicity and persistence and to evaluate the economics of any formulation of garlic essential oil plus DE, too.

A synergistic effect on the mortality of *R. dominica* was noticed for *C. sinensis* essential oil, when it was combined with kaolin, an antagonistic one, when the DE was added to this (Campolo et al., 2014).

Another promising IPM strategy proposed to additional research was that indicated by Michalaki et al. (2007), based on application of DE associated with the enthomopathogenic fungi (EF) such as *Paecilomyces fumosoroseus*, (Wise) Brown, taking into consideration that both substances can be applied by the same technology, as traditional grain protectants. In this context, results obtained by Lord (2001) with *Beauveria bassiana* (Balsamo) Vuillemin, (the most important and common EF) at rates of 11, 33, 100, and 300 mg of conidia per kilogram of grain with and without single rates of DE that killed 10% or less of the target beetles, emphasized the synergism against adult of *R. dominica*, at all applied doses. The author states the followings: taking into account that the two types of treatments are complementary in their optimal environmental action, the formulation of myco insecticides -lipophilic materials is of interest, both for the control of adults and of the immature stages, of different stored - products insect pests.

Through a combined treatment of entomo-pathogenic fungi, *Metarhizium anisopliae* (Metschinkoff) Sorokin and *B. bassiana,* with DE SilicoSec (DE commercial formulation) applied to wheat at 200 mg/kg and 400 mg/kg against *T. castaneum* and *Oryzaephilus surinamensis* L. showed a significantly reduced pathogenicity after 7 days of exposure, excepting *R. dominica*. It was demonstrated that the isolates were virulent to the beetles and the efficacy increased in combination with DE, especially in the case of longer exposure time. At the same time, it was noted that the most resistant species was *T. castaneum*, followed by *R. dominica* (Shafighi et al., 2014).

As excellently reviewed by Batta and Kavallieratos (2018), there are many research reports which present different effects of combinations between EF and other components, including DEs against stored-products insects. In the case of EF and DE a synergistic effect was noticed, but not with natural enemies belonging to arthropods. It was mentioned a true thing, that the action of EF against insects pests, especially for stored grain is compatible with the food safety and environmental regulations, and implicitly maybe a success alternative in the future, to implement the use of these biocontrol agents instead synthetic insecticides.

The utility of DE has also been proven against house pests, in dwellings in urban areas. Hosseini et al. (2014) noticed that DE treatment is a promising procedure against German cockroaches, *Blattella germanica* (L.), the most common roaches in houses and restaurants. Increase of DE doses from 2.5 to 25 g/m^2, respectively for 24, 48 and 72 h exposure period determined after 24 h a proportionally increasing of adults and nymphs mortality rate.

After specimens transfer to the beakers contained food and water, results obtained after 1 week were not significant from the statistical point of view, as regard as the lethality of 50% of DE plus water, on the German cockroach nymphs.

Also, the bed bugs (*Cimex lectularius* L.) still creates major problems in the built environment, as Romero et al. (2017) mentioned, in the recent bibliographic synthesis.

Therefore, even if proactive bed bug management programs prove to be effective, sustainable and economically viable in the long term than reactive and insecticide - only programs, the adoption of good practices can be limited by the budget limitation and interest of affordable multiunit housing providers.

Nowadays, increased attention needs to be paid to the problem of the phenomenon of resistance to insecticides in this case (Dang et al., 2017), or other cases, too. Careful monitoring of the resistance to insecticide is required, also an understanding of the resistance mechanisms can determine a proper use of insecticides and / or timely modification of chemical control strategies can be recommended. Thus, thanks to previous bioassay, genetic, morphological, biochemical and behavioral studies and due to the evolution of investigation approaches, such detailed studies are accessible.

One of the most plausible explanations of DE efficacy is the epicuticular effect for insect dehydration. When the insect moves through DE, the particles are picked up by the insect's body and absorb wax from the insect's cuticle, due to DE adsorptive property. Thanks to its high amounts of amorphous silica particles and as a consequence in water loss, the insect dies by dehydration (Ebeling, 1971; Lupu et al., 2016) and partially through abrasion (Subramanyam and Roesli, 2000).

The toxicological successfully effect on insects is related to amorphous silica content, with uniform particle size (<10 μm), with a high capacity to oil sorption, an extensive and active surface area, next to a lower content of clay and other metal oxides (Korunic, 1997).

Nevertheless, the use of DE involves certain disadvantages, such as those regarding health concerns, the reduction in bulk density, differences in insect species tolerance to the same DE formulation, the effects of grain moisture and temperature on the effectiveness against insects, the influence of various commodities on DE efficacy or the use of DEs in some other fields (Koruniæ, 2016). That is why, to be a wider acceptance of DE as a means to control stored-product insect pests, the studies must be continued, with a view to develop a safe enhanced formulation, with a low DE concentration and as a consequence a minimal adverse effect on bulk density and grain flowability.

ANTIFUNGAL EFFECT OF DIATOMACEOUS EARTH

The use of kaolin and DE based treatments and/ or combined sweet orange [*Citrus sinensis* (L.) Osbeck] peel essential oil has led to the detection of toxic effects on *R. dominica* and

the damage wheat microbial populations. The efficacy of different formulations, application rates and exposure times for insect mortality and progeny production were different. Thus, a synergistic effect on the mortality of *R. dominica* was noticed for *C. sinensis* essential oil, when it was combined with kaolin, and an antagonistic one, when the DE was added to this. As regard as yeasts, moulds, as well as total mesophilic aerobic bacteria growths were reduced when the *C. sinensis* essential oil was applied alone, as compared with the other dusts and dust-essential oil-based treatments (Campolo et al., 2014).
Recent research carried out by Fernández and Bellotti (2017) on the modification of DE on the basis of quaternary ammonium groups has led to obtain hybrid materials, a more effective form of DE, which can be used as antimicrobial filter material.

MYCOTOXIN ADSORBENT PROPERTIES OF DIATOMACEOUS EARTH

The quality of the products is subject to the influence of many abiotic and biotic stress factors (Delian, 2006), so knowing the critical control points during harvesting, drying and storage stages of the grain is essential for the development of effective post-harvest prevention strategies, including those related to mycotoxin contamination (Magan and Aldred, 2007).
Mycotoxins are secondary metabolites produced by pathogenic fungi, accumulated in the field conditions, such as pre-harvest deoxynivalenol produced by *Fusarium graminearum* or post-harvest zearalenone (ZEA) due to *F. poae*; also ochratoxin due to *Penicilium verrucosum* (Magan et al., 2010). There is the risk that a good quality raw material may be contaminated if it is not stored in an appropriate manner, too (Kabak et al., 2006).
In both cases, the presence of mycotoxins causes contamination across the whole food chain and shows toxicity to animals and humans (He and Zhou, 2010; Raiola et al., 2015; Lupu et al., 2017), having a significant economic impact on animal agriculture, also (Smith and Girish, 2012).
To overcome this inconvenient, expertly performed crop protection practices are absolutely necessary (reviewed by Raiola and Ritieni, 2014) and adequate procedures are need to be apply, with a view to reduce the possible mycotoxins incidence (He and Zhou, Besides, because the preventive methods applied during the growing, harvesting and storage periods are only meant to reduce the risk of contamination, post-harvest detoxification procedures are required (He and Zhou, 2010). The results of the recent research carried out in our country (Popescu et al., 2016) led to the production of an eco-friendly bioproduct, through the use of clean technology of green chemistry, based on the well-known DE features and the antimicrobial characteristics of essential oils extracted from medicinal plants. The product has been shown to have a significant repellent potential against adults of *Sitophilus granaries*, as well as an antifungal action of thyme volatile oils coupled with diatomite as insecticide and mycotoxin absorbing agent has been highlighted.
One of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability, by including various mycotoxin-adsorbing agents in the compound feed, which leads to a reduction of mycotoxin uptake, as well as distribution to the blood and target organs. Mycotoxin binders can work in three ways: by physically binding toxins and metal ions by adhesion; by binding toxins through electrostatic charge or cation exchange capacity. They can eliminate the source of the toxins, by increasing cell membrane permeability of the fungi, which are the cause of mycotoxin production. There are a range of commercially available mycotoxin binders and antifungal agents, which have been shown to have varying potency effects at reducing the presence or eliminating the toxicity of mycotoxins. The development of a successful commercial mycotoxin binder incorporates the best of each of these active ingredients at the required concentration, to ensure that the overall function acts to reduce the harmful effects of mycotoxins in animal nutrition (Pearce et al., 2010). There are a number of approaches that can be taken to minimise mycotoxin contamination in animal feeds and these involve prevention of fungal growth and therefore mycotoxin formation, and strategies to reduce or eliminate mycotoxins from

contaminated commodities, especially feed additives. It seems impossible to completely remove such mycotoxins from the animal and human food chain, given that feed and its precursors can be stored and transported throughout a range of time intervals, atmospheric humidity and temperatures. OcraTox (an activated DE) has been tested as a detoxifying agent of ochratoxin A (OTA), a secondary metabolite produced by some strains of *Aspergillus ochraceus* and *Penicillium verrucosum*, in laying hens (Denli et al., 2008). Addition of OcraTox to the contaminated diet alleviated the negative effects resulting from OTA, reaching values not significantly different from the control diet for most of the parameters, except the relative weight of the liver, showing that OcraTox counteracted the deleterious effects caused by OTA (Boudergue et al., 2009). Another important way to improve characteristics of the silica based materials is to combine with other components, organic or inorganic, resulting composite materials or materials with core-shell structure (Florea et al., 2012).

The mode of action of the feed supplements is different: mould inhibitor for animal feeds with a preventive action, mycotoxin binder, or absorption of moisture (http://orbitec.es/feed-additives-feed-ingredients/mold-inhibitor/).

Naturally-balanced feed supplements help to amend the existing deficiencies in major feed elements, with no harmful effect on humans, animal health or the environment. There are such supplement, commercially available (Diatomaceous earth*), based on ground fossil shells from the remains of single-cell algae called diatoms. The product is untreated, unheated, food grade, fresh water variety. The fine drying powder absorbs moisture and dehydrates on contact. It can be used as an animal feed additive, up to 2% of feed ration (http://www.ohioearthfood.com/animal-feed-supplements.html) as FDA recommends (Food and Drug Administration, USA, 1995).

In consequence, prevention of mycotoxicoses in livestock and poultry by different dietary strategies such as inorganic and organic adsorbents, mycotoxin inactivators and nutritional supplements, and knowing their detoxifying, decontaminating mechanism and relative efficacy have been considered. The use of mineral additives, as for instance DE (as a mycotoxin-binding agent) added to animal feed has been shown to reduce the toxic effects of mycotoxins, due to the sequestering (absorbing) in the digestive tract of the animals (Smith and Girish, 2012). Moreover, the use of diatomite along with other based on natural silica materials has proven effective in biomedical applications, such as use in drug delivery as carrier for Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), given its physicochemical and functional characteristics and its good biocompatibility (Krajišnik et al., 2017).

Studies carried out by Sprynskyy et al. (2012) have shown that talc had stronger adsorption properties for ZEA at its hydrophobic surface, than diatomites, that have a more hydrophilic surface, in the case of synthetic gastric fluids. The authors mentioned that the driving force for the physical adsorption process that link ZEA and DE is probably due to the hydrophobic bonding between the surface siloxane groups of DE and the partially positive electrical charges of ZEA molecules. In the same time, the ZEA adsorbtion on DE can be limited by competition of less polar toxin molecules, with the stronger polar water molecules.

There are many concerns to study the DE efficacy. The study of the bioactive properties of DE and essential oils found an innovative approach of feed treatment in the sequence of food chain from storehouse to animal body grown in farm, with ecological products obtained from cheap minerals and renewable vegetal sources, such as DE and essential oils, with insecticidal-fungicidal and adsorbent properties (Lupu et al., 2017).

CONCLUSIONS

Using the diatomaceous earth as protectant for stored products against pest infestations constitutes one of the most efficient naturally occurring dust. Among the main advantages of this treatment are: it is safe, has low toxicity to mammalians, does not affect the final quality of the product, provides long-term protection, is registered as a food additive and is an alternative to the application of synthetic pesticides (Shafighi et al., 2014).

The combination of diatomaceous earth with plants oils provides two important advantages. Firstly, it is more cost-effective as it decreases both the amount of oil and DE needed, and it reduces the adverse effects of the oil, i.e. its strong odor and volatility. Secondly, essential oil and diatomaceous earth are environmentally compatible, an essential consideration in the development of insecticidal treatments for grain (Yang et al., 2010).

The innovative product developed in Romania (Popescu et al., 2017) consists of 15-25% potassium salts of fatty acids from vegetable oils, 3-5% potassium acetate, 1.5-3% glycerin, 1-5% essential oils extracted from aromatic herbs selected from spontaneous or cultivated flora, due their recognized insecticidal and fungicidal properties, a natural insect attractant, 40-65% bioactive mineral vehicle such as insecticidal diatomaceous earth, a biopolymeric adhesive if necessary, 1.5-2% unsaponifiable substances and water. The novel granulated eco-friendly product, based on natural non-toxic and accessible raw materials ensures attracting insects to stored grains, due to ecological attractants including volatile esters, followed by dehydration insects attracted into contact with diatomaceous earth.

Another Romanian invention (Lupu et al., 2017) relates to a method of protecting stored cereals against insects and toxigenic fungi. The method consists in placing, prior to the introduction of the cereals into the storage place, on the basis of the cereal volume, some pellets containing plant debris and diatomite, soaked with volatile oil of thyme, and after the filling of the deposit, dried diatomaceous earth is administered on the cereals surface, in thin layer, as a contact insecticide.

Considering some undesirable effects, to be a wider acceptance of diatomaceous earth as a means to control stored-product insect pests, the studies must be continued, with a view to develop a safe enhanced formulation, with a low DE concentration and as a consequence with a minimal adverse effect on grain (Koruniæ, 2016).

The general conclusion may be that the ecological products are one of the most effective and valuable tools for good practice of risk management in the grain storage and agro veterinary sector, as eco-effective alternatives to chemical treatments (Lupu et al., 2017).

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BIOMASS QUALITY OF SOME *Poaceae* SPECIES AND POSSIBLE USE FOR RENEWABLE ENERGY PRODUCTION IN MOLDOVA

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Abstract

Biomass makes a major contribution to the world and nation's renewable energy portfolio. Plant biomass represents stored energy that may be drawn upon on demand, can be converted into energy by combustion, producing cellulosic ethanol and biogas. Domestication of new species as dedicated energy crops may be necessary. Currently Poaceae species are the most commonly utilized herbaceous plants as raw material for the production heat energy by direct combustion process in Moldova. The objective of this research was to evaluate some physical and mechanical properties of dry biomass of the new Poaceae species: Miscanthus giganteus and Sorghum almum collected from the experimental land of the Botanical Garden (Institute), control variants - wheat straw, Triticum aestivum and corn stalks, Zea mays. The physical and mechanical properties of dry biomass were determined according to European Standards in the State Agrarian University of Moldova, the production of solid fuels - by the equipment developed in the Institute of Agricultural Technique „Mecagro" Chisinau. In the field it has been established that Miscanthus giganteus stems defoliated faster than Zea mays, the stems of Sorghum almum dehydrated rapidly. The bulk density of the milled chaffs by sieve 10 mm and 6 mm of the tested energy crops was 90-167 kg/m^3 and 163-198 kg/m^3, respectively. The specific density of briquettes reached 740-923 kg/m^3, but the specific density333 of pellets – 1007-1262 kg/m^3. The Miscanthus giganteus was distinguished by high density, gross calorific value (19.3 MJ/kg) and low ash content (2.23%); Sorghum almum biomass moderately gross calorific value (18.6 MJ/kg) and ash content (3.71%); Zea mays high specific density of solid fuel (923 and 1124 kg/m^3); wheat straw lowest bulk density and calorific value, and high ash content (4.93%).
Miscanthus giganteus and Sorghum almum are promising energy crops for the production solid biofuel in Moldova.

***Key words**: biomass quality, briquettes, Miscanthus giganteus, pellets, Sorghum almum.*

INTRODUCTION

In the face of fluctuating petroleum costs and a growing demand for energy, the need for an alternative and sustainable energy source has increased. Energy generation from renewable supplies is also important from social and economic standpoint; it also can reduce greenhouse gas emissions. Biomass makes a major contribution to the world and nation's renewable energy portfolio (Hăbăşescu, 2011). In the context of fuel conversion, biomass is commonly sourced from plants, either as a by-product of harvesting, as a dedicated energy crop, or as an excess waste product after processing. Plant biomass represents stored energy that may be drawn upon on demand, can be converted into energy by combustion, producing cellulosic ethanol and biogas. Due to its high moisture content, irregular shape and size and low bulk density, biomass is very difficult to handle, transport, store and utilize in its original form. Densification of biomass into durable compacts (pellets, briquettes) is an effective solution to these problems and it can increase the bulk density of collected biomass from an initial bulk density of 40-200 kg/m^3 to a final compact density of 600-1200 kg/m^3. The knowledge of the engineering properties of biomass, such as bulk density, particle density, particle size, colour, moisture content, ash content, heating value and flowability is important for the design and operation of processing facilities for handling, storage, transportation, and conversion to fuels, heat, and power (Plíštil et. al., 2005; Lisowski et. al., 2010; Hăbăşescu, 2011; Lam, Sokhansanj, 2014; Jackson, 2015; Marian, 2016).

Currently, cereal straw is the most commonly utilized raw material for the production of heat energy by direct combustion process in Moldova. Cereal straw is sometimes used as feed and bedding for animals, as a soil amendment and incorporated into the ploughed layer or used as mulch. The annual area sown with corn crop is about 433 thousand ha, which constitute 29% of the total area (Marian et al. 2013) therefore, it is a widely available biomass resource in Moldova. It has been estimated that approximately 100,000 dry tons of corn stover are available annually to support the biomass industry, but at present the degree of their utilization in the production of solid biofuels is very low.

Domestication of new species as dedicated energy crops may be necessary. High potential lies in perennial herbaceous species for biomass, which offer agro ecological benefits not present in annual row crop production such as increased soil organic carbon, reduced soil erosion, reduced input requirements and higher net energy return. Land use change could be minimized in such systems through the utilization of marginal croplands or abandoned grasslands. One of the most commonly used energy crops from the family *Poaceae* is *Miscanthus giganteus* Greef et Deu., a sterile tetraploid hybrid, parental forms: *Miscanthus sinensis* Andersson and *Miscanthus sacchariflorus* (Maxim.) Franch., C_4 photosynthetic pathway plant group, native to tropical and subtropical regions of Africa and Southeast Asia. *Miscanthus giganteus* is propagated asexually, usually by dividing the rhizomes and by tissue culture. It is characterized by rapid growth and development, ultimate height 4.5 metres, produces a large quantity of biomass under low input levels, is tolerant to soil and environmental conditions, being widely used for fuel production of the end last century (Lewandowski et. al., 2000; Havrland et. al., 2013; Jackson, 2015; Ţîţei, 2015).

The genus *Sorghum* Moench, plant group C_4, includes 31 species, is native to Europe, Asia, North and South America along with Australia. It is among the top five cereals and one of the key crops in global food security efforts, grown in drier areas, being able to exploit the salty soils where the cultivation of cereals is more difficult, is utilized for the production of grain, forage, sugar and, more recently, biofuels (Ţîţei and Teleuţă, 1994; 2011; Getachew et. al., 2016.).

Sorghum almum Parodi, also called Columbus grass or almum sorghum is native to South America, it was first developed in Argentina in 1936 as natural hybrid between *Sorghum bicolor* and *Sorghum halepense,* confirmed as species in 1943 by Parodi L.R. It is a robust, tussocky, short-lived perennial plant. It has numerous tillers and thick short rhizomes, reproduces by seed and rhizomes. Culms are thick and solid and can reach up to 4.5 m. *Sorghum almum* tolerates a wide range of soil types and temperatures and it is drought tolerant, reproduces by seed and rhizomes. The species *Sorghum almum* is studied in scientific centres and universities in different regions of the Earth (Rakhmetov and Rakhmetova, 2008; Heuzé et al., 2015). It has been cultivated as forage crop, in the USA, since 1943. In Romania, it has been studied since 1962 in several scientific centres: Fundulea, Caracal, Lovrin (Popescu and Albu, 1970). The green mass productivity of Columbus grass under the conditions of Uzbekistan reached 211 t/ha (Avutkhonov et al., 2016). Depending on the age and the manner of exploitation of the plantation under the conditions of the Republic of Moldova, the biomass productivity of *Sorghum almum* was about 11-15 t/ha (Ţîţei et. al., 2015), in Ukraine, it reached 20 t/ha (Rakhmetov and Rakhmetova, 2008), the maximum yield, 49.5 t/ha, was attested in Gaudiano di Lavello, southern Italy (Corleto et al., 2009).

The objective of this research was to evaluate some biological features, engineering properties of the dry biomass of the non-native *Poaceae* species: *Miscanthus giganteus* and *Sorghum almum*, as well as the quality of solid biofuel.

MATERIALS AND METHODS

The cultivar *Titan* of *Miscanthus giganteus* and the cultivar *Argentina* of *Sorghum almum*, which were cultivated in the experimental plot of the Botanical Garden (Institute), served as subjects of study, control variants - wheat straw, *Triticum aestivum*, and corn stalks, *Zea mays.*

Miscanthus giganteus and *Sorghum almum* were harvested manually in the first days of February. Corn stalks were collected as whole plants after the ears had been removed. Harvestable stems of *Miscanthus giganteus, Sorghum almum* and *Zea mays* and wheat straw bales were chopped into chaff with the use of stationary forage chopping unit. The obtained chaffs of mean dimension from 7 to 35 mm, were milled in a beater mill equipped with a sieve with diameter of openings of 10 mm (for briquettes), and 6 mm (for pellets). Scientific researches on the biomass for the production of solid biofuel were carried out: the moisture content of the plant material was determined by CEN/TS 15414 in an automatic hot air oven MEMMERT100-800; the content of ash was determined at 550ºC in a muffle furnace HT40AL according to CEN/TS 15403; automatic calorimeter LAGET MS-10A with accessories was used for the determination of the calorific value, according to CEN/TS 15400; the particle size distribution was determined using standard sieves, the collected particles in each sieve were weighed; the cylindrical containers were used for the determination of the bulk density, calculated by dividing the mass over the container volume; the briquetting was carried out by hydraulic piston briquetting press BrikStar model 50-12 and pelleting - by the equipment developed in the Institute of Agricultural Technique „Mecagro"; the mean compressed (specific) density of the briquettes and pellets was determined immediately after removal from the mould as a ratio of measured mass over calculated volume.

RESULTS AND DISCUSSIONS

We could mention that, in the first year of vegetation, the cultivar *Titan* of *Miscanthus giganteus* developed, in the underground part, the root system and new rhizomes, and the 5-7 shoots grew up to 185 cm tall. In the following years, the growing season for *Miscanthus giganteus* began on 10-17 April; 17-35 shoots grew and, by the end of the growing season, reached 3.3-4.2 m in height, the root system reached 2 m depth and the number of rhizomes increased considerably. Analyzing the biological peculiarities of the cultivar *Argentina* of *Sorghum almum*, we could mention that after sowing (in May), during 5-7 days, the seedlings emerged at the soil surface, the rate of growth and development was intensive and, in July, the formation of the panicle started, the shoots reaching 235 cm high, the seed ripening stage finished in the middle of September. The formation of rhizomes determined the long-term cycle of development of this crop. In the second year, shoots developed from the underground rhizomes formed in the previous year. *Sorghum almum* plants needed a sum of active temperatures higher than *Miscanthus giganteus,* the revival of vegetation was observed at the end of April, the growth and development rate of shoots increased after 25-30 days after the revival of vegetation and, until the end of June, the plants were in the panicle formation stage, growing 270-290 cm tall. We may mention that, in the following years, the panicle formation occurred 25-30 days earlier in comparison with the first year of vegetation.

It is known that moisture and leaf share in harvested biomass influence the costs of transport, storage, drying and processing, and the thermophysical properties of solid biofuel reduce the final usable energy and thus the efficiency of the energy system, contributing at the same time to the increased emission of pollutants. There is a practical limit of autogenous combustion at about 67% moisture. High moisture content biomass decreases the heating value of fuel, which in turn reduces the conversion efficiency as a large amount of energy would be used for the initial drying step during the conversion processes.

The results of moisture and leaf contents in harvested biomass of the tested *Poaceae* species are shown in Table 1. It was found that, at the end of the growing season, the stems of all the species contained a lot of moisture 52.1-53.9%, but the leaf and panicle share in the biomass of the tested species varied significantly from 27.9%, *Sorghum almum*, to 47.0%, *Zea mays.*

Under the climatic conditions with temperatures above 0°C and rain in the period October-January, the dehydration of the stems in all studied species was very slow, but the rain and the wind also affected the defoliation rate. Thus, leaf and panicle contents in *Miscanthus giganteus* decreased from 39.0% to 16.4%, in *Sorghum almum* from 27.9% to 11.9% and in *Zea mays* from 47.9% to 29.9%. After the establishment of temperatures below -

12°C in early February, the studied species differed in the pace of dehydration of tissues, *Miscanthus giganteus* in the field dehydrated faster than *Zea mays*. Similar results were presented by other authors, for example, in Poland, the moisture content of *Miscanthus giganteus* plants in November-April decreased from 58.36% to 23.23% (Stolarski et. al., 2014).

Biomass particle size and its distribution is an important parameter used for handling, storage, conversion, dust control systems and the combustion behaviour of biomass fuels.

Table 1. Biomass moisture and leaf contents of the studied *Poaceae* species

Harvesting period	*Zea mays*		*Miscanthus giganteus*		*Sorghum almum*	
	moisture content, %	leaf content, %	moisture content, %	leaf content, %	moisture content, %	leaf content, %
2 October	53.0	47.0	52.1	39.0	53.9	27.9
6 November	50.5	45.6	49.1	38.6	48.4	26.7
21 November	46.3	40.0	48.0	37.7	45.4	23.7
14 December	45.3	39.0	44.7	31.5	35.5	19.3
3 January	42.3	35.0	43.0	21.3	29.7	17.0
16 January	41.0	33.3	33.6	20.4	28.6	12.0
31 January	31.5	29.9	31.6	16.4	27.6	11.9
5 February	28.8	25.4	23.0	12.2	22.0	11.0

Table 2. Particle size distribution of milled chaffs of the studied *Poaceae* species, %

Particle size	*Triticum aestivum*		*Zea mays*		*Miscanthus giganteus*	*Sorghum almum*	
	sieve10 mm	sieve 6 mm	sieve10 mm	sieve 6 mm	sieve 6 mm	sieve 10 mm	sieve 6 mm
<5mm	31.5	0.1	25.7	0.5	2.1	28.7	2.7
4-5mm	17.3	2.9	19.5	1.4	2.9	15.4	3.7
3-4mm	15.2	31.7	14.9	10.4	11.8	19.8	13.3
2-3mm	17.7	31.8	18.0	34.3	26.3	17.6	26.4
1-2mm	12.4	20.6	17.5	32.0	30.1	17.3	33.0
1mm	6.0	12.8	3.9	21.4	26.7	6.2	20.9

Table 3. Some physical and mechanical properties of biomass and solid biofuel of the studied *Poaceae* species

Indices	*Triticum aestivum*	*Zea mays*	*Miscanthus giganteus*	*Sorghum almum*
ash content of biomass,%	4.93	4.40	2.51	3.71
gross calorific value of biomass, MJ/kg	17.4	17.8	19.3	18.6
bulk density of chopped chaffs 7-35 mm, kg/m^3	79	87	146	89
bulk density of milled chaffs 10 mm, kg/m^3	90	100	167	109
bulk density of milled chaffs 6 mm, kg/m^3	163	165	198	163
specific density of briquettes, kg/m^3	740	923	882	783
bulk density of briquettes, kg/m^3	407	501	488	415
specific density of pellets, kg/m^3	1007	1174	1262	1008
bulk density of pellets, kg/m^3	685	701	700	674

It is commonly known that, for the production durable compacts bio fuel the particle shape and size, the density and moisture content are the most essential properties of the comminuted material (Lisowski et al., 2010). In our study, the moisture content of milled chaffs of tested energy crops differed essentially and ranged from 7.9% *Zea mays*, 11.6% *Triticum aestivum*, 13.3% *Sorghum almum*, to 15.4% *Miscanthus giganteus*.

Data on particle size distribution of milled chaffs of the studied *Poaceae* species are shown in Table 2. Analyzing distribution on particle size in milled chaffs, sieve with diameter of openings of 10 mm, it can be stated that the highest content of particles larger than 5 mm in wheat chaffs and the lowest - in corn chaffs. In samples with *Sorghum almum* chaffs contained 60.9% particle size <4 mm, which has positively influenced the increase bulk density of milled chaffs up to 109 kg/m^3, versus wheat straw 51.3% and 90 kg/m^3, respectively.

It was found that milled chaffs, sieve with diameter of openings of 6 mm, contained 65-88% particle size <3 mm for all speeds, which indicated that these milled materials were suitable for effective pelleting. The biomass of *Miscanthus giganteus*, even if it had the highest moisture content (15.4%), was milled very well - about 26.7% particle size 1 mm. These differences result from biometric features of the plant and diameter of their stems, share of leaves and structure of tissues.

The energy content of biomass is determined by its calorific value, which is influenced by biomass elemental composition, moisture and ash content.

Comparing the obtained results on ash content (Table 3), one can add that the highest average amount is contained by *Triticum aestivum* and *Zea mays* biomass (4.93% and 4.40%). The lowest average ash content was found in *Miscanthus giganteus* biomass (2.51%). The greatest ash level reported in research studies conducted by other authors. The ash content in Czech of wheat straw pellets reached 6.33% (Ivanova et al., 2015), in Poland straw and hay briquettes - 7.1-7.3% (Kaleta et al., 2016), which negatively influenced the combustion efficiency.

The gross calorific value of biomass varied significantly (17.4-19.3 MJ/kg). The research showed that *Miscanthus giganteus* had high gross calorific values (19.3 MJ/kg), *Sorghum almum* - moderate (18.6 MJ/kg), but wheat straw - very low ones (17.4 MJ/kg), probably because of the high content of ash. Stolarski et al. (2014) investigated the higher heating value of *Miscanthus giganteus* and obtained a value of 19.09 MJ/kg, while Daraban et al. (2015) found a HHV of 17.7-19.6 MJ/kg, which is in line with the results obtained in this research. Similar research was conducted by Havrland et al. (2013), who saw that the gross calorific value of raw material ranged from 19.1 MJ/kg in the biomass of sweet sorghum to 20.3 MJ/kg in *Miscanthus giganteus*, and Heuze et al. (2015), who found that the gross calorific value of Columbus grass reached 17.8 MJ/kg. The lowest average calorific value 15.7-15.8 MJ/kg is characteristic of hay and straw briquettes (Kaleta et al., 2016).

It was established that the bulk density of the chopped material of *Miscanthus giganteus* constituted 146 kg/m^3, the difference between the other tested species wasn't significant, the lowest bulk density was 79-89 kg/m^3 (Table 3).

Differences were found in the bulk density of chaffs that were chopped and milled in a beater mill equipped with a sieve with diameter of openings of 6 mm. These differences amounted to 52 kg/m^3 in *Miscanthus giganteus* biomass and about 80 kg/m^3 in the other species.

We could mention that the briquettes produced from *Miscanthus giganteus* and corn chaffs in our study were observed as very solid and were not cracking, versus wheat straw, its specific density reaching values 882-923 kg/m^3. The specific density of briquettes from *Sorghum almum* was 783 kg/m^3, but wheat straw -740 kg/m^3.

Plíštil et al. (2005), also, reported similar trends of engineering properties for sorghum briquettes 800-870 kg/m^3 and barley straw 650-730 kg/m^3, destruction force 40-60 N/mm and 6-13 N/mm, respectively.

One of the most economically advantageous methods in densification is compression of biomass in pellet, the density increases of 6-8 times and reaches 1100 to 1250 kg/m^3, while the baled straws have only 120 - 150 kg/m^3. This product is simple to use as energy source in corporate and individual boilers (Hăbăşescu, 2011; Marian, 2016). The our investigation showed that *Miscanthus giganteus* and *Zea mays* pellets have high specific density, 1262 and 1124 kg/m^3, respectively, but bulk density similar values.

According to Tumuluru (2014), that specific and bulk density, and durability of the corn pellets range of 813-1180 kg/m^3 and 445-681 kg/m^3, and 76-96%. The pellets bulk density for miscanthus, wheat straw, and corn stover was 580, 490 and 635 kg/m^3, respectively (Jackson, 2015).

CONCLUSIONS

The studied *Poaceae* species differed significantly in the moisture and leaf contents of plant material at the end of the growing season and in the rate of stem defoliation and dehydration after the establishment of temperatures below -12°C. *Miscanthus giganteus* stems defoliated faster than *Zea mays*, the stems of *Sorghum almum* dehydrated rapidly. The specific density of briquettes made from chopped material (7-35 mm) of *Miscanthus giganteus* was low 594 kg/m^3.

The bulk density of the milled chaffs by sieve 10 mm and 6 mm of the tested energy crops was 90-167 kg/m^3 and 163-198 kg/m^3, respectively.

The specific density of briquettes reached 740-923 kg/m^3, but the specific density of pellets – 1007-1262 kg/m^3.

The *Miscanthus giganteus* was distinguished by high density, gross calorific value (19.3 MJ/kg) and low ash content (2.23%); *Sorghum almum* biomass moderately gross calorific value (18.6 MJ/kg) and ash content (3.71%); *Zea mays* high specific density of solid fuel (923 and1124 kg/m^3); wheat straw lowest bulk density and calorific value, and high ash content (4.93%).

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RESEARCH OF THE COLEOPTERA (*Cerambycidae* and *Lucanidae*) FOUND IN THE NATURAL HABITATS OF THE GATEJESTI-BUNESTI FOREST

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Abstract

The territory that we have been studying (since 2016) is situated in the Valcea County, in Gatejesti-Bunesti forest, part of the basin of Govora river. Referring to the geo-morphological aspect, the research area is part of the Subcarpathian area of Oltenia. The conspectus of the coleoptera in the Bunesti-Gatejesti Forest has been elaborated on the basis of personal researches undertaken since 2016, as well as the little bibliographical information regarding this field. The field researches were made from March till November, on scheduled itineraries. The research area is a forest edified by: Quercus patreae, Fagus sylvatica and Carpinus betulus. Following research in the forest habitats of the Govora river basin, we have identified for species of the Coleoptera (Cerambycidae and Lucanidae), belonging to 5 genera and 3 subfamilies. In this forest we realise 20 transects of de 100 m and we identified 160 specimens. Lucanus cervus species is most common. The Cerambycidae family it is represented by two subfamilies - Cerambycinae and Lamiinae. From the Cerambycinae subfamily was identified two species: Cerambyx cerdo (Linnaeus, 1758) and Rosalia alpina (Linnaeus, 1758) and from Lamiinae subfamily was identified one species - Morimus funereus (Mulsant, 1863). From the Lucanidae family, Lucaninae subfamily was identified two species: Lucanus cervus (Linnaeus, 1758) and Dorcus parallelipipedus (Linnaeus, 1758). Installing species of the Coleoptera in this area on certain tree species is determined by their trophic preferences. Among the species identified, a particular interest is represented by the species: Cerambyx cerdo (Linnaeus, 1758), Rosalia alpina (Linnaeus, 1758), Morimus funereus Mulsant 1863 and Lucanus cervus (Linnaeus, 1758), which are protected species contained in Annex II of the Habitats Directive. We consider the study and knowledge of the entomofauna of this territory, whose boidiversity is going through important changes due to the anthropo-zoogenic factors, to be of great importance nowadays.

***Key words**: Coleoptera, Lucanidae, Cerambycidae, forest habitats, Govora basin.*

INTRODUCTION

The territory under research is located in the Govora river basin (Valcea County) part of the Subcarpathian area of Oltenia.
The researches were made in the forest Gatejesti-Bunesti. This forest is edified by: *Quercus patrea, Fagus sylvatica* and *Carpinus betulus*. In this area we found the next forest habitats: 91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*), 9110 *Luzulo-Fagetum* beech forests, 9170 *Galio-Carpinetum* oakhornbeam forests.

MATERIALS AND METHODS

In this forest we realise 20 transects of the 100 m and we identified 160 specimens.
To characterize their populations, specific methods have been used, summarized below.

Field studies to identify invertebrate species

For the identification of the three invertebrate species, observations were made in the field between March and November. In order to confirm the presence or absence of these key species in the investigated areas, we have inspected old *Quercus*, beech, hornbeam etc., woods with decayed wood residues, old trunks, secular shafts with cracks, fallen trees etc., stacks of wood from the exploitation of trees, litter, paths, forest roads.

Determination of the effective population for each species

Since the area to be investigated is very large and the counting of all individuals is not possible, indirect methods of assessing the effective population were used.

Transects have been delineated, with areas of old trees, trunks, fallen trees, hollows. One transect had the following parameters: L = 100 m and l = 20 m/S = 2000 m^2. Along these were the individuals observed on the trunks and the litter, and after the end of the flight period, for the species *Lucanus cervus* and *Cerambyx cerdo* were considered exoskeletons or scabs of exoskeletons. In the case of *Morimus funereus*, no exoskeleton was observed except isolated, as the species probably entered the diapause.
Observations were made on the gender of the individuals, the height from the soil of the observed species, the wood species, the diameter of the tree, the behaviour of the insect, whether solitary or in couple, the moment of the day, the type of habitat, there were taken into account also the head with the mandibles. The twilight transect method was used, recommended by Harvey et al. (2011b) for *Lucanus cervus* and *Cerambyx cerdo*.

RESULTS AND DISCUSSIONS

The conspectus of the Coleoptera in the Bunesti-Gatejesti forest has been elaborated on the basis of personal researches undertaken since 2016, as well as the little bibliographical information regarding this field. The field researches were made from March till November, on scheduled itineraries.
Following research in the forest habitats of the Govora river basin, we have identified for species of the Coleoptera (Cerambycidae and Lucanidae), belonging to 5 genera and 3 subfamilies.
The Cerambycidae family it is represented by two subfamilies - Cerambycinae and Lamiinae. From the Cerambycinae subfamily was identified two species: *Cerambyx cerdo* (Linnaeus, 1758) and *Rosalia alpina* (Linnaeus, 1758) and from Lamiinae subfamily was identified one species - *Morimus funereus* (Mulsant, 1863).
Cerambyx cerdo (Linnaeus, 1758) is a protected species (Natura 2000 Code: 1088 according to Council Directive 92/43/EEC, Annex II, GEO 57/2007 Annex 3) (figure 1). Following the inventory of trees in this forest stand of the forest part, the species is present in the following type of habitat: 9170 *Galio-Carpinetum* oakhornbeam forests, where they prefer the secular trees isolated in the shingles or at the edge of the forest, especially those partially attacked by various pests. The species was highlighted in trees over 70 years old, but it takes time for the number of trees in this forest to reach the appropriate age and be inhabited by the species. The size of the population is closely correlated with the ecological conditions of the trees stand, so between March and November 36 individuals, males and females, alive or dead, were inventtoried. A negative impact on the species is the cutting of secular trees, especially in the plateau area. Such activities affect the habitat of the species, and secular trees are rarer in the area. Land observations related to the population of the species under the current pressures make us believe that the manifestation of these threats will have a significant impact in the future.
At *Cerambyx cerdo* sexual dimorphism is obvious, according to the data recorded on the ground, the result of the statistical calculation of this indicator resulted in a 63.9% sex ratio having a supraunit value.

Figure 1. *Cerambyx cerdo* in the Gatejesti-Bunesti Forest

Rosalia alpina (Linnaeus, 1758) is also a protected species (Natura 2000 Code: 1087, according to Council Directive 92/43/EEC, Annex II, GEO 57/2007 Annex 3). In the studied territory the population of this species from a numerical point of view is insignificant. One single male specimen was reported during the flight in a beech phytocenosis.
Morimus funereus (Mulsant, 1862) is a protected species (Natura 2000 Code: 1089, according to Council Directive 92/43/EEC, Annex II, GEO 57/2007 Annex 3).
He prefers dry, partially dried or attacked by other pests, such conditions being ensured by

forests over 40 years old. Adults were observed during the day or evening, near the trunks, on the freshly cut trunks, on the logs, on the stems of old trees, with the branches on the lower dry floor or on completely dry trees belonging to different woody species: *Quercus petraea*, *Fagus sylvatica*, *Cerasus avium*. Individuals are located at different heights on the surface of the stems, at the base of these up to 230 cm above the ground. In the forest stands of this forest the population of this species is relatively small, few individuals have been identified. The transects performed showed 1-2 specimens/2000 sq m, sometimes present with individuals of *Lucanus cervus* and *Dorcus parallelipipedus*.

Impacts, current anthropogenic pressures and threats, have a cumulative low or insignificant effect on the species, not significantly affecting its viability in the long run. Several stacks of wood were observed following field research, especially in the Gatejesti area, so that they have been stored for a longer period of time in the forest fund act as traps for the individuals of *Morimus funereus* during mating. Sexual dimorphism is also evident in this species, and for the sex ratio there were taken into account the living individuals observed on the field. Recorded data reveal male dominance.

From the Lucanidae family, Lucaninae subfamily was identified two species: *Lucanus cervus* (Linnaeus, 1758) and *Dorcus parallelipipedus* (Linnaeus, 1758).

Lucanus cervus (Linnaeus, 1758) is a protected species (Natura 2000 Code: 1083, according to Council Directive 92/43/EEC, Annex II, GEO 57/2007 Annex 3) (figure 2).

There are many individuals in rare-leafed areas, preferred by *Lucanus cervus*, but also dense, dark. The forest stands are planted on level ground and slopes with a slope of 10-40%. The species is commonly found in plateau areas in *Quercus petraea* phytocoenoses, rarely on the hills, with hornbeam and beech. In terms of population size: during the field trips in the Gatejesti-Bunesti area in March-November, 64 individuals, males and females were inventoried, many in the form of whole or fragmented exoskeletons. The species is rare or lacking in wet and cold valleys with a lot of grooves and coarse and submarine coats developed.

The cumulative effect of impacts on the species is cumulatively low or insignificant, not significantly affecting the long-term viability of the species under the conditions of an appropriate management of the forest fund in that area. A negative impact is carpinization - after deforestation, the hornbeam installs very slightly at the expense of the *Quercus species*, preferred by *Lucanus cervus*; has the effect of reducing the preferred biodiversity habitat. Field observations surprised gender issues as a result of increased sexual dimorphism. Data reveal males dominance, with a 62.5% sex ratio having a supraunit value. In the course of journeys, many individuals were observed in the substrate or covered with litter in August-September.

Figure 2. *Lucanus cervus* in the Gatejesti-Bunesti Forest

Dorcus parallelipipedus (Linnaeus, 1758) is not a protected species and is frequently found in the forest stands in the studied territory.

In the forest stands of this forest the population of this species is large, a number of 51 individuals have been identified. The transects performed revealed a relatively large number of copies/2000 square meters. Data reveal male dominance, with a 58.8% sex ratio, also having a supraunit value.

CONCLUSIONS

Among the species identified, a particular interest is represented by the species: *Cerambyx cerdo* (Linnaeus, 1758), *Rosalia alpina* (Linnaeus, 1758), *Morimus funereus* (Mulsant, 1863) and *Lucanus cervus* (Linnaeus, 1758), which are protected species contained in Annex II of the Habitats Directive. We consider the study and knowledge of the entomofauna of this territory, whose boidiversity is going through important changes due to the anthropo-zoogenic factors, to be of great importance nowadays.The forests in the studied area abound in rotten trunks, dry trees, wood remains left by tree exploitation. These rotten elements come from different woody species: *Quecus patrea*, *Fagus sylvatica* and *Carpinus betulus*. In the observed forests hygiene and thinning works are done, and the wood is kept in the forest. Numerous trunks of *Quercus petraea*, less rarely *Fagus sylvatiaca*, present drills of *Lucanus cervus, Cerambyx cerdo*. Our research reveals a higher density of individuals of *Lucanus cervus* in the selvages.

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STUDIES ON THE VITALITY AND THE STATE OF HEALTH OF CHARACTERISTIC SPECIES OF TREES FROM THE FOREST HABITATS FOUND IN THE PROTECTED AREA ROSCI 0128 (THE NORTHERN AREA OF EASTERN GORJ COUNTY)

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Abstract

*The thematic area provided in this paper is situated in the protected area ROSCI 0128 (Northern area of Eastern Gorj county), occupying the southern part of Parang Mountains and the west part of the Capatanii Mountains. In the researched area there are the following Natura 2000 habitats: 9410 - Acidophilous Picea forests of the montane to alpine levels (*Vaccinio piceetea*), 91E0*-Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae), 91V0 –Dacian Beech forests (Symphyto Fagion), 9110 - Luzulo-Fagetum beech forests, 9130 - Asperulo-Fagetum beech forests, 9170 - Galio-Carpinetum oak-hornbeam forests, 91Y0 - Dacian oak-hornbeam forests, 91Q0 - Western Carpathian calcicolous Pinus sylvestris forests, 9260 - Castanea sativa woods, 9150 - Medio-European limestone beech forests of the Cephalanthero-Fagion, 91L0 - Illyrian oakhornbeam forests (*Erythronio-Carpinion*). The stability of the forest is determined, in addition to the physiognomy, the composition of the flora, its stage of development, density, structure, and constituent species and especially the vitality of the characteristic species. Forest habitats from the thematic area have a vitality or state of vegetation from normal to low. The health of the trees or plant health is determined by the health and vitality of trees that make up the forest and is expressed by the degree of defoliation of trees or discoloration of the foliage, phenomena of damage or drying trees, tears, windfalls, established by biomonitoring forest ecosystems.*

Key words: *forest habitat, vitality, the state of health, protected area.*

INTRODUCTION

The territory under research is in the protected area ROSCI 0128 (Northern area of Eastern Gorj county), occupying the southern part of Parang Mountains and the west part of the Capatanii Mountains.

The state of health of the trees or the phyto-sanitary state is determined by the health and vitality of the trees that make up the tree stand and it is expressed by the degree of defoliation of the trees or the discoloration of the foliage, tree injury or drying phenomena, breaks, knocks, established through biomonitoring of forest ecosystems.

The stability of a tree is determined, in addition to the physiognomy, the floristic composition, its stage of development, its density, its structure, and the vitality of the component species and especially of the edifying species.

The vitality or vegetation refers to the growth vigor and the weather resistance of a tree stand. Thus, the planning rules in Romania classify vitality in the following categories:

- tree stands with a very active (vigorous) vegetation state when the trees have in recent years increased in diameter and height very much in relation to the age and reliability of the station;
- tree stands with active (vigorous) vegetation, when the last annual increases in diameter and height exceed the normal ones;
- tree stands with a normal vegetation state where the last increases in diameter and height are average in relation to the age and the reliability of the station;
- trees with weak vegetation, when the latest increases in diameter and height are subnormal (lower than average);
- tree stands with a very weak (feeble) vegetation, when the last increases in diameter and height are virtually null.

The analysis of the stands, in the North East Gorj protected area has led to the following results: the stands have a **normal vitality or vegetation state - N**, having the latest increases in diameter and height at middle level in relation to the age and the reliability of the station and **weak vitality or vegetation state**, when the last increases in diameter and height are subnormal (lower than average).

MATERIALS AND METHODS

The field research on the field was carried out between 2014-2017, during all seasons and having clearly defined itineraries. The research underpinned solid bibliographical document-tation with respect to the physical and geogra-phical environment: the relief, geology-lithology, types of rocks, hydrographic net, soils and the general and local climate. The findings included forest habitats was closely analyzed, especially taking into consideration the anthropic factor.

RESULTS AND DISCUSSIONS

Studies on each type of forest habitat according to the health status of edifying tree species

The state of health of the trees or the phytosanitary state is determined by the health and vitality of trees that make up the tree stand and is expressed by the degree of defoliation of the trees or the discoloration of the foliage, phenomena of injury or drying of trees, breaks, knocks, established by the biomonitoring of forest ecosystems.

1.9410 habitat - Acidophilic mountain forests with *Picea abies* (Vaccinio-Piceetea)

Edifying species: *Picea abies*

Analyzing the phytocoenoses in the 9410 habitat was found to be characterized by: weak drying, isolated breaks, isolated break downs, weak hunting.

Within this habitat, the most important injuries are those caused by wind or snow break downs.

Figure 1. Isolated drying of lime spruce (photo M. Niculescu)

Figure 2. Isolated spruce break downs from the 2014 flood (photo M. Niculescu)

Figure 3. Wind break downs of the spruce (photo M. Niculescu)

Figure 4. Wind break downs of the spruce (photo M. Niculescu)

2. 91E0* habitat - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*)

Edifying species: *Alnus glutinosa, A. incana, Salix alba, S. fragilis*

Analyzing the phytocoenoses within the 91E0* habitat it was found to be characterized by: break downs following the 2014 floods, isolated break downs, weak hunting.

Figure 5. Alder break downs
(photo M. Niculescu)

Figure 6. Alder break downs
(photo M. Niculescu)

3. 91V0 habitat - Dacian beech forests (*Symphyto-Fagion*)
Edifying species: *Abies alba, Fagus sylvatica, Acer pseudoplatanus*
Analyzing the phytocoenoses in the 91V0 habitat, it was found that they are characterized by: weak drying, isolated breaks, isolated break downs, weak hunting. The wind break downs were isolated in the *Abies alba* species. At *Fagus sylvatica* there were also encountered isolated attacks involving the the decay of the clover in the deciduous trees (*Phellinus igniarius* Quel.*),* the cancer of the deciduous species (*Nectria galligena* Bres.*),* the bacterial cancer of the deciduous species (*Psudomonas syringae*), *Cryptococcus fagisuga, Xyleborus saxeseni, Trypodendron domesticum, Cerambycidae, Hylecoetus dermestoides, Taphrorynchus bicolor, Xyleborus monographus, Lymantria monacha*.
4. 9110 habitat - *Luzulo-Fagetum* beech forests
Edifying species: *Fagus sylvatica*
Analyzing the phytocoenoses within the 9110 habitat, it was found that they are characterized by: weak drying, isolated breaks, isolated break downs, weak hunting. Isolated attacks have also occurred, which cause: the decay of the clover in the deciduous trees (*Phellinus igniarius* Quel.*)*, the cancer of the deciduous species (*Nectria galligena* Bres.) the bacterial cancer of the deciduous species (*Psudomonas syringae*), *Cryptococcus fagisuga, Xyleborus saxeseni, Trypodendron domesticum, Cerambycidae, Hylecoetus dermestoides, Taphrorynchus bicolor, Xyleborus monographus, Lymantria monacha*.

Figure 7. Isolated beech drying in 91V0 habitat
(photo M. Niculescu)

Figure 8. Weak drying of beech trees
(photo M. Niculescu) (rotting of the clover of deciduous trees (*Phellinus igniarius* Quel.)

Figure 9. Isolated break downs in beech in 9110 habitat (photo M. Niculescu)

5. 9130 habitat - *Asperulo-Fagetum* beech forests Edifying species: *Carpinus betulus, Fagus sylvatica*

Analyzing the phytocoenoses in the 9130 habitat it was found to be characterized by: weak drying, isolated breaks, weak hunting.

In the beech and hornbeam, there have also been isolated attacks that produce: the decay of clover in the deciduous trees (*Phellinus igniarius* Quel.), the cancer of the deciduous species (*Nectria galligena* Bres.) the bacterial cancer of the deciduous species (*Psudomonas syringae*), *Cryptococcus fagisuga*, *Xyleborus saxes*, *Trypodendron domesticum*, *Cerambycidae*.

Figure 10. Breaks to beech trees (photo M. Niculescu)

6. 9170 - Galio-Carpinetum oak-hornbeam forests

Edifying species: *Carpinus betulus, Quercus petraea*

Analyzing the phytocoenoses within the 9170 habitat it was found to be characterized by: weak drying, isolated breaks. In the hornbeam, there have been encountered isolated attacks that produce: the decay of clover in the deciduous trees (*Phellinus igniarius* Quel.), the cancer of the deciduous species (*Nectria galligena* Bres.) the bacterial cancer of the deciduous species (*Psudomonas syringae*). In holm - *Cerambycidae*

Figure 11. Phytopathogenic attack on the habitat 91Y0 tree stand (photo M. Niculescu)

7. 91Y0 habitat - Dacian oak-hornbeam forests Edifying species: *Carpinus betulus, Fagus sylvatica, Quercus robur, Quercus petraea*

Analyzing the phytocoenoses in the 91Y0 habitat it was found to be characterized by: weak drying, isolated breaks, isolated break downs, weak hunting.

Isolated attacks occurred that produce: Defoliator pests of the oak: oak hairy caterpillar (*Limantria dispar* L.), green oak moth (*Tortrix viridana* L.), *Laspeyresia splendana* Hb., *Archips rosana* L., *Eudemis profundana* F, the brown grasshopper (*Erannis defoliaria* Cl.), green grasshopper (*Operophtera brumata* L.), *Erannis aurantiaria* Hb.

Among the specific xylophagous pests of our forests and which are dominant in the complex structure of this category of pests, we can mention species such as Buprestide with two points (*Agrilus biguttatus* F.), *Agrilus viridis* L., the great oak crown (*Cerambyx cerdo* L.), painted oak crown (*Plagionotus detritus* L.), oak bark beetle (*Scolitus intricatus* Ratz).

The most widespread forest diseases are oak firing (*Microsphaera alphitoides* Griff. Et Maubl.), *Armillaria mellea* (Vahl: Fr.) the decay of clover in the deciduous trees (*Phellinus igniarius* Quel.), the cancer of the deciduous species (*Nectria galligena* Bres.) the bacterial cancer of the deciduous species (*Psudomonas syringae*) and *Taphrinia carpini* Rostr.

Figure 12. Break at *Quercus* petraea (photo M. Niculescu)

Figure 13. Rest of *Lucanus cervus* exoskeleton, attack in 91Y0 (photo M. Niculescu)

8. 91Q0 habitat- *Pinus sylvestris* relict forests on limestone
Edifying species: *Pinus sylvestris*
Analyzing the phytocoenoses of *Pinus sylvestris*, within the 91Q0 habitat, it was found that they are characterized by: weak drying, isolated breaks, weak hunting.
9. 9260 habitat - Edible chestnut forests (*Castanea sativa*) [*Castanea sativa* Woods]
Edifying species: *Castanea sativa*
Isolate there has been observed attack of: *Cryptonecria parasitica, Phytophtora cynnamoni* and *P. cambivora*, weak drying, isolated breaks.
10. 9150 habitat - Medio-European limestone beech forests of the *Cephalanthero-Fagion*
Edifying species: *Carpinus betulus, Fagus sylvatica*
Analyzing the phytocoenoses within the 9150 habitat it was found to be characterized by: weak drying, weak injury.
Isolated it has been observed attack of: the the decay of clover in the deciduous trees (*Phellinus igniarius* Quel.), the cancer of the deciduous species (*Nectria galligena* Bres.) the bacterial cancer of the deciduous species (*Psudomonas syringae*).
11. 91L0 habitat - Illyrian oak-hornbeam forests (*Erythronio-Carpiniori*)
Ass. Asperulo taurinae-Carpinetum Soó et Borhidi in Soó, 1962.
Edifying species: *Carpinus betulus*
Analyzing the phytocoenoses in the 91L0 habitat it was found to be characterized by: weak drying, weak injury.
In the hornbeam, there have also been isolated attacks that produce: the decay of clover in the deciduous trees (*Phellinus igniarius* Quel.), the cancer of the deciduous species (*Nectria galligena* Bres.) the bacterial cancer of the deciduous species (*Psudomonas syringae*), Cerambycidae.

Research on the distribution of forest types of treatment applied and applicable to each forest habitat

In a tree stand, an essential condition is to apply the treatments properly, especially when it comes to a protected area.
At the age of exploitation, determined by forest planning studies, the mature tree stand is harvested to create the conditions required for the installation and development of a new generation of trees. The special way in which the mature trees are harvested and the way how the regeneration of the tree stands is ensured under the same regime for the purpose of achieving household purposes is called treatment.
The main source of data on the cutting of main products or secondary products is the arrange-ment, whose field and office work is carried out by specialized bodies, by administrative territorial units: forest districts, associations of owners, councils, communes, simple forest owners or silo-hunting sections.
In the case of the treatment there are differ-rences of the periodic surface in time, the period of regeneration or rotation, in the case of trees treated in gardening, the special regeneration period, being differentiated according to the objectives set for the choice and application of the treatments. The choice of treatment takes into account the biological characteristics of the species, the static conditions, ecological and economic considerations. At the same time, it is necessary to consider whether the tree stands are located in a protected area or National Park. if In relation to these elements, are established: the duration of the regeneration process, the number, the frequency and the intensity of the interventions, the epochs and the deadlines for the execution of the exploitation works.
In the protected area North East Gorj, the following types of applied and applicable forest treatments are met: conservation cuts, helping natural regeneration, hygiene cuts, progressive cuts, cleansing, clearance, cuttings, cuttings in the groves, additions, cuts.
In addition to the above-mentioned works, which are considered to be forest treatments, in the protected North East Gorj area, the

following types of treatments are also provided according to the planning regulations:

- ***Type II (T_{II}):*** forests with special protective functions located on environmentally-friendly stations, as well as the forest stands where it is not possible to harvest wood, requiring only ***special preservation*** works;
- ***Type III (T_{III}):*** forests with special protection functions for which only intensive treatments are allowed - gardening, quasi-gardening.
- ***Type IV (T_{IV}):*** forests with special protection functions for which, in addition to gardening, quasi-gardening are admitted other treatments subject to special restrictions on application;

They apply equally to all the forest habitats in the area, respecting the planning regulations.

CONCLUSIONS

The stability of the forest is determined, in addition to the physiognomy, the composition of the flora, its stage of development, density, structure, and constituent species and especially the vitality of the characteristic species. Forest habitats from the thematic area have a vitality or state of vegetation from normal to low. The health of the trees or plant health is determined by the health and vitality of trees that make up the forest and is expressed by the degree of defoliation of trees or discoloration of the foliage, phenomena of damage or drying trees, tears, windfalls, established by biomonitoring forest ecosystems. In the protected area North East Gorj, the following types of applied and applicable forest treatments are met: conservation cuts, helping natural regeneration, hygiene cuts, progressive cuts, cleansing, clearance, cuttings, cuttings in the groves, additions, cuts.

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DEVELOPMENT OF TAILOR-MADE FOOD WASTE PREVENTION MEASURES BASED ON CONSUMER TYPE ANALYSIS

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Abstract

The paper aims to present the development of tailor-made measures to reduce food waste at household level based on findings of a large-scale household questionnaire conducted in Austria between March to April 2017. Through a cluster analysis of the results it was possible to divide consumers that are to a full or large extent responsible for shopping and cooking at home into four different groups (Eager avoiders, Uninformed but eager, Informed but uninterested, Uninformed Squanderers). Although there can be found large differences among these groups when it comes to attitude, knowledge, food waste prevention behaviours as well as behaviours that might lead to increased food losses, it was not possible to find great differences among the consumer groups when asked about the kind and frequency of materials they want to receive in order to prevent food waste at home. Based on that findings first aid boxes for food have been developed and distributed to 2000 households.

Key words*: food waste prevention, consumer behaviours, tailor-made measures, food waste types*

INTRODUCTION

Looking at food waste amounts in all European countries it is striking that especially at household level a considerable prevention potential can be found that contrasts all efforts towards a sustainable food chain. Schneider et al. (2012) provided a compilation on food waste generation in Austria. Food waste in Austria accounts for 25% of residual waste. This equals to 33.1 kg per person and year or 276,430 tonnes per year. Out of this amount 43% belong to non-avoidable food waste or preparation residues like bones, peels or stalks while 57% are at least potentially avoidable. Important for waste prevention measures are therefore 14.5% of the residual waste or 157,647 tonnes per year or 18.9 kg per inhabitant and year.

The successful implementation of measures at household level has proved to be difficult because the reasons for the generation of food waste at consumer level are often connected with personal life situation, attitudes, behaviours and of course knowledge. Therefore, within this study tailor-made measures for different consumer groups should have been developed.

MATERIALS AND METHODS

Between March and April 2017 a large scale online - household questionnaire on food waste has been conducted in Austria. 28 food waste related questions aimed to bring up a status quo on attitudes towards food waste, knowledge about the handling of food stuff as well as food waste generation and food waste prevention at households. The questionnaire has been answered fully by 2159 participants, 473 did not finish. As consumer participated on a voluntary basis it cannot be expected that the data is statistically significant and will deliver equally meaningful results for consumers of all age groups or all educational levels, but as there is no comparable data available for Austria at the moment this information will help to identify first measures that need to be developed. It must be taken into account that the majority of participants (almost 50%) has a university degree and is interested into this topic at least to some extent.

Subsequently a cluster analysis has been conducted to find groups of consumers that show similar properties within the group but differ as much as possible from other groups. The main focus was laid on consumer that stated to be fully or mostly responsible for shopping and food preparation as these are mainly responsible for food waste prevention or generation.

RESULTS AND DISCUSSIONS

The majority of respondents in this survey are females (71%), which means that many of the clusters are female dominated. It can be seen clearly that the responsibility for purchasing and preparing food is still more often overtaken by women. Following four groups of consumers mainly responsible for food shopping and preparation have been identified: “Eager Avoiders” (Group 1), “Uninformed but eager” (Group 2), “Informed but uninterested” (Group 3) and “Uninformed squanderers” (Group 4). The group of "eager avoiders" is particularly large in this survey. This is related to the fact that participation in the survey was based on a voluntary basis and was likely to have more people participating in it, for hom this topic already has a relatively high priority. This group is very well informed and already implemented a lot of food waste prevention methods at home. Soccio-demographic characteristics show that participants of this group have a lower income and less often have a university degree.

The second group is ideal for the implementtation of measures. This group is very bad informed about all important issues to prevent food waste as expiration dates, food storage and environmental effects but they already started with food waste prevention and have the second best attitude towards food waste.

The third group can be described as "informed but uninterested". Respondents in these group have solid basic knowledge (though not extensive knowledge) of how to handle food and the effects of food waste, yet they are very unconcerned with food and not convinced of the seriousness of the situation - reaching this group seems to be particularly difficult.

Particularly interesting in this regard is group 4 ("uninformed squanderers"). This group states to waste the most and more often than the other groups but is not as disinterested as the group 3. It can therefore also be a very good target group for measures to be developed, where, in particular, information on waste volumes and their effects (on a financial, ecological and social level) should be prepared in order to raise awareness.

Although different groups of consumers could be identified, these hardly differ in terms of the desired frequency of contact, the desired way to be contacted or even the type of information desired.

The survey showed that over 50% of respondents would like to receive more information about proper storage of certain food products, especially fruits and vegetables. Furthermore, leftover-recipes are in demand as well as information about which products can cause health issues after the expiration date.

An interesting aspect is that a majority of consumers surveyed (about 74%) would like to receive information on food waste prevention measures and also tips on proper storage on product packaging itself, about 47% of respondents would like to get this information at supermarkets. It is interesting to see that consumer want that the retail sector carries more responsibilities even about food waste prevention at households. Therefore, it has to be assumed that supermarkets also have a role model function for consumers. Especially fruits and vegetables that should be stored in the refrigerator are often stored at room temperature in supermarkets. This type of presentation could have a negative impact on the handling of certain varieties (for example, apples, pears, oranges, berries, salads) in households.

About 40% of respondents would like to have an app that responds individually to their needs, while free works hops, for example, would be used by just under 20% of respondents.

Concerning the frequency of the desired information all groups prefer to be contacted once so they know where to find the information when they choose to use it. It also turned out that the awareness of and the knowledge about existing campaigns is very much linked to intensive (social) media work.

CONCLUSIONS

Based on the findings of this survey tailored measures meet following consumer needs in Austria. The measure must be suitable for offering at retail stores, measures should contain the most important knowledge about food stuff handling. Additional it must contain basic facts about food waste in Austria and the impacts of food waste. It should provide an overview about all possible food waste measures and give detailed information about some of them, e.g. instructions for food waste prevention techniques like “making a jam” or how to freeze food products. It is helpful when the measures support consumers in a way that they immediately experiment with some contents and apply their newly gathered knowledge.

Already eager avoiders should be kept up to date and also be given the chance to act as multiplier to positively influence other consumers with their showcase behaviour and by distributing materials.

Ideal target groups at the moment are the ones that state to waste much but are generally interested in the topic or just haven’t had this issue in mind so far. It is hard and maybe won’t be possible to reach or create more awareness among consumers, who are not interested in the topic and see no need to change their wasteful behaviours. It may be possible to reach them with humorous contents in a first step to attract their attention.

Nevertheless, in the long run, although consumers stated to prefer to be contacted only once with all necessary information a certain persistence and the presence of the topic in the media will help to improve the situation and be the key to success, because attitudes and habits in particular change slowly.

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This research work was carried out within the framework of the EU-Interreg Project STREFOWA (Strategies to reduce and manage food waste). Based on these findings a first-aid box for food (Figures 1 and 2 below) has been developed and handed out to 2000 consumers in supermarkets in Vienna.

Figure 1. First Aid Box - Exterior View

Figure 2. Content of First Aid Box

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PRECISION OF DROUGHT BASED ON THE TOPSIS METHOD

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Abstract

In this study, Kahramanmaraş Central District is aimed to determine the years which are arid or rainy by taking average of annuals in winter, spring, summer, autumn seasons between the years 1995 and 2014, and to calculate the possibility of temporary dry or rainy in future years. TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) method was chosen to estimate as a creating a model requires 6 parameters (long-term, average minimum temperature and maximum temperature, average minimum and maximum relative humidity, average annual precipitation, total annual precipitation) in this direction. For this purpose, data of Kahramanmaraş Meteorological Station, which has the longest rainfall records in the region, are used. In this study, days with less than 2.5 mm of rainfall were considered dry, and days with 2.5 mm or more of rainfall were considered rainy. TOPSIS method resulted in 6 steps and meteorological droughts were detected in 2002, 2008 and 2011. In 2015, 2016 and 2017, there is the possibility of a gradual drought.

Key words: *TOPSIS method, whether, precipitation, drought.*

INTRODUCTION

The development that lives in every area from the primitive society brings together many problems and continues to bring. Human activities such as population growth, industrialization and the consequent emergence of urbanization, such as the release of carbon dioxide and other greenhouse gases effects on temperature, precipitation and other weather-related events and causes global climate change. Drought is at the forefront of natural disasters brought by global climate change (Özfidaner et al., 2016; Keten, 2016).

Although drought is increasing its influence in the world, its scope has not yet been understood and its effects have not been adequately assessed. As a natural consequence of this, there is no definite definition of drought. According to professions, the definitions made are meteorological, hydrological, agricultural, geographic or industrial, energy production, water supply, maritime, recreation places (Sırdaş and Şen, 2010).

In a given time period, falling below normal values of precipitation is defined as meteorological drought.

As a result of the meteorological drought, it is inevitable that some problems such as the problems of irrigation of agriculture areas and the lack of adequate water collection in dams, inadequacy of drinking water resources, negative influence of environment and social structure are inevitable in terms of engineering (Dinç et al., 2016).

Pre-determination of arid circuits is of great importance in water resource planning. Because the maximum drought that will be called the critical drought is an effect on the economic, political and social situation of an country. In order to be able to take precautionary measures, it is necessary to estimate the time, severity and area under which critical drought circuits are affected (Şen, 1980; Sırdaş and Şen, 1999).

Drought is a meteorological natural hazard that has enormous negative effects on the lives of living things, which limits important activities of the people and causes important ecological problems (Şahin and Sipaoğlu, 2003)

Despite the fact that the climate is constantly repetitive, it is difficult to predict. When climate events occur, the duration, intensity and impact range from year to year. As a result, economic, social and environmental influences are taking place, and these effects are a great danger for humanity from time to time. In the analysis of droughts, the long-term average of the balance between precipitation and evapotranspiration in a region should be considered. Drought is a time-dependent parameter (Graedel et al., 2007).

The Palmer Drought Severity Index developed by Palmer (1965) is the first comprehensive drought index. While previous drought indices generally describe drought as descriptive, Palmer Drought Severity Index; precipitation, temperature and soil moisture parameters. The Palmer Drought Severity Index was used extensively between 1960-1990. Later, many drought indexes were developed (Palmer, 1965). The first step in calculating the Palmer Drought Severity Index is to determine the climatic water balance using input from long-term monthly precipitation and temperature data. Palmer uses a simple approach to define moisture accumulation by separating the soil into two layers (Topçuoğlu et al., 2005). Santos et al. (2011) used monthly rainfall data from 144 stations in the study of regional frequency analysis of drought events in Portugal between 1910-2004. To characterize drought events, the Standardized Rainfall Index's 1, 3, 6 and 12 month time periods were used. The drought amplitudes obtained by these time steps of the Standardized Rainfall Index were subjected to regional frequency analysis using two different approaches. According to the results of the analysis, it was determined that the general character of drought periods is evenly distributed throughout the country. Drought indices are either weak or superior to other indices. The advantages and limitations of some indices have been reported in studies (Heim, 2002; White and Walcott, 2009).

In this study, Kahramanmaraş Central District is aimed to determine the years which are arid or rainy by taking average of annuals in winter, spring, summer, autumn seasons between the years 1995 and 2014, and to calculate the possibility of temporary dry or rainy in future years.

MATERIALS AND METHODS

Materials

Coordinates of the study area are important for the climate data to be used. Kahramanmaraş is located between 37° 35' North Parallel and 36° 55' East Meridian Apartments. The height from the sea level is 568 m. Mediterranean climate is dominant in the region. Kahramanmaras is located in the Eastern Mediterranean region. Summers are hot and dry, winters are warm and rainy (Anonymous, 2015). According to the data taken from the Kahramanmaraş meteorological station, the average annual temperature varies between 16.5°C and 8.9°C (Anonymous, 2015). It is seen that the temperature values of Kahramanmaraş province center are above 0°C. The average lowest temperature (4.9°C) was observed in January. The highest average temperature is seen in August. Continuous temperature increase between January and August, from August until February until the continuous decrease in temperature is noticeable. The minimum temperature is observed in January, and the maximum temperature is observed in July - August (Anonymous, 2015).

Kahramanmaraş province 'Mediterranean Climate and Terrestrial Climate' is a transition area and the sea effect is also found in the 'Degraded Mediterranean Climate' is effective. In terms of temperature and precipitation 'Mediterranean climate is summertime hot and dry, winters are warm and rainy' is dominant. Higher areas are cooler in the summer and colder in the winter. It has a continental climate effect from south to north, west to east (Anonymu, 2015).

The total amount of evaporation in Kahramanmaraş province center is 1530 mm. This amount is more than annual precipitation. The annual precipitation is around 720 mm (Anonymous, 2015).

Kahramanmaraş province in winter and spring in the amount of abundance in the amount of rainfall, the Mediterranean precipitation regime shows that the domination. The weather is dry during the summer season from the end of spring. In September and October, there are occasional short periods of precipitation.

In this study, daily rainfall data measured during Kahramanmaraş Meteorology Station between 1994 and 2014 were used. These values were obtained from computer records archived by the General Directorate of State Meteorology Affairs. The evaluation of the data was carried out by accepting 28 days in February and 365 days in the year.

Methods

TOPSIS (Multiple Criteria Decision Making Method) was developed by Yoon and Hwang in 1980. Decision points are based on the principle of the ideal solution approximation. The TOPSIS method includes a 6-step solution process. The steps of the TOPSIS method are described below.

Step 1: Creating the Decision Matrix (A)

Step 2: Creating the Standard Decision Matrix (R)

Step 3: Creating the Weighted Standard Decision Matrix (V)

Step 4: Creating Ideal (A *) and Negative Ideal (A ~) Solutions

Step 5: Calculation of the separation measures

Step 6: Calculation of Ideal Solving Relative Proximity

Determinant C * Coefficient Value in Topsis Drought Analysis

Table 1. Topsis Method Index Values

Extremely Wet	**0.9**	**< TOPSIS <**	**1**
Severe Wet	0.8	< TOPSIS <	0.9
Medium Wet	0.7	< TOPSIS <	0.8
Weak Wet	0.6	< TOPSIS <	0.7
Normal	0.4	< TOPSIS <	0.6
Weak Drought	0.3	< TOPSIS <	0.4
Medium Dry	0.2	< TOPSIS <	0.3
Serious Dry	0.1	< TOPSIS <	0.2
Extremely drought		TOPSIS <	0

Model Example Application

The selection of the selected model for Kahramanmaraş Central district and the preparation of the excel program used are discussed in this section. Matrices are formed and formulated from the data from the required graphs.

i= 1…m

J= 1…n

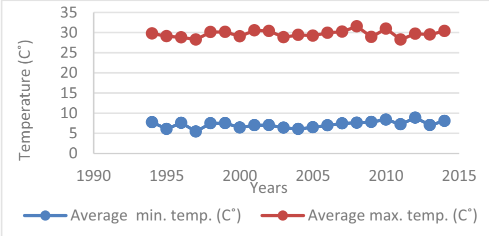

Figure 1. Average minimum and maximum temperatures

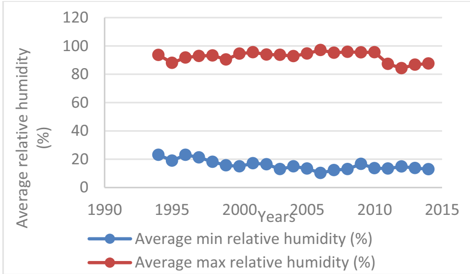

Figure 2. Average minimum and maximum relative humidity

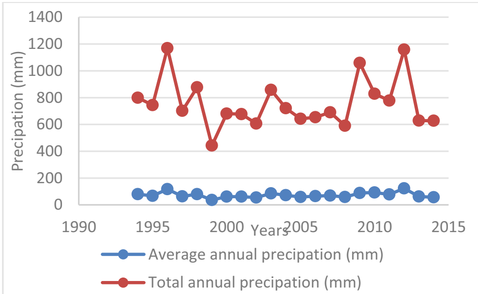

Figure 3. Average and total precipation

Creating the Decision Matrix

$$r_{ij} = \frac{a_{ij}}{\sqrt{\sum_{k=1}^{m} a_{kj}^2}}$$

In the lines of the decision matrix, the years of supremacy are listed (1994-2014). The decision points in the decision points are the assessment factors to be used in decision making: average minimum temperature (C°), average maximum temperature (C°), average minimum relative humidity (%), average maximum relative humidity (%) total annual precipitation (mm).

The matrix A is the initial matrix generated by the decision maker. The decision matrix is constructed as follows Table 2.

Creating the Standard Decision Matrix (R)

The R matrix is formed from the formula results applied separately for each year.

Creating the Weighted Standard Decision Matrix (V)

The weight values (w) for the evaluation factors were determined. The weight values were found by dividing the sum of the numbers in each column by the number of the criterion.

$$\sum_{i=1}^{n} w_i = 1$$

The elements of each column of the R matrix are multiplied by the corresponding wj value to form the V matrix.

Determination of Ideal (A*) and Negative Ideal (A^-) Solution

At this stage, the weighted matrix shows the maximum and minimum values for each column.

Table 2. Climatic Data Observed in Kahramanmaras

Years	Average min temperature (C°)	Average max temperature (C°)	Average min relative humudity (%)	Average max relative humudity (%)	Average annual precipation (mm)	Total annual precipation (mm)
1994	7.75	29.77	23.08	93.58	79.90	799.00
1995	6.08	29.09	19.00	88.00	67.65	744.20
1996	7.59	28.80	23.08	91.75	116.90	1169.00
1997	5.43	28.26	21.25	92.92	63.82	702.00
1998	7.51	30.11	18.17	93.25	79.71	876.80
1999	7.54	30.15	15.67	90.42	36.89	442.70
2000	6.43	29.06	15.00	94.50	61.85	680.30
2001	7.01	30.53	17.17	95.58	61.56	677.20
2002	7.04	30.37	16.42	93.92	55.12	606.30
2003	6.41	28.83	13.00	93.67	85.75	857.50
2004	6.09	29.40	15.00	92.75	72.15	721.50
2005	6.49	29.21	13.42	94.58	58.37	642.10
2006	6.97	29.94	10.33	97.08	65.33	653.30
2007	7.45	30.21	12.33	95.17	69.05	690.50
2008	7.61	31.53	13.08	95.83	58.98	589.80
2009	7.83	28.91	16.67	95.42	88.28	1059.30
2010	8.39	30.96	13.67	95.58	92.12	829.10
2011	7.23	28.25	13.42	87.33	77.75	777.50
2012	8.89	29.71	14.83	84.25	123.10	1158.00
2013	7.03	29.51	13.75	86.75	62.91	629.10
2014	8.07	30.36	12.92	87.58	57.10	628.10

Calculation of Distance Measures Between Alternatives

After identifying the ideal points, the maximum and minimum ideal point distance values are calculated.

$$S_i^* = \sqrt{\sum_{j=1}^{n}(v_{ij} - v_j^*)^2} \qquad \text{i= 1, 2,…, m}$$

$$S_i^- = \sqrt{\sum_{j=1}^{n}(v_{ij} - v_j^-)^2} \qquad \text{i= 1, 2,…, n}$$

Calculation of Ideal Solving Relative Proximity

The criterion used here is the share of the negative ideal difference measure within the total difference measure. The calculation of the ideal solution relative affinity value is shown in the following formula.

$$C_i^* = \frac{S_i^-}{S_i^- + S_i^*} \qquad \text{i= 1, 2,…, m}$$

The ideal solution relative proximity (C) of each decision point is calculated using the equation given earlier.

RESULTS AND DISCUSSIONS

In this study, climate data for Kahramanmaraş Central district was used. In this study, days with less than 2.5 mm of precipitation and dry days of 2.5 mm and more were evaluated as rainy. Based on this feature, the probabilities calculated by the TOPSIS method and the actual probabilities represented by the observational data for the winter, spring, summer and autumn seasons of annual precipitation between 1995 and 2015 have been determined.

Within the scope of analysis within 20 years; no year was not very dry, four years were medium dry, five years were weak dry, seven years were at normal climate, two years were poor wet and two years were extremely wet.

When the 'C' values in the scope of the analysis are examined, the years of 2002, 2008, 2009 and 2011 medium dry, 2003, 2004, 2005, 2007 and 2010 weak arid, 1996, 1998, 2000, 2001, 2006, 1995 and 2012 are wet wet, 1997 and 1999 are extremely wet past.

Table 3. Topsis Outcome Values

1995	0.62126	C1	2005	0.31867	C11
1996	0.45146	C2	2006	0.48927	C12
1997	0.91077	C3	2007	0.39368	C13
1998	0.45146	C4	2008	0.27408	C14
1999	0.91077	C5	2009	0.29526	C15
2000	0.44964	C6	2010	0.35944	C16
2001	0.58279	C7	2011	0.29525	C17
2002	0.23485	C8	2012	0.66832	C18
2003	0.33265	C9	2013	0.55671	C19
2004	0.38798	C10	2014	0.43982	C20

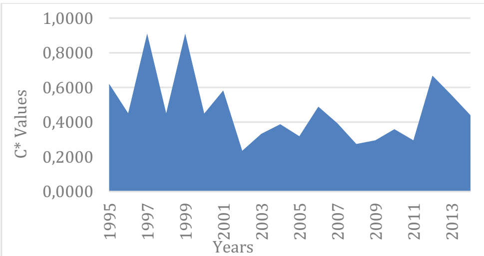

Figure 4. C * Values of Years

Within the information observed in this study; there are two years between two very wet periods and the climate returns to its normal level afterwards.

The closest wet period is between 1 year and the longest wet period is 15 years.

The period between the nearest middle dry period is 1 year, and the longest medium dry period is 9 years.

In this study, where the drought analysis is performed with the TOPSIS method, it is highly likely that a drought or a wet (wet) period is experienced near the same level for two consecutive years. Kahramanmaraş Central has been in the district since 1999, after a normal level and then to a moderate dry level.

CONCLUSIONS

According to various meteorological data and calculated drought method, Kahramanmaraş Central District is experiencing meteorological drought in 1999-2008 period. The drought that we have been living in recently has been turned from meteorological drought to agricultural and hydrological drought with a considerable decrease in winter precipitation. As a result of this study, which is aimed at predicting the next generation analysis, determining the product design to be trained and taking precautions for the future disasters, a drought should be expected at a weak level near 2015, 2016 and 2017. Along with climate change in the near future, these droughts are expected to become a part of our everyday life by becoming a repetitive nature event in the long run.

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