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THE INFLUENCE OF THE AGRICULTURAL PRACTICES AND ENVIRONMENTAL CONDITIONS ON THE SOIL MICROBIAL COMMUNITY IN *Camelina sativa* CULTURE

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Abstract

Camelina sativa, belonging to the Brassicaceae family, is a plant with high potential to be cultivated for biomass for biofuel production, including on less fertile soils. In Moara Domneasca farm (of UASMV Bucharest-Ilfov County) different camelina cultures were conducted using 'Mădălina' new variety. The aim of the study was to evaluate de microbial community in different stages of the camelina cultivation. The analysis targeted the level of the total aerobic bacteria, fungi, actinomycetes and lactic bacteria. The cultural practices have less affected the total bacterial content during an entire culture cycle, while the addition of initial nitrogen fertilisation decreased significantly (three logarithmic units) the level of the total bacteria, including the actinomycetes. No significant inhibition of the initial fertilisation was noticed in the case of fungi and lactic bacteria.

***Key words**: Camelina sativa, soil microbiology, fertilisation, fungi, actinomycetes, lactic bacteria.*

INTRODUCTION

Nowadays, environmental conditions and agricultural practices are very important, having an important influence on the soil microbial community.

Camelina (*Camelina sativa* L. Crantz) has the popular names gold of pleasure and false flax. It is an annual member of the *Brassicaceae* family (Putnam et al., 1995; Zubr., 2003) from south-eastern Europe and south-western Asian steppe region (Dobre et al., 2014).

It is a very good source of animal feed products and human food because *Camelina sativa* contains essential fatty acids, particularly n-3 (omega-3) fatty acids (Waraich et al., 2013; Belayneh et al., 2018). The oil obtained from camelina seeds has a high content of fatty acids with 50-60% unsaturated fatty acids, 35-40% omega 3-fatty acids and 15-20% omega 6-fatty acids. The main attractive features are: drought and frost tolerance, disease and pest resistance, a considerably high seed oil content (Belayneh et al., 2015).

Romania presents a considerable risk to climate change, its effects being clearly reflected by changes in temperature and precipitation, mainly since 1961 and until now. The most affected areas, according to relevant international reports and analyzes climatological data series for the period 1901-2020 conducted by the National Meteorological Administration, being located in the southern, south-eastern and eastern part of the country. The main risks that Romania faces in the short and medium term consist in the significant increase of the average annual temperature, the decrease of precipitations and the general occurrence of extreme climatic events.

Severe pedological drought coupled with high water consumption between July and August lead to a reserve of water in the soil often below the wilting point on large agricultural areas.

Romania is characterised by a temperate continental climate and the precipitations fall in different forms. Most of the year there is liquid precipitation, especially in the hot season. In the cold season, especially in winter, the precipitations are in solid state, but there are also mixed precipitations (sleet, drizzle, etc.).

Soil biodiversity can have a major impact on the resistance and resilience of the ecosystem to climate change, which is particularly relevant for the management of future crops. Current approaches to anticipating the effects of climate change in agriculture completely neglect underground biodiversity (Lewis et al., 2018).

Different groups of microorganisms have a varying degree of resistance to drought. Thus, bacteria and fungi are more sensitive, compared to actinomycetes, the low water content of the soil favouring both vegetative development and sporulation of the latter (Wardle et al., 1999).

Under arid conditions, factors such as the availability of nutrients, organic matter, soil texture and temperature are unfavourable to the development of soil microorganisms. Among the stressors, short-term drought could be a major stressor affecting the diversity and activity of bacterial populations, due to reduced substrate diffusion in dry soils and increased needs for C and N. In soils affected by drought an important role mesophilic bacteria also play thermophilic bacteria (Wardle et al., 1999).

Humidity has a high influence on soil microorganisms, excessive water levels decreasing both the frequency and activity of microorganisms, which is due to the reduction of the possibility of oxygen supply.

Excessive water levels in the soil decrease both the frequency and the activity of microorganisms, which is due to the reduced possibility of oxygen supply. The enzymatic activities in the soil are the result of the integration of complex synthesis processes, of the persistence, stabilization and catalytic regulation as well as of their location (intracellular or extracellular) inside or on the surface of the micro-aggregates. All these processes can be dynamically influenced by changes in the physical, chemical and biological composition of the soil (Sassenrath et al., 2018).

Modern, intensive, highly productive agriculture exerts significant demands on the soil and an insufficient knowledge of how the soil reacts to such increased demands can have negative consequences, manifested by processes of degradation, even destruction of its production capacity (Hamza et al., 2005). When approaching a certain type of tillage system, the soil, plant and climate conditions that can influence or can be influenced by that system must be taken into account (Franzluelbers, 2002). The beneficial action of the tillage system on a crop factor must keep the other factors at an acceptable level, so that the increase of agricultural production, the decrease of fuel consumption or the increase of the soil production capacity can be possible through economic optimization solutions. Soil works, in addition to the unique and direct effects, beneficial in plant cultivation technologies, induce in the soil and lasting effects, which act on the physical and physical-mechanical, chemical and biological properties of the soil, modifying them (Canarache, 1991).

Climate change predicts rainfall changes with increased annual rainfall in some regions and more intense rainfall events. Higher rainfall is often correlated with increased nutrient leaching (Austin and Vitousek, 1998), which exacerbates the risk of human and environmental health problems, as highlighted by the Millennium Ecosystem Assessment.

Therefore, it is crucial to reduce nutrient losses through leaching in order to preserve the environment and protect human health. A number of recent studies suggest that soil biota, including arbuscular mycorrhizal (AM) fungi, improves the nutrient cycle in agroecosystems and reduces leaching losses (Bender & Van der Heijden, 2015; Cavagnaro et al., 2015). However, it is not known whether the fungal capacity of AM to reduce nutrient losses is maintained in different precipitation scenarios.

Good management of soil biota can mitigate the negative consequences of climate change and, in particular, of high rainfall. Previous studies have shown that mushrooms have an impact on nutrient leaching (Gounani et al., 2011).

MATERIALS AND METHODS

In order to characterize the influence of the agricultural practices and environmental conditions on the soil microbial community in *Camelina sativa* culture our interest was focused on evaluating the level of the total aerobic bacteria, fungi, actinomycetes and lactic bacteria in the plots soil. The period analysed in this study was between November 2019 and October 2020.

Location characteristics

The experiments were conduct in Moara Domnească farm, Călărași County. Moara Domnească farm belongs to Belciugatele experimental unit of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The type of soil is reddish preluvosoil, having loam-clay texture.

The extreme months from a thermal point of view are July and January. July is the warmest month of the year, with an average temperature of 24.25°C. January is the coldest month of the year, with the lowest temperature values, with an average temperature of 0.95°C.

The extreme months from a precipitation point of view are January and June. January is the poor month in precipitation of the year, with totally precipitation 1.60 mm. June is the richest month in precipitation of the year, with the highest precipitation values, with totally precipitation 131.40 mm.

The meteorological data of the location were collected by an automated meteo-station WatchDog 2900 ET and its Software Pro9, implanted in the farm.

Experimental plots and sampling

The aim of the study was to evaluate de microbial community in different stages of the camelina cultivation. For the trials was used the new camelina variety, Madalina, patented in 2018 by the UASMV Bucharest. The employed technology for camelina was the following: double culture, on an initial surface of 2108 m^2; the used tractor was U 683 DT. In june 2020, the first step was to straw grain harvester - rotary mower (1.2 m) then gathered plants with rotary rake and then voted with round ballot socket.

After that was prepare the land by milling - 1.5 m - one passage), fertilization (small 300) with ammonium nitrate - 100 kg (475 kg/ha), then sowing (SUP 21) - A1 - (3.2 kg/2108 m^2) the Mădălina variety with a seeding rate of 10-12 kg/ha.

After two weeks the soil was irrigated with U683 DT + EEP 600 20 m^3/ha because there wasn't precipitation. After another two weeks we observe that the weed begun to sprout and was applied Panther herbicide 0.5 l/ha. In September 2020 was started an autumn culture, by the use of herbicide with Glyphothim 3-4 l/ha (p = 5 atm., N = 300 l/ha).

The field was prepared as follows: destruction of vegetation with U683 DT + GD 3.4 and tilling the soil with U683 DT + PP 3X30. It was used a complex fertilizer to fertilize the soil with NP 20:20 with U683 DT + MIC-300 machine (Figure 1) in a dose of 250 kg/ha.

Figure 1. Fertilization of soil with U683 DT + MIC-300 machine

In the same day it was prepared the land with U683 DT + GD 3.4 machine and then we prepared the germination bed with U683 DT + C 3.9 machine. After that was sowed with U683 DT + SUP 21; gearbox diagram A2; B = 2.625 m the Mădălina variety (2018); G = 60%; N = 12 kg/ha. Later on, in October, a roller with U683 DT + TN 3 (Bl = 3 m) machine was used in the technology.

At different periods (before and after culture), we have collected 5 soil samples of 200 grams from different 4 corners of the field and one from the middle of it. In that 4 corners we have collected soil from one meter distance from the margins of the field. All the soil samples were collected from 20 centimetres deep, then put in sterile container and then transport in optimal conditions to the laboratory.

Physico-chemical analysis of the soil

After that the analysis of all the soil samples were conducted in the agrochemistry laboratory of UASVMB. Simultaneously, the nutrients and agrochemical indices from the culture substrates from the experimental variants were analyzed. The analyses performed were pH in aqueous extract 1:2.5 and potentiometric dosing (SR ISO 10523-2012), soluble salt content extraction in water 1:5 and conductometric dosing (SR EN 27888-1997), N, P, K extraction in distilled water 1:5 and colorimetric dosing with AFDS for nitrates, colorimetric dosing with Nessler reagent for N-NH_4^+, P-PO_4^{3-} colorimetric dosing with Duval reagent and K + flame-photometric dosing (STAS 7184/19-82).

The analysis methodology and the interpretation of the results were performed according to the existing standards and norms in our country (ICPA soil and plant analysis methodology, 1980).

Microbiological analysis

In the analysis were targeted the following microorganism groups: total aerobic bacteria, total fungi, actinomycetes and lactic acid bacteria. Specific cultivation media were used for each microbial group, respectively: Nutrient Agar, DRBC, Gausse and MRS + Cycloheximide.

After preparing decimal dilutions from the soil samples, the suspension was spread in Petri Dishes, followed by the cultivation during 24-48 hours at 27ºC. The developed colonies were counted and the CFU/ml was calculated accordingly.

RESULTS AND DISCUSSIONS

In Romania, at Moara Domnească was an extremely dry period with 580.20 mm per year, according to November 2019 - October 2020 studies. Temperature influences almost every aspect, in special the activity of enzymes, according to Figure 2.

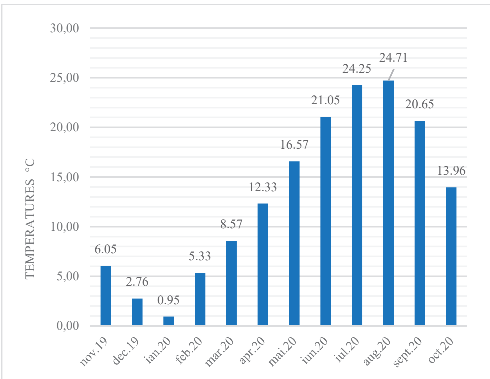

Figure 2. Average temperatures from Moara Domnească farm

Soil moisture is another abiotic factor that decisively influences soil respiration. In conditions of extreme drought, metabolic activity of microorganisms become latency states. The intensity of precipitation had a strong positive effect on the amount of nutrients lost from the soil by leaching (Figure 3). The results on climatic parameters show a year with dry rainfall and air and soil temperatures that have exceeded normal values. In most months of the year, there was a precipitation deficit. The air temperature registered, in all the months of the year, values higher than the calculated multiannual normal. Influence of temperature, humidity and pH in the soil is very important.

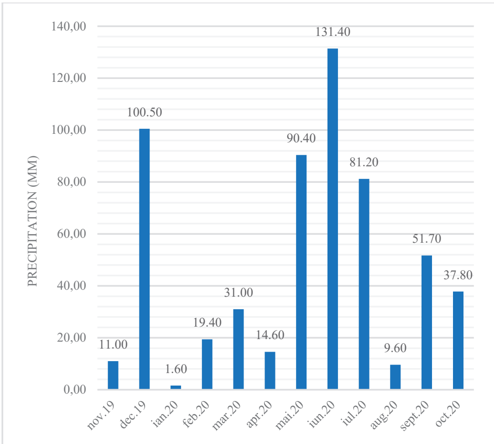

Figure 3. Monthly precipitation from Moara Domnească farm

According to Table 1, it was realized that there is a basic pH nearly to 8, a good quantity of hummus and a negligible amount of salts. The main inorganic forms of N in soils are ammonium (NH_4) and nitrate (NO_3), which are both useable by plants. Organic nitrogen may be present as a living organism, as humus, or as the intermediate products of organic matter decomposition. The level of ammonium in soil is between 4.25 and 8.50 ppm. The normal background level of nitrates in soil not fertilized or used for commercial crops ranges from 5 to 10 parts per 1 million (ppm), in our study was found the lowest value 2.25 ppm and the highest value 9.00 ppm. In conclusion, N is present in our soil in a good concentration. Agrochemical indices from the culture substrates from the experimental variants that we analysed are in a constant concentration.

Table 1. Soil analyses from Moara Domnească farm

Variant	pH	H, %	Soluble salts, %	ppm content						
				$N\text{-}NH_4$	$N\text{-}NO_3$	Σ N	P_{AL}	K_{AL}	Ca	Mg
1	7.9	1.320	0.011	4.25	2.25	6.50	1.95	69	20	11
2	7.9	1.322	0.058	6.40	4.50	10.90	2.06	70	18	12
3	7.9	1.340	0.084	4.25	9.00	13.25	2.10	70	15	12
4	7.6	1.128	0.026	8.50	9.00	17.50	2.98	56	21	10

The research aimed at determining if the *Camelina sativa* employed technology has the capacity to change soil microbial community.

According to average temperatures and monthly precipitation collected from Moara Domnească farm, the results of the studies (Figure 4) describe the total aerobic bacteria, fungi, actinomycetes and lactic bacteria during different stages of camelina culture, respectively November 2019, June 2020, September 2020 and October 2020.

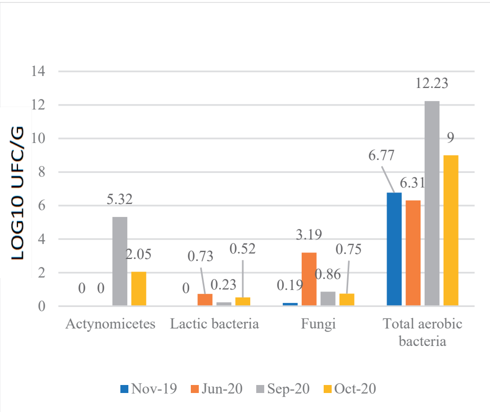

Figure 4. Microbial evolution of camelina stages in different periods

Actynomicetes level increased in September, after the first cycle of *Camelina sativa*, Mădălina to 10^5CFU/g. They play major roles in the cycling of organic matter, has major contribution for soil and environment by nitrogen fixation (Absar et al., 2017). However, after the fertilisation, one month later, their level decreased to 10^2CFU/g, due to an inhibitory mechanism which could not be explained. Lactic bacteria have less significant levels in soil after the first cycle, which was expected (10 CFU/g); however, their presence may demonstrated an increase of fertiliser solubility, very good at improving pore space in soil ventilation, improve nutrient availability from compost or other organic material.

Very important is that lactic bacteria can directly promote plant growth (Higa et al., 2004).

Fungi are in a relatively low level in soil (10^1-10^3 CFU/g) because in most of case they decompose recalcitrant organic residues high in lignin and cellulose (Boer et al., 2010).

Regarding the influence of autumn plowing on different groups of microorganisms, it is found that microscopic fungi are more numerous, with the proliferation primarily of those of the genera *Fusarium* and *Aspergillus*. Of these, the largest share is *Aspergillus niger*. In spring soils, *Aspergillus ochraceus* and *Trichoderma viridis* predominate, as well as some fungi that are antagonistic to some pathogens which is in accordance to other authors (Abdel-Azeem et al., 2016).

Total aerobic bacteria increase in September 2020 and October 2020, after the first cycle of Mădălina culture because they grow in many different microenvironments and expand rapidly, reaching a level of 10^{12} CFU/g. They could be more competitive when in soil are digestible simple sugars that are ready available in rhizosphere. Root exudates, dead plant debris, simple sugars, and complex polysaccharides are abundant is this region (Sylvia et al., 2005). During other studies in China (Hu et al., 2019) in similar conditions noticed that not exist significant inhibition of fungi and lactic bacteria.

CONCLUSIONS

The cultural practices have less affected the total bacterial content during an entire culture cycle, while the addition of initial nitrogen fertilisation decreased significantly (three logarithmic units) the level of the total bacteria, including the actinomycetes. No significant inhibition of the initial fertilisation was noticed in the case of fungi and lactic bacteria.

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