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ASSESSMENT OF THE PRODUCTIVE POTENTIAL AND ESSENTIAL OIL QUALITY OF *SALVIA SCLAREA* AFTER LEAF TREATMENT

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Abstract

In line with the needs of the Green Deal, Europa's Common Agricultural Policy follows the strategy for sustainable agriculture. The biostimulators, which are gaining increasing popularity, are an essential tool for ensuring an integrated approach of all agricultural raw materials. The aim of the present study is to investigate the effect of the biostimulators Speed$^{b®}$ and Amino Expert® Impuls, as well as of the foliar fertilizer and immunomodulator Acramet Ultra® on the elements of productivity, yield of fresh inflorescences, essential oil content and composition of Salvia sclarea. The experiment was arranged according to the randomized block method in four replications with plot size of 15 m^2. The used products affected positively the productivity, as the treatment with Speed$^{b®}$ led to highest increase in the yield of fresh flower spikes with 25% when comparing with the control. Only the treatment with both biostimulators increased the essential oil content, as the highest values were obtained when using Amino Expert® Impuls. Based on the results, the products could be recommended and successfully used depending on the production direction.

Key words*: biostimulators, essential oil composition, Salvia sclarea, yield.*

INTRODUCTION

The negative effects of global warming and continuous climate change have a detrimental effect not only on the development and yield of agricultural plants, but also on the quality of production (Saddiq et al., 2021). For decades, the misuse of chemicals aimed at higher productivity has upset the balance of the environment (Delitte et al., 2021). In natural conditions, plants are continuously exposed to stress of different nature: drought, salinization, heavy metals, extreme temperatures (Srivastava et al., 2021). Not all plants have the ability to develop their plastic responses and to adapt to the unfavourable environmental conditions (Shah et al., 2021). U.S. National Climate Assessment has estimated that environmental stress is responsible for loses in crop yield up to 50% (Ma et al., 2022). Various agrochemical approaches involving the use of synthetic fertilisers and pesticides are perceived as key agricultural inputs developed to alleviate adverse environmental impacts (Manzoor et al., 2021; Silva et al., 2022). Agrochemicals increase crop productivity, but their imbalanced application damage the environment and negatively affect the human health (Ma et al., 2022). The main challenge facing the agro sector is to find alternative approaches that preserve the environment, people, and plants. The application of biostimulators is a novel approach, which can ensure sustainable food production. One of the pillars of the climate-smart agriculture is the sustainable crop management. By means of some approaches like plant breeding, right choice of variety, ecosystem management the crop production can adapt to changes in climate. Composed of organic, inorganic compound or microorganisms the biostimulators stimulate processes in plants, which result in improved growth, enhanced productivity, and stress tolerance (Franzoni et al., 2022). According to some authors (Bulgari et al., 2017; Lau et al., 2022) the market for these compounds increases on annual base with 12% and will reach probably 5.6 billion dollars in 2026. These natural substances are non-toxic and do not accumulate in long term (Sangiorgio et al., 2020). Based on the source of raw material biostimulators are classified in six groups: plant extracts, seaweed, humic substances, protein hydrolysates, inorganic compounds, and microorganisms (Franzoni et al., 2021). Most studied and commercialized are the biostimulators composed of seaweeds (Cristiano et al., 2018). In this group are included also marine algae belonging to different taxonomic groups (Bulgari et al., 2017). Hormones, polyphenols, polysaccharides and kahydrin in the composition of the algae promote the growth processes and activate the plant defence system (Baltazar et al., 2021). Bioactive compounds extracted from different plant structures and parts can also activate physiological processes and improve the performance of the plants (Franzoni et al., 2022; Zulfiqar et al., 2020). Hydrolysed proteins include amino acids, polypeptides or oligopeptides obtained through process of hydrolyses of proteins delivered from plants or animals (Cristofano et al., 2021). Using by-products of agriculture and industry for production of hydrolysed proteins contributes to sustainable waste management and circular economy (Colla et al., 2017). Humic substances, the major component of lignite, soil, and peat can also serve as e resource for biostimulators production because of the content of humic and fluvic acid (Popa et al., 2022). The group of the biostimulators based on microorganisms is represented of bacteria, fungi, and arbuscular mycorrhizal fungi, which are isolated from soil, plants, or organic materials (Baltazar et al., 2021). These lining organisms interact with the plants and establish symbiotic relationships or improve indirectly the nutrient availability of the plants (Franzoni et al., 2021). Despite the great interest of the scientific community and the studies carried out, some aspect about biostimulants need to be further investigated, like for example their mechanism of action, especially under stress conditions (Ma et al., 2022). It is necessary to test different biostimulators by variety of crops and under various climatic conditions to be able to track their effectiveness and recommend them for industrial application (Ma et al., 2022). As some authors like Mossi et al. (2011) and Tuttolomondo et al. (2020) indicated there is no sufficient information about the agronomic performance of *Salvia sclarea*. In this connection the presents study aiming to test the effectiveness of different biostimulators on the productivity and essential oil quality of *Salvia sclarea* is very important and relevant.

MATERIALS AND METHODS

Field experiment

For the aims of the study a biennial field experiment was established in the region of the village Tuzha (42°39'0"N, 25°5'0"E) Southern Bulgaria with a clary sage (*Salvia sclarea* L.) variety Boyana. The experiment was arranged according to the randomized complete block design in four replications with a plot size of 15 m^2. Three different products were included in the study: Speed$^{b®}$, Amino Expert® Impuls and Acramet Ultra®. Their chemical composition and application rates are specified in Table 1.

Table 1. Description of the applied biostimulators and foliar fertilizer

Product name	Manufacturer	Chemical composition	Applied amount
Speed$^{b®}$	Ledra Ltd Agrochemicals	Huminic acids - 2.2%; Amino acids -2.2%; N- 6.1%; K_2O - 4.2%; B-0.65%; Cu-0.02%; Fe-0.02%; Mn-0.01%; Zn-0.002%; Mo- 0.001%; Co-0.002%	3 l/ ha
Amino Expert® Impuls	Ecofol Ltd	Organic matter – 85.5%; Amino acids - 11%; Total nitrogen – 2.9%; MgO-0.59%; SO_3-4.66 %; Zn -0.90%; Mn -0.45%; B - 0.61%; Fe -0.45%; Cu - 0.46%; Mo - 0.09%, phytohormons-0.00035%	2 l /ha
Acramet Ultra®	Plantis Ltd	N – 12.5%, P_2O_5 - 5.7 % K_2O - 11%; S-2.9%; B-0.35%; Cu- 0.025%; Mg-0.48%; Mn-0.028%; Zn-0.125%; Fe - 0.026%; Mo-0.024%; ultra trace elements – cobalt, chromium, vanadium	2% solution

The products were applied as leaf treatments twice in 15 days before flowering. The effectiveness of the products was compared to the untreated control. The structural elements of the yield were reported based on 20 randomly selected plants from each variant. Following parameters were estimated: plant height (cm), canopy spread (cm), number of leaves, number of branches, inflorescence length (cm), fresh flower yield (g/ha), essential oil content (%), essential oil yield (kg/ha), essential oil composition. The soils in the region belong to the Fluvisols and are represented by deluvial noncalcareous sediments (Todorova et al., 2020). Soil samples have been taken annually form the layer 0-30 cm in the autumn. The reaction of the soil was acid (5.21-5.67), which is characteristic for this soil type. The content of available mineral nitrogen ranged from 15.88 to 16.62 mg/kg soil. According to the available P_2O_5 the values varied between 12.67 and 13.85 mg/100 g of soil. The available K_2O was ranging from 17.30 to 18.20 mg/100 g of soil.

Weather conditions

Climate conditions during the year of the investigation are presented in Figure 1. The air temperatures in the first and the second year during the vegetation period of *Salvia sclarea* are above those established for the long-term period. The sum of the monthly average temperatures in 2023 exceeds not only the values from the previous year, but also those of the long-term period. For this reason, in 2023, the highest yields of essential oil and the highest percentage of oil in flowers are also reported. On an annual basis, there are also differences in the amount of rainfall, as well as its distribution by months. The total amount of rainfall in the first year is with 541.7 mm below the norm of the region. Although the clary sage has a pronounced dry resistance, the lack of sufficient moisture negatively affects the yield and its elements. The amount of rainfall in the second year exceeds the climatic norm by 9.8 mm. From the climatic characteristics it can be concluded that 2023 is more favorable for the development of the crop.

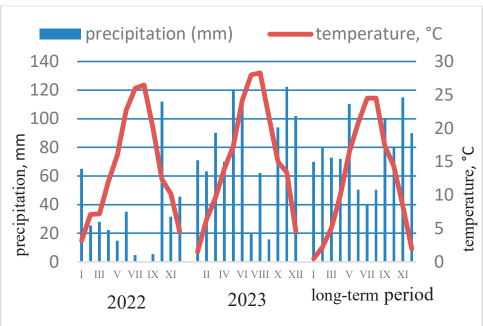

Figure 1. Climatogram during the vegetation period of *Salvia sclarea*

Essential oil extraction

Essential oils were extracted from 1 kg fresh flowers via hydrodistillation in Clavenger apparatus for 3 h and dried over anhydros sodium sulfate (Mastelic et al., 2003; Singh et al., 2008). Representative samples from every field plot were analyzed.

Gas chromatography- mass spectrometry analysis (GC-MS)

The Gas Chromatography-Mass Spectrometry Analysis of *Salvia sclarea* essential oil was performed using an Agilent 7890A equipped with an Agilent 5975C mass spectrometer detector. The electron ionization energy was 70 eV, scan range of m/z 45-550. Chromatographic separation of compounds was performed on a DB-5MS capillary column with the following parameters: film thickness of 0.25 µm, a length of 30 m, and internal diameter 0.32 mm. Helium was used as carrier gas with a flow rate of 1.0 ml/minute, split ratio 30:1. Inlet temperature was 250ºC. The GC oven temperature program was used as follows: 40ºC initial temperature, hold for 2 min., raised at 5ºC/min. to 300ºC and finally hold at 300^0C for 10 min. The identification of compounds was performed by comparing their mass spectra with data from Adams Library and US National Institute of Standards and Technology (NIST, USA).

Statistics

The statistical processing of the results was performed using the method of analysis of variance (ANOVA), and the differences between the variants were established by the multi-rank LSD test.

RESULTS AND DISCUSSIONS

The foliar application of the biostimulators and the fertilizer increased the growth parameters of the *Salvia sclarea* (Table 2). The insufficient amount of precipitation during the first year of the investigation was the reason for the overall lower presentation of the plant, expressed in lower growth, lower yield of fresh inflorescences, as well as lower yield of essential oil. By the control plants the plant height varied from 98.3 cm for the first year to 109.6 cm for the second year of the investigation. Due to the favorable climate conditions in 2023 an increase in the values up to 10% has been observed. The application of the biostimulators affected in greater extent the elements of the growth and productivity compared to the foliar fertilizer Acramet Ultra®. After the treatment with Speed$^{b®}$ the plants reached the greatest height of 108.1 cm for 2022 and 120.5 cm for 2023 year and the values exceeded the control with an average 10% for the study period. The lowest increase in the values is observed after the application of the foliar fertilizer, as the values varied between 103.2 cm and 115.1 cm respectively for the period. Because of its positive correlation with other production parameters as number of inflorescences per plant, inflorescence length and oil yields, plant height is an important feature when selecting accessions (Yassen et al., 2014). Moreover, the same author reported the positive direct effect of the plant height on the essential oil content. According to the canopy spread in the first year by the control plant were observed the lowest values of 44.5 cm. During the second year the values increased up to 14%, because of the favourable climate conditions. The highest values of the parameter of 48.5 cm for 2022 and 56.2 cm for 2023 were recorded after the treatment with the biostimulator Speed$^{b®}$. The second biostimulator increased the canopy spread on average up to 6%, and by the fertilizer Acramet Ultra® the average increase was only 3%.

The parameter number of leaves has also been positively influenced by the applied products, as on average for the period the biostimulator Speed$^{b®}$ increased the values with up to 8%, followed by Amino Expert® Impuls with an average increase of 7%. The fertilizer Acramet Ultra® exceeded the values of the control with an average 3%. The number of branches varied between 7 (2022) and 9 (2023) by the untreated control, as well as by the variant treated with the leaf fertilizer. Because of the application of the biostimulators the number of branches increased to maximal 13 for the second year of the study. The inflorescence length is an important characteristic determining *Salvia sclarea* flowers yield. According to Tuttolomondo et al. (2020) the inflorescence length could successfully be used as a characteristic when selecting biotypes with high content of essential oil. The same authors reported that in years with lower precipitation are produced longer inflorescences with also higher essential oil content, which is not in line with the results from the present study.

Table 2. Effect of the biostimulators and foliar fertilizer on *Salvia sclarea* growth parameters

Growth parameter	Treatment	Values for 2022	Values for 2023	Average for the period
Plant height (cm)	Control	98.3^{a}	109.6^{a}	103.95^{a}
	Speed$^{b®}$	108.1^{c}	120.5^{b}	114.3^{bc}
	Amino Expert® Impuls	106.2^{b}	118.3^{b}	112.25^{b}
	Acramet Ultra®	103.2^{b}	115.1^{b}	109.15^{b}
	LCD 5%	*4.9*	*5.5*	*5.2*
Canopy spread (cm)	Control	44.5^{a}	50.7^{a}	47.6^{a}
	Speed$^{b®}$	48.5^{d}	56.2^{d}	52.35^{d}
	Amino Expert® Impuls	47.1^{c}	53.7^{c}	50.4^{c}
	Acramet Ultra®	45.8^{b}	52.2^{b}	49.0^{b}
	LCD 5%	*1.3*	*1.5*	*1.4*
Nr. of leaves	Control	31^{a}	38^{a}	34.5^{a}
	Speed$^{b®}$	33^{c}	41^{d}	37.0^{cd}
	Amino Expert® Impuls	32^{b}	40^{c}	36.0^{bc}
	Acramet Ultra®	32^{b}	39^{b}	35.5^{b}
	LCD 5%	*0.89*	*0.89*	*0.89*
Nr. of branches	Control	7^{a}	9^{a}	8^{a}
	Speed$^{b®}$	9^{b}	13^{c}	11^{b}
	Amino Expert® Impuls	9^{b}	12^{bc}	10.5^{b}
	Acramet Ultra®	7^{a}	9^{a}	8^{a}
	LSD 5%	*1.6*	*1.8*	*1.7*

[a] Means within columns followed by different lowercase letters are significantly different (P<0.05)

The highest values were determined in the year with the higher amount of precipitation when the average temperature values were also higher (Table 3). This parameter is influenced not only by the different climatic conditions during the years of the investigation, but also by the applied products. The lowest values of that indicator were observed in 2022 by the untreated control (55.3 cm), when the climatic conditions for the growth and development of *Salvia sclarea* were less favorable. All the treated variants exceeded the control by 7% to

11%. The largest inflorescence length was reported when applying Speed^{b}® – 61.4 cm, followed by the variant treated with Amino Expert® Impuls – 60.2 cm and the smallest values of 59.1 cm were recorder after the treatment with Acramet Ultra®. The results obtained are statistically significant. In the second experimental year the inflorescence length varied from 60.2 cm for the untreated control to 66.8 cm for the variant treated with Speed^{b}®. The percentage increase in the indicator because of more favourable conditions is within 9%. In the first experimental year (2022) the yield of fresh flowers in the treated variants varied from 7.0 t/ha to 7.8 t/ha versus 6.2 t/ha for the control. During the second year an average increase in the productivity of the *Salvia sclarea* was observed, because of the better environmental conditions. The yield of fresh flowers varied from 7.7 t/ha for the control to 10.8 t/ha for the variant treated with Speed^{b}®. On average for the period this product increased the productivity of the clary sage with 25%. The application of Acramet Ultra® and Amino Expert® Impuls exceeded the control with 11-20% respectively.

Table 3. Effect of the biostimulators and foliar fertilizer on *Salvia sclarea* productivity

Parameter	Treatment	Values for 2022	Values for 2023	Average for the period
Inflorescence length (cm)	Control	55.3^{a}	60.2^{a}	57.75^{a}
	Speed^{b}®	61.4^{c}	66.8^{c}	64.1^{cd}
	Amino Expert® Impuls	60.2^{b}	65.6^{c}	62.9^{c}
	Acramet Ultra®	59.1^{b}	64.4^{b}	61.75^{b}
	LCD 5%	*1.8*	*2.7*	*2.25*
Fresh flower spike yield (t /ha)	Control	6.2^{a}	8.7^{a}	7.45^{a}
	Speed^{b}®	7.8^{c}	10.8^{c}	9.3^{c}
	Amino Expert® Impuls	7.5^{b}	10.4^{c}	8.95^{b}
	Acramet Ultra®	7.0^{b}	9.5^{b}	8.25^{b}
	LCD 5%	*0.78*	*0.80*	*0.79*
Essential oil yield (kg/ha)	Control	7.2^{a}	8^{a}	7.65^{a}
	Speed^{b}®	8.1^{b}	8.8^{b}	8.45^{b}
	Amino Expert® Impuls	8.4^{b}	9.2^{b}	8.8^{b}
	Acramet Ultra®	7.5^{a}	8.1^{a}	7.8^{a}
	LSD 5 %	*0.81*	*0.75*	*0.78*

[a]Means within columns followed by different lowercase letters are significantly different (P<0.05)

Tuttolomondo et al. (2020) reported that the floral spike yield was greater in the year with more precipitation, while the content of essential oil in percentage term was higher during the year with less precipitation. In the present study the second year combined sufficient precipitation with higher average temperatures, and this is the reason for the better productivity of the clary sage not only in term of fresh flowers, but also in term of essential oil yield.

The essential oil yield by the untreated control varied between 7. 2 and 8 kg/ha or the first and the second year respectively. The applied foliar fertilizer has no influence on the parameter and there is no statistically significant difference between both variants. The treatment with Speed^{b}® increased the yield of essential oil with on average 10% for the study period. The highest yield of essential oil (9.2 kg/ha) was achieved in 2023 after the application of Amino Expert® Impuls, which compared to the control is an increase of 15%. The content of essential oil was also affected by the conditions of the year, as well as by the applied products (Figure 2). The lowest values were recorded in 2022 ranging from 0.24% to 0.31%. The application of the foliar fertilizer had no statistically significant influence on the parameter. For the tested period the product Amino Expert® Impuls resulted in the highest amount of essential oil in the fresh flowers of 0.31% for the first and 0.37% for the second year of the experiment. The percentage of essential oil reported from Tuttolomondo et al. (2020) was higher (0.58-1.8%).

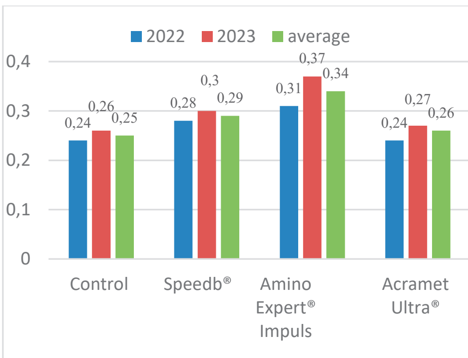

Figure 2. Essential oil content of *Salvia sclarea* (%)

Other authors also estimated greater content of essential oil ranging between 0.31-0.65% (Pesic et al., 2003) and 0.78-0.83% (Rajabi et al., 2014). The path coefficient analysis for oil content performed by Yaseen et al. (2015) found that the plant height has a positive direct effect on the oil content.

Table 4. Chemical composition of *Salvia sclarea* essential oil through GC-MS

Compound	RT (min)[a]	%[b]			
		Control	Speed[b®]	Amino Expert® Impuls	Acramet Ultra®
β- Myrcene	997	1.55	1.64	1.60	1.52
Limonene	1032	0.62	0.65	0.58	0.55
Trans-β-Ocimene	1051	1.12	1.22	1.17	1.18
Linalool	1105	**23.02**	**24.70**	**28.30**	**23.20**
Linalyl acetate	1135	**52.41**	**50.22**	**55.70**	**53.01**
α-Terpineol	1185	**2.08**	**1.95**	**2.11**	**1.98**
Nerol	1235	0.90	0.98	1.1	0.92
Neryl acetate	1360	1.69	1.77	1.78	1.72
Geranyl acetate	1387	**2.62**	**2.58**	**2.66**	**2.64**
Geraniol	1402	0.45	0.52	0.50	0.41
β-caryophyllene	1426	**2.56**	**2.54**	**2.63**	**2.58**
Sclareol oxide	1480	0.10	0.11	0.10	0.12

[a]Retention time.
[b]Relative proportions of the essential oil constituents.

The performed GC-MS analysis identified 12 essential oil constituents (Table 4). The main compounds are linalyl acetate, linalool, geranyl acetate, β-caryophyllene and α-Terpineol representing more than 80% from the total essential oil content. The values of the linalyl acetate, the main compound were ranging from 50.22% for the variant treated with Speed[b®] to 55.70% for the variant treated with Amino Expert® Impuls. According to the linalool content the values varied between 23.02% and 28.30% depending on the treatment. Many species from the fam. *Lamiaceae* demonstrate chemical variability in the essential oils due to exogenous or endogenous factors (La Bella et al., 2021; Virga et al., 2020). The higher content of linalyl acetate and linalool, which are the main volatile terpenoids present in the *Salvia sclarea* essential oil, also determine its good quality as a raw material for the cosmetic industry (Yuce et al., 2014; Saeidnia et al., 2014). The main constituents of the essential oil of *Salvia sclarea* according Rajabi et al. (2014) are linalool (12.17-21.41%), linalyl acetate (13.06-52.61%) and germacrene D (up to 17.69%). The last compound was not detected in the present investigation. The values of linalool and linalyl acetate approximate those reported by Dzamic et al. (2009), where the value of linalyl acetate was 52.83% and those of linalool 18.18%. The application of the products led to a slight variation in the chemical composition of the essential oil. It is worth noting that as a result of the application of the Amino Expert® Impuls the amount of linalyl acetate increased by 3.29% and those of linalool by 5.28% compared to the control variant.

CONCLUSIONS

The products used have a positive effect on the parameters studied and have been shown to increase the productivity of the crop. As a result of the application of biostimulators, a higher percentage of essential oil in fresh inflorescences is reported, as well as increased yield of essential oil. Based on the observations made, the product Speed[b®] can be recommended for higher productivity of fresh inflorescences, and the biostimulator Amino Expert® Impuls for higher quality and yield of essential oil.

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