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Research Article

The effect of the use of biostimulant on the yield and quality of lavandin and lavender

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Abstract

Lavandula species are one of the most popular aromatic plants in the world and have a high content of high-quality essential oil (EO). Although there are many species in this genus, only lavandin (*Lavandula intermedia* Emeric ex Loisel.) and lavender (*L. angustifolia* Mill.) are highly valued worldwide. The quality and yield of lavandin and lavender depend on genetic factors, environmental conditions and cultivation methods. Therefore, the aim of this paper is to research the effects of the application of biostimulant on the inflorescence yield and the quality of lavandin and lavender. The treatments used in this research consisted of a combination of different species (lavandin and lavender) and biostimulant (applied and unapplied). The research results show that all the research traits significantly depended on the used species and the applied biostimulant. The inflorescence yield, the content of total flavonoids, and the content of EO were higher in the lavandin species (477.3 g plant^{-1}, 17.21 mg CAE g^{-1}, 8.57 mL 100 g^{-1}, respectively) than in the lavender species (180.5 g plant^{-1}, 13.41 mg CAE g^{-1}, and 3.69 mL 100 g^{-1}, respectively). EOs of lavandin and lavender were rich in linalool and linalyl acetate. The use of biostimulators had a positive effect on the inflorescence yield and the content of essential oil. Furthermore, the applied biostimulant increased the linalool content in the essential oil of both researched species, i.e. it positively affected its quality.

Keywords: antioxidant capacity; biostimulant; essential oil; *Lavandula*; linalool

Introduction

Lavandula species are attractive plants because of their healing and fragrant properties. Although there are 39 species in *Lavandula* genus, only three have a high commercial value. These species are lavandin (*Lavandula intermedia* Emeric ex Loisel.), lavender (*Lavandula angustifolia* Mill.), and spike lavender (*Lavandula latifolia* Medik.) (Sönmez *et al*., 2018). Often, all three of these species have the common name lavender. Cultivation of lavender as aromatic and medicinal plants has increased significantly recently (Gavrić

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et al., 2023). Currently, lavender is mainly grown in France, Spain, Bulgaria, Italy, Turkey, Croatia, Greece, South Africa, Kashmir, and small regions in North Africa (Vijulie *et al*., 2022). The primary purpose of growing lavender is to obtain essential oil (EO). This EO is highly valued in cosmetics, food preservation, agro-industry and traditional medicine etc (Mambrí *et al*., 2018; Wells *et al*., 2018; Białoń *et al*., 2019). The content of various constituents greatly influences the quality of the EO. Lavender EO can contain more than 100 constituents, most of which are terpene compounds (Sałata *et al*., 2020). Previous research has shown that the following constituents have the largest participation in the EO: linalyl acetate, linalool, and γ-cadinene, *p*-cymen-8-ol, borneol, lavandulol, *o*-cymene, bornyl acetate, (E)-caryophyllene, eucalyptol, camphor (Mambrí *et al*., 2018; Białoń *et al*., 2019; Sałata *et al*., 2020; Minji *et al*., 2022). However, the levels of these constituents show significant variations depending on species and environmental conditions (Wells *et al*., 2018; Białoń *et al*., 2019). Lavender EO typically consists of 17.8-50.0% linalool, 10.1-54.0% linalyl acetate, and a small amount of camphor. On the other hand, lavandin EO contains approximately 22.5-35.5% linalool, 23.6-35.4% linalyl acetate and 6.3-12.2% camphor (Lesage-Meessen *et al*., 2015). Furthermore, EO composition has been found to depend on the environmental conditions in the growing region. For example, the concentration of linalool, the primary constituent of lavender oil, increases when the average daily temperature increases but decreases during the rainy season (Hassiotis *et al*., 2014).

On the other hand, different agricultural practices, such as fertilisation, tillage, planting date, mulching, irrigation, etc., affect the change in environmental conditions, which can indirectly lead to changes in the quality and yield (Rakic and Johnson, 2008; Svecova and Neugebauerová, 2010; Al-Huqail *et al*., 2020; Gavrić *et al*., 2022). One of the newer agronomic practices in plant production is biostimulant' application (Martínez-Gutiérrez *et al*., 2022). Biostimulants are substances applied on plants to increase nutrition efficiency, resist environmental conditions and improve product quality (Mackiewicz-Walec and Olszewska, 2023). The composition of biostimulants is very different. They usually contain humic acids, hormones, vitamins, and extracts of bacteria and algae that stimulate plant growth (Du Jardin, 2015; Martínez-Gutiérrez *et al*., 2022).

In recent years, various research studies have been conducted on the influence of biostimulants on the growth and development of multiple crops. However, most of these studies have focused on horticultural and field crops, while a limited number of research studies have been conducted on aromatic plants. In the context of all the above, we hypothesise that applying biostimulant can increase the yield and quality of lavandin and lavender. Therefore, this work aims to study the impact of biostimulant application on yield inflorescence, phytochemicals content, antioxidant activity and constituent content in the EOs lavandin and lavender.

Materials and Methods

Research location

A field experiment was conducted at Butmir, Bosnia and Herzegovina (B&H). The location coordinates are 43°49'34.42" N, 18°19'18.48" E, and the elevation is 500 meters above sea level. An analysis of the fertility of the soil from the research area was done before setting up the experiment. Soil samples were taken from the upper horizon (up to 20 cm deep) before the field experiment was set up. The collected samples were analysed to determine soil pH (ISO 10390, 1994), organic matter content (ISO 14235, 1998), and P_2O_5 and K_2O content (Egnér *et al*., 1960). Based on the analysis, the soil had the following chemical properties: pH of 7.0, 4.3% organic matter, P_2O_5 = 29.0 mg 100 g^{-1}, K_2O = 31.0 mg 100 g^{-1}. The soil was classified as an alluvial type.

Weather conditions

The weather station was positioned 120 meters away from the experiment. Data on monthly average air temperature and precipitation amounts were collected in the research. According to the data presented in Figure 1, average monthly air temperatures during the field research varied greatly. The lowest average monthly temperature was recorded in February 2023 (0.9 °C) and the highest in July (22.8 °C). The recorded amounts of precipitation during the research period were relatively high. Specifically, the least rain fell in February and October 2023 (43 mm each). On the other hand, high amounts of precipitation were recorded in January (149 mm), May (191 mm) and November (258 mm).

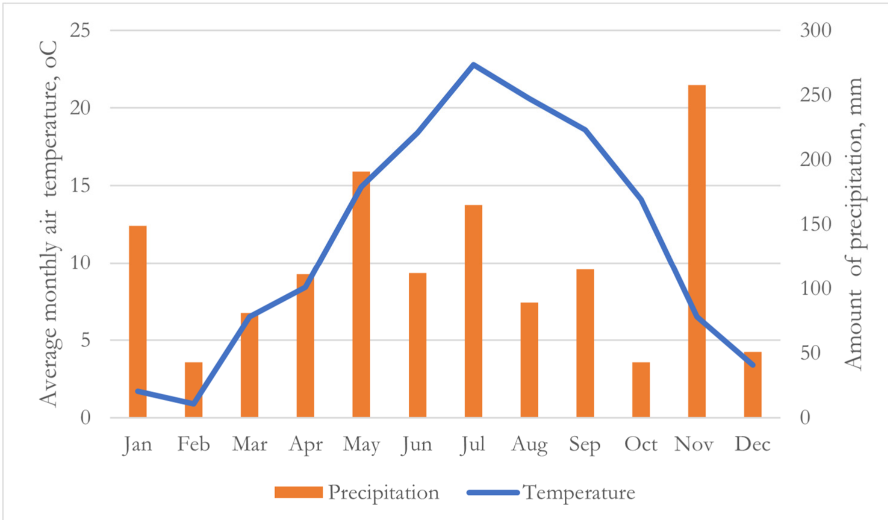

Figure 1. Average monthly temperature and monthly rainfall in 2023

Experimental design

This research's experimental design was a complete split-plot design. The treatments used in this research consisted of a combination of different species (lavender and lavendin cv. 'Grosso') and biostimulant (applied and unapplied). Biostimulant (Poly-Amin, Haifa) was foliarly used three times (07, 14 and 21 April 2023) in the recommended concentration (1%).

Materials for analysis

Inflorescence samples were collected to evaluate yield, bioactive component content and EO isolation. The samples were collected during the flowering phase (July 06, 2023). The samples were dried under low light conditions for 30 days at room temperature. The samples were weighed, and the inflorescence yield per plant was recorded after drying. The total phenolic content, flavonoid content, antioxidant activity, and EO contents were determined in inflorescence samples.

Extraction for antioxidant activity determination

The plant water-alcohol extract was prepared as follows: 1 g of dried and ground lavender flowers (size of 1 mm) was added to the flask. Then, 100 mL of 60% ethanol was added, homogenised and mixed gently. After 24 hours, the mixture was homogenised and filtered through quantitative filter paper (blue ribbon) (Figure 2). The obtained extracts were used to analyse the total phenolics, flavonoid and antioxidant activity assay.

Determination of total phenolic content

The total phenolic content of the ethanol extracts was determined by using the Folin–Ciocalteu reagent, according to the procedure described by Gavrić *et al.* (2023). A total of 0.5 mL of extract, blank, or standard was mixed with 0.25 mL of the Folin–Ciocalteu reagent. The mixture was shaken before adding 0.75 mL of 20% Na_2CO_3, and then the flask volume was filled to 5 mL with distilled water. After incubation in the dark for 120 min, the absorbance of extracts at 765 nm was measured using UV-VIS spectrophotometer (Ultrospec

2100 pro). The total phenolic content was expressed as mg of gallic acid equivalents per gram of dry weight through a calibration curve prepared with gallic acid (mg GAE 100 g^{-1}).

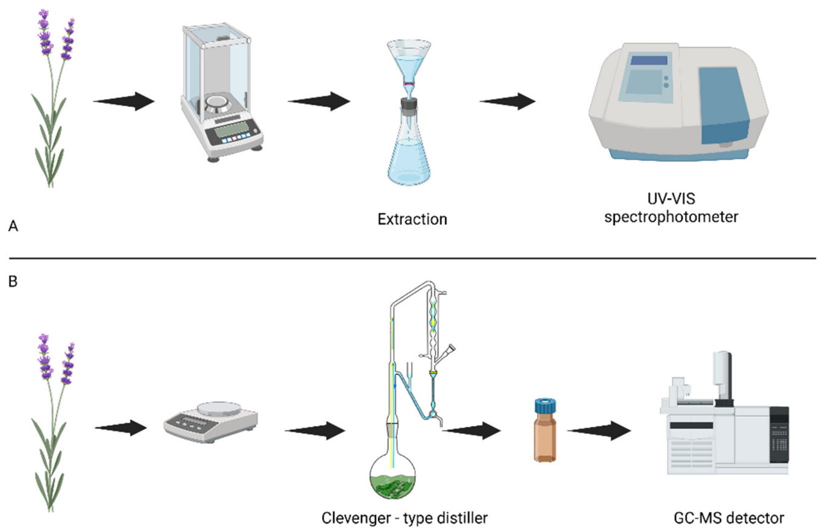

Figure 2. A: Analysis of total phenolics, flavonoids, and antioxidant activity; B: Isolation and determination of EO constituents. Figure created on BioRender.com

Determination of total flavonoid content

The content of flavonoids was determined by the spectrophotometric method based on the coloured reaction of flavonoids with aluminium chloride ($AlCl_3$) (Gavrić *et al.*, 2018). Briefly, 0.5 mL of ethanolic extract, blank or standard, was added to a 5 mL volumetric flask containing 2 mL of distilled water. After that, the flask was then filled with 0.15 mL of 5% $NaNO_2$. After 6 min, 0.15 mL of 10% $AlCl_3$ and 1 mL of 1 M NaOH were added. The flask was filled up to 5 mL with H_2O. After mixing the solution, the absorbance at 510 nm was determined. The total flavonoid content was determined using the calibration curve generated using (+)-catechin as a standard.

Antioxidant activity assay

The total antioxidant capacity of the extract was measured using the FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (Benzie and Strain, 1996). FRAP reagent was prepared by mixing 0.3 M acetate buffer, 20 mM $FeCl_3$, and 10 mM TPTZ in the ratio 10:1:1. After that, 240 μL of H_2O, 80 μL of extract, blank or standard, and 2.080 μL of FRAP reagent were mixed in a flask and incubated for 5 minutes at 37 °C. After incubation, the absorbance was measured at 595 nm. The total antioxidant capacity was evaluated by creating a calibration curve using an aqueous solution of $FeSO_4$ as the standard.

Isolation and determination of essential oil (EO) composition

Isolation and quantification of EOs from lavender flower samples were performed with a Clevenger-type apparatus (Clevenger, 1928). Briefly, samples of dried inflorescence were weighed (20 g) and then transferred to a flask with a volume of 1000 mL. After that, 500 mL of distilled water was added to the flask, and the distillation process was started. The samples were distilled for 120 minutes. Then, the volume of EO is quantified. After that, the EOs were dehydrated using anhydrous sodium sulfate, transferred into dark vials (2 mL), and stored in a refrigerator (4 °C) until the constituents' content was determined.

Gas Chromatography/Mass Spectrometry Analysis

The EO' constituents were measured using a gas chromatograph with an autosampler (Agilent Technologies, 7820 A) that had a capillary column (Agilent HP5-ms ultra-inert, 30 m × 320 μm × 0.25 μm) connected to an Agilent Technologies Mass Selective Detector (MSD-5977E). The temperature program employed was as follows: starting at 60 °C with a 1-minute hold, the temperature was increased at 3 °C per minute until reaching 246 °C, with no hold time. Subsequently, the temperature was further increased at 10 °C per minute until reaching 280 °C. The injection was performed using a volume of 1 μL in splitless injection mode at a temperature of 220 °C and a pressure of 58.328 kPa. The carrier gas utilised was helium, with a purity of 99.9995%.

Statistical methods

Data were analysed using the SPSS 22.0 software program (IBM, Armonk, New York, USA). Statistical analyses of the data between treatments were performed by analysis of variance (ANOVA test) with a significance level of 5% ($P<0.05$). All analysed results are shown as mean.

Results

The research recorded that all the examined traits significantly depended on the used species and the applied biostimulator (Table 1). The yield per plant, the content of total flavonoids, and the content of EOs were higher in the lavandin species (477.3 g plant^{-1}, 17.21 mg CAE g^{-1}, 8.57 mL 100 g^{-1}, respectively) than in the lavender species (180.5 g plant^{-1}, 13.41 mg CAE g^{-1}, and 3.69 mL 100 g^{-1}, respectively). In contrast, the lavender species (134.79 mg GAE g^{-1}) had a higher total phenolic content than lavender (118.67 mg GAE g^{-1}). On the other hand, applying biostimulant had different effects on lavandin and lavender. For example, in the lavandin species, the yield, content of total phenolics, flavonoids and antioxidant capacity (553.0 g plant^{-1}, 118.8 mg GAE g^{-1}, 71.0 μM Fe^{2+} g^{-1}, respectively) were higher with biostimulant application compared to the variant without biostimulant application (401.6 g plant^{-1}, 118.5 mg GAE g^{-1}, 70.67 μM Fe^{2+} g^{-1}, respectively). In lavender species, the highest content of total phenolics, flavonoids and antioxidant capacity was recorded in the treatment without biostimulant (149.6 mg GAE g^{-1}, 19.3 mg CAE g^{-1}, 101.0 μM Fe^{2+} g^{-1}, respectively). This species recorded higher yield in the treatment with biostimulant (205.6 g plant^{-1}) than without biostimulant (155.4 g plant^{-1}).

Table 1. Effect of species and biostimulant on total phenolic, flavonoid, and antioxidant capacity

Species	Biostimulant	Yield	Total phenolic	Total flavonoids	Antioxidant capacity	EO contents
		g plant^{-1}	mg GAE g^{-1}	mg CAE g^{-1}	μM Fe^{2+} g^{-1}	mL 100 g^{-1}
Lavandin	Applied	553.0a	118.82a	16.47b	71.07a	8.57
	Unapplied	401.6b	118.52b	17.95a	70.67b	7.14
	Average	477.3A	118.67B	17.21A	70.87ns	7.85
Lavender	Applied	205.6a	119.91b	7.51b	49.66b	4.60
	Unapplied	155.4b	149.67a	19.31a	101.09a	2.79
	Average	180.5B	134.79A	13.41B	75.37ns	3.69
Average		**328.9**	**126.73**	**15.31**	**73.12**	**5.77**

Different small letters (a, b and c) indicate significant differences between different biostimulant treatments at the 0.05 level; Different capital letters (A and B) indicate significant differences between lavender species at the 0.05 level; ns: nonsignificant differences. GAE is the equivalent of gallic acid, and CAE is the equivalent of cathetin acid.

Based on the data presented in Table 2, it can be seen that there is a positive correlation between some research traits. Data show a strong correlation between total phenolic content and antioxidant capacity (0.849) and total flavonoid content and antioxidant capacity (0.790). Furthermore, a negative correlation was recorded between the yield and the content of total phenolics (-0.646).

Table 2. The correlation between yield, total phenolics content, total flavonoids content and antioxidant capacity

Correlated traits	Yield	Total phenolics content	Total flavonoids content	Antioxidant capacity
Yield	1			
Total phenolics content	-0.646*	1		
Total flavonoids	0.224	0.431	1	
Antioxidant capacity	-0.242	0.849**	0.790**	1

*. Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

The data from Table 3 show that 38 different constituents were isolated in lavandin and lavender EO, depending on the biostimulant application. Furthermore, it is important to note that lavandin and lavender EOs differed. In the EO of lavandin main isolated constituents were linalool (22.68-25.86%), linalyl acetate (21.06-23.48%), (+)-2-bornanone (9.29-9.68%), eucalyptol (8.26-9.08%), lavandulol acetate (4.46-5.32%) and other constituents in a smaller amount (Figure 3). The main constituents in the EO of lavender were linalool (38.03-47.03%), linalyl acetate (17.90-23.04%), lavandulol acetate (8.69-10.14%), lavandulol (3.53-5.44%), *β*-caryophyllene (2.32-4.29%) and other compounds (Figure 4). On the other hand, minor differences in the content of constituents were due to the application of biostimulant.

Table 3. The content of constituents in lavandin and lavender EOs depending on the application of biostimulant

No	Constituents name	Lavandin EO, % (VV)		Lavander EO, % (VV)	
		Applied	Unapplied	Applied	Unapplied
1	*α*-pinene	0.47	0.73		
2	camphene		0.25		
3	β-pinene	0.58	0.80		
4	1-octen-3-ol			0.86	0.47
5	*n*-hexyl acetate			0.94	
6	*β*-myrcene	1.36	1.56	0.69	0.74
7	eucalyptol	8.26	9.08		1.78
8	trans-β-ocimene			0.78	
9	*β*-ocimene	0.38	0.45		
10	*cis*-sabinene hydrate	0.31	0.35		
11	*trans*-linalool oxide				1.02
12	*cis*-linalool oxide	0.30	0.30		0.52
13	terpinolene		0.24		
14	linalool	25.86	22.68	47.03	38.03
15	(-)-camphor				0.62
16	*1*-octen-3-ol acetate			1.31	1.85
17	(+)-2-bornanone	9.68	9.29		
18	endoborneol	3.83	3.49		1.66
19	lavandulol			5.44	3.53
20	(-)-4-terpineol	2.42	2.55	1.68	6.19
21	crypton			0.74	0.40
22	*α*-terpineol	4.08	3.95	2.90	1.93
23	*cis*-geraniol	0.54	0.54		
24	cuminaldehyde			0.50	
25	linalyl acetate	21.06	23.48	17.90	23.04
26	lavandulol acetate	5.32	4.46	10.14	8.69
27	neryl acetate	1.46	1.38	0.88	0.86
28	geranyl acetate	2.77	2.63		1.65
29	*β*-caryophyllene	1.35	1.61	4.29	2.32

30	*β*-trans-farnsene	0.96	1.13		1.14
31	germacrene D		0.45	1.64	
32	*β*-bisabolene	0.23	0.29		
33	lavandulyl isovalerate	0.70	0.59		
34	*γ*-cadinene	0.50	0.50		
35	*β*-sesquiphellandrene		0.26		
36	caryophyllene oxide	0.83	0.62	2.28	3.59
37	tau-cadinol	2.40	2.24		
38	*α*-bisabolol	4.40	4.13		
	Σ (%)	100.00	100.00	100.00	100.00

Below are sample chromatograms of lavandin and lavender essential oils, featuring major peaks and the corresponding compounds identified using mass spectrometry (MS) libraries.

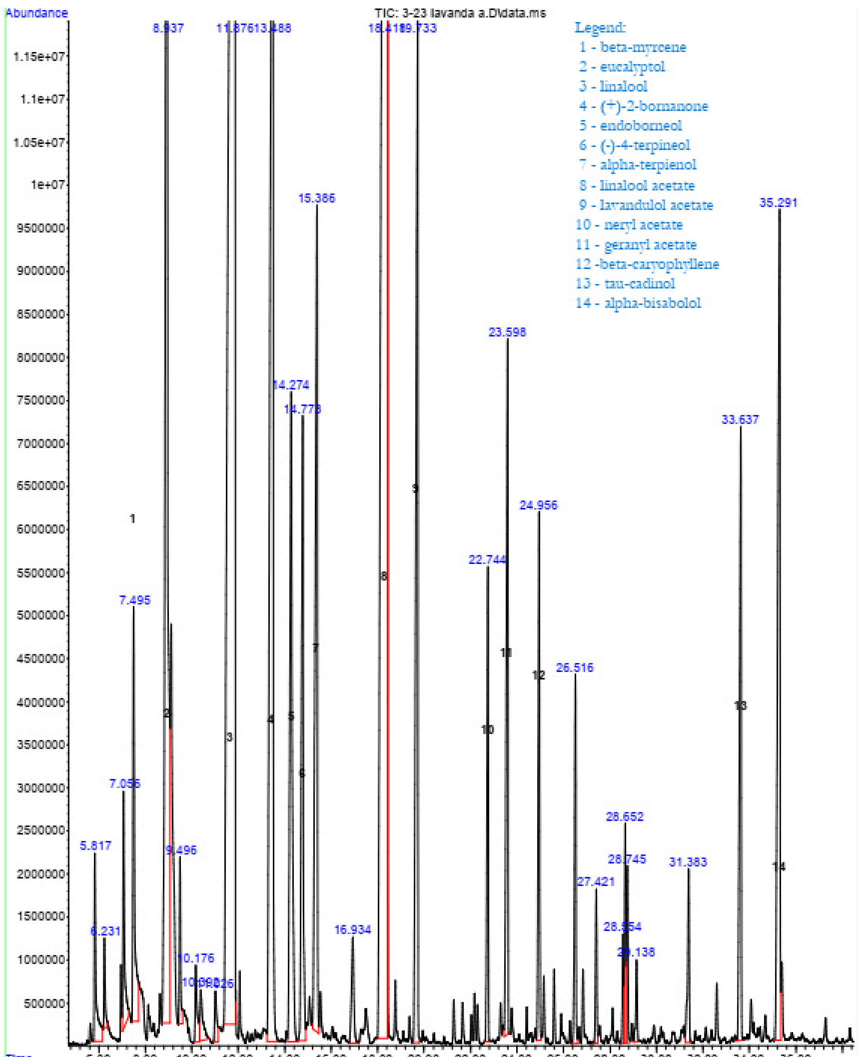

Figure 3. Chromatogram of lavandin essential oil

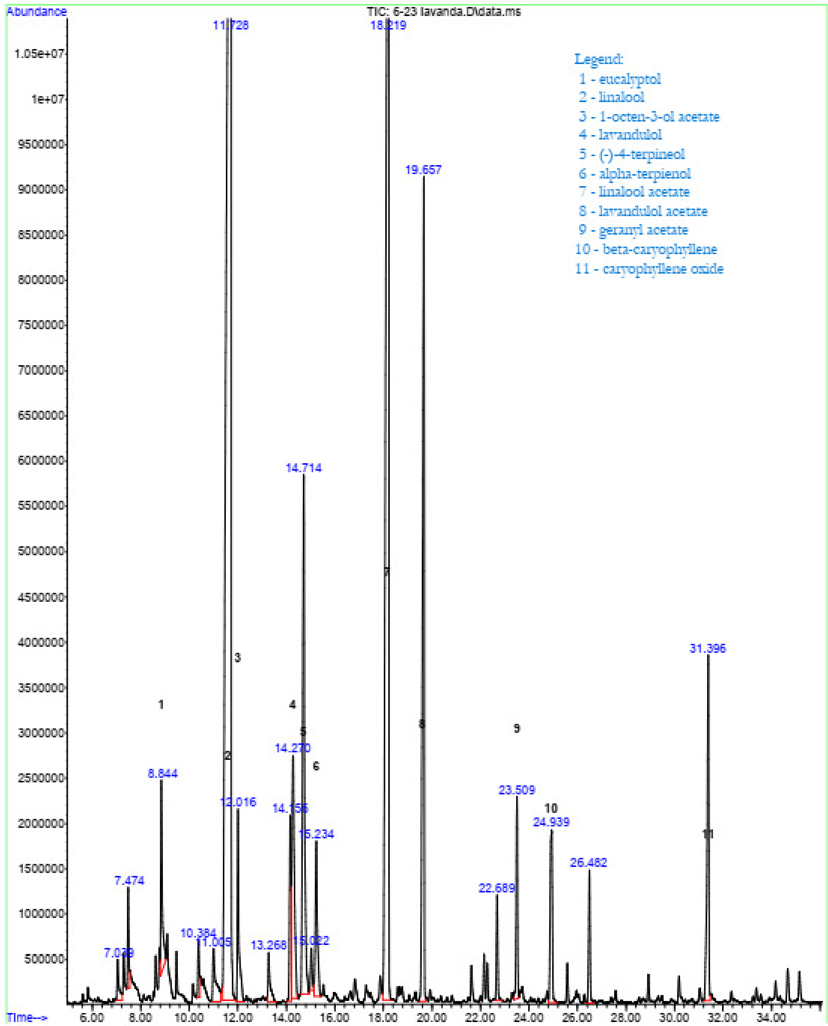

Figure 4. Chromatogram of lavender essential oil

Discussion

Lavandin and lavender are aromatic plant species whose productivity and EO quality depend on various factors. The quality of EO aromatic plants, including these species, is primarily determined by genetic factors, environmental conditions and the type of agronomic practices (Nurzyńska-Wierdak *et al.*, 2013; Tsasi *et al.*, 2017; Sharma *et al.*, 2019; Tsusaka *et al.*, 2019; Copolovici *et al.*, 2021; Gavrić *et al.*, 2023; Solouki *et al.*, 2023). The results of the experiment showed that species and biostimulant application significantly affect the research traits. Specifically, the lavandin species showed superior inflorescence yield per plant yield, total flavonoid content and EO yield compared to the lavender species. In contrast, the lavender species showed a higher total phenolic content and antioxidant activity. Thus, this research has determined that all examined traits depended significantly on the species used. The observed differences between lavender and lavandin are probably the result of genetic variations in the plants (Gavrić *et al.*, 2023). The findings of this research are consistent with those of Bajalan *et al.* (2016), who studied 30 different lavender cultivars. Their research showed that flavonoid content ranged from 28.19 to 71.62 mg CAE g^{-1} dry matter.

On the other hand, this research showed that the inflorescence yield depends significantly on applying biostimulant. In particular, the yield of both species was significantly higher when using a biostimulant compared to the control. A similar observation was presented by other researchers (Giannoulis *et al*., 2020; Truzzi *et al*., 2021; Caccialupi *et al*., 2022; Yordanova *et al*., 2022) who studies about the influence of different types of biostimulants on the yield and quality of lavandin. They found that biostimulants application increases the yield of fresh and dry inflorescence by 22 - 47%. These results can be explained by the plants receiving additional nutrients for growth from the applied biostimulant. Namely, biostimulants contain various compounds, such as micronutrients, humic acids, hormones, and vitamins, that stimulate plant growth (Du Jardin, 2015; Martínez-Gutiérrez *et al*., 2022) and increase nutrition efficiency and resistance to adverse environmental conditions (Martínez-Gutiérrez *et al*., 2022).

In this research, biostimulant application had a different effect on the phytochemicals content and antioxidant activity of lavandin and lavender. More specifically, lavandin had a significantly higher phenolic content and antioxidant capacity when a biostimulant was applied, while it was the opposite for lavender. Therefore, the species reacted differently to the application of biostimulant. These variations can be attributed to the fact that the total content of phenolics and other bioactive compounds is determined by many genes and their interactions (Czyczyło-Mysza *et al*., 2019; Taheri *et al*., 2023). Furthermore, the phenolic content and antioxidant capacity are strongly influenced by abiotic factors, such as cultivation location, altitude, weather conditions, UV radiation, soil fertility, etc. (Gavrić *et al*., 2023). However, in this research, a positive correlation was recorded between the total content of phenolics and antioxidant capacity and the total content of flavonoids and antioxidant capacity, which is consistent with earlier research (Dobrinas *et al*., 2021; Muflihah *et al*., 2021)

The EO content and its composition are the most important indicators of the quality of aromatic plants, including lavender. These research results suggested that lavandin had a significantly higher EO content than lavender. According to Mkaddem Mounira *et al*. (2022) variations in EO content can result from genetic factors, stage of development, environmental conditions, cultivation practices, harvesting methods, methods of drying, extraction, and analysis type. In this experiment, all factors influencing the oil content were uniform except for the species and application of biostimulant. Therefore, the resulting differences can be attributed to these factors. The findings of this research support the examination of different authors. For example, Renaud *et al*. (2001) found that the EO content varies between 7.1-9.9% in lavandin and 2.8-5.0% in lavender. On the other hand, Giannoulis *et al*. (2020) believe that biostimulants can increase EO production by up to 20% compared to the control.

Another important property that determines the quality of the EO is the content of constituents. This research determined that linalool is the main constituent in both EOs. By comparing this ingredient among species, it can be said that the highest proportion was recorded in lavender. In contrast, a lower content of linalool was recorded in lavandin. The results of this work are consistent with many authors (Lesage-Meessen *et al*., 2015; Gavrić *et al*., 2023) who found a higher linalool content in lavender than in lavandin species. However, this research found that the linalool content in lavender EO was slightly higher than the results mentioned by the authors. According to Alizadeh and Aghaee (2016), these differences may be due to variations in species, environmental conditions, harvest time, and oil extraction methodology.

On the other hand, this research recorded that the application of biostimulant positively affected the linalool content of both species. Consentino *et al*. (2023) had a similar observation. They studied the effect of biostimulants on basil quality. They observed that its application caused an increase in the content of phenolics, vitamin C, 1-octen-3-ol, *β-cis*-ocimene, linalool and eugenol, compared to the control. Considering, as reported by Aktsoglou *et al*. (2021), that nutrition has an effect on the composition of EOs, and given that biostimulants contain different micronutrients, humic acids and hormones (Du Jardin, 2015; Martínez-Gutiérrez *et al*., 2022), the use of biostimulants has led to significant variations in the content of EO constituents. Furthermore, in addition to the content of linalool, linalyl acetate is the crucial ingredient that determines the quality of lavender EO (Pokajewicz *et al*., 2021). In this research, its share did not depend on the species and the application of biostimulant, and its values followed previous research (Lesage-Meessen *et al*., 2015).

Although this research provided insight into the impact of biostimulant on the yield and content of lavandin and lavender EO, this research has certain limitations. A limitation of this study is that only one biostimulant was used. Therefore, future research should consider the potential influence of different biostimulants on the quality of medicinal plants. Despite this limitation of this study, this research has shown certain advantages that can be applied in practice. Namely, the researched biostimulant positively affected the inflorescence yield, EO and linalool content. Therefore, its more intensive application in sustainable agriculture could be a cheap alternative to expensive, often environmentally unfavourable mineral fertilisers.

Conclusions

The experiment results showed that used biostimulant positively affected the inflorescence yield and the EO content. Furthermore, the applied biostimulant increased the linalool content in the EO of both researched species, i.e. it positively affected its quality. Therefore, this agricultural practice could increase yields and quality of lavender and lavender, which is especially important for growing plants according to sustainable agriculture principles.

Authors' Contributions

T. G., D. G. and R. E. conducted a field experiment. T. G. and K. H V. collaborated in laboratory research and analysed the data. T. G., D. G. R. E. and K. H V. contributed to the preparation of the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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