

Research Article

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Effect of natural boron mineral use on the essential oil ratio and components of Musk Sage (*Salvia sclarea* L.)

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Abstract: This study was aimed to determine the effect of different boron doses (boron free, pure boron with 8 liters per decare and in 1/8 ratio diluted boron) on the rate and quality of volatile oil in musk grown in Kütahya-Gediz conditions. Essential oil from Musk Sage was obtained by hydrodistillation method (GC-MS/FID). In the analysis carried out in 2017, the rate of essential oil was found to be 0.11% in the plant grown without boron, while it was 0.44% in the plant grown with pure boron. However, this rate was found as 0.23% in the 1/8 boron ratio application. The main components of volatile oil were found as follows: for the boron-free application—spathulenol 23.75%, caryophyllene oxide 19.41%, linalool 10.10%, and sclareoloxide 9.92%; for the pure dose application—spathulenol 26.67%, sclareoloxide 18.81%, and caryophyllene oxide 16.13%; for in 1/8 ratio diluted boron dose application—spathulenol 24.82%, sclareoloxide 16.68%, and caryophyllene oxide 14.86%. It has been observed that pure boron dose has a positive effect on the essential oil ratio and components of Musk Sage.

Keywords: boron doses, GC-MS/FID, volatile oil

1 Introduction

The plants of the *Lamiaceae* family are herbaceous or bushy plants, which are rich in volatile oil, distributed in regions dominated by the Mediterranean climate. *Salvia* species are mainly medicinal and aromatic plants grown in the hot, humid regions of the world [29,7]. The types of *Salvia* were used before 1,400 and have been used since then. Then, the

Spanish and Moroccan scientists used it in traditional medicine. Spanish and Moroccan scientists used names such as “salima” or “asphacus” for a few species of *Salvia* [12]. The leaves of *Salvia* species have been used since the ancient years in traditional folk medicine as a domestic or mouthwash (for common cold, nervous fatigue, pharyngitis, gingivitis, and similar diseases) to prevent excessive sweating and to increase lactation [3,11]. *Salvia* species showed biological activities such as antispasmodic, hallucinogenic, antibacterial, antifungal, antiviral, antiseptic, analgesic, antioxidant, anticancer, tuberculostatic, cardiovascular activity, and insecticide [4,28]. *Salvia sclarea* L. is an important medicinal and aromatic plant that has been cultivated worldwide, and the essential oil of *Salvia sclarea* L. is a volatile oil known as “Clary Sage” in the world market [9]. The *Lamiaceae* family is rich in medicinal and aromatic plant species. Most of these species produce and store essential oils of specialized epidermal and sebaceous glands, which are responsible for their particular flavors. *Salvia sclarea* L. has two types of glands: peltate and capitate glands, which produce essential oil and have different morphological structures. In one study, the contents of the individual sebaceous glands were sampled using SPME fibers from different positions on the plant (corolla, calyx, and leaf) and analyzed by gas chromatography to examine the variability of the volatile oil composition. The composition of terpenoids was found to be highly variable in a single plant. The capitate sebaceous glands mainly produce three essential oil compounds: monoterpene linalool and linalyl acetate and diterpene sclareol. In addition, the fat composition varies according to the plant organ and each type of secretion. Linalool and linalyl acetate are characteristic substances of flowers, whereas sesquiterpenes occur at higher rates in leaves [30]. *Salvia sclarea* L., which is among the *Salvia* species, has a significant economic value in the perfume and cosmetics industry. *Salvia sclarea* L. has an important place in world trade. The drugs of *Salvia sclarea* L. have been mentioned in the supplement of French Pharmacopoeia’s “The French Explanatory Note,” which was published in

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1998 [5]. *Salvia sclarea* L. grows naturally in northern Africa, Anatolia, and southern regions of Europe. This type of culture, which has been cultivated in Spain, Russia, Morocco, France, Switzerland, Germany, Italy, Hungary, Yugoslavia, Bulgaria, America, England, Israel, South Africa, and India, has economic importance [5,33]. Boron has a quite narrow range between deficiency and toxicity, and inadequate boron supply exhibits a detrimental effect on the yield of agricultural plants. The deficiency problem can be solved by fertilization, whereas soil boron toxicity can be ameliorated using various procedures; however, these approaches are costly and time-consuming, and they often show temporary effects. Plant species, as well as the genotypes within the species, dramatically differ in terms of boron requirements; thus, the available soil boron that is deficient for one crop may exhibit toxic effects on another [6]. It is one of the essential oils approved by the FDA in the US for use in foods and generally accepted as reliable by FEMA [7]. *Salvia sclarea* L. generally contains the following terpenes belonging to different types such as monoterpenes, oxygenated monoterpenes, aliphatic alcohols, esters, and sesquiterpenes. The terpenes that are monoterpenes include α -pinene, kamfen, β -pinene, mirsen, limonene, osimen isomers, p-simen, and α -terpinolene. The terpenes that are oxygenated monoterpenes include α -terpineol, linalool, carvacrol, geraniol, nerol, 1,8-cineol, *cis*-linalol oxide, geranyl acetone, and α -mono-farnesyl acetone. The terpenes that are aliphatic alcohols include (*z*)-3-hekzen-1-ol and *l*-okten-3-ol. The terpenes that are esters include neryl acetate, linalyl acetate, and geranyl acetate. The terpenes that are sesquiterpenes include β -bourbonene, β -caryophyllene, α -humulene, germachren-*D*, β -kubeben, α -kopaen, γ -elemen, spatulenol, and olmesmol isomers [35]. The main producers of *Salvia sclarea* L. essential oil are Russia, USA, Bulgaria, France, India, Switzerland, and Morocco. Countries that produce small amounts of essential oil are Israel, South Africa, Hungary, the former Yugoslavia, and Italy. The total production of *Salvia sclarea* L. essential oil is approximately 45 tons globally [7]. Some species belonging to the genus *Salvia* are of commercial importance both in our country and in the world. For this reason, a large number of studies have been carried out on sage species in Turkey and abroad. In addition to making use of raw boron minerals from ancient times to the present, demand and need for boron minerals have been increasing with the industrialization and development of technology in the last century. The use of boron in the industrial sector is aimed to produce and obtain boron compounds for different industrial areas such as glass sector, energy field, photography field, pharmaceutical and cosmetic sector, communication field, construction field, etc. Boron mineral is used

in as the manufacturing of biological development and control chemicals, fertilizers, insecticides, herbicides, etc., in the agricultural sector [36]. Since boron is very suitable for bonding with oxygen, it forms a number of different oxygen compounds. Due to this feature, boron has 230 different minerals that have been identified until now. Seven of these minerals have a high commercial value. These high-value minerals are boron salts, such as tinkal and kernite, which are water-soluble minerals, and colemanite, Ulexite, pandermite, boracite, and sassolite, which are insoluble in water. Boron minerals with a high-grade content of “tenor” are more valuable, and they are demanded more when compared with the other boron minerals [36]. This is a study examining how boron mineral affects the volatile oil composition of Musk Sage (*Salvia sclarea* L.) which is grown in the inner parts of the Aegean region. Up to now, boron mineral study on the type of *Salvia sclarea* L. was not found in the literature. Therefore, this study aims to determine how the boron mineral affects the volatile oil composition, to increase the product variety for the people of the region, and to contribute to the pharmaceutical, food, and perfume sectors.

2 Materials and methods

2.1 Plant material

In this study, the seeds that were used as materials were obtained from Ankara University, Faculty of Agriculture, Department of Field Crops. The research was carried out in the application area of Medical and Aromatic Plants Department of Dumlupınar University, Gediz Vocational School, in 2016–2017. Seeds were planted in seed containers in February 2016. Seed germination lasted for 20–25 days. The rooted seedlings were transferred to the field from April 2016 and they were given water. Field trials were carried out with a randomized block design with three replications. Each parcel was designed with a 40 cm width \times 30 cm length row space. Drying plants were replaced with new ones, and so it was granted that there were at least 60 plants in each parcel. After the second week, watering was carried out once a week. In the experiment, three different boron doses (boron free, pure boron with 8 liters per decare, and 1/8 ratio diluted boron) were applied to the medicinal sage plant. The boron mineral was given in the second year after the plant height was 15 cm in all parcels. The boron mineral, which was supplied without processing, damaged leaves of the plants. In the experiment, irrigation was done considering the

Table 1: Some chemical analysis results of soil samples of the trial area

Analysis type – Kutahya-Gediz	Result	Status
Potassium (K ₂ O) kg; da	20.0123	Medium
Phosphorus (P ₂ O ₅) kg; da	6.231	Medium
Lime (%)	4.0318	Limy
Organic substance (%)	0.7862	Very little
Total salt (%)	0.0035	Salt-free
PH	7.14	Neutral
Saturation (%)	53.3	Clay and loam

rainfall, air temperature, and humidity in the soil. The observations and measurements were made on flower samples obtained from nine plants selected for each application from 60 healthy plants in each parcel. In the first year, the plant has just completed its development, and in the second year, one harvest was made. The beginning of the flowering period in which the highest rate of volatile oil can be obtained was preferred at the time of the harvest. Soil analysis of the trial area in the Gediz district of Kutahya is given in Table 1. According to Table 1, potassium and phosphorus are moderately low, lime content is high, organic substances are low, and the saturation was determined to be clayey [1].

Boron is not present as a single element in nature, but as compounds with multiple elements. The most common compounds are Na, Ca, and Mg. boron with Na origin is called tinkal (borax), with Ca origin is called colemanite, and with Na and Ca origin is called Ulexite [36].

The chemical analysis of the natural boron mineral to be used in field trials was performed (Table 2). According to the results of the analysis, the most common mineral was found to be Ca 108.9 mg kg⁻¹, while the least found minerals are as follows: Cu <10 µg kg⁻¹, Ni <10 µg kg⁻¹, Cd 10 µg kg⁻¹, and Co <10 µg kg⁻¹ [2]. Boron compounds of calcium origin are called Colemanite. The boron type used in this study is Colemanite.

2.2 Preparation of boron extracts

The extract used in our study was prepared from powdered (pulvarized) boron. The boron element was

obtained from the Emet region. The boron element taken from the area was pulverized. Then, 20 g of powdered boron mineral was weighed and then shaken with 100 mL of pure water. And then, it was homogenized for 5 min for the precipitation process. The homogenate boron was centrifuged at 3,500 rpm for 5 min. The residue was weighed as 0.0260 g from the mixture of 100 mL of water and 20 g of powdered boron element. The supernatant portion was stored in the refrigerator. This extract was used either in absolute form (a mixture of 100 mL water and 20 g powdered boron) or diluted with pure water in 1/8 ratio [16,14].

2.3 Essential oil isolation

At the beginning of the trial volatile oil analysis, 20 g of dried flower material was weighed and taken into a 500 mL flask. Two hundred mL (depending on the amount of sample, about 10 times) of pure water was added and shaken. Hydrodistillation was carried out for 2 h to obtain a volatile oil. After the system cools down, the volatile oil collected in the graduated portion was separated from the aqueous phase and the amount (mL) is determined. According to the amount of sample (g) weighed, the amount of volatile oil in the 100 g sample was calculated as the percentage of volatile oil [32].

2.4 Determination of essential oil composition by GC-MS

Analysis of essential oil components was carried out at the Research Laboratory of the Western Mediterranean Agricultural Research Institute. Samples were diluted with 1% hexane and injected in 1 µL with 40:1 split ratios to gas chromatography (Agilent 7890 Å). Capillary columns (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm) were used to separate the components. The column was split into two fractions at a rate of 1:1 using a splitter to the FID and mass spectrometry detector (Agilent 5975°C). In the analysis, helium was used as carrier gas at a flowrate of 0.8 mL/min.

Table 2: The results of chemical analysis in the sample of boron mineral

Ca Mg kg ⁻¹	K Mg kg ⁻¹	Mg Mg kg ⁻¹	Na Mg kg ⁻¹	Fe Mg kg ⁻¹	Mn Mg kg ⁻¹	Zn Mg kg ⁻¹	Cu µg kg ⁻¹	Ni µg kg ⁻¹	Cd µg kg ⁻¹	Cr µg kg ⁻¹	Co µg kg ⁻¹
108.9	19.66	33.22	58.68	0.680	0.042	0.10	<10	<10	<10	0.034	<10

The injector temperature was maintained at 250°C, and the column temperature program was 10 minutes at 60°C, 4°C/minute (40 minutes) at 60°C and 220°C, and 10 minutes at 220°C. It is set to be 60 minutes. The scan range (m/z) for the mass detector is 35–450 atomic mass units, and the electron bombardment ionization energy is 70 eV. The diagnosis of volatile oil components is based on the data from OIL ADAMS, WILEY, and NIST libraries. The data of the FID detector were used for the volatile oil component ratios [22].

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

This study was conducted to compare the effect of different doses of boron mineral applied on *Salvia sclarea* L. species on volatile oil and components. In this study, it was determined to what degree do three different applications (boron-free, pure boron dose, and 1/8 diluted boron doses) affect the essential oil ratio and the components of the plant. The highest percentage of essential oil was determined in pure dose application with 0.44%. As the boron mineral dose increased, the volatile oil content increased. “Bicyclogermacrene, δ -cadinene, and salvial-4-en-1-one” are the essential oil components acquired after the administration of pure dose and 1/8 dose. In addition, “linalool, linalyl acetate, α -terpineol, neryl acetate, geranyl acetate, and geraniol” are the compounds that are lost after the application of both the pure dose and the 1/8 dose. The main component of the essential oil obtained from *Salvia sclarea* L. species was determined to be spathulenol with 23.75% in the experiment of no boron use whereas it was found to be Spathulenol with 26.67% in the experiment of pure boron. And spathulenol with 24.82% was found as the main component in the experiment of 1/8 boron dose. As in the volatile oil ratio, as the boron dose increased, some of the volatile oil components also increased and different components were obtained. The pure dose may be recommended for volatile oil and its components. Boron doses may also be recommended for the yield and quality of *Salvia sclarea* L. species. It will be useful to carry out further studies on species that can be valuable in terms of medicinal and aromatic plants. Volatile oil ratio and the compositions of essential oil obtained in this study were presented in the following sections.

4 Discussion

4.1 Volatile oil ratio (%)

The volatile oil ratios in the Musk Sage (*Salvia sclarea* L.), which was grown under Kütahya-Gediz conditions and exposed to different boron doses (boron free, pure boron with 8 liters per decare, and 1/8 ratio diluted boron), were measured as 0.11–0.44–0.23%, respectively. When boron mineral was applied in the pure dose and 1/8 dose, no death was observed in plants although plant growth was low. On the contrary, the development of the plant was observed to be healthier as a result of the measurements carried out in the second year.

The results that were found in different studies about volatile oil ratio are as follows: while the flowers were in the bud stage, the oil yield was found to be 0.08% in fresh plant phase, it reached its peak level with 0.18% in full bloom stage, and then it was determined as 0.07% after a sharp decline in maturation [17]. The components of 22 different essential oils belonging to *Salvia sclarea* L. and nine other salvia species originating from Iran were examined. Their essential oil yield ranged between 0.07 and 0.71% [25]. Researchers have investigated the *in vitro* antimicrobial, antioxidant, antiviral activities, and volatile oil content of various extracts and essential oils of *Salvia sclarea* L. plant. At the end of the study, the rate of volatile oil was determined as 0.20% [21]. The amount of essential oil obtained from *Salvia sclarea* L. species at Çanakkale location was found to be 0.02% [15]. When compared with the values obtained in other studies with the same plant in different places, the ratio of essential oil obtained by using pure boron was found to be high. Some studies related to boron use on plants show that excessive external boron reduces water transport and transpiration in *Arabidopsis*, which could act as a mechanism of boron tolerance [18]. Moreover, it was also pointed out that boron is easily leached from the leaves by rain [26,27].

4.2 Composition of essential oil (%)

As a result of the analysis of the volatile oil found in the Musk Sage (*Salvia sclarea* L.) exposed to different boron doses (boron free, pure boron with 8 liters per decare, and in 1/8 ratio diluted boron); 20, 17, and 17 components were defined in the dried flower for the second year, respectively

which means a total of 23 different components were obtained at the end of all three applications (Table 3). Furthermore, the proportions of 14 common components of all doses were presented in Figure 2. These components are 100–100–92.17% of the total fat in the dry leaves, respectively. The values of the volatile oil components obtained from the flowers of *Salvia sclarea* L. species were determined by the sample taken from the mixture of the flowers harvested in the second year. The values of the volatile oil components of the species *Salvia sclarea* L. are shown separately in Table 3. The main components obtained from the dried flowers of *Salvia sclarea* L. species are as follows: when no boron was used, the volatile oil components were as follows: spathulenol 23.75%, caryophyllene oxide 19.41%, linalool 10.10%, and sclareoloxide 9.92%. When pure boron was used, the volatile oil components were as follows: spathulenol 26.67%, sclareoloxide 18.81%, and caryophyllene oxide 16.13%. When 1/8 diluted boron was used, the volatile oil components were as follows: spathulenol 24.82%, sclareoloxide 16.68%, and caryophyllene oxide 14.86% (Figure 1). According to the doses applied to the species *Salvia sclarea* L., the volatile oil components vary considerably. The main component of the essential oil obtained from *Salvia sclarea* L. species was

determined to be spathulenol with 23.75% in the experiment of no boron use, whereas it was found to be spathulenol with 26.67% in the experiment of pure boron. And spathulenol with 24.82% was found as the main component in the experiment of 1/8 boron dose. Similar to our study, researchers have investigated the *in vitro* antimicrobial, antioxidant, and antiviral activities and volatile oil content of various extracts and essential oils of *Salvia sclarea* L. plant. The main essential components of the essential oil of *Salvia sclarea* L. species were reported to be germacrene-D 24.72%, bicyclogermacrene 9.63%, β -caryophyllene 16.24%, and linalyl acetate 5.52% [21]. A total of 52 different compounds were identified representing 98.25% of the total fat content. Linalyl acetate 56.88% and linalool 20.75% were identified as major essential oil components, followed by germacrene-D 5.08% and β -caryophyllene 3.41% [13]. Fifty components were obtained in the essential oil of the Musk Sage cultivated at the time of full flowering. Linalyl acetate 35.9%, germacrene-D 13.3%, linalool 12.8%, and sclareol 9.27% were obtained as main components. Forty-five components were detected in its wild form. The main components were linalyl acetate 34.0%, linalool 18.5%, germacrene-D 10.0%, and sclareol 8.7% [20]. A total of 59 compounds of the wild-growing *Salvia sclarea* L. species

Table 3: Changes in the amount of the essential oil (%) obtained from the flower of *Salvia sclarea* L. species according to boron doses

No	Component name	Boron-free	Pure dose	1/8 boron dose
1	α -Copaene	2.60 \pm 0.014	4.37 \pm 0.007	4.22 \pm 0.042
2	Linalool	10.10 \pm 0.014	—	—
3	Linalyl acetate	5.95 \pm 0.028	—	—
4	β -Caryophyllene	3.43 \pm 0.007	4.18 \pm 0.014	3.96 \pm 0.014
5	α -Terpineol	2.68 \pm 0.0014	—	—
6	Germacrene	1.87 \pm 0.007	3.34 \pm 0.014	3.24 \pm 0.063
7	Neryl acetate	0.90 \pm 0.014	—	—
8	Geranyl acetate	1.74 \pm 0.014	—	—
9	Geraniol	1.63 \pm 0.014	—	—
10	1,5-Epoxy-salvial-4-ene	0.89 \pm 0.049	3.74 \pm 0.007	3.68 \pm 0.028
11	Caryophyllene oxide	19.41 \pm 0.049	16.13 \pm 0.141	14.86 \pm 0.028
12	Humulene epoxide	0.86 \pm 0.014	0.74 \pm 0.141	0.68 \pm 0.007
13	Spathulenol	23.75 \pm 0.035	26.67 \pm 0.056	24.82 \pm 0.028
14	Isospathulenol	1.15 \pm 0	1.64 \pm 0.007	1.52 \pm 0.028
15	Sclareoloxide	9.92 \pm 0.028	18.81 \pm 0.035	16.68 \pm 0.028
16	Mustakone	0.91 \pm 0.014	1.29 \pm 0.021	1.23 \pm 0.028
17	14-Hydroxy- α -muurolene	0.86 \pm 0.014	1.51 \pm 0.042	1.45 \pm 0.021
18	Caryophylla-4,8-dien-5b-ol	0.94 \pm 0.014	0.96 \pm 0.007	0.94 \pm 0.028
19	Eudesma-4,7-dien-1-b-ol	1.32 \pm 0.014	1.91 \pm 0.021	1.86 \pm 0.021
20	Unidentified	9.09 \pm 0.007	11.61 \pm 0.049	10.22 \pm 0.070
21	Bicyclogermacrene	—	0.73 \pm 0.028	0.64 \pm 0.064
22	δ -Cadinene	—	1.29 \pm 0.049	1.17 \pm 0.028
23	Salvial-4-en-1-one	—	1.08 \pm 0.014	1.02 \pm 0.007
	Total (%)	100%	100%	92.17%

*Each value in the table represents the mean \pm standard deviation of triple analyses.

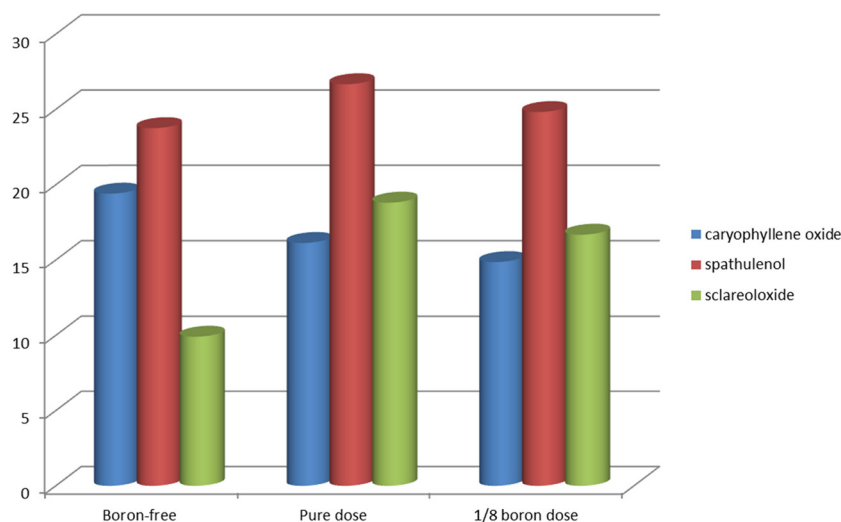


Figure 1: Proportion of the highest major constituents of *Salvia sclarea* L.

were obtained in Tajikistan, representing 94.2% of the total fat composition. The main components of essential oil were determined to be linalyl acetate 39.2%, linalool 12.5%, germacrene-D 11.4%, α -terpineol 5.5%, geranyl acetate 3.5%, and (*E*)-caryophyllene 2.4% [31]. In another study, it was reported that *Salvia sclarea* L. was a species native to Southern Europe and cultivated around the world. Investigators analyzed the essential oil of the same species as a potential antifungal agent and identified linalyl acetate 52.83% and linalool 18.18% as the main compounds [10].

Essential oils of *Salvia sclarea* growing in two regions in Greece were analyzed by GC-MS. Sixty-six compounds representing 93.26–98.1% of the essential oils were identified. Linalyl acetate 19.75–31.05%, linalool 18.46–30.43%, geranyl acetate 4.45–12.1%, and α -terpineol 5.08–7.56% were determined as the main components [24]. The composition of 22 essential oils of *Salvia sclarea* L. and nine different *Salvia* species grown in Iran was evaluated. The compounds of the essential oils were analyzed by GC-MS, yielding 73 compounds. The main components detected

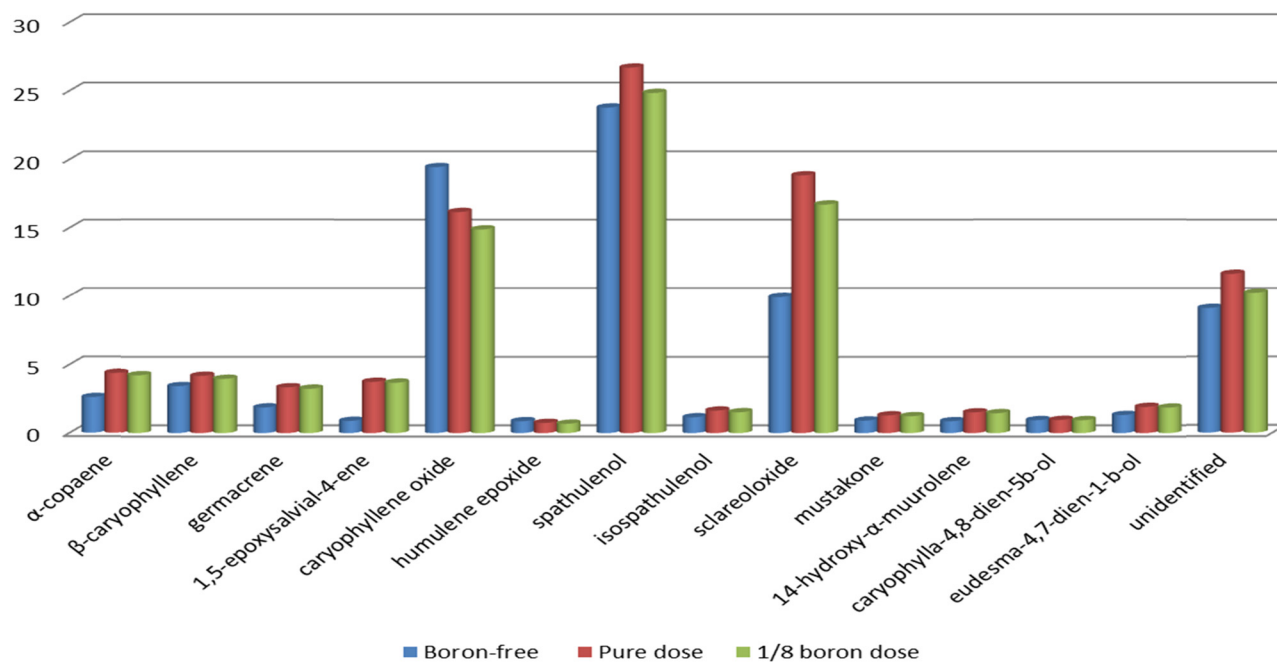


Figure 2: Proportion of common components of all doses used in *Salvia sclarea* L. species.

are linalool 0.6–51.58%, *e*-caryophyllene 3.08–60.58%, germacrene-D 0–25.16%, and spathulenol 0–35.4%. Chemical variability may be due to genetic and environmental factors. The analysis indicated that some of the contributions from the south presented more essential oil yields than other regions [22]. Some researchers analyzed the components of eight *Salvia* (*Salvia sclarea* L.) species' volatile oil with GC-MS in their study in Romania. As a result of the study, they found that the main active substances of the essential oils consisted of β -pinene and germacrene-D in different ratios [23]. In this study, essential oils of *Salvia sclarea* were analyzed by GC and GC-MS method using a Clevenger distiller device.

In a study by Verma (2010), a total of 20 components were identified representing 96.45–99.53% of the total essential oils. The main components of the essential oils were linalool 27.08–62.51%, linalyl acetate 43.01%, α -terpineol 0.12–0.25%, 0.74–4.84%, (*E*)- β -osimene 1.19–4.83%, and geranyl acetate 0.36–3.11% [34].

Researchers stated in another study that *Salvia sclarea* L. is a natural product with a high value for the perfume industry and the majority of commercially produced sclareol is derived from the extraction of *Salvia sclarea* L. plant material [8]. In the essential oil of *Salvia sclarea* L. grown in the Çanakkale location, germacrene-D 20.78%, phytol 17.81%, pentadecane 6.92%, and sclareoloxide 5.36% were found to be the main constituents [15].

Spathulenol, the main component of the essential oil obtained by using the pure dose, was determined as 26.67%, and this value was higher when compared with the value obtained in the boron-free application in this study which was 23.75%. In a study carried out by Lewis, it was stated that since a toxic element cannot have “deficiency symptoms,” those previously so-called are postulated to be largely due to the expressed toxicity of phenylpropanoids. A principal requirement for the otherwise toxic boron is to nullify using its indirect chemical and physical sequestration, such expression [18].

Conflict of interest: Authors declare no conflict of interest.

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