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Effects of natural boron mineral on the essential oil ratio and components in medicinal sage (Salvia officinalis L.)

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Abstract

The effects of different boron doses (boron-free, pure boron, diluted in 1/2 and1/8 ratios) on the ratio and quality of essential oil in medicinal sage (*Salvia officinalis* L.) during the years 2016 and 2017 were investigated. Field trials were conducted with the randomized block design in triplicates. The essential oil of *S. officinalis* L. was obtained by gas chromatography/mass spectrometry (GC–MS) with flame ionization detection (FID). The required measurement could not be taken at the pure dose application because the plants were damaged. In the analysis carried out on dry leaf, the main components of essential oil were found as follows: α-thujone 32.99%; 1.8-cineol 17.41% for the boron-free dose; viridiflorol 24.25%, manool 15.28% for the 1/2 boron dose, and lastly, α-thujone 32.31%, camphor 14.23% for the 1/8 boron dose. Some boron doses had a positive effect on the essential oil yield and essential oil components. In this study, different compounds were obtained with different boron dose applications. The dose to be recommended is the 1/8 boron dose since it has the minimum toxic effect on the plants and a positive effect on the essential oil yield and quality as general.

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1. Introduction

The Salvia genus, which is commonly called sage, has approximately 900 species, which are naturally distributed in the world. Turkey is very rich in different types of sage and 89 Salvia species are naturally found in Turkey 46 of which are endemic [1]. Despite the development of medical science in the 20th century, the use of plants in traditional medicine has not lost its importance. Developed countries have focused more on herbal products. Natural remedies constitute a large part of drugs used in treatment. Natural source drugs constitute 60% of the total drug quantity in developed countries and 4% in developing countries [2]. The refractive indices of essential oils, which are lighter than water and optically active, are generally high. The interest in aromatherapy, which is considered a new field of medicine recently, has expanded the alternative uses of essential oils [3]. Essential oils that are considered medically valuable include α, β-thujone, 1, 8-cineol, camphor and borneol. The essential oils of some sage species also reportedly contain thymol and carvacrol [4]. Essential oils have different uses. In addition to the use of essential oils in the perfume, food, medicine and cosmetics industries, their use in aromatherapy and agricultural production has increased the demand for essential oils. Recently, essential oils have been used in agricultural research, animal husbandry and beekeeping [5]. Plants need fundamental macro and micronutrients for their normal development in their growth period. The nutrient stress, whether it may cause deficiency or toxicity, influences the development of the plant and may cause losses of yield and quality in agricultural plants [6]. The deficiency and toxicity range of boron is remarkably narrow. Fertilization may be the solution of the deficiency problem, while a set of procedures can be utilized to ameliorate soil boron toxicity. However, these approaches are costly and time-consuming, and they do not have permanent effects most of the time. Plant species and also the genotypes within species are highly different in terms of their boron requirements. So, a sort of soil boron which is accepted deficient for one crop may exhibit toxic effects on another [6]. In the agricultural sector, boron is used as fertilizers, insecticides and herbicides [7]. In a study on essential oil percentages, it was found that ecology and soil structure were affected by the development of the plant and the amount of essential oil [8]. A shortage of boron supply may lead to a set of biochemical, physiological and anatomic changes. Boron deficiency reduces male fertility by decreasing

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microsporogenesis, germination, and elongation of the pollen tube [9]. Boron is the only element which cannot be taken up from the soil as an ion. In conditions of sufficient supply, this element is transported by passive diffusion, and during this process, no protein catalysis and energy consumption are required. Passive diffusion was considered as the only mechanism of transport since the cell is extremely permeable to boron, characteristic patterns of flux along the transpiration stream, and accumulation in the tips of the leaves [10 -11].

This study is a type of comparison of essential oil composition of S. officinalis L. species which grow in the Central Aegean Region and also an evaluation of essential oils. Accordingly, it was aimed to compare the essential oils of S. officinalis L. species according to different boron doses and to determine the effect of boron doses on essential oil ratio and essential oil components. It is also aimed to increase the product range and contribute to the pharmaceutical and food sector for the people of the region.

2. Materials and Methods

2.1. Study site and experimental material

This study was conducted in the application area of the Dumlupinar University (74.74°E, 29.24°N; altitude 804 m asl) during the years 2016–2017. Seed germination lasted 20–25 days. The rooted seedlings began to be transferred to the farm in April 2016. Soil analysis of the trial area in Kutahya is given in Table 1.

2.2. Preparation of boron extracts

The powdered boron mineral was weighed in 20 g and then shaken in 100 ml pure water. Subsequently, it was homogenized for five minutes for precipitation. Then, the homogenate boron was centrifuged at 3500 rpm for five minutes. The residue was weighed as 0.0260 g from a mixture of 100 ml water and 20 g powdered boron. The supernatant was stored in a refrigerator. This extract was then used either in absolute form or diluted with pure water at ratios of 1/2 and 1/8 (Table 2) [12].

Table 1: Pre-sowing chemical analysis of experimental soil

Soil variables	Values	Status
Potassium (K_2O; kg ha^{-1})	20.01	Medium
Phosphorus (P_2O_5; kg ha^{-1})	6.23	Medium
Lime (%)	4.03	Limy
Organic matter (%)	0.78	Very Little
Total salts (%)	0.003	Salt-free
pH	7.14	Neutral
Saturation (%)	53.3	Clay and Loam

Table 2: Chemical analysis of pure boron mineral

Nutrient/element	Ca	K	Mg	Na	Fe	Mn	Zn	Cu	Ni	Cd	Cr	Co
Units	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}
Values	108.9	19.66	33.22	58.68	0.680	0.042	0.10	<10	<10	<10	0.034	<10

Ca: calcium, K: potassium, Mg: magnesium, Na: sodium, Fe: ıron, Mn: manganese, Zn: zinc, Cu: copper, Ni: nickel, Cd: cadmium, Cr: chromium, Co: cobalt

2.3. Experimental details

In the experiment, four different boron doses [boron-free, pure boron (80 L ha^{-1}), and boron diluted in 1/2 and 1/8 ratios] were applied to the medicinal sage plant. After the plant reached 20 cm in length, the prepared boron mineral was supplied in all treatments; it was administered as 80 liters ha^{-1} after 1 month. In both years, experiment was conducted with a randomized complete block design in triplicates. The dimensions of each planting area were 40 cm × 30 cm. A total of 72 plants were planted by 24 plants per plot. There were 3 rows of plants in each plot, and each plot had an area of 1.6 m × 3 m The plants were watered according to their water needs. New ones were planted in place of the dried plants, and there were 72 plants in each plot. Experiment was irrigated considering rainfall, air temperature, and humidity in the soil to save plants from moisture stress. No other additives or fertilizer were applied to the plant neither before nor during the planting session except for boron mineral. The weeds were observed and they were cleared manually.

2.4. Data recorded

Observations and measurements were made on leaf samples obtained from 9 plants labeled in 72 healthy plants in each plot. Since the first year was the plantation year and the plant growth was too low, no measurement could be taken and no doses were applied. In the second year, two harvests were made, and the beginning of flowering was preferred as the harvest time. After the second week, the plants were watered once a week.

2.5. Isolation of essential oil

The samples were diluted 1:100 with hexane for analysis. At the beginning of the trial essential oil analysis, 20 g of dry material was weighed and taken in a 500 ml flask. The samples were diluted with 1% hexane and injected into gas Chromatography in 1 µl with 40:1 split ratios. Agilent 7890 A Capillary columns (HP Innowa x Capillary: 60.0 m × 0.25 mm × 0.25 µm) were used to separate the components. The scan range (m/z) for the mass detector was 35-450 atomic mass units, and the electron bombardment ionization energy was 70 eV. The data of the WILEY and OIL ADAMS libraries were taken as a basis in the diagnosis of the components of the essential oil. The data from the FID detector were used for the essential oil component ratios [13].

2.6. Determination of essential oil composition by GC–MS

The samples were diluted with 1% hexane and injected with 40:1 split ratio to gas chromatography (Agilent 7890 A). Capillary columns were used to separate the components. The column was split into two fractions at a ratio of 1:1 using a splitter to the FID and MS detector (Agilent 5975 ^{0}C). In the analysis, helium was used as the carrier gas at a flow rate of 0.8 ml min^{-1}. The scan range (m/z) for the mass detector was 35–450 atomic mass units, and the electron bombardment ionization energy was 70 eV. The data of the FID detector were used for determining essential oil component ratios [14].

2.7. Statistical analysis

Two harvests for aerial parts of plants at flowering periods were made on 28.05.2017 and 02.09.2017 in the second year. Statistical analysis of the data obtained in the experiment was performed using the JUMP package program according to the random blocks experiment pattern. The significance levels of the investigated features were determined by random blocks experimental model and by variance analysis. The differences between meaningful applications are grouped according to the calculated LSD value (Table 3).

3. Results and Discussion

Different boron doses (0, Pure dose, 1/2 boron dose, 1/8 boron dose) were applied to the type of medical sage (*Salvia officinalis* L.) examined in this study. While natural boron mineral applied without extraction has a toxic effect on the plant causing the plant to dry out, extracted pure boron doses also has the same effect on the plant. However, no complete drying or death occurred in the plant in 1/2 and 1/8 boron dose applications. According to analysis of variance, the effects of applications on yield and essential oil parameters were found statistically significant (Table 3). The medical sage species started flowering by the date of 20 May 2017, and till the end of the month, it arrived at the harvest period by completing 50% flowering. Considering the flowering dates of the plant based on the doses, it was determined that the boron-free dose reached the first and complete flowering dates earlier than the other doses. The plants under the treatment of the 1/8 boron dose started flowering earlier, while those lower than 1/2 dose started later. In this context, in our study, the boron-free dose and the 1/8 boron dose provided more than 1.5% essential oil contents (Table 3). The lowest essential oil ratio was 0.67% in the 1/2 boron dose application, while the highest one was 2.05% in the boron-free dose application (Table 3). According to the GC-FID/MS analyses on the boron doses (boron free dose, 1/2 boron dose, 1/8 boron dose), the essential oils of medical sage contained respectively 17, 16, 25 different components.

The ratios of the total essential oil components in dry leaf for the boron-free dose, 1/2 boron dose and 1/8 boron dose were respectively 97.78%, 98.94% and 99.07% (Table 3). Among these components, (boron free dose, 1/2 boron dose, 1/8 boron dose) especially α-thujone, camphor, 1,8-sineol, manool and viridiflorol were compounds found at high ratios (Figure 1). Viridiflorol, which was one of the main components obtained in the 1/2 boron dose application, was found as 24.25% and higher than the values obtained in all other dose applicatios. Natural factors may have a positive or negative effect on essential oils. The viridiflorol component has vitro anti-inflammatory, antioxidant, anti-tuberculosis effects [15]. If the viridiflorol component is required pharmacologically, the 1/2 boron dose is recommended.

Table 3. Effect of different doses of boron mineral on essential oil componnents (%) obtained from the leaves of *S. officinalis* L.

Essential oil components (%)	RI	RT	Boron-free	1/2 of pure boron mineral	1/8 of pure boron mineral
Cis-salvene	944	8.90	0.72±0.029b	-	0.82±0.016a
α-pinene	977	11.42	4.40±0.101b	2.31 ±0.197c	5.53±0.013a
Camphene	1026	13.13	4.65±0.178b	3.72±0.099c	6.82±0.02a
β-pinene	1068	14.85	3.30±0.068c	3.82±0.017a	3.52±0.013b
1,8-cineol	1171	19.39	17.41±0.625a	9.2±0.223b	2.33±0.013c
α-thujone	1383	28.23	32.99±0.467a	4.23±0.085c	32.31±0.026b
β-thujone	1400	28.87	6.35±0.219b	0.44±0.012c	7.74±0.019a
Camphor	1477	31.54	10.22±0.424b	8.89±0.024c	14.23±0.021a
Bornyl-acetate	1477	33.39	2.32±0.146c	4.80±0.024a	2.94±0.030b
β-caryophyllene	1552	34.00	1.62±0.225b	5.24±0.039a	1.62±0.028b
α-humulene	1623	36.23	4.30±0.187a	4.08±0.026b	2.07±0.025c
Borneol	1646	36.92	1.74±0.030c	5.17±0.024a	4.25±0.023b
Humulene epoxide II	1994	46.40	0.29±0.039c	2.75±0.028a	0.65±0.013b
Viridiflorol	2026	47.20	3.17±0.109c	24.25±0.037a	4.83±0.017b
Manool	2589	64.87	0.77±0.049c	15.28±0.057a	4.18±0.022b
Limonene	1160	18.89	1.36±0.115b	0.94±0.014c	1.92±0.014a
Tricyclene	958	10.92	-	-	0.31±0.023a
Myrcene	1123	17.24	-	-	1.04±0.021a
α-terpinene	1141	18.03	-	-	0.30±0.019a
Cis-β-ocimene	1192	20.36	-	-	0.34±0.045a
ϒ-terpinene	1204	20.89	-	-	0.37±0.011a
P-cymene	1230	21.99	-	-	0.72±0.019a
Terpinolene	1241	22.46	-	-	0.26±0.016a
Caryophyllene oxide	1939	45.01	-	3.82±0.042a	0.25±0.025b
β-myrcene	1123	16.2	2.17±0.071a	-	-
Linalool	1490	31.97	-	-	0.26±0.023a
Total (%)			97.78	98.94	99.07
Essential oil rate (%)			2.05a	0.67c	1.54b

Means±standard deviation with different letters are significantly different from each other p≤0.01
The value of the compounds with no value is zero

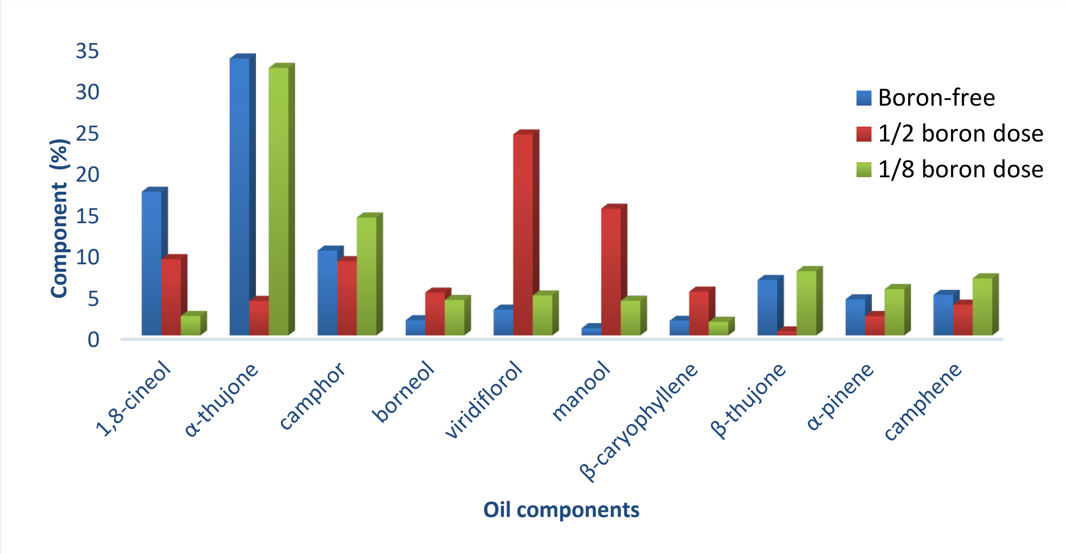

Figure 1. Changes in significant components of essential oil obtained from the leaves of *Salvia officinalis* L. species according to (%)

Since natural boron mineral which was used in this study is rich in Ca, it had a positive effect on *Salvia officinalis* L. species as expected (Table 2). Consequently, it is obvious that Ca along with other elements, is a crucial nutrient for *Salvia species*. The cis-salvene component was a common essential oil component in boron-free and 1/8 dose applications, but no cis-salvene component was obtained in the 1/2 dose application. While the β-myrcene component was found in the boron-free application, this component was not obtained in the 1/2 and 1/8 dose applications (Table 3). The highest number of components were obtained as a result of the 1/8 dose application with 25 components. Moreover, the compounds "linalool, tricyclene, myrcene, α-terpinene, cis-β-ocimene, ΄ϒ-terpinene, p-cymene, terpinolene, and caryophyllene oxide" were obtained only in the 1/8 dose application. As the boron dose was diluted as in the essential oil ratio, the essential oil components increased, and different components were obtained (Table 3). Viridiflorol, which was one of the main components obtained in the 1/2 boron döşe application, was found as 24.25% and higher than the values obtained in all applications (Table 3). Consequently, it was projected in this study that boron doses may be assessed as a priority in medical sage species breeding with drug-leaf yield and essential oil quality. The essential oil of medical sage is mainly composed of monoterpenes consisting of bicyclic, monocyclic and acyclic carbon frames. However, it was reported that thujones may be toxic on both brain and liver cells in humans, and especially α-thujone is more toxic in comparison to β-thujone [16 -17]. The essential oil component ranges in *S. officinalis* L. which is known as a rich source of essential oil with prevalent usage in folk medicine were found as follows: α-thujone (21.43%–40.10%), β-thujone (2.06%–7.41%), camphor (11.31%–37.67%), 1,8-cineol (4.47%–9.17%), camphene (1.89%–7.04%), viridiflorol (2.14%–5.56%), β-caryophyllene (1.06%–5.59%). It was reported that some populations had chemotypes of the camphor type, while some others had those of the thujone type [18]. The effect of different water and nitrogen applications on some yield parameters and antioxidant activity on sage (*Salvia officinalis* L. var.) was investigated in a study. The highest essential oil ratio was obtained from the S3 water dose as 2.07%. The main component in the essential oils recovered by both experiments were α+β thujone showing a large variation. Considering the N fertilization combined with the S2 irrigation had positive effect on the characteristics measured [19]. Some researchers studied the effects of boron fertilization on chemical composition, of *Arnica montana* L. and *A. chamissonis* L. It was determined that essential oil contents ranged between 0.174% and 0.200%, between 0.158% and 0.188%, respectively [20]. In another study, the main components of the essential oil of *S. officinalis* L. found as follows: cis-thujone (19.8%–42.5%), manool (3.6%–15.1%), viridiflorol (3.1%–12.8%), 1,8-cineol (2.8%–13.8%), camphor (1.4%–22.1%), borneol (0.9%–4.8%) whereas it was reported that some populations had chemo-types of the camphor type, while some others had those of the thujone type [21]. These findings coincide with the results in this study. In terms of the boron doses and essential oil components in our study, this situation indicates that some medical sage plants contains different chemo-types. For example, high camphor at the 1/8 boron dose and low α-thujone at the 1/2 boron dose were significant chemo-types (Table 3).

Therefore, studies in this field and specifically on this species, particularly focusing on the use of natural boron mineral, would be necessary and invaluable.

4. Conclusions

The following inferences were made based on the results obtained from this study. In terms of essential oil yield, the most effective dose was the 1/8 boron dose. In general, to obtain different essential oil ratios and different essential oil components in a plant, either different parts of the plant are used, or a genotype-environment study is necessary. On the other hand, with this study, it was determined that we could obtain some important components with the 1/2 and 1/8 boron doses without needing studies conducted at different locations (Table 3). In this context, in our study, the 1/8 boron dose provided more than 1.5% essential oil contents (Table 3). The dose to be recommended is the 1/8 boron dose since it has the minimum toxic effect on the plants and a positive effect on the essential oil yield and quality as general.

Conflicts of Interest

No conflict of interest

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