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Effects Of Natural Boron Mineral On The Yield And Quality İn Medicinal Sage (*Salvia Officinalis* L.)

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Abstract— *The effects of different boron doses (boron-free, pure boro, boron diluted in 1/2 and 1/8 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). Besides, different boron doses (boron-free, 1/2 diluted, 1/8 diluted doses) had a positive effect on the essential oil yield and essential oil components. The required measurement could not be taken in 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. In this study, different compounds were obtained at different boron dose applications. The maximum yields based on the boron doses were obtained as fresh herbage yield: 1506.87 kg ha^{-1}, drug herbage yield: 708.52 kg ha^{-1}, drug leaf yield: 349.18 kg ha^{-1}and essential oil yield: 7.89 L ha^{-1} with 1/2 boron dose treatment while maximum plant height was obtained with 1/8 boron dose application by 59.33 cm The growth and yield parameters of the plant were affected differently by different boron doses. The dose to be recommended is the 1/8 boron dose since it has the minimum toxic effect on the plant as general.*
Keywords— *Boron; GC–MS; Medical sage; Natural fertilizer; Viridiflorold*

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I. Introductıon

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, and 46 of them are endemic [6]. Despite 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 [8]. 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 [11]. 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 [20]. 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 [1]. 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 [2]. In the agricultural sector, boron is used as fertilizers, insecticides and herbicides [18]. Because boron is very suitable for bonding with oxygen, it forms many different oxygen compounds. Owing to this feature, boron has 230 different minerals that have been identified so far. Seven of these minerals have a high commercial value [18]. These high-value minerals are boron salts, which are water-soluble minerals, while, for example, tincal, kernite, colemanite, ulexite, pandermite, boracite and sassolite are insoluble in water. Boron minerals with a high-grade content are more valuable, and they are more in demand in comparison to other boron minerals [18]. 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 [10]. A shortage of boron supply may lead to a set of biochemical, physiological and anatomic changes. Boron deficiency reduces male fertility by decreasing microsporogenesis, germination, and elongation of the pollen tube [2]; [4]. 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 [14]; [13]; [2]. 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 [2]. To best of my knowledge, very little information is available about the effects of different doses of pure boron minearl on yield and oil quality of sage plant. Therefore, this study was carried out to determine the effects of boron mineral doses on yield and quality in medical sage plants. Additionally, it is aimed to increase the product variety for the people of the region and contribute to the pharmaceutical and food sector.

II. Materials And Methods

Study site and experimental material This study was conducted in the application area of the Medical and Aromatic Plants Department of Dumlupınar University Gediz Vocational School (74.74°E, 29.24°N; altitude 804 m asl) during 2016–2017. In this study, the seeds were obtained from Department of Field Crops, Faculty of Agriculture, Ege University. Seed germination lasted 20–25 days. The rooted seedlings began to be transferred to the farm from April 2016. Soil analysis of the trial area in the Gediz district of Kütahya is given in Table 1.

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

Analysis Type–Kütahya-Gediz	Result	Status
Potassium (K_2O) kg/ ha^{-1}	20.0123	Medium
Phosphorus (P_2O_5) kg/ ha^{-1}	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

2.1. Preparation of boron extracts

The natural boron minerals to be used in the field trials were chemically analyzed, and their composition is given in Table 2. This study used boron compounds of calcium origin, which are called colemanite. The extract used in this study was prepared from powdered (pulverized) boron. The boron element was obtained from the Emet region and was pulverized. Then, the powdered boron mineral was weighed in 20 g and then shaken in 100 mL pure water. Subsequently, it was homogenized for 5 min for precipitation. The homogenate boron was centrifuged at 3500 rpm for 5 min. 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 (a mixture of 100 mL water and 20 g powdered boron) or diluted with pure water at ratios of 1/2 and 1/8 [9].

Table 2. The results of chemical analysis in the sample of Boron Mineral

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

calcium potassium magnesium sodium ıron manganese zinc copper nickel cadmium chromium cobalt

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, 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.

2.3. 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 yield and 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.

2.4. Isolation of essential oil and Determination of essential oil composition by GC–MS

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 7890A Capillary columns (HP InnowaxCapillary: 60.0 m × 0.25 mm × 0.25 μm) were used to separate the components. The column was split into two fractions at a ratio of 1:1 using a splitter in the FID and mass spectrometry detector (Agilent 5975C). Helium was used as carrier gas at a flow rate of 0.8 mL/min. The injector temperature was maintained at 250 °C; the column temperature program was 10 minutes at 60 °C, raised at 4 °C/minute (40 minutes) at 60 °C and 220 °C and 10 minutes at 220 °C. The detector was set for 60 minutes. 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 [15].

2.5. Statistical analysis

No measurements were made to determine the yield and measuremenin the first year (2016) because sage plants cannot fully adapt to the soil and ecological environment and plant development is low. Two harvests were made on 28.05.2017 and 02.09.2017 in the second year. Statistical analysis of the data obtained in the experiment was calculated 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 (Tables 3-4).

III. Results And Discussion

Very different results were encountered in the ratio of essential oil and essential oil components in this study. Essential oil ratios and components of essential oils obtained from dry leaves of Salvia officinalis L. species were determined using boron-free and boron dosing applications. It was determined that the highest essential oil rate was on the boron-free leaf with the rate of 2.05%. As the dose of boron mine increases, the essential oil rate (0.67%) decreases. As the dose is diluted, the rate of essential oil (1.54%) increases. When the boron dose was diluted, the essential oil rate was found to increase. In addition, when the boron dose was diluted, the number of essential oil components increased, as well. Although the use of boron reduced the essential oil yield, different components were obtained thanks to this application. In fact, if this study is carried out in different locations, perhaps different results can be obtained. According to the variance analysis, the effect of the applications on the yield parameters was found statistically significant ($P < 0.05$) and the averages were subjected to LSD multiple comparison test (Table 3). The average values of the essential oil main components of the 1st and 2nd harvests carried out at the dose of boron-free application are respectively as follows; α-thujone 32.99, 1,8-cineol 17.41, camphor 10.22. The data obtained from the harvest of 1/2 boron dose are as follows; viridiflorol 24.25, manool 15.28, 1,8-cineol 9.2-9. Finally, the data obtained as a result of 1/8 boron dosing application is as follows; α-thujone 32.31, camphor 14.23, β-thujone 7.74. The cis-salvene component is a common essential oil component at 1/8 dose and 1/8 dose applications, but no cis-salvene component was obtained in 1/2 dose application. While the β-myrcene component was found in the boron-free application, this component was not obtained in 1/2 dose and 1/8 dose applications. The highest number of components were obtained as a result of 1/8 dose application with 25 components. Also; the compounds "linalool, tricyclene, myrcene, α-terpinene, cis-β-ocimene, ϒ-terpinene, p-cymene, terpinolene, caryophylleneoxide" were obtained in only 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. Viridiflorol, which is one of the main components obtained in 1/2 boron dose, was found as 24.25% and was found higher than the values obtained in all doses. Natural factors can have a positive or negative effect on essential oil. Viridiflorol component, antiinflammatory, antioxidant, tuberculosis has an in vitro effect [16]. If a viridiflorol component is required pharmacologically, a 1/2 boron dose is recommended. There fore, studies on this field and specifically on this species, particularly focusing on the use of natural boron mineral, would be necessary and invaluable.

3.1. Agronomic Characteristics

3.1.1. Plant Height

Table 3 presents the plant height values for 2016 and 2017. As a result of the statistical analysis made for the figures belonging to this feature, the harvest was made in the first year but no measurements were made due to low plant growth. In the second year, no statistically significant difference was observed (Table 3). When the average of the two 2017 harvests was evaluated, the shortest plant height was 41.38 cm without boron, the highest value was 46.33 cm with a 1/2 boron dose. In 2017, the plant heights increased in the second harvest compared to the first. For two harvest averages, the highest plant height (46.33 cm) was recorded at the 1/2 boron dose. In a study carried out by some researchers in the Adana region between 1986-1987 for the medicinal sage, the plant height varied in the 55.40-71.13 cm range [19]. A study was conducted to determine the effect of different nitrogen ratios (0, 5, 10, and 15 kg/ha-1) on *Salvia officinalis* L. species in the conditions of Kazova. According to this study, plant height ranged between 31.53-51.70 cm [12]. In the study carried out using Salvia officinalis L. species in Ankara conditions between 2002 and 2004, it was found that the plant height ranged between 18.4-28.3 cm [7]. Our findings were similar to the findings of the researchers above. The average of 1/2 boron dose was found to be high in the study conducted by [7].

Table 3. The yield-average groups for *Salvia officinalis* L. species

2017 harvest			
Agronomic Characteristics	Without boron	1/2 boron dose	1/8 boron dose
Plant height 1st harvest	27.00a	31.55a	29.33a
Plant height 2nd harvest	55.77a	61.11a	61.44a
Average plant height	**41.38**	**46.33**	**45.38**
Fresh herb yield 1st harvest	466.33ab	515.42a	336.73b
Fresh herb yield 2nd harvest	509.40a	525.68a	509.40a
Average fresh herb yield	**487.86**	**520.55**	**423.06**
Drug herb yield 1st harvest	227.18a	261.10a	212.59a
Drug herb yield 2nd harvest	246.48a	469.63a	370.30ab
Average drug herb yield	**236.83**	**365.36**	**291.44**
Drug leaf yield 1st harvest	117.66a	123.63a	113.33a
Drug leaf yield 2nd harvest	144.55b	237.99a	193.47ab
Average drug leaf yield	**131.10**	**180.81**	**153.4**
Essential oil yield 1st and 2nd harvest	4.7b	6.44a	4.8b

P<0.01 ** (Significant at 1%) P<0.05 * (Significant at 5%)

3.1.2. Fresh Herb Yield

In terms of fresh herb yield, when the results of variance analysis for 2016 and 2017 were evaluated separately, the first harvest in the second year was found to be significant at $P<0.05$ level. In the second harvests, the differences between doses were not significant. As a result of the statistical analysis made for the figures belonging to this feature, the harvest was made in the first year but no measurements were made due to low plant growth; In the second year, the average fresh herb yield was the lowest at a dose of 1/8 boron (423.06 kg/ha^{-1}) and the highest at 1/2 dose (520.55 kg/ha^{-1}). In a study carried out using Salvia officinalis L. species between 2002 and 2004 in Ankara conditions, it was found that in 2003, the average values of the three harvests varied in the range of 682.2-973.9 kg/ha^{-1} [7], in terms of fresh herb yield. In a study, the effects of six different planting distances on the agronomic and technological properties of sage (*Salvia officinalis* L.) were investigated in the Menemen, Bornova, and Aydın-Çakmar locations. The researchers reported that the total fresh herb yield varied between 3577-3964 kg/ha-1 [3]. The green herb yield of Salvia officinalis L. was found to vary between 321-1336 kg/ha-1 [17]. Our findings were similar to those of the researchers summarized above.

3.1.3. Drug Herb Yield

In terms of dry herb yield, when the variance analysis results of 2016 and 2017 were evaluated separately, the differences between the doses in the Gediz location were not found to be significant in the first harvest, and statistically significant at the level of $P<0.05$ in the second harvest. As presented in Table 3, at the Gediz location, in the second year, the highest average dry herb yield in terms of doses was obtained as 365.36 kg/ha^{-1}at 1/2 boron dose and the lowest as 236.83 kg/ha^{-1}without boron. In a study conducted in the Ege University Faculty of Agriculture in 2005-2006, dry herb yield was determined in the range of 712.7-1494.7 kg/ha^{-1} [5]. In the study carried out using Salvia officinalis L. species between 2002 and 2004 in Ankara conditions, three harvests were made for herb yield in the second and third years. Dry herb yield was found to be 257.2-264.2 kg/ha^{-1}as the average value of the three harvests in 2003 [7]. In the study, researching the effects of six different planting distances on the agronomic and technological properties of sage (*Salvia officinalis* L.) in the Menemen, Bornova, and Aydın-Çakmar locations, researchers have determined that the total drug herb yield varied between 638-1461 kg/ha^{-1}by years [3]. Our findings were similar to those of the researchers summarized above.

3.1.4. Drug Leaf Yield

When the variance analysis results of 2016 and 2017 for dry leaf yield were evaluated separately, the differences between the doses in the Gediz location were not statistically significant in the first harvest; The difference between the doses in the second harvest was found to be statistically significant at the level of $P <0.05$. As presented in Table 3, in the second year at the Gediz location, the average dry leaf yield was the lowest without boron (131.10 kg/ha^{-1}) and the highest with the dose of 1/2 boron (180.81 kg/ha^{-1}). In the study, researching the effects of six different planting distances on the agronomic and technological properties of sage (*Salvia officinalis* L.) in the Menemen, Bornova, and Aydın-Çakmar locations, the researchers found that total drug leaf yield varied between 758-950 kg/ha^{-1}according to locations [3]. In the study carried out using *Salvia officinalis* L. species between 2002 and 2004 in Ankara conditions, three harvests were made for herb yield in the second and third years. In 2003, the dry leaf yield for three harvests was found to vary in the range of 170.2-181.9 kg/ha^{-1} [7]. In the research conducted in the Ege University Faculty of Agriculture in 2005-2006, two species of Swiss origin were used and the plants were harvested in three different distances (5, 10, 15 cm). In this study, dry leaf yield was in the range of 527.4–1072.9 kg/ha^{-1} [5] Our findings are similar to the studies conducted.

IV. Conclusions

The following inferences were made based on the results obtained from this study: In the height development of the plant, the 1/8 boron dose showed a positive effect. In fresh herbage yield, drug herbage yield, drug leaf yield and essential oil yield, the most effective dose was the ½ 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, we have determined that we could obtain some important components with the 1/2 and 1/8 boron doses without needing studies conducted at different locations. The dose to be recommended is the 1/8 boron dose. The reason for not recommending the 1/2 boron dose was that some leaves of the plant were damaged (part of the lower leaves of the plant). It will be useful to carry out further studies on species that can be valuable in terms of medicinal and aromatic plants.

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