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Research Article

Short-term effect of biochar on the improvement of calcareous soil biological properties and marjoram (*Origanum majorana* L.) growth under greenhouse conditions in a Mediterranean climate

Ismail Emrah TAVALI

Akdeniz University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 07070 Antalya, Turkey; etavali@akdeniz.edu.tr

Abstract

In the cultivation of medicinal and aromatic plants may be possible to improve the biological properties of the soil and to grow plants with properties close to those in the natural environment by using the valuable organic matter resources obtained by special methods such as biochar in an economical. In this study, oak wood biochar (OBC) was used as fertilizing material for aromatic marjoram cultivation and applied to soil as follows: 0 t ha^{-1} OBC-control (OBC-0), 20 t ha^{-1} OBC (OBC-2), 40 t ha^{-1} OBC (OBC-4), 60 t ha^{-1} OBC (OBC-6), 0 t ha^{-1} OBC + chemical fertilizer (OBC-0+CF), 20 t ha^{-1} biochar + chemical fertilizer (OBC-2+CF), 40 t ha^{-1} biochar + chemical fertilizer (OBC-4+CF), 60 t ha^{-1} biochar + chemical fertilizer (OBC-6+CF). Afterwards, the effects of the applications on the biological properties of the soil where marjoram is grown and the physical properties of the plant, volatile oil yield and oil components were investigated. Accordingly, it was determined that the number of bacteria and enzyme activities (dehydrogenase, urease, alkaline phosphatase, β-glycosidase) of the soil reached with the biochar applications alone. It was determined that the application that increased the green herb yield the most with the fresh and dry weight of marjoram was the application of the biochar with chemical fertilizer added. On the other hand, in contrast to the addition of chemical fertilizers, it was determined that the application of biochar alone increased the volatile oil rate and the amount of carvacrol and thymol in the plant.

Keywords: aromatic plant; carvacrol; p-cymene; soil quality; thymol

Introduction

Volatile oils obtained from medicinal and aromatic plants are generally used as natural raw materials in the cosmetic, food and pharmaceutical industries. Sweet marjoram (*Origanum majorana* L.) is an aromatic plant that is widely used around the world and is economically important (Bhardwaj and Dubey, 2020). This plant is used primarily as a tea and spice in the form of fresh or dried herba. Since some marjoram species are rich in volatile oil components, a volatile oil called marjoram oil is obtained from this plant (Çinbilgel and Kurt, 2019). This oil is used as a gas remover, wound healer and antiseptic within the scope of aromatherapy. In addition, its antimicrobial, antihypertensive, blood sugar lowering and spasmolytic effects are also used (Farrar and Farrar, 2020). In many countries with a Mediterranean climate, medicinal and aromatic plants are collected

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from nature or cultivated and exported to industrial countries that process them. However, it is known that naturally distributed species of these plants are adversely affected by the aforementioned collection activities, especially due to excessive and unconscious collections (Göktaş and Gıdık, 2019).

Turkey is the world leader in thyme and marjoram exports, and annual plant exports are around 17 thousand tons. The species most commonly traded and exported in Turkey and used in volatileoil production are *Origanum onites*, *Origanum vulgare* subsp. *hirtum*, *Origanum minutiflorum*, *Origanum majorana*, *Origanum syriacum* var. *bevanii* (Pakdemirli, 2020). The common feature of all these species is that they contain a high amount of volatile oil (at least 2.5%), as well as the main components carvacrol, thymol, p-cymene, 1,8 cinaol, linalool, etc. At the same time, the content of carvacrol and thymol are the most important parameters that determine the price of plant. One of the most collected aromatic plant species from nature in the Mediterranean climate regions of Turkey is marjoram (*Origanum majorana* L.). Since it has high volatile oil yield, this type of marjoram is collected from nature located in the east of Antalya (Akseki, Alanya, Gazipaşa) and exported to Europe as a raw material in the herbal medicine industry (Aytaç, 2020). Although it is organic and has a high-quality value because it is collected from nature and exported, there is a danger of disruptions in the continuation of the export and even the extinction of the species due to excessive and unconscious collection.

In studies, it was reported that environmental factors such as direction, altitude, seasonal changes as well as factors such as plant collection time, storage conditions, drying times and temperatures are effective on volatileoil yields and components of medicinal and aromatic plants (Kızıl *et al.*, 2015). In marjoram cultivation, climate, soil characteristics, agronomic factors (plant sowing frequency, fertilization, irrigation, etc.) and harvest time are among the most important factors affecting plant volatile oil yield and oil quality (Nikou *et al.*, 2019). In addition, fertilization has an important place among the agronomic practices that most affect the number of harvests, oil yield and oil component ratios in a vegetation period in the cultivation of volatile oil-containing plants (Mota *et al.*, 2020). As a matter of fact, because it is known that chemical fertilizers adversely affect the secondary metabolites of the plant organic fertilizers are recommended rather than chemical fertilizers to increase the amount of dry matter in medicinal and aromatic plant cultivation for volatile oil yield (Chrysargyris *et al.*, 2021).

By using the pyrolysis method, plant biomass (tree wood) can be converted into a very valuable input rich in organic matter called 'biochar', which can be used as a soil conditioner or organic fertilizer for plant production (Alhashimi and Aktas, 2017). Biochar is a material with a porous structure, large surface area, high water holding and cation exchange capacity. Due to these properties, it keeps nutrients such as calcium, magnesium and potassium by enhancing the cationic activities in the soil (Akgül, 2017). They are rich in aromatic and humic substances (Lorenz and Lal, 2014). In addition, it is reported that biochar accelerates biological activity by improving soil microbial biomass (Egamberdieva *et al.*, 2022). On the other hand, it is reported that the application of biochar suppresses diseases in many plants, increases the yield with biomass in the plant, and the mineral nutrition of the plant reaches the desired levels (Graber *et al.*, 2010; Islami *et al.*, 2011; Liu *et al.*, 2013; Alburquerque *et al.*, 2014). For these reasons, biochar in agriculture can be used as a soil conditioner and/or organic fertilizer (Sun *et al.*, 2020). In this study, the effects of biochar on both the biological properties of the calcareous soil and the physical properties, volatile oil yield and oil components such as carvacrol, thymol, p-cymene of the marjoram plant were tried to be determined.

Materials and Methods

Design of experiment

Since it is known that the environmental conditions have positive effects on the volatile oil yield and quality (Mehalaine and Chenchouni, 2019), this was taken into consideration in the selection of the experiment area. Accordingly, study was carried out as a greenhouse experiment (36° 21′ 3" N; 32° 15′ 47" E, altitude 185 m) located in Alanya, Turkey. The experiment covers the months of March-August 2020, and the climate data of the greenhouse between these dates were collected. Accordingly, the highest temperature in the experimental area was 42.5 °C, the lowest temperature was 22.7 °C, and the average temperature was 32.6 °C. The highest relative humidity was 78%, the lowest relative humidity was 45%, and the average relative humidity was 63%. It was determined that the average solar radiation during the trial months was 6.85 kWh/m^2/day. In addition, it was determined that the average precipitation amount in the experiment area was around 380 mm.

Oak tree biochar (OBC) was used as an organic fertilizing material in the experiment. The material was obtained from a local charcoal facility that produces biochar from the woods of oak trees by the prolysis method in the forest area managed by the Alanya District Agriculture and Forestry Directorate. The analysis results of the biochar used in the study and the soil in the area where the experiment was established are shown in Table 1. While planning the application rates of biochar to the soil, the application rates (20-40 t ha^{-1}) recommended by Baydar (2019) for farm manure in marjoram cultivation were taken into account. In addition, in order to meet the nutrients needed by the marjoram plant, 15.15.15 N.P.K. composite-chemical fertilizer (CF) was applied in the amounts recommended by Baydar (2019), but by calculating the missing nutrients of the experiment soil according to the analysis result. The study includes 8 biochar applications with 3 replications planned as factorial in a randomized plot design. Biochar applications are as follows: [0 t ha^{-1} OBC- control (OBC-0), 20 t ha^{-1} OBC (OBC-2), 40 t ha^{-1} OBC (OBC-4), 60 t ha^{-1} OBC (OBC-6), 0 t ha^{-1} OBC + chemical fertilizer (OBC-0+CF), 20 t ha^{-1} biochar + chemical fertilizer (OBC-2+CF), 40 t ha^{-1} biochar + chemical fertilizer (OBC-4+CF), 60 t ha^{-1} biochar + chemical fertilizer (OBC-6+CF)].

Table 1. Properties of the soil and biochar used in the experiment

Analysis parameters		Soil	Biochar
Texture		Loam	-
pH		8.1	10.1
EC (μS cm^{-1})		875	3158
Lime (%)		27.0	-
Moisture (%)		3	22
Organic matter (%)		1.52	76
Carbon/nitrogen ratio		8/1	28/1
Total (%)	C	0.88	44.08
	N	0.11	1.6
	P	0.005	0.8
	K	0.004	3.6
	Ca	0.4	0.3
	Mg	0.02	0.1
Number of bacteria (cfu g^{-1} dw)		1.9×10^6	2.1×10^8
Dehydrogenase activity (μg TPF g^{-1} dw h^{-1})		16.12	89.57
Nitrification activity (μg NO_2^--N g^{-1} dw h^{-1})		21.18	85.12
Urease activity (μg NH_4^+-N g^{-1} dw h^{-1})		51.27	183.28
Alkaline phosphatase activity (μg PNP g^{-1} dw h^{-1})		98.17	134.34
β-glycosidase activity (μg PNG g^{-1} dw h^{-1})		29.52	167.33

Experiment management and collection of samples

In the experiment, seeds of a regional marjoram (*Origanum majorona* L.) variety, which were previously collected and dried from the 150-300 altitude regions of Alanya, where marjoram is naturally distributed, were used. Afterwards, marjoram seedlings were obtained by using these seeds. Peat, perlite and vermiculite, which are widely used for seedling growing media, were preferred. The ratios of these materials to each other were mixed to be 3:1:1, respectively, and moistened around 80%. This prepared moist mixture was transferred to viols with 216 holes and it was ensured that all the holes were filled with the mixture. Then, marjoram seeds were placed at a depth of about 0.5-1 cm and at least 15-20 seeds were placed in each hole. After the seeds were placed, they were covered with vermiculite again and watered to moisten them well. After the viols prepared in this way were covered with cling film, they were left to germinate for 1-2 days in a dark room (20-25 °C). When the first seedling emergence was observed, the viols were moved to a 100 m^2 seedling growing greenhouse. Irrigation, fertilization and other practices were carried out by paying attention to the optimum level of some parameters such as root: stem ratio, root length, stem thickness, the root tightly surrounding the growing medium and the number of true leaves. Accordingly, it was ensured that the seedlings were ready in about 30 days.

Before the greenhouse experiment, the soil was cleared of weeds by tilling and the preliminary preparation was completed after leveling. Then, in accordance with the experiment plan, 2.5 m^2 plots (0.5 m wide, 5 m long and approximately 30 cm high) were created. In order to minimize the interaction of the experiment subjects with each other, approximately 1 m gaps were left between the plots. The chemical fertilizer and biochar (according to the dry matter calculation) were weighed, adhering to the applications detailed above. Afterwards, biochar and chemical fertilizer applications were homogeneously mixed into the plots by using a shovel and rake. The previously obtained seedlings were planted in such a way that at least 20 plants (25 cm on rows, 50 cm between rows) were planted in each plot, and the experiment process was started by giving first water through the drip irrigation system. All agronomic practices were carried out meticulously during the approximately 140-day vegetation period from seedling planting to harvest. In this context, weed cleaning was carried out on the plots regularly. Drip irrigation was applied to the plants equally in each plot until each flowering and before harvesting. For this, after calculating the total amount of water missing from the precipitation, the moisture status of the soil, the wilting symptoms of the plant and the solar radiation data were monitored and irrigation was done every 4-5 days. The first harvest was carried out approximately 40 days after planting and a total of 4 harvests were made. As reported by Baydar (2019), mowing's were carried out 8-10 cm above the soil surface when the plants reached the 80% flowering period, when the amount of volatile oil is highest. Both soil and plant samples were taken at each harvest, and measurements and analyzes were made on them. The greenhouse experiment was terminated after all harvests, sampling and measurements were completed.

Analysis of biochar and soil

The following analyzes were carried out on the biochar used as organic fertilization material in the experiment. The organic matter content was determined according to the oven drying method, and the total organic carbon (C) was determined according to the wet oxidation method (Black, 1965). pH and EC values were determined in a 1:10 ratio by biochar: distilled water mixture with a pH-EC meter (Jackson, 1967; Consort C1010). Total nitrogen (N) determination was determined according to the modified Kjeldahl method (Kacar, 1995; Gerhardt Vapodest 300). Total phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined according to the wet decomposition method (Kacar and İnal, 2008). Accordingly, after the filtrates were obtained the concentrations of the mentioned elements were determined by ICP-OES (Perkin Elmer 7100DV).

At the beginning of the greenhouse experiment, fertility analyzes were made in the soil sample taken from 0-30 cm depth. After the test soil was air-dried for chemical analysis, it was sieved through a 2 mm sieve (10 mesh) and made ready for analyzes. The analyzes made are as follows: Texture (Bouyoucos, 1951; Hamilton Beach-CS), lime (Çağlar, 1949; Eijkelkamp C-08.53), pH and EC (Jackson, 1967; Consort C1010). The determination of soil total organic carbon and organic matter were based on the Walkley-Black chromic acid wet oxidation method (Black, 1965). Total N was calculated as described above for biochar. Available P was determined by the Olsen method (Olsen and Sommers, 1982; Perkin Elmer Lambda 25 UV-VIS). The exchangeable K, Ca, Mg were determined by the ammonium acetate method (Kacar, 1995; Perkin Elmer ICP-OES 7100DV).

Biological properties were analyzed in soil samples taken from 0-15 cm depth in each harvest. The samples taken for the analyzes were brought to the laboratory quickly without losing moisture and were kept under protection at +4 °C. The following analyzes were carried out to determine the biological properties of the soils obtained during the harvests. Dilution-plate counting method, which is a cultural counting method, was used to determine the total number of aerobic mesophilic heterotrophic bacteria in the seedling growing media (Parkinson et al., 1971). For dehydrogenase activity, 5 ml of TTC solution was added onto 5 g of moist soil and incubated at 30 °C for 24 hours. After incubation, 40 ml of acetone was added to the mixture and left in the dark for 2 hours, and the intensity of the red color that emerged after the suspension was filtered and measured at 546 nm (Thalmann, 1968). For nitrification activity, 0.1 mL of sodium chlorate solution and 20 mL of ammonium sulfate solution were added onto 5 g of moist soil. Then, after 5 hours of incubation at 25 °C, 5 mL of KCl solution was added and filtered. The resulting color intensity after adding the color solution was measured at 520 nm (Schinner *et al.*, 1995).

For urease activity, 0.2 mL of toluene, 7.5 ml of citrate buffer solution and 10 ml of urea solution were added onto 10 g of soil sample. This mixture was incubated for 3 hours at 37 °C. The final volume was made up to 100 mL and filtered. The released ammonium after adding the color solution was measured at 578 nm (Hoffman and Teicher, 1961). For alkaline phosphatase activity, 0.2 ml of toluene on 1 g of soil, 4 ml of MUB and 1 ml ρ-nitrophenyl phosphate were added on 1 g of soil. This mixture was incubated for 1 hour at 37 °C, after incubation, 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH were added and filtered. The intensity of yellow color formed was determined at 410 nm (Tabatabai and Bremner, 1969). To determine the β-glucosidase activity, 0.2 ml of toluene, 4 ml of MUB (12.1 g tris, 11.6 g maleic acid, 14.0 g citric acid and 6.3 g boric acid / 1 L, pH 6) and 1 ml PNG (ρ-nitrophenyl-β-D-glucoside: 0.654 g β-D glucoside / 50 ml MUB) solution were added on 1 g of soil. After incubation at 37 °C for 1 hour, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.1 M THAM buffer solution were added to the sample and filtered. The intensity of yellow color was determined at 410 nm (Eivazi and Tabatabai, 1988). Sample readings on all enzyme activities were performed on a spectrophotometer (Perkin Elmer Lambda 25 UV-VIS). Data were expressed on the basis of the oven-dry weight of the soil.

Measurement and analysis of plant

The foreign materials on the plant were removed by pre-cleaning the plant materials obtained during the harvests. Drying processes were carried out in a drying oven (Venticell-404) with air circulation (7.272 m3/hour) adjusted to 40 °C in 48 hours. Then, the physical properties of the plant, the details of which are given below, and the volatile oil ratio and oil components of the plant samples were determined. For this, transactions (measurement, weighing, calculation, etc.) were performed on 5 plants obtained from each replication plot and the results were given as their average. According to this; physical properties such as the number of branches (pieces), flower spike length (cm), plant height (cm), fresh weight (g), dry weight (g) and green herb yield (kg ha^{-1}) were determined.

To obtain volatile oil, 100 g of dry plant material was distilled for 90 minutes using a clavenger apparatus and the yield of oil was recorded at every 5 min. After hydrodistillation, water was removed by decantation and the volatile oil obtained was stored at 4 °C in a dark-colored container to prevent light-sensitive decomposition. The obtained volatile oil rate was given as %. Quantitation of essential oil components was performed by gas chromatography (GC; Agilent 7890A). Analyzes were performed on an HP Innowax Capillary MS (30 Meters 0.25 mm ID 0.25 µm df). The temperature program was set as follows: 40-180 °C (3 °C min-1), 180-240 °C (20 °C min-1), 240 °C (20 min). Helium (0.4 µL injection volume, diluted 1:10 in n-hexane) was used as the carrier gas in the process and the injector temperature was set to 250 °C. Mass spectrometry (GC-MS; Agilent 5975C) was used as a detector for the chemical characterization of essential oils of carvacrol, thymol and p-cymene. For this, the ion source temperature was set to 200 °C, the interface temperature to 250 °C, and the solvent cut-off time was set to 4 minutes (Özek *et al.*, 2010).

Statistical analysis

The laboratory analyses results of the soil and plant samples were statistically evaluated using the SPSS 17.0 package program. In this context, the significance of the results (at the level of 5%) was determined by repeated measurement analysis (*r*ANOVA). Significant results were graded by lettering with Duncan's multiple comparison test. In addition, the relationships between soil and plant parameters were determined by the Pearson correlation test (SPSS, 2008).

Results and Discussion

Number of bacteria

The changes in the bacterial numbers of the soil samples taken during the harvest periods in the soil where marjoram is grown are shown in Figure 1. Accordingly, visible changes were observed in the number of bacteria depending on the harvest period and the applications. In this context, as of the second harvest, it was observed that all applications increased the number of bacteria more than the control. On the other hand, the effect of the interaction between the applications and the harvests on the number of bacteria was found to be statistically significant at the $p < 0.05$ level. At this point, it was determined that OBC-4 and OBC-6 applications increased the number of bacteria more than all other applications (Table 2). The low humus content (1.52%) of the experiment soil appears to be poor characteristics for microbial biomass and microorganism diversity. However, as the biochar used is a good source of organic matter (36%), contains a high amount of bacteria ($2.1\text{x}10^8$ cfu g^{-1} dw), and the C/N ratio is wide (28/1), the number of bacteria in the soil has increased in a short time. The number of bacteria present in the soil is an indicator that significantly affects soil fertility and the availability of nutrients required for plants. Adding organic matter to the soil affects this parameter very positively. As a matter of fact, it was reported that soil microbial biomass or bacterial numbers increase with various organic matter sources such as biochar (Uz *et al.*, 2016; Sheng and Zhu, 2018; Cooper *et al.*, 2020).

Table 2. Effects of applications on soil biological properties

Treatment	Number of bacteria (cfu g^{-1} dw x10^6)	Dehydrogenase activity (µg TPF g^{-1} dw h^{-1})	Nitrification activity (µg NO_2^--N g^{-1} dw h^{-1})	Urease (µg NH_4^+-N g^{-1} dw h^{-1})	Alkaline phosphatase activity (µg PNP g^{-1} dw h^{-1})	β-glycosidase activity (µg PNG g^{-1} dw h^{-1})
OBC-0	1.3c[1]	6.63c	4.28d	6.82c	18.36d	8.08d
OBC-2	2.0b	6.72c	7.14b	30.96a	51.79a	16.95c
OBC-4	2.6a	8.03a	9.36a	30.65a	39.74c	17.04c
OBC-6	2.5a	6.91b	7.52b	25.59b	38.41c	26.24a
OBC-0+CF	2.0b	6.92b	7.19b	25.14b	38.03c	22.36ab
OBC-2+CF	1.9b	6.87bc	7.10b	25.17b	42.53b	19.61b
OBC-4+CF	2.1b	6.88bc	5.90c	23.80bc	43.12b	18.97bc
OBC-6+CF	1.9b	6.95b	6.97bc	25.65b	43.42b	20.82b
*r*ANOVA (LSD %5)						
Harvest time (H)	8.182**	1.387*	22.598***[2]	5.637**[3]	51.386***	11.027**
Treatment (T)	3.241*	5.029*	29.176***	7.596*[4]	26.751***	8.367**
H x T	1.135*	Ns	18.038***	Ns[5]	9.384**	7.367**

[1] Means in the same column followed by the same letter are not significantly different (LSD test, $p < 0.05$), [2] ***$p < 0.001$,

[3] **$p < 0.01$, [4] *$p < 0.05$, [5] Ns: Not significant.

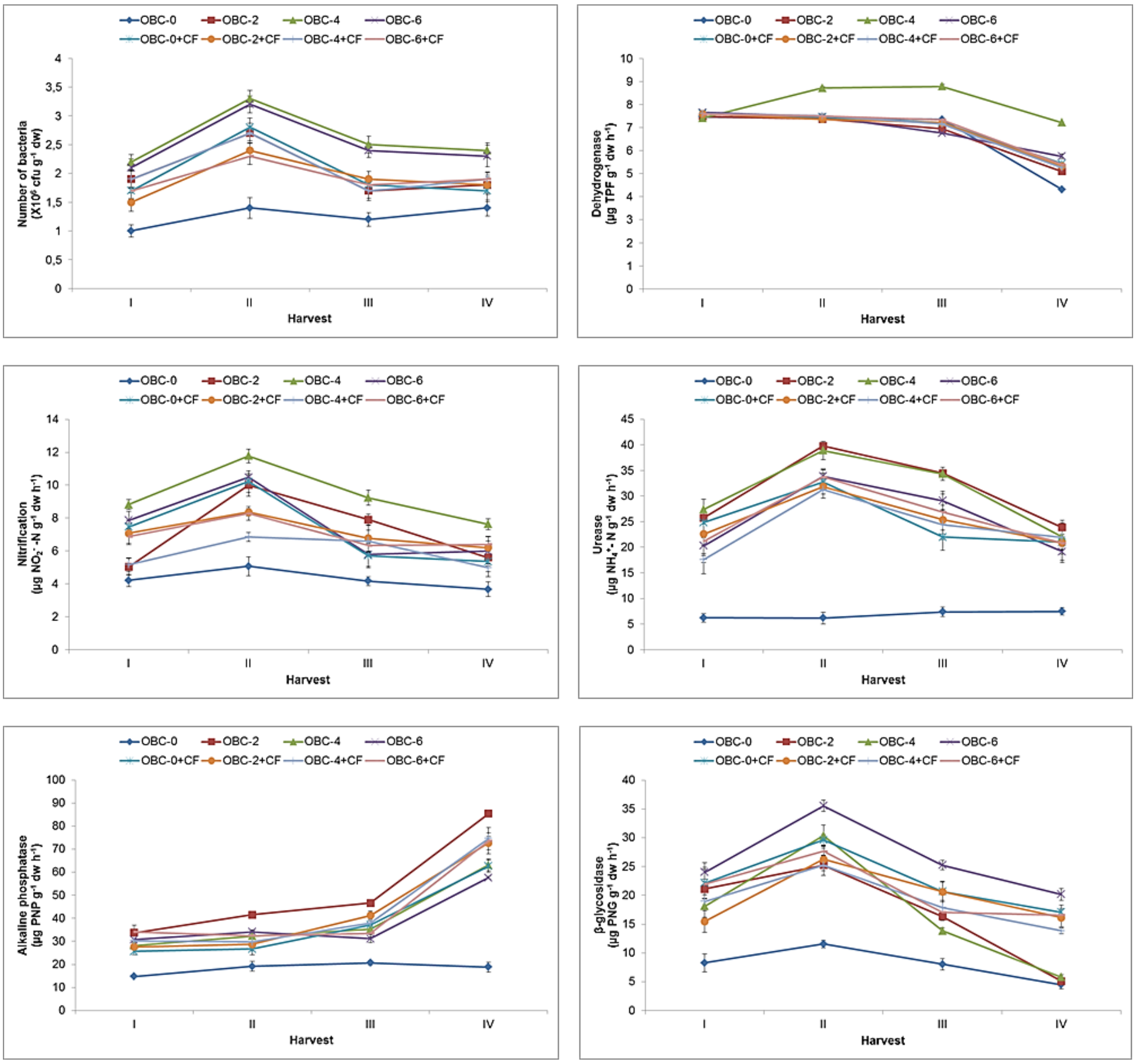

Figure 1. Changes in the soil biological properties during the growth season. Error bars represent standard errors based on three replicates

Dehydrogenase activity

It was observed that all applications, except one (OBC-4), had a similar effect on the dehydrogenase activity of the soil (Figure 1). However, it was observed that dehydrogenase activity showed a significant decrease in the fourth harvest, unlike the other harvests. The effects of the interaction between applications and harvests on the dehydrogenase activity of the soil were found to be statistically insignificant. However, it was determined that OBC-4 application increased the dehydrogenase activity statistically more than other applications according to the average values of the applications related to the harvest periods (Table 2). Among the important factors that direct soil fertility are the enzymes secreted by soil microorganisms and the activities of these enzymes (Patel *et al.*, 2015). Dehydrogenase is an intracellular enzyme that displays the total amount of oxidative activity of soil microflora. By measuring this enzyme activity, the living microbiological activity of the soil can be determined (Tiwari *et al.*, 2019). In the experiment, it was observed that biochar applications also increased the dehydrogenase activity similar to the number of bacteria. The high carbon content (44.08%) and high enzyme activity (89.57 µg TPF g^{-1} dw h^{-1}) of the biochar applied to the test soil with low productivity in terms of many chemical and biological properties can explain the situation. On the other hand, the decreasing trend of enzyme activity as the harvesting period progresses may be due to the increasing mineral nutrients despite the decreasing organic matter amount. As a matter of fact, it is reported that the increase in mineral nutrients in the soil inhibits dehydrogenase activity (Futa *et al.*, 2021).

Nitrification activity

According to the course of the data obtained in the harvests, it was observed that all applications in the second harvest increased the nitrification activity of the soil compared to the control (Figure 1). On the other hand, it was determined that the effects of the interaction between applications and harvests on the nitrification activity were statistically significant at the $p < 0.001$ level. At this point, considering the average values of the harvest periods, it was determined that OBC-4 application increased the nitrification activity of the soil more than the others (Table 2). Nitrification activity, which directs nitrate availability in soil, is in close relationship with important soil properties such as organic matter, texture, pH, O_2 and available water (Norton and Ouyang, 2019). In addition, it is known that organic matter sources added to soils increase nitrification significantly (Leptin *et al.*, 2021). In addition to the wide C/N ratio (28/1) of the biochar used in the experiment, the pH (8.1) and texture (loam) properties of the experiment soil, especially suitable for bacterial activities, may have increased the nitrification activity. It is also noteworthy that the current nitrification activity of biochar is approximately 4 times higher than that of the experiment soil. It was reported that organic nitrogen mineralization and nitrification increase in soil with biochar application (Shah *et al.*, 2020). In this study, it was determined that the addition of biochar increased the nitrification activity of the soil, and this increase was mostly caused by biochar. However, it was reported that biochar at high application rate reduces nitrification in soil due to its wide C/N ratio and soil N content (Tsai and Chang, 2021).

Urease activity

The urease activity values of the soil were measured higher than the others in the second harvest for all applications except the control (Figure 1). The effects of the interaction between applications and harvests on urease activity were found to be statistically insignificant. However, it was determined that OBC-2 and OBC-4 increased the urease activity the most according to the average values of the harvests (Table 2). The increase in soil enzyme activity is in parallel with the increase in soil organic matter. This proves that the enzymes are linked to the population dynamics of the soil biota (Simon and Czako, 2014). It was reported that the increase in urease activity with the addition of organic matter source to the soil is closely related to the nitrogen content of the added material, and that nitrogen acts as a substrate for the urease enzyme and promotes the activity of this enzyme (Jing *et al.*, 2020). The fact that the biochar used in this study is rich in both nitrogen (1.6%) and organic matter (76%) reveals that the increase in the urease activity of the soil is due to biochar. In addition, the fact that the current urease activity of the material is approximately 3.5 times higher than that of soil may also be an important factor. Accordingly, it is reported that biochar application significantly increases the urease activity of the soil and is responsible for nitrogen enrichment in the soil (Jatav *et al.*, 2021). On the other hand, it can be seen that biochar applications with the addition of chemical fertilizers suppress the enzyme activity.

Alkaline phosphatase activity

It was observed that the alkaline phosphatase activity of the soil was higher with biochar applications than the control during all harvests. In addition, it is observed that alkaline phosphatase activity of the soil reaches the highest levels in the fourth harvest (Figure 1). The interaction between the applications and harvests on alkaline phosphatase activity was determined to be statistically significant at the $p < 0.01$ level. At this point, it was determined that the OBC-2 application increased the alkaline phosphatase activity more than the other applications, according to the average values of the harvest periods (Table 2). There is a close relationship between soil organic matter and phosphatase activity (Oliveira and Ferreira, 2014). In addition, it is known that organic fertilizers, which are widely used to increase soil organic matter, increase the activity of this enzyme by acting as a substrate for the phosphatase enzyme (Tamilselvi *et al.*, 2015). In addition to having a wide C/N ratio (28/1) of the biochar used in this study, it is seen that it is a material rich in total P (0.8%) and organic matter (76%). In addition, the pH (8.1) of the soil may have a positive effect on the enzyme's activity. With the application of biochar, the increasing organic P in the soil stimulated the microorganisms and the enzyme activity seems to have increased accordingly. In line with this result, studies reporting that biochar is responsible for the increase in alkaline phosphatase activity of the soil are available in the literature (Trupiano *et al.*, 2017; Oladele, 2019; Dai *et al.*, 2021).

β-glycosidase activity

It was observed that the β-glycosidase activity of the soil was at its highest values in the second harvest (Figure 1). However, the effect of the interaction between applications and harvest periods on β-glycosidase activity was found to be statistically significant at the $p < 0.01$ level. In addition, considering the average values of the applications regarding the harvest periods, it was determined that the OBC-6 application increased the β-glycosidase activity of the soil more than the other applications (Table 2). By measuring soil enzyme activities, the effects of fertilization, other agronomic activities in the soil and climate factors on soil fertility can be evaluated (Zhen *et al.*, 2014). As with other extracellular enzymes, β-glycosidase activity also affects the organic matter, pH, temperature, humidity, microorganism activities, etc. in the soil (Almeida *et al.*, 2015). The β-glucosidase enzyme is an extracellular secretion synthesized by soil microorganisms in order to convert a complex organic compound such as cellulose into glucose, a form suitable for plants or other organisms (Srivastava *et al.*, 2019). In the study, it is thought that biochar's wide C/N ratio (28/1) as well as being rich in organic matter (76%) rapidly stimulate the activity of this enzyme in the soil. In addition, the fact that the enzyme activity of biochar is approximately 5.5 times higher than the experimental soil may also be effective in this. Indeed, results were reported that biochar and similar materials increase β-glycosidase activity (Martins Filho *et al.*, 2021; Becagli *et al.*, 2022). On the other hand, the drastic decrease in enzyme activity in advanced harvests may have resulted from increased mineral nutrients or toxic compounds (alkaloids, phenolics, etc.) in the soil.

Physical properties of plant

The effect of the applications on the physical properties of the plant is seen in Figure 2. The effects of the applications on the properties of the plant such as the number of branches, flower spike length and plant height were found to be statistically insignificant (Table 3). On the other hand, a similar course was observed between the parameters related to the fresh weight, dry weight and green herb yield of the plant during the harvest periods. Accordingly, the highest values were measured in the second harvest in all three parameters (Figure 2). According to the statistical results, it was observed that the interaction between harvests and applications on the mentioned parameters was significant at the $p < 0.05$ level, and OBC-4+CF application increased these parameters the most (Table 3).

When organic matter sources are added to the soil in sufficient quantities, they primarily help to improve the physical properties of the soil such as structure, aeration, water retention, heating and permeability (Ninh *et al.*, 2015). Afterwards, they increase the adsorption of nutrients by expanding the surface area under the combined influence of the biological and chemical properties of the soil. At the same time, they increase the availability of nutrients by regulating the soil pH, and thus, they help the plant growing on it to develop its physical properties better by providing a balanced nutrition (Hewidy *et al.*, 2015). It provides a basis for the correct functioning of the physicochemical and biochemical processes within the plant, with good physical properties for many cultivated plants. In this way, productive plants and quality products emerge (Hu *et al.*, 2014). In this context, it is reported that the application of biochar to the soil positively affects the physical properties of many plants and increases the yield (Ding *et al.*, 2016; Oni *et al.*, 2019; Usevičiūtė *et al.*, 2022). In this study, it was determined that the physical properties of the plant were positively affected by the application of biochar, in accordance with previous studies. Although this material has a wide C/N ratio (28/1) and a relatively high pH (8.1), it was shown to be beneficial for plant growth because it is nutrient rich and balanced. However, it was determined that the effect of this material on plant growth and yield increased by 1.2 times when supplemented with chemical fertilization. Undoubtedly, the fact that not only soil fertility parameters but also abiotic factors such as climate are suitable for this situation is undoubtedly a major factor. As a matter of fact, it is known that increased temperature and relative humidity positively stimulate the development of many plants (Islam *et al.*, 2021).

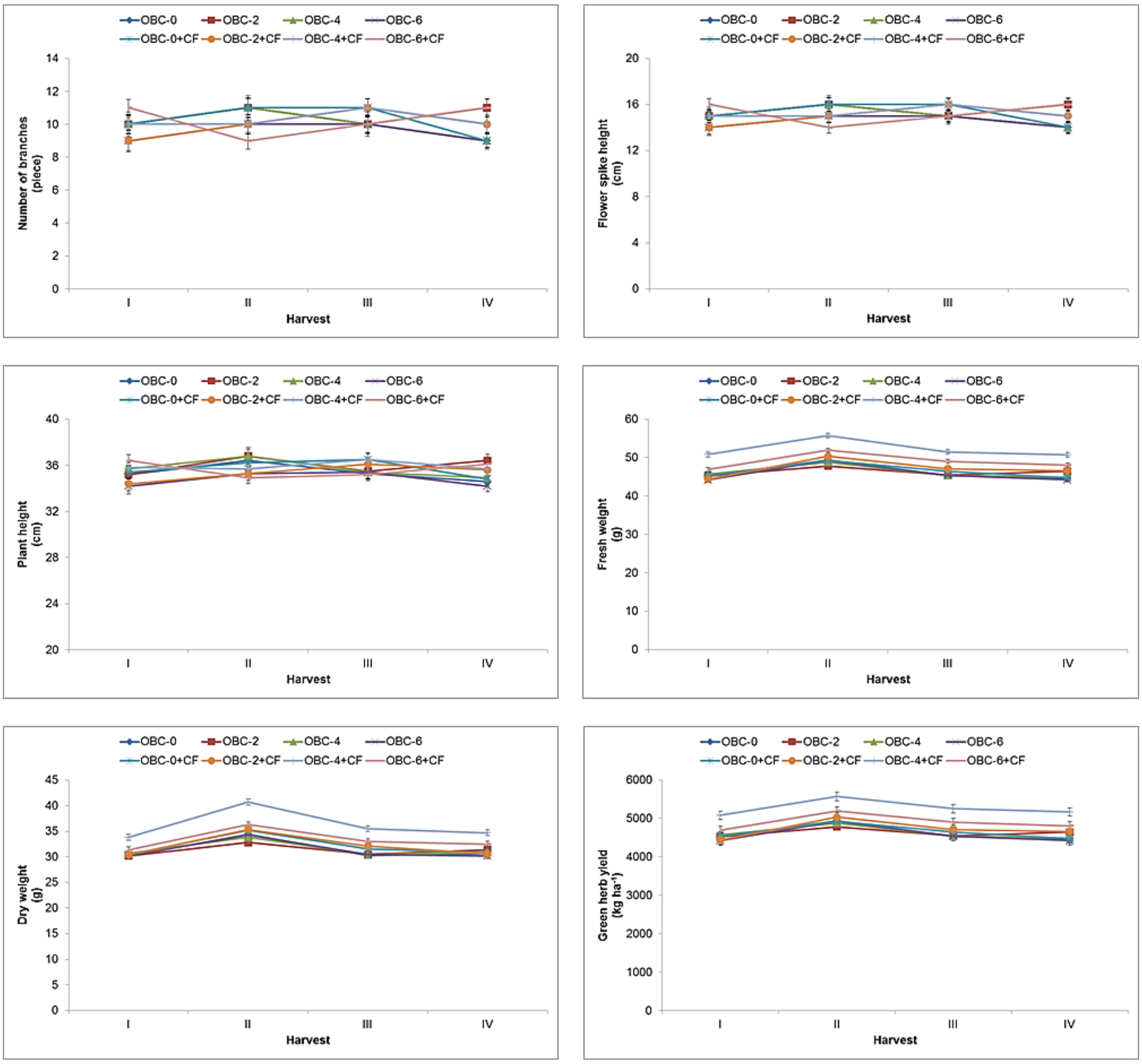

Figure 2. Changes in the plant physical properties during the growth season. Error bars represent standard errors based on three replicates

Volatile oil yield and oil components

The effect of the applications on the volatile oil yield and oil components of the plant is shown in Figure 3. According to this, although the volatile oil ratios do not show a significant change trend between the harvest periods, the highest volatile oil ratio was obtained in the third harvest. On the other hand, the interactions of harvests and applications were found to be statistically significant at the $p < 0.05$ level. Accordingly, it was determined that the OBC-4 application was the most effective on the volatile oil yield of marjoram (Table 4). Similarly, it was reported that organic fertilization is beneficial on volatile oil yield in many medicinal and aromatic plants (Singh *et al.*, 2015; Mota *et al.*, 2020).

Table 3. Effects of applications on the physical properties of the plant

Treatments	Number of branches (piece)	Flower spike height (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Green herb yield (kg ha^{-1})
OBC-0	10.00	15.00	35.38	46.13b[1]	31.38c	4612.50bc
OBC-2	10.50	15.50	35.98	46.23b	31.23c	4622.50bc
OBC-4	10.00	15.00	35.70	46.20b	31.45c	4620.00bc
OBC-6	9.50	14.50	34.78	45.78c	31.23c	4577.50c
OBC-0+CF	10.25	15.25	35.73	46.48b	31.98c	4647.50bc
OBC-2+CF	10.00	15.00	35.35	47.10b	32.10bc	4710.00b
OBC-4+CF	10.25	15.25	35.93	52.18a	36.18a	5267.50a
OBC-6+CF	10.25	15.25	35.65	48.97b	33.30b	4897.50b
*r*ANOVA (LSD %5)						
Harvest time (H)	Ns	1.113*	1.075*	3.012*	9.675**[2]	4.153*[3]
Treatment (T)	Ns	Ns	Ns	2.156*	2.472*	12.346**
H x T	Ns	Ns	Ns[4]	1.897*	2.157*	3.861*

[1] Means in the same column followed by the same letter are not significantly different (LSD test, $p < 0.05$),

[2] **$p < 0.01$, [3] *$p < 0.05$,

[4] Ns: Not significant.

However, it was reported that nutrition with cations such as K, Ca and Mg rather than N and P increases the ratio of volatile oil in aromatic plants and is beneficial in optimizing the concentrations of oil components (NurzyĔska-Wierdak, 2013). From this point of view, the biochar used in the experiment is a material rich in the mentioned cations (K: 3.6%, Ca: 0.3%, Mg: 0.1%). In addition, the fact that the soil on which biochar is applied has supportive properties [texture (loam), pH (8.1), lime (27%)] in the uptake of the mentioned nutrients to the plant explains this situation.

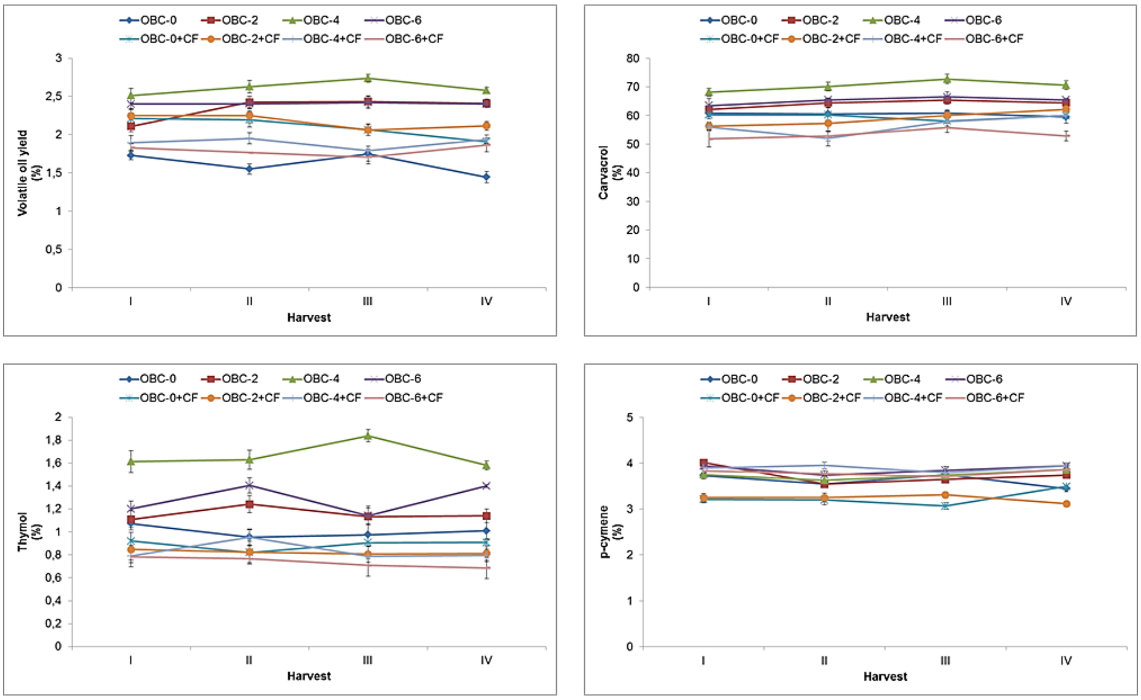

Figure 3. Changes in the volatile oil yield and oil components during the growth season. Error bars represent standard errors based on three replicates

Table 4. The effects of the applications on the volatile oil yield and components of the volatile oil

Treatments	Volatile oil ratio (%)	Carvacrol (%)	Thymol (%)	p-cymene (%)
OBC-0	1.62d[1]	60.37bc	1.00bc	3.62
OBC-2	2.35ab	64.10b	1.16b	3.73
OBC-4	2.62a	70.38a	1.67a	3.74
OBC-6	2.41ab	65.16b	1.29b	3.87
OBC-0+CF	2.10b	59.60c	0.89c	3.24
OBC-2+CF	2.17b	58.92c	0.82c	3.23
OBC-4+CF	1.89c	56.39c	0.83c	3.89
OBC-6+CF	1.79c	53.29d	0.74d	3.79
*r*ANOVA (LSD %5)				
Harvest time (H)	8.781**	15.562***[2]	3.472*[4]	Ns[5]
Treatment (T)	3.489*	3.457*	2.451*	Ns
H x T	2.783*	9.123**[3]	1.387*	Ns

[1] Means in the same column followed by the same letter are not significantly different (LSD test, p < 0.05), [2] ***p < 0.001, [3] **p < 0.01,
[4] *p < 0.05, [5] Ns: Not significant.

It was determined that the interactions of harvesting periods and applications on volatile oil components were important for carvacrol and thymol (p < 0.01, *P* < 0.05, respectively). At this point, as in the volatile oil ratio, OBC-4 was the application that increased carvacrol and thymol the most (Table 4). In addition, with this application, the highest carvacrol and thymol values were observed in the third harvest (Figure 3). On the other hand, the effects of the interactions of the treatments alone or during the harvest periods on p-cymene were found to be statistically insignificant. It is known that not only the oil content, but also the components of the volatile oil are important in the plants from which volatile oil is obtained (Göze *et al.*, 2016). As a matter of fact, the components of the marjoram such as carvacrol, thymol, p-cymene are used for phytotherapy and aromatherapy (Lombrea *et al.*, 2020). In addition, it is reported that it is important for marjoram volatile oil to be around 2-2.5% and to contain 60-75% carvacrol, 1-2% thymol, 3-5% p-cymene (Başer *et al.*, 1993). In this context, it was seen that the application of biochar has the potential to reach the desired quality of the volatile oil components.

Correlations between the biological properties of the soil and the physical properties of marjoram, volatile oil content and oil components were analyzed. According to this; a positive correlation was found between the urease activity of the soil and the green herb yield of the plant (r=0.215 and r=0.217, respectively; p < 0.001). In addition, a positive correlation was found between the β-glycosidase activity of the soil and the dry weight of the plant (r=0.242 and r=0.257, respectively; p < 0.01). On the other hand, positive correlations (r=0.253 and r=0.298, r=0.286, p < 0.001, respectively) were found between the bacterial count and dehydrogenase activity of the soil and the volatile oil content of the plant.

Conclusions

Due to the negative effects of excessive vegetative growth on secondary metabolites, the use of organic fertilizers comes to the forefront rather than the use of chemical fertilizers in medicinal and aromatic plant cultivation. Especially if aromatic plant cultivation is carried out for volatile oil yield and oil quality, the use of chemical fertilizers should either be limited or avoided. However, the time, amount and characteristics of organic fertilizer use should also be evaluated in accordance with the demands of these plants. In this context, it may be possible to improve the biological properties of the soil and to grow plants close to the natural environment by using important organic material resources such as biochar in the cultivation of medicinal and aromatic plants in an economical way. In the study carried out with this approach, biochar was applied to the soil at low, medium and high rates alone and by adding chemical fertilizers. Afterwards, the effects of the applications on the biological properties of the soil where marjoram is grown and the physical properties of the plant, volatile oil yield and oil components were investigated. Accordingly, it was determined that the number of bacteria and enzyme activities (dehydrogenase, urease, alkaline phosphatase, β-glycosidase) of the soil reached with biochar applications (2, 4, 6 t ha^{-1} OBC) alone. It was determined that the application that increased the marjoram fresh weight, green herb yield and dry weight the most was OBC-4+CF. On the other hand, it was determined that OBC-4 application increased the volatile oil ratio and volatile oil components the most. According to the correlation analysis, as the soil biological properties improved, the volatile oil yield and oil quality of the plant also increased. These results reveal that in calcareous soil conditions dominated by the Mediterranean climate, the application of biochar alone and at a medium level (40 t ha^{-1}) may be sufficient to improve soil biological properties, increase volatile oil yield and obtain optimized volatile oil components. On the other hand, it is clear that there is a need for long-term studies in which factors such as climate, soil and production model differ in order to increase the reliability of the results and to expand the use of biochars with different properties.

Authors' Contributions

The experimental design, management, experiments and data analysis of this article, its preparation according to the journal writing rules, and its editing were done by IET. The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The author declares that there are no conflicts of interest related to this article.

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