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# Effect of bio-fertilizers on some physiological traits and biochemical indicators of mint *(Mentha spp.)* grown in two soil textures

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**Abstract**
The following study was conducted in the wooden shade of the forestry department, College of agriculture and forestry, university of Mosul, in the agricultural season of 2025-2026. To estimate the effect of bio-fertilizers on some physiological and biochemical processes in two types of mint (*Mentha*) planted in two different soil types (clay and sand). The results of this study showed that treating mint with bio-fertilizers increased the Characteristics of the plant as in: (plant height, leaf area, soluble sugars content, chlorophyll A content, chlorophyll B content, and percentage of volatile oil), with an increase of the percentage as: (52.5%, 44.1%, 215.5%, 93.5%, 75.8%, and 66.4%), respectively. Bio-fertilizer treatment also helps getting rid of negative effects of antioxidant enzyme activity (SOD and POD) in mint for both types, with a reduction rate of 22.2% for superoxide dismutase and 45.7% for peroxidase. Furthermore, plants grown in clay loam soil showed excellent results in most physiological, and biochemical Characteristics compared to the plants that were grown in sandy soils, while the results showed a clear superiority of the domestic type over the wild type in most of the studied traits.

**Keywords:** Bio-fertilizers, *Mentha*, clay loam soil, sandy soil

## Introduction

A bio-fertilizer is a substance that contains beneficial microbial organisms such as bacteria, fungi, or algae which improves the fertility and the ability of the soil to support the growth of plants when implanted in it, it also increases the amount of water and nutrients absorbed from the soil (Giri *et al*, 2019) [14]. In addition, bio-fertilizers are environmentally friendly compared to chemical fertilizers, because they significantly reduce environmental pollution and also maintain soil fertility (Areej *et al*, 2024) [8]. The excessive use of chemical fertilizers to increase food production has harmful effects to the ecological balance and human health, in addition to their high cost for many farmers (Fasusi *et al*, 2021) [12]. Plants benefit from only small proportions of the nutrients present in chemical fertilizers, while a larger proportion precipitates due to metallic cations present in the soil.

Moreover, the excessive and inappropriate use of the chemical fertilizers can lead to environmental problems that are a major concern for farmers, thus there is a great need to adopt harmless agricultural practices (Babalola, 2010) [7]. The production and transportation of chemical fertilizers, also requires the use, and combustion of fossil fuels resulting in the emission of ($CO_2$ and N) into the air which then deposit in terrestrial ecosystems (Giri *et al*, 2019) [14]. As the micro-organisms produces auxins they are used bio-fertilizers Because of the ability of the substance to resist harsh environmental conditions such as salinity and drought, auxins are also used as growth promoting substances to stimulate root growth (Abdallah *et al*, 2009) [2]. Among the advantages of using biofertilizers is that they reduce the use of chemical fertilizers, which have negative side effects on the soil (Woźniak & Siebielec, 2025) [32]. Bio-fertilizers increase microbial activity, and improves soil texture, therefore they contribute to the physical and biological properties of the soil, leading to an increased agricultural crop productivity, and making the plans more resistant to environmental conditions (Mahanty *et al*, 2017) [22]. These fertilizers consist of well-chosen strains of beneficial microorganisms such as mycorrhizae which lives in a symbiotic state with plant roots, forming hyphae that attaches to the roots and extends farther into the soil more than the roots themselves to absorb larger amounts of water and nutrients such as phosphorus; in return, the plant provides carbohydrates to the fungus (Pooja *et al*, 2025) [27]. In addition to mycorrhizae Azotobacter and Rhizobium bacteria which are nitrogen fixing microorganisms that converts the nitrogen present in the soil to simpler compounds such as ammonia that the plants can absorb are also present in bio-fertilizers to increase soil fertility (Aasfar *et al*, 2024) [8]. Pseudomonas and Bacillus (bacteria), Penicillium and Aspergillus (fungi) are phosphorus-solubilizing microorganisms that converts or dissolves inorganic phosphorus present in the soil, which is un-absorbable for plants, into phosphate ions that plants can easily absorb (Alori *et al*, 2017) [5].

As for the potassium solubilizing fertilizers, they are known to be containing microorganisms that are capable of liberating potassium found in the soil, and converting it to a compound that can be easily absorbed by plants, like Frateuria and Bacillus (Dobhal *et al*, 2019) [11]. As for the mint, some studies showed that bio-fertilizers can affect the physiological traits that support the plan's growth, and its ability to tolerate environmental conditions. Bio-fertilizers significantly affect chlorophyll (A&B), they improve the relative water content of leaves, increase the efficiency of antioxidant enzymes, and stimulate the biochemical pathways responsible for the formation of essential oils, thereby raising the economic value of the crop (Ostadi *et al*, 2023) [13]. The fungal bio-fertilizer increases the number of aromatic glands on the leaves, which are responsible for oil storage, thus raises the content of secondary metabolite compounds. The bacterial bio-fertilizer on the other hand, stimulates the gene expression responsible for the biosynthetic pathway of secondary metabolites, also stimulates important enzymes such as menthone reductase which participates in the construction of these compounds, and acts synergistically with plant hormones to increase the quantity of these compounds (Israel *et al*, 2022) [19]. As for the effect of soil texture, mint grows better in sandy soil as their texture provides suitable conditions for plant growth, including maintaining soil moisture, and aeration which enhances root growth, increases vegetative production, and increase the production of essential oils. Clay soil on the other hand blocks root growth and makes it easier for the root to get infected due to poor drainage and waterlogging, while sandy soils cause a significant decrease in essential oil production, and vegetative growth due to the rapid drainage of the water, and nutrients (Suryavanshi *et al*, 2021; Bghbani-Arani & Poureisa, 2024) [8, 28]. Soil porosity, and bulk density play an important role in the ability of plant roots to absorb nutrients, especially nitrogen (N) which is the primary element for vegetative growth. Studies have confirmed that soil mixed with bio-fertilizers outperforms soils devoid of these fertilizers in stimulating the growth of mint shoots, increasing their length, number of branches, and increase their ability to absorb nitrogen (N), due to the improvement of the soil's physical and biological structure (Hasanah *et al*, 2019) [15].

## Materials and Methods

### Experimental Design

The field experiment was carried out in the wooden shade of the Forestry Department / College of Agriculture and Forestry / University of Mosul. It involved planting two types of mint (domestic and wild) in two different soil types (clay loam and sandy loam) during the season of (2025 [27]-2026) to estimate the effect of adding bio-fertilizers (WSG, Bacillus strain) and Trichoderma at a rate of 20 g/kg. The effect of the treatments individually, and in combination on the growth traits, and some physiological, biochemical, and chemical traits of the mint was studied.

### The Wooden Shade Experiment

A factorial experiment of (2 × 2 × 4 × 3) was used according to the Completely Randomized Design (C.R.D.) to assess the effect of bio-fertilizers addition, and soil type on some physiological, and chemical traits and oil yield of two typed of mint.

Plastic pots were used with a diameter of 25 centimeters, a height of 18 centimeters, and a capacity of 5 kilograms. Two different types of soil were used: clay loam (Yarimja) and sandy loam (Sada wa Ba'wiza). The soil was sterilized by an autoclave. The two types of soil were inoculated with a commercially prepared bacteria bio-fertilizer (WSG, Bacillus strain) and a fungal bio-fertilizer (Trichoderma), in which 20 grams of each were mixed with the surface layer of the soil according to the studied treatments. The treatment included:

1. Addition of 20 g/kg of bacterial bio-fertilizer.
2. Addition of 20 g/kg of fungal bio-fertilizer.
3. Addition of 20 g/kg of combined bio-fertilizer (fungal and bacterial).
4. Control treatment (without bio-fertilizer addition).

The treatments included both types of soil.

Good seeds of similar size from both mint types were planted on 2/9/2025 [27]. And after 15 days the number of seedlings was sent to five seedlings / pot. The plants irrigated by regular water to reach 75% of field capacity, and the irrigation was adjusted to maintain the soil moisture levels using daily weighing of the pots with soil. 45 days after germination, two replicates of each treatment were used to study some growth traits, physiological traits, and chemical content of mint, the last replicate was kept in the wooden shade to complete the remaining experiments, and determine oil yield.

### First: The Studied Traits in the Wooden Shade Experiment

1. **Plant Height (cm):** The height of mint was measured from its point of attachment to the soil, to the tip of the stem using a graduated ruler.

2. **Leaf Area (cm²/plant):** This was determined based on the method used by Scott *et al*. (1973), which involved calculating the ratio of the dry weight of disks of known area to the total dry weight of the mint leaves according to the equation of Roy *et al*. (1981).

Area of disks (cm²) × [Dry weight of disks (g) + Dry weight of plant leaves (g)] / Dry weight of disks (g)

### Second: Physiological Indicators

1. **Estimation of Relative Water Content (RWC):** It was calculated using the method of Turner (1981), which is a very popular method and been used by most researchers such as Schonfeld *et al*. (1988).

Relative Water Content (RWC) = [(Fresh weight – Dry weight) / (Turgid weight – Dry weight)] × 100

2. **The Estimation of Carbohydrates:** Soluble carbohydrates in the leaves were calculated according to the method of Otls (2011). The intensity of the brown color that appeared in the test solution was measured using a spectrophotometer at a wavelength of 490 nm.

### Third: Biochemical Indicators

1. **Non-Enzymatic Antioxidant Systems**

1.1 **Estimation of Proline Content:** The concentration of proline acid in the leaves of mint was calculated according to the method of Bates *et al*. (1973) using a spectrophotometer at a wavelength of 520 nm.

2. **Enzymatic Antioxidant Systems**

2.1 **easurement of Peroxidase (POD) Enzyme Activity:** Peroxidase enzyme (POD) activity in mint was calculated using Kim and Yoo (1996) method, which is a colorimetric method based on the formation of a colored complex from the plant leaves, which can be measured using a spectrophotometer at a wavelength of 470 nm.

2.2 **Measurement of superoxide dismutase (SOD) enzyme activity:** the concentration of superoxide dismutase (SOD) enzyme in the leaves of mint was measured based on the method of Calatayud *et al*. (2002), using a spectrophotometer at a wavelength of 560 nm.

**2.3 Estimation of the percentage of volatile oil in leaves and stems:** The percentage of volatile oil in leaves was done by the use of 100 g of shredded stems and leaves of mint, which were added to a glass flask that contain 2 liters of distilled water. The mixture was then boiled at 100°C, and this process took approximately 12 hours until the oil was completely extracted from the plant parts. The extracted oil was then placed in opaque, tightly sealed glass bottles, these bottles were stored in a refrigerator at 4°C until they become of use. This method is known as steam distillation method which requires a (Clevenger apparatus), according to the study done by Wagner & Bladt (2009).

## Results

### Plant Height

As shown in Table (1) the mint that grows in clay soil were significantly taller than those grown in sandy soil, with an increase in plant height of (3.6%).

As for the effect of the type of bio-fertilizer, the results showed that the treatment with the mixed bio-fertilizer (fungal + bacterial) was better in increasing stem height, as the plants recorded a significant increase of (16.5%) compared to the control.

It was also noted that the wild type was significantly shorter compared to the domestic type, by a percentage of (20.08%).

It was found from the results of the two-way interaction (soil + bio-fertilizer) that the types grown in clay soil showed a significant increase in plant height compared to those grown in the sandy soil, with a percentage of (22.01%).

Regarding the three-way interaction (plant type+ soil + bio-fertilizer), it was concluded that the domestic type of mint which was grown in clay soil, and had a mixed bio-fertilizer (fungal + bacterial) showed better results in regards of plant height, when compared to the control, with a percentage of (52.5%).

**Table 1:** The effect of soil texture and bio-fertilizer on plant height of two types of mint

| Plant type | Soil type | Types of bio- fertilizer | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + bacterial | | | |
| Wild mint | Clay | 43.80 i | 47.30 gh | 46.50 ghi | 50.70 eif | 47.08 b | | |
| | Sandy | 40.50 j | 46.00 ghi | 44.60 hi | 48.50 fg | 44.90 c | | |
| Domestic mint | Clay | 54.00 de | 59.30 abc | 58.20 bc | 61.80 a | 58.33 a | | |
| | Sandy | 51.70 ef | 58.00 bc | 56.90 cd | 60.50 ab | 56.78 a | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 42.15 f | 46.65 e | 45.55 e | 49.60 d | | 45.99 b | |
| | Domestic mint | 52.85 c | 58.65 b | 57.55 b | 61.15 a | | 57.55 a | |
| Interaction between soil type and bio-fertilizers | Clay | 48.90 e | 53.30 bc | 52.35 bcd | 56.25 a | | | 52.70 a |
| | Sandy | 46.10 f | 52.00 cd | 50.75 de | 54.50 ab | | | 50.84 b |
| Effect of bio-fertilizer type | | 47.50 c | 52.65 b | 51.55 b | 55.38 a | | | |

### Estimation of leaf area

As shown in table (2), mint treated with mixed bio-fertilizer (fungal + bacterial) showed a significant increase in leaf area by 18.3% compared to the rest of treatments

As for the domestic type of mint, it showed a significant decrease in leaf area, with a reduction percentage of (-15.7%) compared to the wild type of mint

From the table, it can be observed that mint planted in sandy soil has a greater leaf area than that grown in clay soil, by a percentage of (5%)

It was found from the results of the two-way interaction (plant + soil) on leaf area, it was shown that there is no significant effect of soil type on this trait.

Regarding the three-way interaction (plant + soil + addition), It was concluded that the domestic mint type grown in clay soil without any bio-fertilizer added, showed a significant decrease in leaf area compared to the other treatments, with a reduction percentage of (30.6%).

**Table 2:** Effect of soil texture and bio-fertilizer on leaf area of the two types of mint

| Plant type | Soil type | Types of bio- fertilizer | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + bacterial | | | |
| Wild mint | Clay | 16.10 e-i | 18.59 a-d | 17.67 b-f | 19.71 ab | 18.02 a | | |
| | Sandy | 16.95 d-h | 19.62 abc | 18.34 a-e | 20.50 a | 18.85 a | | |
| Domestic mint | Clay | 14.22 i | 15.75 f-i | 14.50 hi | 16.02 f-i | 15.12 b | | |
| | Sandy | 14.80 ghi | 16.46 d-i | 15.31 f-i | 17.25 c-g | 15.96 b | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 16.53 cd | 19.11 ab | 18.01 bc | 20.11 a | | 18.44 a | |
| | Domestic mint | 14.51 e | 16.11 de | 14.91 de | 16.64 cd | | 15.54 b | |
| Interaction between soil type and bio-fertilizers | Clay | 15.16 d | 17.17 bc | 16.09 cd | 17.87 ab | | | 16.57 b |
| | Sandy | 15.88 cd | 18.04 ab | 16.83 bcd | 18.88 a | | | 17.40 a |
| Effect of bio-fertilizer type | | 15.52 b | 17.61 a | 16.46 b | 18.37 a | | | |

### Chlorophyll A

As shown in table (3), Many types that were grown in clay soil exhibited a significant advancement in chlorophyll A content, more than those grown in sandy soil, with a percentage increase of (10.1%)

As for the effect of plant type on chlorophyll A content, it was noticed that there was a significant decrease in chlorophyll A in the wild mint compared to the domestic mint, with a reduction percentage of (21.5%).

When observing the effect of bio-fertilizer type on the mint, it's found that the plant treated with a mixed bio-fertilizer

(bacterial + fungal) showed a significant increase in chlorophyll A content compared to the control, with an increase percentage of (39.5%).
It was found from the results of the two-way interaction (plant + bio-fertilizer) the wild type of mint without any addition (control) showed a significant decrease in chlorophyll A content compared to the rest of the treatments, with a reduction percentage of (44.3%).

Regarding the three-way interaction (plant + soil + bio-fertilizer) for chlorophyll A content it was concluded that the domestic type of mint that was grown in clay soil and treated with the mixed bio-fertilizer (fungal + bacterial) Having way more content that other treatments, with a percentage of (93.5%).

**Table 3:** Effect of soil texture and bio-fertilizer on chlorophyll A content of mint

| Plant type / Factor | Soil type | Bio- fertilizer type | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + Bacterial | | | |
| Wild mint | Clay | 5.35 fg | 7.30 b-e | 6.50 c-g | 7.92 a-d | 6.77 b | | |
| | Sandy | 4.95 g | 6.43 c-g | 5.82 e-g | 6.88 e-f | 6.02 bc | | |
| Domestic mint | Clay | 7.37 b-e | 8.82 ab | 8.11 a-d | 9.58 a | 8.47 a | | |
| | Sandy | 6.19 d-g | 8.32 abc | 7.85 a-d | 8.93 ab | 7.82 a | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 5.15 e | 6.87 cd | 6.16 de | 7.40 bcd | | 6.39 b | |
| | Domestic mint | 6.78 cd | 8.57 ab | 7.98 bc | 9.26 a | | 8.15 a | |
| Interaction between soil type and bio-fertilizer | Clay | 6.36 cd | 8.06 ab | 7.31 bc | 8.75 a | | | 7.62 a |
| | Sandy | 5.57 d | 7.38 bc | 6.84 bc | 7.91 ab | | | 6.92 b |
| Effect of bio-fertilizer | | 5.97 c | 7.72 ab | 7.07 b | 8.33 a | | | |

## Chlorophyll B

As shown in (table 4) chlorophyll B content in mint that were treated with the mixed bio-fertilizer (fungal + bacterial) was way more than other treatments, with a percentage increase of (38.5%) compared to the control.
It was also observed that the wild type of mint plant showed a significant decrease in chlorophyll B content compared to the domestic type, with a reduction percentage of (16.6%).
The data from table also stated that mint types showed a significant increase in chlorophyll B content in sandy soil, compared to the other treatments, with a percentage of (8.08%).

It was found from the results of the two-way interaction (soil + bio-fertilizer) that the mint grown in clay soil, and treated with mixed bio-fertilizer (fungal + bacterial) has an increase in chlorophyll B content more than other treatments, with a percentage of (49.3%).
Regarding the three-way interaction (plant + soil + bio-fertilizer), it was found that the wild type of mint grown in sandy soil without any addition (control) showed a significant decrease in chlorophyll B content compared to the rest of the treatments, with a reduction percentage of (43.1%).

**Table 4:** Effect of soil texture and bio-fertilizer on chlorophyll B content of mint

| Plant type / Factor | Soil type | Bio- fertilizer type | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + Bacterial | | | |
| Wild mint | Clay | 4.03 cd | 5.21 a-d | 4.70 bcd | 5.97 abc | 4.98 bc | | |
| | Sandy | 3.90 d | 4.92 a-d | 4.28 bcd | 5.33 a-d | 4.61 c | | |
| Domestic mint | Clay | 5.02 a-d | 6.32 ab | 5.73 a-d | 6.86 ab | 5.98 a | | |
| | Sandy | 4.70 bcd | 5.95 abc | 5.19 a-d | 6.26 ab | 5.53 ab | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 3.97 d | 5.07 bcd | 4.49 cd | 5.65 abc | | 4.79 b | |
| | Domestic mint | 4.86 bcd | 6.14 ab | 5.46 abc | 6.56 a | | 5.75 a | |
| Interaction between soil type and bio-fertilizer | Clay | 4.53 bc | 5.77 ab | 5.22 abc | 6.42 a | | | 5.48 a |
| | Sandy | 4.30 c | 5.44 abc | 4.74 bc | 5.80 abc | | | 4.87 b |
| Effect of bio-fertilizer | | 4.41 c | 5.60 ab | 4.98 bc | 6.11 a | | | |

## Estimation of carbohydrate content (soluble sugars percentage)

As shown in (Table 5) the domestic type of mint had significantly more soluble sugars percentage compared to the wild type, with an increase in percentage of (13.04%).
It was observed that mint without any addition (control) showed a significant decrease in soluble sugars compared to the rest of the treatments, with a reduction percentage of (56.2%).
As for the effect of soil on carbohydrate content, its concluded that the mint grown in sandy soil has a significant increase in carbohydrate content compared to those grown in clay soil, with a percentage of (20.7%).
It was found from the results of the two-way interaction (plant + soil), the domestic mint grown in sandy soil has a greater concentration of carbohydrate content compared to other treatments, with an increase in percentage of (36.7%).
Regarding the three-way interaction (plant + soil + bio-fertilizer), it was concluded that the wild mint grown in clay soil without any addition (control) showed a significant decrease in soluble sugars percentage compared to the rest of the treatments, with a reduction percentage of (68.3%).

**Table 5:** Effect of soil texture and bio-fertilizer on carbohydrate content of mint

| Plant type / Factor | Soil type | Bio- fertilizer type | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + Bacterial | | | |
| Wild mint | Clay | 1.03 i | 1.87 e | 1.40 f–i | 2.35 cd | 1.66 c | | |
| | Sandy | 1.46 fgh | 2.03 de | 1.75 ef | 2.85 bc | 2.02 bc | | |
| Domestic mint | Clay | 1.10 hi | 2.01 de | 1.68 efg | 2.77 bc | 1.89 bc | | |
| | Sandy | 1.31 ghi | 2.53 bc | 2.00 de | 3.25 a | 2.27 a | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 1.25 f | 1.95 d | 1.58 e | 2.60 bc | | 1.84 b | |
| | Domestic mint | 1.21 f | 2.27 c | 1.84 d | 3.01 a | | 2.08 a | |
| Interaction between soil type and bio-fertilizer | Clay | 1.07 f | 1.94 d | 1.54 e | 2.56 bc | | | 1.78 b |
| | Sandy | 1.39 e | 2.28 c | 1.88 d | 3.05 a | | | 2.15 a |
| Effect of bio-fertilizer | | 1.23 d | 2.11 b | 1.71 c | 2.81 a | | | |

**Estimation of peroxidase (POD) enzyme concentration**

As shown in (Table 6) there is a notable significant increase in terms of the effect of plant type on (POD) in the wild mint, with a percentage of (22.5%) compared to the domestic mint.

It was also noted that the mint grown in clay soil showed more POD enzyme activity compared to these which were grown in sandy soil, with an increased percentage of (20.5%).

As for the effect of bio-fertilizer's type on (POD), there was a significant decrease of (POD) in the mint treated with the mixed bio-fertilizer (Trichoderma 10g and Bacillus 10g), with a percentage of (26.9%) compared to the control.

It was found from the results of the two-way interaction (plant + soil), there was a significant increase in (POD) of the wild mint grown in clay soil, with a percentage of (31.5%) compared to the rest of the treatments.

Regarding the three-way interaction (Plant + soil + bio-fertilizer), the wild mint type grown in clay soil without any addition (control) recorded a notable increase compared to the rest of the treatments, with a percentage of (84.3%).

**Table 6:** Effect of soil texture and bio-fertilizer on peroxidase enzyme (POD) concentration of two type of mint

| Plant type / Factor | Soil type | Bio- fertilizer type | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + Bacterial | | | |
| Wild mint | Clay | 0.059 a | 0.047 a–e | 0.053 abc | 0.042 bc–e | 0.050 a | | |
| | Sandy | 0.056 ab | 0.046 a–e | 0.051 a–d | 0.040 cde | 0.048 ab | | |
| Domestic mint | Clay | 0.049 a–d | 0.041 b–e | 0.045 a–e | 0.037 de | 0.043 bc | | |
| | Sandy | 0.044 a–e | 0.036 de | 0.039 cde | 0.032 e | 0.038 c | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 0.058 a | 0.047 bc | 0.052 ab | 0.041 cd | | 0.049 a | |
| | Domestic mint | 0.047 bc | 0.039 cd | 0.042 bcd | 0.035 d | | 0.040 b | |
| Interaction between soil type and bio-fertilizer | Clay | 0.054 a | 0.044 abc | 0.049 ab | 0.040 bc | | | 0.047 a |
| | Sandy | 0.050 ab | 0.041 bc | 0.045 abc | 0.036 c | | | 0.039 b |
| Effect of bio-fertilizer | | 0.052 a | 0.043 bc | 0.047 ab | 0.038 c | | | |

**Estimation of superoxide dismutase (SOD) concentration**

As shown in (table 7) there is a significant increase in (SOD) concentration in mint grown in clay soil, with a percentage of (4.7%) compared to those grown in sandy soil. In addition, the domestic mint showed a significant decrease in superoxide dismutase (SOD) concentration compared to the wild type, with a reduction percentage of (9.4%).

It can also be observed that (SOD) enzyme activity showed a significant increase in terms of the effect of bio-fertilizer type in mint without any addition (control), with a percentage of (17.5%) compared to the rest of the treatments.

It was found from the results of the two-way interaction (plant + soil), that the domestic mint type grown in sandy soil showed a significant decrease in (SOD) concentration compared to the rest of the treatments, with a percentage of (13.8%).

Regarding the three-way interaction (soil + plant + bio-fertilizer), it was shown that the wild mint grown in clay soil without any addition (control) recorded a notable increase compared to the rest of the treatments, with a percentage of (34.06%).

**Table 7:** Effect of soil texture and bio-fertilizer on superoxide dismutase (SOD) concentration of two types of mint

| Plant type / Factor | Soil type | Bio- fertilizer type | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + Bacterial | | | |
| Wild mint | Clay | 0.551 a | 0.490 bcd | 0.521 abc | 0.460 de | 0.506 a | | |
| | Sandy | 0.533 ab | 0.470 cde | 0.495 bcd | 0.440 de | 0.485 ab | | |
| Domestic mint | Clay | 0.490 bcd | 0.451 de | 0.470 cde | 0.425 e | 0.459 bc | | |
| | Sandy | 0.465 cde | 0.421 e | 0.447 de | 0.411 e | 0.436 c | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 0.542 a | 0.480 bc | 0.508 abc | 0.450 cde | | 0.495 a | |
| | Domestic mint | 0.478 bc | 0.436 de | 0.459 cd | 0.418 e | | 0.448 b | |
| Interaction between soil type and bio-fertilizer | Clay | 0.521 a | 0.471 bc | 0.496 ab | 0.443 cd | | | 0.482 a |
| | Sandy | 0.499 ab | 0.446 cd | 0.471 bc | 0.426 d | | | 0.450 b |
| Effect of bio-fertilizer | | 0.510 a | 0.458 bc | 0.483 b | 0.434 c | | | |

**Estimation of the percentage of volatile oils**

As shown in (table 8) the mint which was treated with the mixed bio-fertilizer (fungal + bacterial) showed a significant increase in the percentage of volatile oil compared to the control, with an increase in percentage of (35.01%).

As for the effect of plant kind, the wild type showed a decrease in the percentage of volatile oils by (9.1%) compared to the domestic type.

Regarding the effect of soil type, it was observed that the mint grown in clay soil produced more volatile oil than those grown in the sandy soil, with a percentage of (7.8%).

It was found from the results of the two-way interaction (soil + bio-fertilizer) That the mint grown in sandy soil produce more volatile oils than all the other treatments, with a percentage of (46.76%).

Regarding the three-way interaction (plant + soil + bio-fertilizer), it was found that the domestic mint grown in sandy soil, and treated with the mixed bio-fertilizer (fungal + bacterial) had greater percentage of volatile oil, with a percentage of (66.4%).

**Table 8:** Effect of soil texture and bio-fertilizer on the percentage of volatile oils of two types of mint

| Plant type / Factor | Soil type | Bio- fertilizer type | | | | Interaction between plant type and soil | Effect of plant type | Effect of soil type |
|---|---|---|---|---|---|---|---|---|
| | | Comparison | Fungal | Bacterial | Fungal + Bacterial | | | |
| Wild mint | Clay | 3.10 g | 3.78 c–g | 3.44 d–g | 4.03 b–f | 3.59 b | | |
| | Sandy | 3.32 fg | 4.18 b–e | 3.91 b–f | 4.37 be | 3.95 a | | |
| Domestic mint | Clay | 3.39 efg | 4.21 bcd | 3.85 b–g | 4.65 ab | 4.03 a | | |
| | Sandy | 3.65 c–g | 4.30 bc | 4.02 b–f | 5.16 a | 4.28 a | | |
| Interaction between plant type and bio-fertilizer | Wild mint | 3.21 e | 3.98 bcd | 3.68 cde | 4.20 be | | 3.77 b | |
| | Domestic mint | 3.52 de | 4.26 b | 3.94 bed | 4.91 a | | 4.15 a | |
| Interaction between soil type and bio-fertilizer | Clay | 3.25 d | 4.00 bc | 3.65 cd | 4.34 ab | | | 3.81 b |
| | Sandy | 3.49 cd | 4.24 b | 3.97 bc | 4.77 a | | | 4.11 a |
| Effect of bio-fertilizer | | 3.37 c | 4.12 b | 3.81 b | 4.55 a | | | |

**Discussion**

Many studies have shown that soil texture directly affects the wellbeing of plants belonging to the Lamiaceae family like mint. In terms of soil aeration, water retention capacity, and the availability of nutrients, which is reflected in the vegetative growth of the plant, such as plant height, number of leaves, and root and shoot dry weight (Hendawy *et al*, 2017) [17].

The results in (table 1) showed that mint plants which were grown in clay soil were taller than other mint types that were grown in other kind of soils, which is due to improved water and nutrient absorption and increased photosynthesis. These results align with what Malaka *et al*. (2022) [23] stipulated. It was also found that stem height showed a significant improvement when plants were treated with bio-fertilizers compared to other treatments.

Microorganisms such as Azotobacter and mycorrhizal fungi present in bio-fertilizers improve the availability of compounds needed by the plant and increase the efficiency of their absorption by roots, they also produce growth regulators such as auxins, and gibberellins which reflects the increase in plant height, and vegetative growth. These microorganisms also have the ability to fix nitrogen, and convert it into ammonia for easy plant absorption, and to dissolve phosphorus in the soil by secreting organic acids, and specific enzymes such as phosphatase enzymes (Kumar *et al*, 2023) [21]. The results this study also agrees with the findings of Abd El-Hadi *et al*. (2009) [2].

The results of (Table 2) showed that the leaf area of mint recorded the highest rate in the those that were grown in sandy soil, with the percentage of (5%), due to the wide pores in the soil, which provided a good aeration situation for the roots, and prevented oxygen deficiency. These results agree with what Tursun (2022) [30] stipulated in his study. Regarding the effect of bio-fertilizers, it was found that treating the mint with bio-fertilizers led to a significant improvement in leaf areas. These results were constant with what Dasgan *et al*. (2022) [10] stipulated.

The results of (Table 3 & 4) Showed that pigments in mint grown in clay soil recorded the highest value compared to those grown in sandy soil, because clay soil the ability to retain water and nutrients. This also agrees with what Kahkashan *et al*. (2016) [20] stipulated.

The results of (Table 5) showed that mint planted in sandy soil had more soluble sugars (carbohydrates) compared to those grown in clay soil. This soil texture positively affects the carbohydrate content in plant leaves due to its wider pores for aeration, and its ability to retain moisture. The results of this study align with the findings of Nawaz *et al*. (2024) [25].

The results of (table 6 and 7) shows that the activity of antioxidant enzymes was notable in mint grown in clay soil compared to the others, clay soil causes poor aeration, or root suffocation, leading to an increased activity of antioxidant enzymes (POD, SOD). These results also align with what Brahmi *et al*. (2022) [9] stipulated.

The results in (table 8) showed that mint that were grown in sandy soil gave the highest percentage of volatile oils compared to those grown in clay soil. Sandy soil is exposed to significant environmental stresses such as Low water and nutrient availability. Which stimulated the formation of essential oils, and secondary compounds. These results agree with the findings of Tursun (2022) [23].

Bio-fertilizers are considered as one of the modern agricultural alternatives, that are used instead of chemical fertilizers to improve soil fertility, increase root and shoot dry weight, improve the percentage of essential oil and its components, in addition to producing auxins and solubilizing phosphorus, which contributes to increasing nutrients and stimulate plant growth (Swaefy *et al*, 2007) [29].

The results in (Tables 1 and 2) indicate that treating mint plants with bio-fertilizers led to an increase in physiological traits, including plant height, and leaf area. These compounds have microorganisms that can fix atmospheric nitrogen, improve the photosynthesis process, and secrete growth-regulating compounds (Ghotbi-Ravandi *et al*, 2023) [13].

The results in (Tables 3 and 4) showed that the addition of bio-fertilizers to mint led to an increase in chlorophyll A & B content, and enhanced the vegetative growth of the plant. The microorganisms found in these compounds plays an important role in improving water, and nutrient absorption in the root, and enhance nitrogen uptake, leading to an increase in the production of photosynthetic pigments (Nadjafi *et al*, 2014) [24].

The results of the present study for measuring carbohydrate content, as shown in (Table 5), indicated that bio-fertilizers increased it compared to the other treatments. Bio-fertilizers enhanced the plant's ability to absorb nutrients which in turn raised chlorophyll pigment levels, and increases the efficiency of photosynthesis, causing an elevated carbon fixation rates, and its conversion into carbohydrates (Ait Elallem *et al*, 2024) [4].

Regarding antioxidant enzymes (POD, SOD), the results from (Tables 6 and 7) indicated that the mint which was treated with bio-fertilizers showed a decrease in the activity of these enzymes. This decrease is considered a positive indicator, rather than a negative one. Because of the fact that bio-fertilizers provide the nutrients that the plant needs, and reduce its need to produce defensive enzymes that are secreted when exposed to oxidative stress (Hekmati *et al*, 2023) [16].

As for the volatile oils, as shown in (table 8), the mint which were treated with bio-fertilizer showed a greater percentage than the control. As the process of volatile oil formation is directly affected by the physiological state of the plant, and the efficiency of water and nutrient uptake. As bio-treatments lead to enhanced accumulation of phenolic, and terpenoid compounds responsible for the quality and chemical composition of the volatile oil (Hudz *et al*, 2023) [18].

**Conclusions**

Exposing the two types of mint (domestic and wild) to different types of bio-fertilizers (fungal, bacterial, mixed) resulted in various responses depending on the treatment. Clay soil was the optimal texture for the growth of both mint types. The results varied between the domestic and wild types in most of the studied traits. the domestic mint showed better results for plant height, while the wild mint had better results in leaf area. The interaction between the two factors (soil texture and bio-fertilizers) had a positive effect, contributing to the improvement of most of the studied traits.

**References**


1. Aasfar A, Meftah Kadmiri I, Azaroual SE, Lemriss S, Mernissi NE, Bargaz A, *et al*. Agronomic advantage of bacterial biological nitrogen fixation on wheat plant growth under contrasting nitrogen and phosphorus regimes. Frontiers in Plant Science,2024:15:1388775. https://doi.org/10.3389/fpls.2024.1388775
2. Abd El-Hadi NIM, Abo El-Ala HK, Abd El-Azim WM. Response of some *Mentha* species to plant growth promoting bacteria (PGPB) isolated from soil rhizosphere. Australian Journal of Basic and Applied Sciences,2009:3(4):4437-4448.
3. Abdallah AR, Safwat MSA, Moharram TM, Ahmed SFM, Soliman HS. Biofertilizers and their importance in environmental and sustainable agriculture. Journal of Agricultural Chemistry and Biotechnology,2009:34(2):769-779. https://doi.org/10.21608/jacb.2009.90281
4. Ait Elallem K, Ben Bakrim W, Yasri A, Boularbah A. Growth, biochemical traits, antioxidant enzymes, and essential oils of four aromatic and medicinal plants cultivated in phosphate-mine residues. Plants,2024:13(18):2656. https://doi.org/10.3390/plants13182656
5. Alori ET, Glick BR, Babalola OO. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology,2017:8:971. https://doi.org/10.3389/fmicb.2017.00971
6. Areej A, Usama M, Zulfiqar U, Sarwar F, Maryam, Ashiq A. Sustainable agriculture development: The role of biofertilizers in soil fertility and crop yield improvement. Applied Agriculture Sciences,2024:2(1):1-5. https://doi.org/10.25163/agriculture.2110005
7. Babalola OO. Beneficial bacteria of agricultural importance. Biotechnology Letters,2010:32(11):1559-1570. https://doi.org/10.1007/s10529-010-0347-0
8. Bghbani-Arani A, Poureisa M. Soil Properties and Yield of Peppermint (*Mentha piperita* L.) in Response to Different Nitrogen Fertilizers Under Water-Deficit Conditions. Communications in Soil Science and Plant Analysis,2024:55(10):1445-1462. https://doi.org/10.1080/00103624.2024.2317852
9. Brahmi F, Lounis N, Mebarakou S, Guendouze N, Yalaoui-Guellal D, Madani K, *et al*. Impact of Growth Sites on the Phenolic Contents and Antioxidant Activities of Three Algerian *Mentha* Species (*M. pulegium* L, *M. rotundifolia* (L.) Huds, and *M. spicata* L.). Frontiers in Pharmacology,2022:13:886337. https://doi.org/10.3389/fphar.2022.886337
10. Dasgan HY, Aldiyab A, Elgudayem F, Ikiz B, Gruda NS. Effect of biofertilizers on leaf yield, nitrate amount, mineral content and antioxidants of basil (*Ocimum basilicum* L.) in a floating culture. Scientific Reports,2022:12(1):20917. https://doi.org/10.1038/s41598-022-24799-x
11. Dobhal S, Larrea-Sarmiento A, Alvarez AM, Arif M. Development of a loop-mediated isothermal amplification assay for specific detection of all known subspecies of *Clavibacter michiganensis*. Journal of Applied Microbiology,2019:126(2):388-401. https://doi.org/10.1111/jam.14128
12. Fasusi OA, Cruz C, Babalola OO. Agricultural Sustainability: Microbial Biofertilizers in Rhizosphere Management. Agriculture,2021:11(2):163. https://doi.org/10.3390/agriculture11020163
13. Ghotbi-Ravandi AA, Shariatmadari Z, Riahi H, Hassani SB, Heidari F, Ghorbani Nohooji M. Enhancement of Essential Oil Production and Expression of Some Menthol Biosynthesis-Related Genes in *Mentha piperita* Using Cyanobacteria. Iranian Journal of Biotechnology,2023:21(4):3550. https://doi.org/10.30498/ijb.2023.368377.3550
14. Giri B, Varma A, Prasad R, editors. Biofertilizers for sustainable agriculture and environment. Springer Nature Switzerland AG, 2019. https://doi.org/10.1007/978-3-030-18933-4

15. Hasanah Y, Sitepu FE, Butar Butar RR. Morphological characters and N uptake of mint (*Mentha piperita*) with the growing media composition treatment. IOP Conference Series: Earth and Environmental Science,2019:260(1):012147. https://doi.org/10.1088/1755-1315/260/1/012147
16. Hekmati J, Hamidoghli Y, Esmaielpour B, Ghasemnezhad M. The effect of bio-fertilizers and phosphorus on physiological characteristics, antioxidant enzymes activity and the essential oil content of green mint (*Mentha spicata* L.) under arsenic stress condition. 2023.
17. Hendawy SF, Hussein MS, Amer HM, El-Gohary AE, Soliman WSA. Effect of soil type on growth, productivity and essential oil constituents of rosemary (*Rosmarinus officinalis*). Asian Journal of Agricultural Biology,2017:5(4):303-311.
18. Hudz N, Kobylinska L, Pokajewicz K, Horčinová Sedláčková V, Fedin R, Voloshyn M, *et al*. *Mentha piperita*: Essential oil and extracts, their biological activities, and perspectives on the development of new medicinal and cosmetic products. Molecules,2023:28(21):7444. https://doi.org/10.3390/molecules28217444
19. Israel A, Langrand J, Fontaine J, Lounès-Hadj Sahraoui A. Significance of arbuscular mycorrhizal fungi in mitigating abiotic environmental stress in medicinal and aromatic plants: A review. Foods,2022:11(17):2591. https://doi.org/10.3390/foods11172591
20. Kahkashan P, Najat B, Iram S, Iffat S. Influence of Soil Type on the Growth Parameters, Essential Oil Yield and Biochemical Contents of *Mentha arvensis* L. Journal of Essential Oil-Bearing Plants,2016:19(1):76-81. https://doi.org/10.1080/0972060X.2015.1086285
21. Kumar V, Walia SS, Kalra VP, Kaur T, Alfagham AT, Al-Humaid LA, *et al*. Effects of organic manures and bio-fertilizer on yield, oil content, and soil health of Japanese *Mentha* (*Mentha arvensis* L.), 2023.
22. Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, *et al*. Biofertilizers: a potential approach for sustainable agriculture development. Environmental Science and Pollution Research International,2017:24(4):3315-3335. https://doi.org/10.1007/s11356-016-8104-0
23. Malaka MJ, Araya NA, Soundy P, du Plooy CP, Araya HT, Jansen Van Rensburg WS, *et al*. Biomass, Essential Oil Yield, and Composition of Marjoram as Influenced by Interactions of Different Agronomic Practices under Controlled Conditions. Plants,2022:12(1):173. https://doi.org/10.3390/plants12010173
24. Nadjafi F, Damghani M, Tabrizi L, Nejad Ebrahimi S. Effect of biofertilizers on growth, yield and essential oil content of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). Journal of Essential Oil-Bearing Plants, 2014, 17(2). https://doi.org/10.1080/0972060X.2013.813235
25. Nawaz AM, Ramazan S, Majid A, Ubairah, Khan NU, Rasool W. Exogenous application of carbohydrate sources positively regulate growth and carbohydrate accumulation in spearmint (*Mentha spicata*). International Journal of Agriculture Innovation and Cutting-Edge Research,2024:2(3):20-25. https://doi.org/10.5281/zenodo.14905903
26. Ostadi A, Javanmard A, Amani Machiani M, Kakaei K. Optimizing Antioxidant Activity and Phytochemical Properties of Peppermint (*Mentha piperita* L.) by Integrative Application of Biofertilizer and Stress-Modulating Nanoparticles under Drought Stress Conditions. Plants,2023:12(1):151. https://doi.org/10.3390/plants12010151
27. Pooja P, Tallapragada S, Saini S, Punia M, Janaagal M, Kumar V, *et al*. Exploring the potential of arbuscular mycorrhizal fungi as biofertilizers to enhance growth, nutrient acquisition and yield in chickpea genotypes under salinity stress. Journal of Soil Science and Plant Nutrition, 2025, 25. https://doi.org/10.1007/s42729-025-02497-7
28. Suryavanshi P, Kumar D, Mazeed A. Menthol mint cultivation – Step towards Atmanirbhar Bharat, 2021, 10-13.
29. Swaefy HM, Sakr WRA, Sabh AZ, Ragab AA. Effect of some chemical and bio-fertilizers on peppermint plants grown in sandy soil. I. Effect on vegetative growth as well as essential oil percentage and constituents. Annals of Agricultural Science,2007:52(2):451-463.
30. Tursun AO. Impact of soil types on chemical composition of essential oil of purple basil. Saudi Journal of Biological Sciences,2022:29:103314. https://doi.org/10.1016/j.sjbs.2022.103314
31. Tursun AO. Impact of soil types on chemical composition of essential oil of purple basil. Saudi Journal of Biological Sciences,2022:29:103314. https://doi.org/10.1016/j.sjbs.2022.103314
32. Woźniak M, Siebielec S. Agriculturally important groups of microorganisms – microbial enhancement of nutrient availability. Current Agronomy,2025:54(2):17-29. https://doi.org/10.2478/cag-2025-0002