Volume (6) Issue (2)

25 May 2026

The International Journal
of Scientific Publishing

Effect of organic manure and some amino acids on productivity of Peppermint (Mentha piperita L.) plants

Amina. A. Zorab
(Biology Dep.t,/ Botany, Fac. of Science, Misurata,Univ., Libya)

Salem. M. Ghwaji
(Plant production Dept, Fac. of Agric, Misurata, Univ., Libya)

Abobaker E. Alsanose
(Plant production Dept, Fac. of Agric, Sirte, Univ., Libya)

Essam A. Hassan
(Horticulture Dept., Fac. Agric., Al-Azhar University, Assiut, Egypt)

https://doi.org/10.65723/RMSP1902

Abstract:

The current experiment was established to assess the response of Peppermint (Mentha piperita L.) growth, herb yield and volatile oil production to organic manure (humic acid), some amino acids, and their interactions. This experiment was done during From February to October 2025 at a private farm in Misurata, Libya. The tallest plants and more branch number were achieved when Peppermint plants treated with 24 ml/L of Organic nutrient as soil drench and 15 ml/L of amino acids as foliar application compared to the other treatments under study. Likewise, increasing organic nutrient or amino acids rates gradually increased fresh and dry herb yield per plant. The highest volatile oil yield per plant and per hectare was obtained with the combination treatment of 24 ml/L(HU4) of organic nutrient and 15 ml/l (AM4) of amino acids compared to the other combination treatments. According to volatile oil compounds, HU4+AM4 combination treatment recorded the highest values of Limonene, Menthone, Mentofuran, iso-Menthone, neo-Menthol, Menthol, Pulegone and 1,8-cineole. Also, the highest values of iso-Menthol and ρ-cymene acetate were obtained with HU2+AM1 treatment. Compared to the other combinations under study. In general, it could recommend that this treatment, produce a uniform response of Peppermint growth and herb yield and enhancing volatile oil production under sandy soil conditions.

Keywords: Peppermint (Mentha piperita L.), humic acid, amino acids, growth, herb yield, volatile oil compounds.

Introduction:

Medicinal herbs are a source of many chemical contents that are used as medicinal compounds. Products from the secondary metabolism of the plants are among the most expensive plant chemical compounds. The value of global markets for herbal medicines, such as medicinal plants and their products, has always been growing significantly. Due to the fact that most of the world market for medicinal plants is related to the output and supply of secondary metabolites derived from these plants, biologically active compounds from herbs have very high economic values **(Shabani *et al.* 2015; Yusupova *et al*. 2023; Wang *et al.* 2020).** Medicinal and aromatic plants use by 80% of global population for their medicinal therapeutic effects as reported by **(WHO, 2008).** Many of these plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Others contain alkaloids, glycosides, saponins and many secondary metabolites **(Naguib, 2011).** The role of natural products on drug development has been increasing, not only when the bioactive compounds are directly used as therapeutic agents but also when they are used as raw material for drug synthesis, or as a base model for new biologically active compounds (**Mendonça 2006).** However, validating and using plants, as a phytopharmaceutical requires a great deal of basic and applied research, in order to set this resource at the same level of importance of conventional pharmaceutical products **(Batanouny et al 1999**). Essential oils (EO) are volatile, lipophilic mixtures of secondary plant compounds, mostly consisting of monoterpenes, sesquiterpenes and phenylproponoids. The qualitative and quantitative improvement of essential oil production presents an area of high commercial interest **(Khaosaad et al., 2006).**

Mentha piperita is particularly noted for its high concentration of essential oil, reaching up to 2 %. This oil is predominantly used in chewing gum (55 %) and oral care products (34 %)**.** Globally, it ranks as the second most significant essential oil, following citrus oils, with an annual production of around 14,000 tons, generating an estimated income of USD 300 million. The primary source of revenue is menthol. Essential oils from Mentha contain numerous volatile compounds such as anthocyanins, carvone, menthyl acetate, D-limonene, and 1,8-cineole, which contribute to the plant's fragrance and exhibit various biological properties, including antioxidant, antibacterial, anticancer, and anti-inflammatory activities (**.** The commercial cultivation of essential oil-bearing plants, including Mentha, is vital for the development of natural antioxidants and antibacterial agents, which are crucial for food preservation and health-enhancing products. Given the significant importance of essential oils, understanding the factors that influence their production, and composition is essential for optimizing yields. *Mentha* (mint) is a genus of an aromatic perennial herb belonging to Lamiaceae family **(Arzani et. al. 2007).** Owing to the abundant content of phenolic compounds **(Riachi, and Maria, 2015),** For instance, peppermint EO was found to be an effective alternative short-term treatment of irritable bowel syndrome in humans, of which the effect is considered to be mediated through its antioxidant and anti-inflammatory activities **(Khanna et.al. 2014).**

Humic acid (HA), a naturally occurring chemical in the soil as well as a byproduct of the breakdown of organic materials, has been employed successfully in the growth of a number of crops. There are several well described direct effects of HA on plant development, including improved macronutrient and micronutrient absorption and root growth **(Guo Gao et al., 2015)**. The humic substances addition in medicinal plants has been evaluated in various studies and it has been found that these substances can enhance the production of secondary metabolites and increase the activity of bioactive substances such as flavonoids, coumarins, phenylpropanoids, total phenols, and anthocyanins. There are various methods of application, including through the soil, as a spray, or through fertigation. Regardless of the method used, the application of humic substances has been shown to significantly increase the biosynthesis of metabolism and production of biologically and pharmacologically active metabolites in medicinal plants **(Pereira et al., 2019).** In the last few decades, the application of organic fertilization is very important for medicinal and aromatic plants to obtain high quality and quantity of their products. Organic agriculture is based on minimizing the utilize of external inputs and avoiding applying synthetic fertilizers and pesticides **(Galal and Ali, 2004) .**

The utilization of amino acids in agriculture practices had become attention because they are necessity compounds which act an important positive, biological and physiological roles namely, biosynthesis of pigments, terpenoids, enzymes, co- enzymes, vitamins, alkaloids, purine and pyrimidine bases **(Kamar and Omar, 1987).** They are precursors, or activators of phytohormones and growth substances (**Taiz and Zeiger, 2002**). Amino acids stimulated cell growth (Smith, 1982). Amino acids caused an enhance in plant growth, yield and mitigates the injuries by a biotic stress. Amino acids play beneficial roles in nutrition to obtain high production (quality and quantity) and shortening productive cycle with better dry material (**Kowalczyk and Zielony, 2008).**

Amino acids have significant functions in catalyzing secondary metabolic reactions in plants, as stated by **Pratelli and Pilot (2014).** It was shown that tryptophan is linked to the manufacture of camalexin, auxins, phenylpropanoids, the phytoalexin and other related natural compounds in plants.

Amino acids as organic nitrogenous compounds are the building blocks in protein synthesis. They promoted cell growth and capable to protect the plants from the toxicity of ammonia **(Smith, 1982**). This work aims to use safe alternatives in plant nutrition to achieve clean and pollutant-free agricultural production and determine the impact of organic nutrition and some amino acids as well as their interactions on the plant growth traits and essential oil of Peppermint (*Mentha piperita* L.) plants to find out the most suitable treatment for enhancing these characteristics.

Materials and Methods

Experimental site and treatments description.

This study be conducted from March to September 2025 on a private farm in Misrata, Libya, to investigate the effects of an organic nutrient source, some amino acids, and their interactions on plant growth characteristics and essential oil of Peppermint (*Mentha piperita* L.) plants. A separate plot design with four eplicates was used in this study. Humic acid (HU) occupied four main plots (A), while the four amino acid (AM) treatments (B) were distributed among subplots. Thus, 16 interactions (A × B) were achieved. Peppermint stems were planted on March 25 in a plot divided into 16 groups, each containing six plants.

1. Humic acid levels at rates of 0, 8, 16, and 24 ml/L were added to the soil in which the plants were planted three times during the study period.

2. The amino acid treatments were as follows: control group (no sprayed plants), amino acid mixture at concentrations of 5, 10, and 15 ml/L. The plants were foliar sprayed with these concentrations three times as follows: May 15, May 31, and June 15 for the first, second, and third sprays, respectively. The plants were foliar sprayed until the surface water runoff reached the plants. All other agricultural operations were carried out as usual during the study period.

Table 1. Some physical and chemical analysis of the experimental soil

<table>
<tr><th colspan="12">Physical analysis</th><th colspan="2">Soil texture</th></tr>
<tr><td colspan="3">Clay (%)</td><td colspan="3">Silt (%)</td><td colspan="3">Fine sand (%)</td><td colspan="3">Coarse sand (%)</td><td colspan="2" rowspan="2">Sandy</td></tr>
<tr><td colspan="3">6.87</td><td colspan="3">9.79</td><td colspan="3">79.12</td><td colspan="3">4.22</td></tr>
<tr><th colspan="14">Chemical analysis</th></tr>
<tr><th rowspan="2">pH</th><th rowspan="2">E C m.mohs /Cm</th><th rowspan="2">Organic mater (%)</th><th colspan="4">Soluble cations (Meq. / L)</th><th colspan="3">Soluble anions (meq. /L)</th><th colspan="3">Available (ppm)</th><th></th></tr>
<tr><th>Mg^{++}</th><th>Ca^{++}</th><th>K^{+}</th><th>Na^{+}</th><th>Cl^{-}</th><th>HCO_3^{-}</th><th>SO_4^{--}</th><th>N</th><th>P</th><th>K</th><th></th></tr>
<tr><td>8.11</td><td>0.84</td><td>0.74</td><td>1.3</td><td>1.4</td><td>3.1</td><td>4.3</td><td>5.6</td><td>2.3</td><td>2.2</td><td>53.5</td><td>75.3</td><td>7.2</td><td></td></tr>
</table>

Harvest:

Plants harvested at the end of August. Harvesting done by cutting the plants at a height of 10 cm above the soil surface. The following data will be recorded: plant height (cm), number of branches/plants, fresh weight of plant (g), dry weight of plant (g), and essential oil content of air-dried herb according to the British Pharmacopoeia (1963) method .

Volatile oil isolation from herb:

100 g of each replicate of all treatments were weighed, hydro-distilled (HD) for 3 hours using a Clevenger apparatus (Clevenger, 1928). The essential oil content was calculated as a relative percentage (volume/weight). In addition, the total essential oils were calculated as ml/100 plants using dry weight. The essential oils extracted from peppermint plants were collected for each treatment and dried with anhydrous sodium sulphate for chemical identification.

Volatile oil isolation from herb:

100 g of each replicate of all treatments were weighed, hydro-distilled (HD) for 3 hours using a Clevenger apparatus **(Clevenger, 1928).** The essential oil content was calculated as a relative percentage (volume/weight). In addition, the total essential oils were calculated as ml/100 plants using dry weight. The essential oils extracted from sage plants were collected for each treatment and dried with anhydrous sodium sulphate for chemical identification.

Sample preparation:

The sample was dissolved in dichloromethane and injected into GC.

Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with VF-624ms column (30 m x 250 µm internal diameter and 1.4 µm film thickness). Analysis was carried out using Hydrogen as the carrier gas at a flow rate of 1.00 ml/min at a splitless, injection volume of 1.0 µl and the following temperature program: 40 °C for 0 min; rising at 6 °C /min to 200 °C and held for 0 min ; rising at 15 °C /min to 220 °C and held for 0 min ; rising at 30 °C /min to 250 °C and held for 2 min . The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 33-600 and solvent delay 3.50 min. The mass temperature was 230°C and Quad 150 °C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Statistical analysis of the data:

The data obtained will be statistically analyzed according to the **MSTATE-C model (1986)** using a 5% LSD test to determine the differences between all treatments according to **Mead et al. (1993).**

Results and discussion

Effect of organic manure (HU) and amino acid on growth traits:

According to the data in Table 2 the amount of organic manure (HU) applied to the soil had a substantial impact on plant height and the number of branches per plant. Furthermore, HU4 of organic manure (HU) was shown to have the highest values in mint development traits. The two most important characteristics affecting plant growth in Mentha piperita are plant height and branch count. The stimulatory effects of organic manure (HU) seem to be directly related to enhanced absorption of micronutrients and macronutrients such as potassium, phosphorus, and nitrogen. Similarly, plants treated with 6 Litre.fed-1 of humic acid had the highest mint height and number of main branches per plant. **(El-Alakmy et al. 2017)**

As for amino acid treatments, the obtained results in Table (2) pointed out that plant height and the number of branches per plant of mint was significantly augmented as a result of all concentrations of these natural substances comparing to control plants, Obviously, the use of the high concentration of amino acid registered the tallest plant height and branch count. All tested biofertilizer

Table 2. Influence of organic manure (HU), amino acid and their combination treatments on plant height (cm) and number of branches per plant of Mentha piperita.

Organic manure	Natural Substances				
	AM1	AM2	AM3	AM4	Means (B)
	Plant height (cm)				
HU1	40.00	49.00	52.00	55.00	49.00
HU2	44.00	51.00	53.67	58.00	51.67
HU3	45.00	52.33	55.67	61.33	53.58
HU4	48.67	56.67	59.67	65.67	57.67
Mean (A)	44.42	52.25	55.25	60.00	
L.S.D. at 5 %	For A= 3.28		For B= 4.39		For AB= 8.77
	Number of branches per plant				
HU1	7.17	8.83	11.50	12.83	10.08
HU2	8.17	10.00	12.50	14.00	11.17
HU3	8.67	11.00	13.33	15.00	12.00
HU4	9.33	12.00	14.67	16.33	13.08
Mean (A)	8.33	10.46	13.00	14.54	
L.S.D. at 5 %	For A= 1.71		For B= 1.83		For AB= 3.65

Control, with the highest concentrations treatment producing the most pronounced improvements. Under this treatment. These results are in accordance with those obtained by **(Roshanpour et al. 2014 Saburi et al., 2014 and Hassan and Rabie, 2020)** on basil plant, **Ali et al. (2014)** on Cassia acutifolia. On chia (Salvia hispanica) plants, **(Abou El Ghait et al. 2021**).

Regarding the combination of the different rates of organic manure (HU) and amino acid treatments , the data on mint plant growth parameters are contained in Table (2) The results demonstrated the prevalence of using different combination treatments to improve vegetative development traits such as branch count and plant height per papermint plant. However, giving plants

Organic manure at HU4 as a soil treatment along with amino acid AM4 had a significant impact on plant characteristics. As was already indicated, the mint plant's growth metrics were improved by both the organic manure (HU) and amino acid (each separately); when combined, they may have the greatest effect, resulting in higher and more branches.

Effect of organic manure (HU) and amino acid on herb fresh and dry yield per plant:

Using any rate of organic manure greatly increased herb fresh and dry yield per plant when compared to the untreated plants, according to results tabulated in Table 3 regarding the effect of organic manure on herb fresh and dry weight per plant (as herb yield traits) of mint plants. Additionally, greater values were obtained in this regard when HU was applied to the soil at a rate of HU4 as opposed to the control and the other rates (HU1 and HU2) that were studied. HU improves and accelerates the absorption of nutrients through the leaves and their transfer to other parts of the plant by increasing the permeability of cell membranes. Furthermore, there are several well-described direct effects of HA on plant development, including improved macronutrient and micronutrient absorption and root growth **(Guo Gao et al., 2015).**

In respect to amino acid treatments, the listed data in Table (3) enhanced herb fresh and dry weight compared to untreated plants. All SW and BC treatments increased herb fresh and dry weight, with the SW and BC treatment being most effective. Under this treatment. These results confirm the efficacy of foliar applied AM in boosting plant growth, which is consistent with findings in species such as mint **(Abdou et al., 2012),** Nigella sativa **(Abd El-Khalek et al., 2023),** and Gladiolus **(Saja and Ammar, 2020).**

As for the interaction, it was statistically significant effect on herb fresh and dry weight / plant of mint. Obviously, the use of most combined treatments resulted in a significant augment in herb fresh and dry weight /plant comparing to untreated plants. It appears that plants grown in organic manure at the high level (HU4) plus the foliar application with the amino acid (AM4), proved higher effect in increasing herb fresh and dry weight /plant than those revealed by other combined treatments, as clearly shown in Table (3).

Table 3. Influence of organic manure (HU), amino acid and their combination treatments on herb fresh and dry weight /plant of Mentha piperita.

Organic manure	Natural Substances				
	AM1	**AM2**	**AM3**	**AM4**	**Means (B)**
	Herb fresh weight /plant (gm)				
HU1	158.33	177.33	153.67	195.33	**171.17**
HU2	163.33	181.00	192.33	202.33	**184.75**
HU3	168.33	185.00	195.00	209.00	**189.33**
HU4	179.33	190.00	205.33	214.67	**197.33**
Means (A)	**167.33**	**183.33**	**186.58**	**205.33**	
L.S.D. at 5 %	**For A= 17.47**	**For B= 12.12**	**For AB= 12.12**		
	Herb dry weight /plant (gm)				
HU1	29.67	33.33	34.83	37.00	**33.71**
HU2	30.67	34.50	36.33	39.33	**35.21**
HU3	31.67	35.83	37.33	41.83	**36.67**
HU4	32.67	36.83	38.17	43.33	**37.75**
Means (A)	**31.17**	**35.13**	**36.67**	**40.38**	
L.S.D. at 5 %	**For A= 12.12**	**For B= 1.98**	**For AB= 3.96**		

Effect of organic manure (HU) and amino acid treatments on volatile oil production:

The presented data in Table (4) cleared that volatile oil %, its yield per plant as well as per hectare of mint was positively responded to the application of humic acid as an organic manure. Obviously, the addition of humic acid HU at all levels, led to a significant increase in volatile oil production, except for the lowest level (HU1) for volatile oil % of such manure, as compared to unfertilized plants. As regard, increasing the levels of organic manure such parameters were gradually significantly elevated. There are various methods of application, including through the soil, as a spray, or through fertigation. Regardless of the method used, the application of humic substances has been shown to significantly increase the biosynthesis of metabolism and production of biologically and pharmacologically active metabolites in medicinal plants **(Pereira et al., 2019)**. These results are in line with those reported by **Jamali et al.,2015). Sardashti et al. (2012)** on (Artemisia herbalba) and (Semenovia suffruticosa) and **(Sharaf-El-Deen et al. 2012 and Mohamed et al., 2022)** on fennel.

Table 4. Effect of organic manure (HU), amino acid (AM) and their combination treatments on volatile oil production of Mentha piperita.

Organic manure	Natural Substances				
	AM1	**AM2**	**AM3**	**AM4**	**Means (B)**
	Volatile oil (%)				
HU1	1.090	1.160	1.190	1.260	**1.175**
HU2	1.150	1.200	1.247	1.330	**1.232**
HU3	1.170	1.220	1.287	1.367	**1.261**
HU4	1.197	1.250	1.320	1.430	**1.299**
Means (A)	**1.152**	**1.208**	**1.261**	**1.347**	
L.S.D. at 5 %	**For A= 0.062**		**For B= 0.054**		**For AB= 0.109**
	Volatile oil yield per plant (ml)				
HU1	0.324	0.392	0.422	0.473	**0.403**
HU2	0.353	0.415	0.454	0.524	**0.437**
HU3	0.371	0.438	0.481	0.573	**0.466**
HU4	0.392	0.463	0.507	0.627	**0.497**
Means (A)	**0.360**	**0.427**	**0.466**	**0.549**	
L.S.D. at 5 %	**For A= 0.046**		**For B= 0.044**		**For AB= 0.088**
	Volatile oil yield per hectare (L)				
HU1	12.947	15.691	16.866	18.933	**16.109**
HU2	14.133	16.607	18.148	20.972	**17.465**
HU3	14.840	17.533	19.249	22.905	**18.632**
HU4	15.669	18.529	20.273	25.081	**19.888**
Means (A)	**14.397**	**17.090**	**18.634**	**21.973**	
L.S.D. at 5 %	**For A= 1.828**		**For B= 1.758**		**For AB= 3.516**

In addition, foliar application of amino acid (AM) significantly enhanced essential oil production. Although most AM concentrations improved the essential oil percentage relative to the control, the 15 ml/L treatment was the most effective. Under 15 ml/L AM, essential oil percentages were 1.375 and 0.489 for volatile oil yield per plant (ml), with corresponding volatile oil yield per hectare (L) of 19.58 L/hectare/season. In contrast, the control treatments yielded lower percentages and yields. These results are consistent with previous findings in aromatic plants such as coriander (**Hassan and Ali, 2010**; **Rekaby, 2013**) and Nigella sativa (**Abd El-Khalek et al., 2023**).

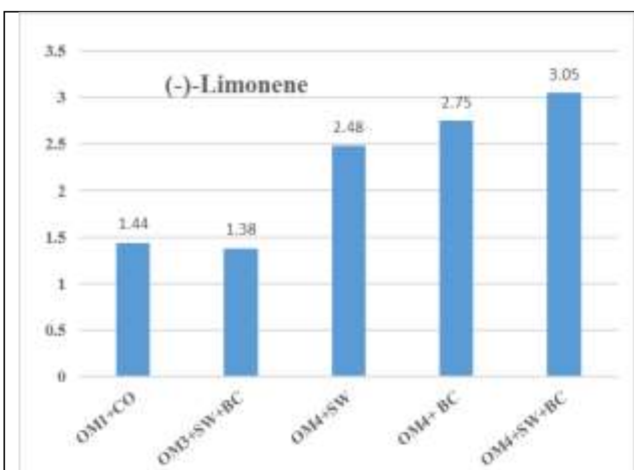
With respect to the interaction, the listed data in (Table 4) pointed out that significant effect on volatile oil production of mint, Clearly, supplemented the plants with the most combined treatments caused a significant elevate of volatile oil production, compared to control plants. Apparently, the utilization of humic acid at the high level (HU4) + (AM4) treatment, proved to be more effective in augmenting volatile oil percentage, its yield per plant as well as per hectare than those noticed by other combination treatments.

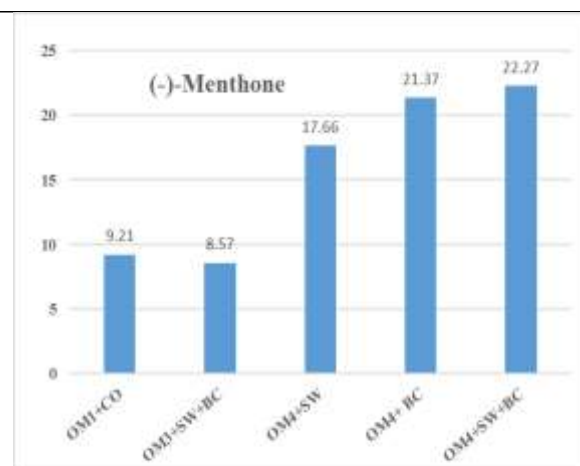
Effect of organic manure (HU) and amino acid treatments on volatile oil compounds:

According to volatile oil compounds, HU4+AM4 combination treatment recorded the highest values of Limonene, Menthone, Mentofuran, iso-Menthone, neo-Menthol, Menthol, Pulegone and 1,8-cineole. Also, the highest values of iso-Menthol and ρ-cymene acetate were obtained with HU2+AM1 treatment. Compared to the other combinations under study (Table 5 and Figs. 1 and 2).

Table 5. Effect of natural nutrients on essential oil components (%) of papermint (*Mentha piperita* L.) plants

Treatments / E.O components	HU1+AM1	HU3+AM4	HU4+AM3	HU4+AM2	HU4+AM4
(-)-Limonene	1.44	1.38	2.48	2.75	3.05
(-)-Menthone	9.21	8.57	17.66	21.37	22.27
(+)-Mentofuran	2.05	1.88	3.55	5.63	6.11
(+)-iso-Menthone	1.38	1.25	2.28	2.16	2.33
(+)-neo-Menthol	3.44	3.22	3.81	4.86	4.95
(-)-Menthol	38.25	37.00	39.22	39.75	40.15
(+)-iso-Menthol	1.11	1.04	0.85	0.77	0.88
(+)-Pulegone	1.45	1.36	2.18	4.18	4.37
1,8-cineole	1.27	1.21	1.37	1.47	1.67
ρ-cymene	1.26	0.23	0.31	0.33	0.37





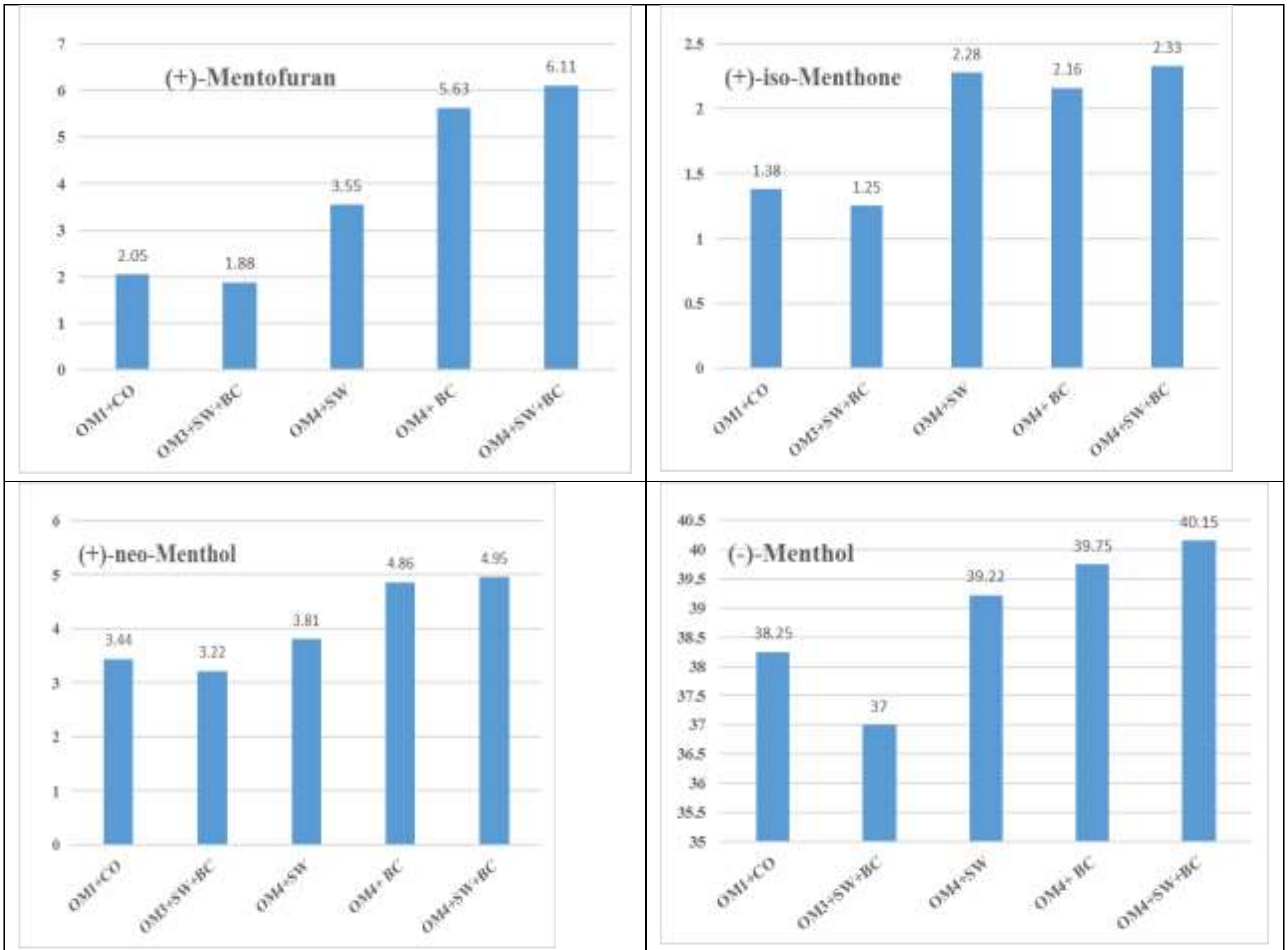
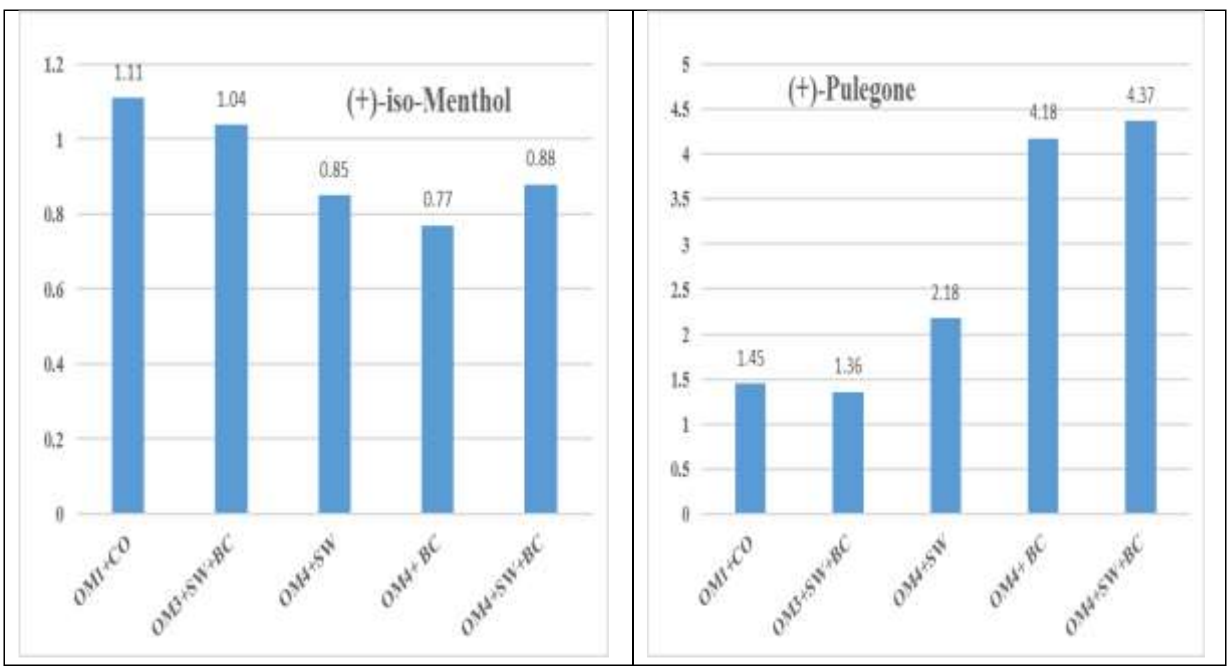


Fig. 1. Influence of organic manure and amino acid treatments on Limonene, Menthone, Mentofuran, iso-Menthone, neo-Menthol and Menthol of Mentha piperita.



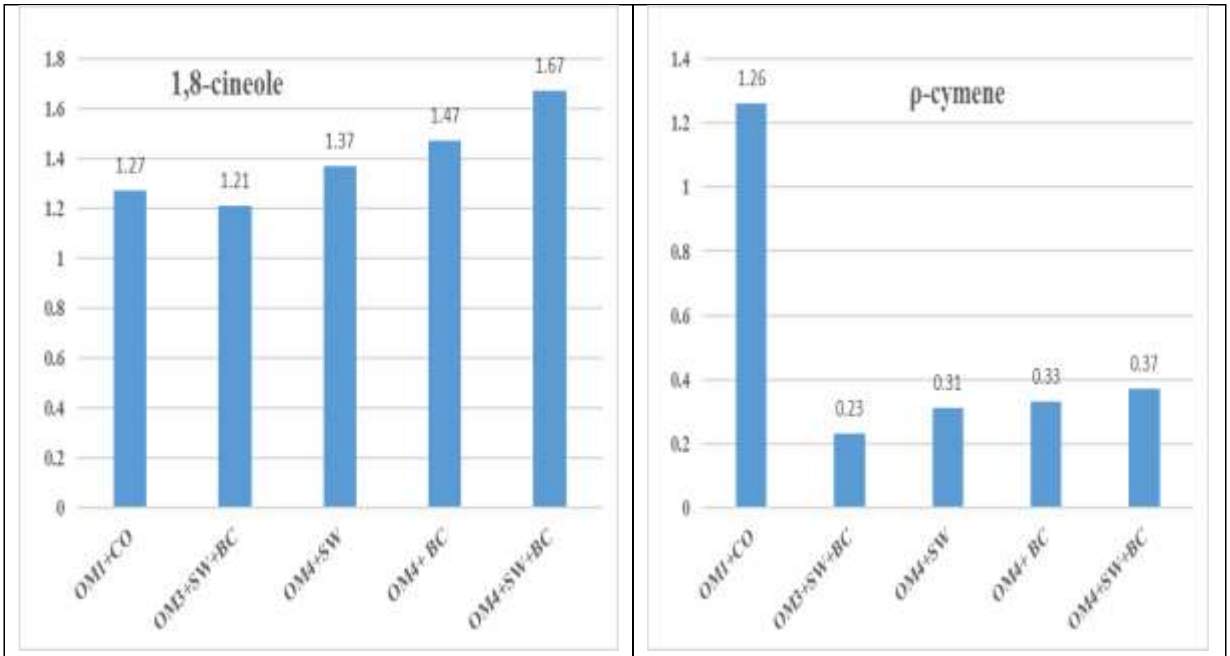


Fig. 2. Influence of humic acid and amino acid treatments on iso-Menthol, Pulegone, 1,8-cineole and ρ-cymene of Mentha piperita.

Conclusion

The study's most significant finding is that the productivity of the mint (Mentha piperita L.) plants increased as a result of the varying organic manure utilized. When compared to the control, the mint plants' growth, herb yield, and oil yield all markedly enhanced with amino acid treatments. Growth, yield, and the oil index were all significantly impacted by the interaction between organic manure and amino acid treatments. Organic manure at (HU4) with highy concentratio at amino acid (AM4) were the most effective treatments

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