

FOLIAR FERTILIZER APPLICATION: EFFECTS ON AGRONOMIC PERFORMANCE AND ESSENTIAL OIL PROFILE ACROSS DIFFERENT *MENTHA* SPECIES

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ABSTRACT

This study investigated the effects of various foliar organic fertilizers (plant-based amino acid, animal-based amino acid, and seaweed extract) on the agronomic performance and essential oil composition of commercially important Mint (*Mentha*) plant species: *Mentha arvensis*, *Mentha × piperita*, and *Mentha spicata*. The experiment was laid out under randomized complete block design (RCBD) with three replications during the 2021 and 2022 growing seasons. Significant variations in dried leaf yield, essential oil content, and composition have been observed among the mint species used in the study and the different foliar fertilizer treatments. Species × fertilizer combinations resulted in dried leaf yield ranging from 1.7 to 4.1 t ha⁻¹ and essential oil content ranging from 1.88% to 5.54%. The essential oil composition exhibited notable variations depending on the species. The menthol content ranged from 58.75% to 66.29% in *Mentha arvensis*, from 30.40% to 52.23% in *Mentha × piperita* (Mitcham, Multimentha, Swiss), and carvone varied between 31.70% and 44.12% in *Mentha spicata*. Pearson correlation analysis revealed significant positive correlations among yield traits and a strong correlation between essential oil content and essential oil yield. Principal component analysis (PCA) explained 97.4% of the total variance and clearly separated *Mentha* species × fertilizer combinations based on agronomic performance, essential oil content, and yield. *Mentha arvensis* × fertilizer combinations excelled in essential oil content and yield, while *Mentha × piperita* Multimentha × fertilizer combinations excelled in agronomic traits. The highest essential oil yield was obtained from *Mentha arvensis* X T3 combination, while the highest dried leaf yield was recorded for *Mentha × piperita* Multimentha X T4 combination. These findings emphasize the necessity of selecting appropriate mint species and foliar fertilizer treatments to optimize yield and essential oil quality, thereby enhancing the economic potential of mint cultivation.

Keywords: *Mentha*, foliar fertilizer, agronomic traits, yield, essential oil, multivariate analysis

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INTRODUCTION

Mint plants (*Mentha* spp.) are recognized as one of the oldest and most widely adored herbs worldwide. *Mentha*, a genus within the Lamiaceae family, encompasses plants known for their widespread distribution in diverse global environments, distinguished by their aromatic qualities (Šarić-Kundalić *et al.*, 2009). The *Mentha* genus holds a prominent position within the Lamiaceae family, encompassing 25–30 species distributed globally, particularly in South Africa, Australia, and the temperate regions of Eurasia (Dorman *et al.*, 2003). This taxon holds immense significance, both commercially and medicinally. With diverse applications, various parts of *Mentha* plants, such as leaves, flowers, and stems, find frequent use in herbal medicine, teas, and as aromatic and flavorful additives in spice blends for a range of foods (McKay and Blumberg, 2006; Nieto,

2017). According to Dorman *et al.* (2003), Oudhia (2003), and Telci *et al.* (2011) the mint species that hold notable commercial importance are primarily spearmint (*Mentha viridis* L., syn. *Mentha spicata* L.), peppermint (*Mentha × piperita* L.), and corn mint (*Mentha arvensis* L., syn. *Mentha canadensis* L.).

Mint (*Mentha* spp.) cultivation area and production in Türkiye increased significantly, reaching 14.970 ha and 26.322 tons in 2024 compared to 886 ha and 6.500 tons in 2004 (TUIK, 2025). Despite this growth, the country still depends on imports for menthol and essential oil. In mint cultivation, selecting appropriate genotypes and enhancing agronomic practices are essential for Türkiye to meet its demand for mint essential oil and menthol production and become a major global producer, leveraging its agricultural resources, climate and ecological conditions, industry, and strategic geographical position (Yilmaz and Telci, 2022). Moreover, in response to climate change and

limited arable land, developing adaptive cultivation strategies, including optimized nutrient and fertilizer management tailored to arid and semi-arid regions, is crucial for sustainable mint production.

Fertilization, a key agronomic practice in plant production, has a substantial impact on yield and its components (Moniruzzaman *et al.*, 2014; Hussain *et al.*, 2016). The impact of fertilization on the yield of medicinal and aromatic plants is noteworthy. Fertilization can be divided into two main methods: root and foliar fertilization. Foliar fertilization can be absorbed directly through the leaves and can be transported more quickly and efficiently to the other plant organs compared to root fertilization (Kentelky and Szekely-Varga, 2021; Niu *et al.*, 2021). Foliar nutrient application is a key strategy for optimizing crop yields, serving to complement soil fertilization. Unlike soil application, where nutrients are absorbed by plant roots and translocated to aerial parts, foliar application involves the direct penetration of nutrients through the leaf cuticle or stomata into plant cells. As a result, crops respond more rapidly to foliar application compared to soil application, leading to quicker improvements in growth and yield (Fageria *et al.*, 2009; Fernández and Eichert, 2009; Haider *et al.*, 2020). Moreover, foliar feeding significantly contributes to optimizing plant health and productivity by ensuring efficient nutrient translocation and supporting vital physiological processes such as photosynthesis (Franke, 1967; Patil and Cheatan, 2018).

Among various foliar organic fertilizers, amino acid and seaweed emerge as an eco-friendly fertilizer (Shehata *et al.*, 2011; Tabbara *et al.*, 2018; Tursun, 2022). Amino acids and peptide blends are derived through chemical and enzymatic hydrolysis of proteins from agro-industrial by-products, sourced from both plant origins such as crop residues, and animal sources like collagen and epithelial tissues (Kamar and Omar, 1987; du Jardin, 2012; Halpern *et al.*, 2015). Seaweed extract is composed of a range of bioactive compounds from marine algae, trace elements, vitamins and growth substances including cytokinins, gibberellins and auxins; which show a significant effect on plant growth (Crouch and Staden, 1993; Khan *et al.*, 2009). Numerous products

containing seaweed extract and amino acids are commercially available for application on various crops worldwide under different trade names (Bai *et al.*, 2007). In response to the imperative to reduce chemical inputs in sustainable MAPs production, there has been significant interest in organic inputs and plant growth stimulants (Asadi *et al.*, 2023).

Effective nutrient management is essential for sustainable mint cultivation to maximize biomass production and improve essential oil yield and quality (Cano-Gallego *et al.*, 2023). Previous studies have demonstrated that amino acids and seaweed extracts (Hendawy *et al.*, 2015; Hakimzadeh *et al.*, 2022; Shalaby *et al.*, 2025) enhance growth, stress tolerance, and yield in mint. However, limited information is available on the combined or comparative effects of foliar fertilizers containing amino acids and seaweed extracts on different mint species under field conditions. Therefore, this study aims to evaluate the effects of foliar applications of plant-derived amino acids, animal-derived amino acids, and seaweed extract (alginic acid) on the agronomic performance, essential oil content, and essential oil yield of commercially important mint species (*Mentha arvensis*, *Mentha × piperita*, and *Mentha spicata*) under field conditions.

MATERIALS AND METHODS

Experimental location: The study was conducted in Elmacık village, situated in the Gumusova district of Duzce province, Türkiye, during 2021 and 2022. The trial site is positioned at approximately 40°50'23.2" north latitude and 30°58'24.3" east longitude, with an elevation of 160 meters above sea level. Duzce province is located in the Western Black Sea Region, and its climate is characterized by the general features and influences of the Black Sea climate. The climatic conditions during the experimental years (2021-22) and long years are outlined in Fig. 1. In the first year of the experiment, the average temperature (14.5 °C) and total rainfall (1027.5 mm) were higher than in the second year (14.2 °C and 911.0 mm) (TSMS, 2025).

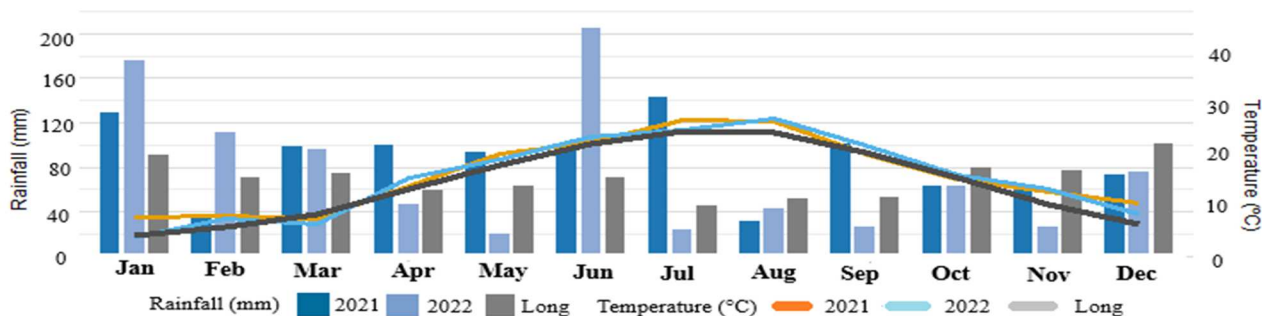


Figure 1. Meteorological data of the experimental field during years 2021-2022 and long years

The experimental area was characterized by clayey soil with a slightly acidic pH. The soil was found to be free of salt, with very low lime content. Additionally, it exhibited limited phosphorus availability

for plants, low potassium levels, and high nitrogen and organic matter content. The soil properties of the experimental area are provided in Table 1.

Table 1. Physico-chemical properties of experimental field soil at 30 cm depth

Texture	pH	E.C. (mmhos cm ⁻¹)	CaCO ₃ (%)	O. M. (%)	N (%)	P ₂ O ₅ (ppm)	K ₂ O (ppm)
Clayey (86)	6.41	0.033	0.000	4.874	0.244	0.57	26

Plant and fertilizer material, treatments, and experimental design: In the experiment, plant materials consisted of *Mentha arvensis* (clone), *Mentha × piperita*

(varieties including Mitcham, Multimentha, and Swiss), and *Mentha spicata* (clone) (Fig. 2). These mint species were sourced from the private sector for use in this study.



Figure 2. a: *M. arvensis* b: *M. × piperita* Mitcham c: *M. × piperita* Multimentha d: *M. × piperita* Swiss e: *M. spicata*

The trial area underwent plowing at a depth of 20-30 cm during the autumn season, followed by superficial plowing in spring prior to planting. Subsequently, plots were prepared through the use of a disk-harrow. Plant materials for the study were propagated in a greenhouse, and the experiment was initiated in 2021 using seedlings (10-15 cm) derived from rooted cuttings. The experimental design employed a split-plot arrangement with three replications in randomized blocks. Main plots were designated for control and fertilizer applications, while sub-plots were allocated for different species. Each plot had a length of 2.4 m, and a width of 1.6 m, with a 1.5 m distance

between blocks and 0.8 m between plots. Planting was conducted in a manner ensuring each plot comprised four rows, with a 0.4 m gap between rows and 0.3 m between plants. Before planting, the trial area received fertilizer application at a rate of 50 N kg ha⁻¹ and 50 P₂O₅ kg ha⁻¹. Additionally, 50 N kg ha⁻¹ was applied after each cutting.

In the experiment, a control (CK) and four liquid fertilizers (T1, T2, T3, and T4), all sourced from the private sector, were applied as treatments. Their compositional details are presented in Table 2. T1 and T3 contain plant-based amino acids, T2 contains animal-based amino acids, and T4 is formulated with seaweed extract, specifically rich in alginic acid (Table 2).

Table 2. The fertilizers used in the experiment and their contents

Fertilizer	Organic Matter (%)	Organic Carbon (%)	Organic Nitrogen (%)	Water Soluble Potassium Oxide (%)	Free Amino acid (%)	pH	Alginic acid (%)
Plant-based Amino Acid Fertilizer I (T1)	25	11	1.5	6	7	4.6-6.6	-
Animal-based Amino Acid Fertilizer (T2)	62	29	8	-	10	5.5-7.5	-
Plant-based Amino Acid Fertilizer II (T3)	47	23	5	5	12	7.0-9.0	-
Seaweed Extract-based Fertilizer (T4)	5	-	-	2	-	8.3-10.3	0.3

The rooted *Mentha* species were planted during the first week of June. During the first year of experimentation, liquid fertilizers were applied when the plants reached a height of 10-15 cm, followed by a second application 20 days later. After the first cutting, the first application was made when the plants reached 10-15 cm, followed by the second application 20-25 days later. In the second year, the same procedure was repeated four times. Plots were irrigated using a flood irrigation system as needed and protected from weeds by hand hoeing.

Harvesting and measurements: Harvests were conducted twice in each of the 2021 and 2022 growing seasons. In 2021, the first harvest was from July 28-30, and the second from October 18-20. In 2022, the first harvest occurred from July 20-22 and the second from October 15-18. Before harvesting, plant height (PH) was measured for 10 randomly selected plants in each plot. The fresh weights of all plots were promptly recorded to evaluate fresh herb (FH) yield, while the dried weights of 500 g fresh herb samples were measured after drying (at 35 °C in a drier cabin) to determine dried herb (DH) yield. Following this, the leaves were separated from the dried herb samples, and the leaf content within the samples was calculated as dried leaf yield (DLY). Subsequently, the dried leaf yield was determined using the dried herb yield and the calculated leaf content (Telci *et al.*, 2011).

Essential oil content and yield: The essential oil content (EOC) in dried leaves was determined via the Neo-Clevenger apparatus using a volumetric method (Clevenger, 1928). 50 g of dried leaves were extracted with 500 mL of distilled water using the same apparatus, followed by 3 hours of hydro-distillation. The resulting essential oils were dried with anhydrous sodium sulfate and stored in amber vials at +4°C. Essential oil yields (EOY) per hectare were determined by utilizing the essential oil content in dried leaves and the dried leaf yields per hectare.

Essential oil content (%) = (Distilled essential oil (g) / 50g) × 100

Essential oil yield (L ha⁻¹) = Dried leaf (t ha⁻¹) × Essential oil content (%)

Essential oil composition: Essential oil composition obtained via steam distillation was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Samples were initially diluted with hexane (1:100 ratio) and injected into the GC/GC-MS apparatus with a capillary column (HP InnowaxCapillary). Helium was used as the carrier gas, and samples were injected at a 40:1 split ratio. The injector temperature was set at 250 °C. The analysis involved a temperature program from 60 °C to 250 °C, with a total duration of 30 minutes. Mass detection ranged from 35 to 450 atomic mass units with 70 eV

electron impact ionization. Component identification relied on the WILEY and OIL ADAMS library data, with percentages determined using the Flame Ionization Detector (FID) and identification performed by the Mass Spectrometry (MS) detector. Relative abundance (% area) was calculated based on the ratio between the peak area of each compound and the sum of areas of all compounds.

Statistical analyses: Throughout each vegetation cycle of the mint plant, multiple yields were obtained, necessitating separate analyses for each harvest. Additionally, the combined results of harvests conducted throughout the year were utilized to calculate total yields and average values, which underwent subsequent statistical analysis. The homogeneity test results showed no significant difference between years ($p > 0.05$); accordingly, the two-way analysis of variance (ANOVA) was performed by pooling the two years data (Levene, 1960). Following variance analyses, significant features were grouped using the LSD test. Principal component analysis based on pooled data was evaluated using biplot visualization. All statistical analyses, including Pearson correlation coefficients, were performed using JMP software (JMP 15.1, SAS Institute Inc., 2020). Data visualizations were generated in R using the 'ggplot2' package (Wickham, 2009).

RESULTS

Plant height: Plant height was significantly influenced by species, fertilizer treatments (Table 3), and their interaction (Table 4). Among the species, the highest plant height was observed in the *Mentha* × *piperita* Multimentha (45.31 cm), and the lowest in the *M. arvensis* (30.86 cm). The highest plant height among fertilizer treatments was recorded under T3 treatment (39.34 cm), whereas the lowest was observed in the control (CK) treatment (36.94 cm) (Table 3). Regarding species × fertilizer combinations, the highest value was measured in the *M.* × *piperita* Multimentha X T4 combination (47.03 cm), and the lowest in the *M. arvensis* X CK combination (28.53 cm) (Table 4).

Fresh herb yield: Significant differences were observed in fresh herb yield among species, fertilizer treatments (Table 3), and their interactions (Table 4). Among the species, the highest fresh herb yield was obtained from *Mentha* × *piperita* Multimentha (21.5 t ha⁻¹), while the lowest was recorded in *M. arvensis* (12.5 t ha⁻¹). Across fertilizer treatments, the highest fresh herb yield was obtained under T3 treatment (18.0 t ha⁻¹), while the lowest was recorded in the CK treatment (13.5 t ha⁻¹) (Table 3). Considering species × fertilizer interactions, fresh herb yield was highest in the *M.* × *piperita* Multimentha X T4 combination (24.4 t ha⁻¹), while the

lowest was recorded in the *M. arvensis* X CK combination (9.9 t ha⁻¹) (Table 4).

Dried herb yield: Dried herb yield was significantly influenced by species, fertilizer treatments (Table 3), and their interaction (Table 4). Regarding the species, the highest dried herb yield was obtained from *M. × piperita* Multimentha (5.9 t ha⁻¹), and the lowest from *M. arvensis* (3.4 t ha⁻¹). Considering fertilizer treatments, the maximum dried herb yield was obtained under T3 treatment (4.9 t ha⁻¹), while the lowest was recorded under the CK treatment (3.7 t ha⁻¹) (Table 3). In terms of species × fertilizer combinations, the maximum yield was observed in the *M. × piperita* Multimentha X T4 combination (6.9 t ha⁻¹), whereas the minimum was noted in the *M. arvensis* X CK combination (2.8 t ha⁻¹) (Table 4).

Dried Leaf Yield: Significant differences in dried leaf yield were observed across species, fertilizer treatments (Table 3), as well as in species × fertilizer interactions (Table 4). Dried leaf yield ranged from 2.0 t ha⁻¹ in *M. arvensis* to 3.5 t ha⁻¹ in *M. × piperita* Multimentha. Regarding fertilizer treatments, the highest dried leaf yield was obtained under T3 treatment (2.9 t ha⁻¹), whereas the lowest was recorded in the CK treatment (2.2 t ha⁻¹) (Table 3). In species × fertilizer interactions, the *M. × piperita* Multimentha X T4 combination (4.1 t ha⁻¹) showed the highest dried leaf yield, while the

lowest was observed in the *M. arvensis* × CK combination (1.7 t ha⁻¹) (Table 4).

Essential oil content: Essential oil content was significantly influenced by species, fertilizer treatments (Table 3), and their interaction (Table 4). The highest essential oil content among species was observed in *M. arvensis* (5.35%), while the lowest was found in *M. spicata* (2.03%). Among fertilizer treatments, the highest essential oil content was obtained under T4 treatment (3.03%), and the lowest under the CK treatment (2.72%) (Table 3). In species × fertilizer combinations, the highest content was recorded in the *M. arvensis* X T4 combination (5.54%), whereas the lowest was noted in the *M. spicata* X T2 combination (1.88%) (Table 4).

Essential oil yield: Essential oil yield was significantly affected by species, fertilizer treatments (Table 3), and their interaction (Table 4). Across the species, the highest yield was obtained from *M. arvensis* (135.9 L ha⁻¹), whereas the lowest was recorded in *M. × piperita* Mitcham (65.7 L ha⁻¹). Among fertilizer treatments, the highest essential oil yield was obtained under T3 treatment (96.2 L ha⁻¹), whereas the lowest essential oil yield (67.9 L ha⁻¹) was recorded under the CK treatment (Table 3). In species × fertilizer combinations, the highest yield was observed in the *M. arvensis* X T3 combination (150.31 L ha⁻¹), whereas the lowest (50.17 L ha⁻¹) was found in the *M. × piperita* Swiss X CK combination (Table 4).

Table 3. Effects of species and fertilizer on agronomic and essential oil traits

Treatments	PH (cm)	FH (t ha ⁻¹)	DH (t ha ⁻¹)	DLY (t ha ⁻¹)	EOC (%)	EOY (L ha ⁻¹)
Species						
<i>M. arvensis</i>	30.86±0.57 ^d	12.5±0.38 ^c	3.4±0.14 ^c	2.0±0.07 ^d	5.35±0.04 ^a	135.9±3.45 ^a
<i>M. × piperita</i> Mitcham	34.25±0.57 ^c	13.5±0.38 ^c	3.9±0.14 ^d	2.4±0.07 ^c	2.41±0.04 ^c	65.7±3.45 ^d
<i>M. × piperita</i> Multimentha	45.31±0.57 ^a	21.5±0.38 ^a	5.9±0.14 ^a	3.5±0.07 ^a	2.23±0.04 ^d	87.3±3.45 ^b
<i>M. × piperita</i> Swiss	40.52±0.57 ^b	16.0±0.38 ^b	4.2±0.14 ^c	2.4±0.07 ^c	2.61±0.04 ^b	74.2±3.45 ^c
<i>M. spicata</i>	39.34±0.57 ^b	16.9±0.38 ^b	4.7±0.14 ^b	2.8±0.07 ^b	2.03±0.04 ^e	67.9±3.45 ^{cd}
LSD value at 5%	1.62	1.06	0.26	0.22	0.12	8.0
Fertilizer						
CK	36.94±0.57 ^c	13.5±0.38 ^d	3.7±0.14 ^d	2.2±0.07 ^c	2.72±0.04 ^b	67.9±3.45 ^c
T1	37.06±0.57 ^{bc}	15.4±0.38 ^c	4.1±0.14 ^c	2.5±0.07 ^b	3.00±0.04 ^a	84.4±3.45 ^b
T2	38.16±0.57 ^{a,c}	16.7±0.38 ^b	4.6±0.14 ^{ab}	2.7±0.07 ^{ab}	2.94±0.04 ^a	89.1±3.45 ^{ab}
T3	39.44±0.57 ^a	18.0±0.38 ^a	4.9±0.14 ^a	2.9±0.07 ^a	2.95±0.04 ^a	96.2±3.45 ^a
T4	38.68±0.57 ^{ab}	16.9±0.38 ^b	4.6±0.14 ^b	2.8±0.07 ^a	3.03±0.04 ^a	93.3±3.45 ^a
LSD value at 5%	1.62	1.06	0.40	0.22	0.12	8.00
CV	8.14	6.60	10.78	11.38	7.99	18.32

Means followed by different letters in a column are statistically different at p<0.05 by LSD test. CK: Control, T1: Plant-based Amino Acid Fertilizer I, T2: Animal-based Amino Acid, T3: Plant-based Amino Acid Fertilizer II, T4: Seaweed Extract-based Fertilizer. PH: Plant height, FH: Fresh herb, DH: Dried herb, DLY: Dried leaf yield, EOC: Essential oil content, EOY: Essential oil yield, NS: Non-significant

Table 4. The effects of species × fertilizer interactions on agronomic and essential oil traits

Species × Fertilizer	PH (cm)	FH (t ha ⁻¹)	DH (t ha ⁻¹)	DLY (t ha ⁻¹)	EOC (%)	EOY (L ha ⁻¹)
<i>M. arvensis</i> X CK	28.53±1.83 ^k	9.9±0.85 ^j	2.8±0.32 ^m	1.7±0.18 ^m	4.95±0.09 ^b	110.02±7.72 ^b
<i>M. arvensis</i> X T1	28.62±1.83 ^k	12.1±0.85 ^j	3.3±0.32 ^{lm}	1.9±0.18 ^{k-m}	5.51±0.09 ^a	130.60±7.72 ^a
<i>M. arvensis</i> X T2	30.33±1.83 ^{jk}	12.4±0.85 ^{hi}	3.5±0.32 ^{kl}	2.1±0.18 ^{j-l}	5.38±0.09 ^a	140.01±7.72 ^a
<i>M. arvensis</i> X T3	33.52±1.83 ^{i-j}	14.6±0.85 ^{d-h}	4.1±0.32 ^{g-j}	2.3±0.18 ^{h-j}	5.39±0.09 ^a	150.31±7.72 ^a
<i>M. arvensis</i> X T4	33.33±1.83 ^{i-j}	13.3±0.85 ^{g-i}	3.4±0.32 ^{k-m}	2.0±0.18 ^{j-l}	5.54±0.09 ^a	140.01±7.72 ^a
<i>M. × pip.</i> Mitcham X CK	34.38±1.83 ^{h-i}	12.4±0.85 ^{ij}	3.5±0.32 ^{kl}	2.1±0.18 ^{j-l}	2.08±0.09 ^{i-l}	50.48±7.72 ^{f-h}
<i>M. × pip.</i> Mitcham X T1	32.47±1.83 ^{jk}	13.1±0.85 ^{hi}	3.5±0.32 ^{j-l}	2.2±0.18 ^{i-k}	2.53±0.09 ^{e-g}	60.47±7.72 ^{f-h}
<i>M. × pip.</i> Mitcham X T2	33.92±1.83 ^{i-j}	12.5±0.85 ^{g-i}	3.7±0.32 ^l	2.3±0.18 ^{g-j}	2.45±0.09 ^{d-g}	60.50±7.72 ^{f-h}
<i>M. × pip.</i> Mitcham X T3	37.01±1.83 ^{gh}	15.6±0.85 ^{d-h}	4.3±0.32 ^{f-i}	2.6±0.18 ^{d-g}	2.39±0.09 ^{e-h}	70.15±7.72 ^{c-h}
<i>M. × pip.</i> Mitcham X T4	33.47±1.83 ^{i-j}	14.1±0.85 ^{f-i}	4.0±0.32 ^{f-k}	2.6±0.18 ^{d-h}	2.42±0.09 ^{e-g}	70.26±7.72 ^{d-h}
<i>M. × pip.</i> Multimentha X CK	43.31±1.83 ^{a-d}	17.9±0.85 ^{bcd}	4.8±0.32 ^{c-f}	2.8±0.18 ^{cde}	2.28±0.09 ^{g-j}	60.41±7.72 ^{f-h}
<i>M. × pip.</i> Multimentha X T1	44.60±1.83 ^{a-d}	18.7±0.85 ^b	5.1±0.32 ^{cd}	3.0±0.18 ^c	2.31±0.09 ^{g-j}	70.89±7.72 ^{c-f}
<i>M. × pip.</i> Multimentha X T2	44.87±1.83 ^{a-c}	22.7±0.85 ^a	6.3±0.32 ^b	3.7±0.18 ^b	2.30±0.09 ^{g-j}	90.48±7.72 ^{bc}
<i>M. × pip.</i> Multimentha X T3	46.75±1.83 ^{ab}	23.8±0.85 ^a	6.6±0.32 ^{ab}	3.9±0.18 ^{ab}	2.15±0.09 ^{h-l}	90.33±7.72 ^{bcd}
<i>M. × pip.</i> Multimentha X T4	47.03±1.83 ^a	24.4±0.85 ^a	6.9±0.32 ^a	4.1±0.18 ^a	2.33±0.09 ^{f-h}	100.56±7.72 ^b
<i>M. × pip.</i> Swiss X CK	39.53±1.83 ^{e-g}	12.0±0.85 ^j	3.3±0.32 ^{lm}	1.9±0.18 ^{lm}	2.37±0.09 ^{e-h}	50.17±7.72 ^h
<i>M. × pip.</i> Swiss X T1	41.18±1.83 ^{c-f}	16.7±0.85 ^{b-e}	4.5±0.32 ^{e-h}	2.6±0.18 ^{d-h}	2.63±0.09 ^{c-e}	70.95±7.72 ^{c-f}
<i>M. × pip.</i> Swiss X T2	39.92±1.83 ^{d-g}	16.2±0.85 ^{c-f}	4.4±0.32 ^{f-h}	2.8±0.18 ^{c-e}	2.71±0.09 ^{cd}	70.71±7.72 ^{c-f}
<i>M. × pip.</i> Swiss X T3	40.83±1.83 ^{ef}	18.2±0.85 ^c	5.0±0.32 ^{c-e}	2.8±0.18 ^{c-e}	2.77±0.09 ^c	80.88±7.72 ^{b-e}
<i>M. × pip.</i> Swiss X T4	41.13±1.83 ^{d-f}	16.8±0.85 ^{b-e}	4.1±0.32 ^{g-i}	2.5±0.18 ^{f-i}	2.59±0.09 ^{c-f}	70.41±7.72 ^{c-g}
<i>M. spicata</i> X CK	38.98±1.83 ^{e-g}	15.6±0.85 ^{d-g}	4.3±0.32 ^{g-i}	2.6±0.18 ^{d-h}	1.94±0.09 ^{kl}	50.89±7.72 ^{f-h}
<i>M. spicata</i> X T1	38.42±1.83 ^{f-g}	16.6±0.85 ^{b-e}	4.3±0.32 ^{f-h}	2.7±0.18 ^c	2.01±0.09 ^{kl}	60.31±7.72 ^{fgh}
<i>M. spicata</i> X T2	41.78±1.83 ^{c-f}	19.0±0.85 ^b	5.3±0.32 ^c	3.0±0.18 ^c	1.88±0.09 ^l	60.88±7.72 ^{e-h}
<i>M. spicata</i> X T3	39.10±1.83 ^{fg}	17.3±0.85 ^{b-d}	4.8±0.32 ^{c-f}	3.1±0.18 ^c	2.05±0.09 ^{j-l}	70.45±7.72 ^{c-h}
<i>M. spicata</i> X T4	38.43±1.83 ^{fg}	16.1±0.85 ^{c-f}	4.6±0.32 ^{d-g}	2.8±0.18 ^{cd}	2.26±0.09 ^{g-k}	70.42±7.72 ^{c-g}
LSD	3.63	2.38	0.90	0.49	0.26	21.6

Means followed by different letters in a column are statistically different at $p < 0.05$ by LSD test. PH: Plant height, FH: Fresh herb, DH: Dried herb, DLY: Dried leaf yield, EOC: Essential oil content, EOY: Essential oil yield. CK: Control (No Fertilizer), T1: Plant-based Amino Acid Fertilizer-I, T2: Animal-based Amino Acid, T3: Plant-based Amino Acid Fertilizer-II, T4: Seaweed Extract-based Fertilizer.

Essential oil composition: The essential oil analysis revealed that compounds such as 3-Octanol, Bicyclol, D-Germacrene, D-Limonene, Isopulegol, Menthol, Menthone, Piperitone, α -Pinene, β -Caryophyllene, β -Cubebene, β -Myrcene, and β -Pinene were present in relatively high proportions among other constituents (Fig. 3a). The study also found that menthol content in *M. arvensis* ranged from 58.75% to 61.02% in the first year and increased to 63.73%–66.26% in the second year. The menthone content in the *Mentha arvensis* ranged from 10.05–21.02% in the first year and 9.61–10.71% in the second year (Fig. 3b). *M. × piperita* Mitcham exhibited major essential oil components including 1,8-Cineol, Bicyclol, Germacrene, Menthofuran, Menthol, Menthone, Veridiflorol, α -Pinene, β -Caryophyllene, and β -Pinene (Fig. 4a). Menthol content ranged from 38.64% to 49.48% in the first year and from 31.33% to 41.41% in the second year. Menthone content ranged from 0.0% to 23.41% in the first year and from 19.82% to 23.52% in the second year (Fig. 4b). *M. × piperita* Multimentha essential oil comprised major compounds such as α -Pinene, β -Pinene, 1,8-Cineol, Menthone, Menthofuran, Menthol, Bicyclol, β -Caryophyllene, Germacrene, and

Veridiflorol (Fig. 5a). Menthol content ranged from 35.73% to 52.43% in the first year, decreasing to 30.40%–36.43% in the second year. Menthone content ranged from 4.78% to 24.61% in the first year, increasing to 14.13%–27.53% in the second year (Fig. 5b). *M. × piperita* Swiss essential oil contained major compounds including α -Pinene, β -Pinene, Pulegone, Piperitone, Menthofuran, Menthyl acetate, Menthone, Menthol, D-Limonene, Bicyclol, and 1,8-Cineol (Fig. 6a). Menthol content ranged from 35.50% to 39.95% in the first year and increased to 39.53%–45.07% in the second year. Menthone content ranged from 14.41% to 16.64% in the first year and rose to 18.43%–25.40% in the second year (Fig. 6b). Major essential oil constituents identified in *Mentha spicata* included β -Pinene, β -Myrcene, β -Caryophyllene, Menthone, Menthol, Dihydrocarvone, Dihydrocarvyl acetate, D-Limonene, Carvone, Carveol acetate, Carvcol, 4-Terpinol, and 1,8-Cineol (Fig. 7a). Carvone content ranged from 31.70% to 35.34% in the first year and increased to 33.74%–44.12% in the second year, while carveol content varied between 9.34% and 14.57% in the first year and between 6.29% and 16.75% in the second year (Fig. 7b).

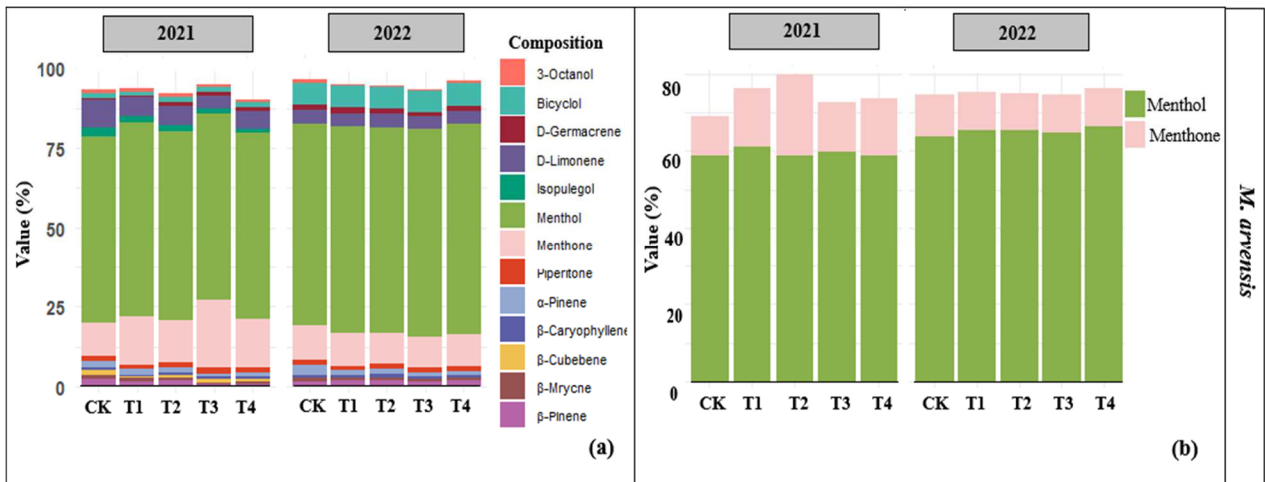


Fig. 3. Essential oil composition (a) and Menthol and Menthone content (b) in *M. arvensis* across treatments and years

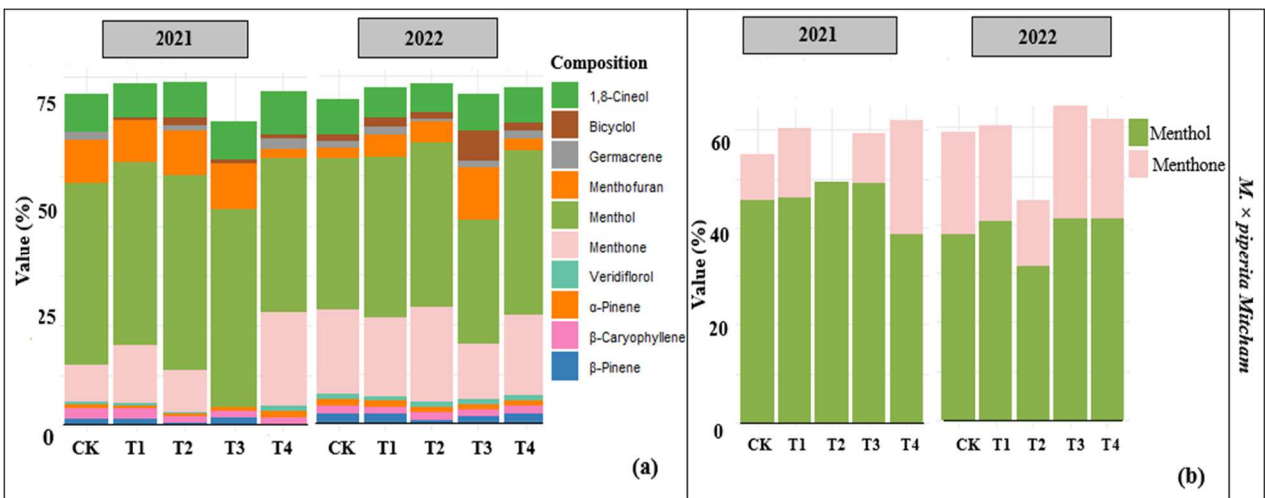


Fig. 4. Essential oil composition (a) and Menthol and Menthone content (b) in *M. x piperita Mitcham* across treatments and years

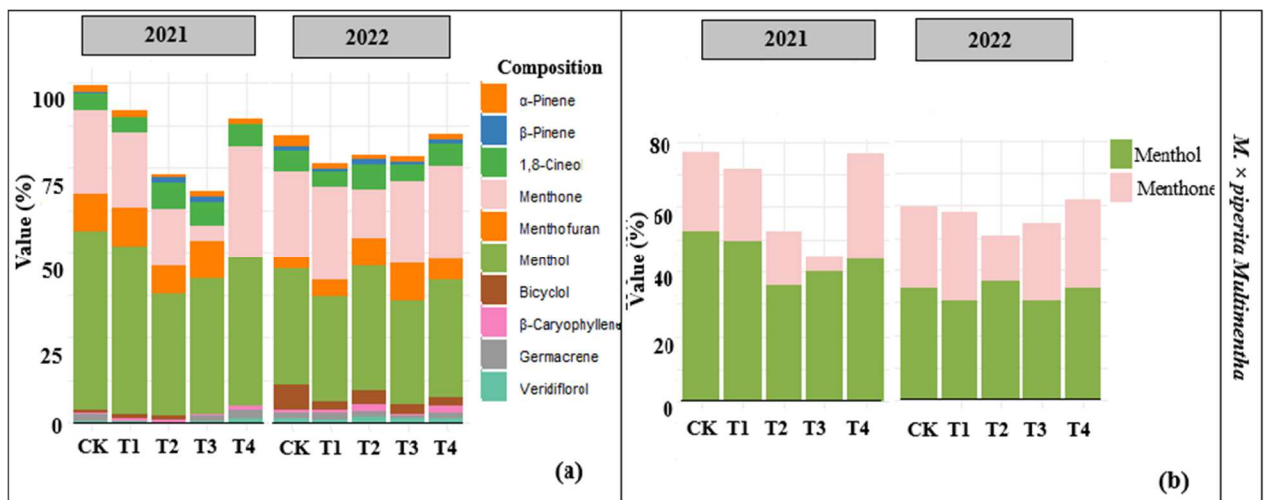


Fig. 5. Essential oil composition (a) and Menthol and Menthone content (b) in *M. x piperita Multimentha* across treatments and years

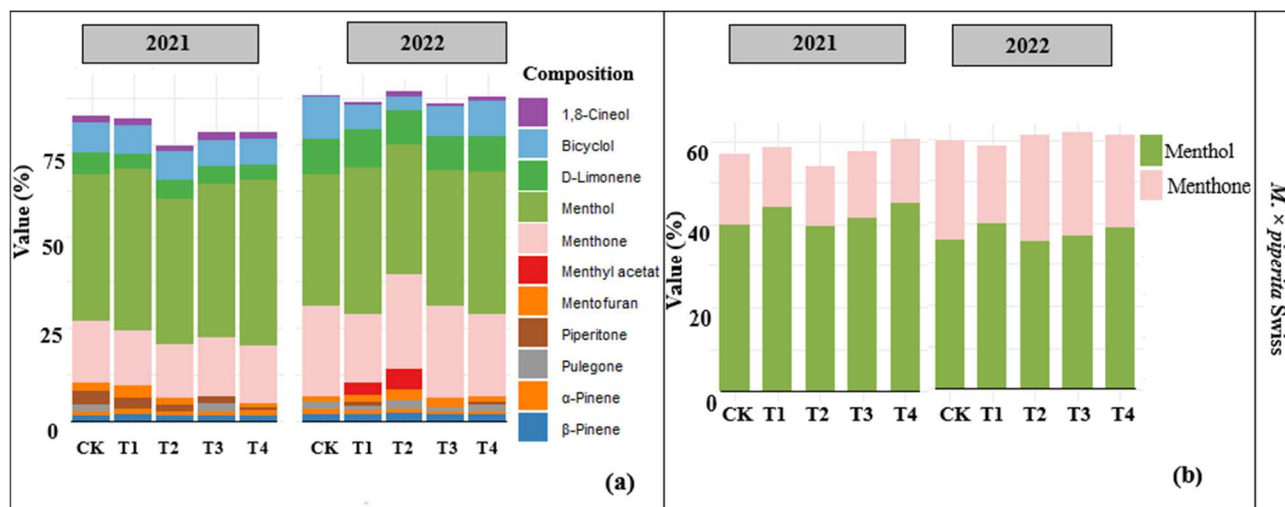


Fig. 6. Essential oil composition (a) and Menthol and Menthone content (b) in *M. × piperita* Swiss across treatments and years

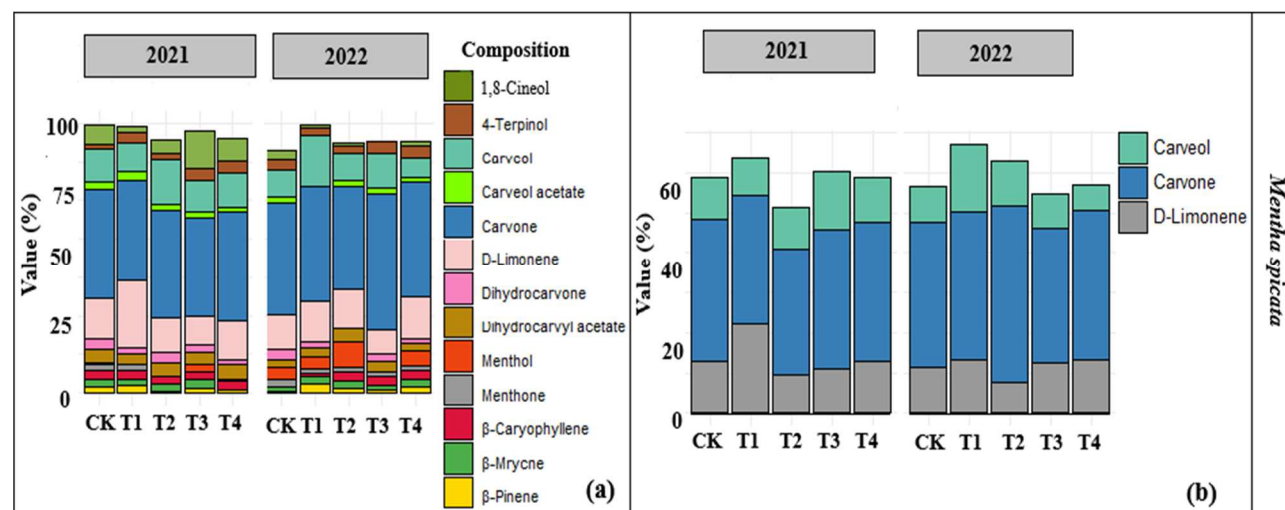


Fig. 7. Essential oil composition (a) and Carveol, Carvone and D-Limonen (b) in *M. spicata* across treatments and years

Correlation and Principal Component Analysis: The correlation analysis revealed strong positive relationships among key agronomic traits. Plant height (PH) was highly correlated with fresh herb yield (FH) ($r = 0.86^{**}$), dried herb yield (DH) ($r = 0.87^{**}$), and dried leaf yield (DLY) ($r = 0.88^{**}$). Additionally, strong and significant correlations were observed between FH and DH ($r = 0.97^{**}$), FH and DLY ($r = 0.96^{**}$), and DH and DLY ($r = 0.99^{**}$). Essential oil content (EOC) showed weak but significant positive correlations with DLY ($r = 0.31^{**}$), DH ($r = 0.32^{**}$), and FH ($r = 0.33^{**}$). Essential oil yield (EOY) was moderately to strongly correlated with FH ($r = 0.75^{**}$), DH ($r = 0.77^{**}$), and DLY ($r = 0.76^{**}$), and also showed a strong correlation with essential oil content ($r = 0.82^{**}$) (Fig. 8a).

Principal Component Analysis (PCA) was conducted to explore the combined effects of species ×

fertilizer interaction on agronomic and essential oil traits. The first two principal components (PC1 and PC2) explained a cumulative 97.4% of the total variation, with PC1 accounting for 71.8% and PC2 for 25.6%. The PCA biplot revealed distinct clustering of species × fertilizer combinations. *Mentha arvensis* (Ma) samples treated with T2, T3 and T4 fertilizers clustered in the positive PC2 region, which was associated with higher essential oil yield (EOY) and essential oil content (EOC). *M. × piperita* Multimentha (Mu) combined with T2, T3, and T4 treatments showed strong positive associations with fresh herb yield (FH), dried herb yield (DH), and dried leaf yield (DLY). Other species × fertilizer combinations, including *M. × piperita* Mitcham (Mi), *M. × piperita* Swiss (Sw), and *M. spicata* (Ms), exhibited highly similar clusters, indicating similar trait expressions across these treatments (Fig. 8b).

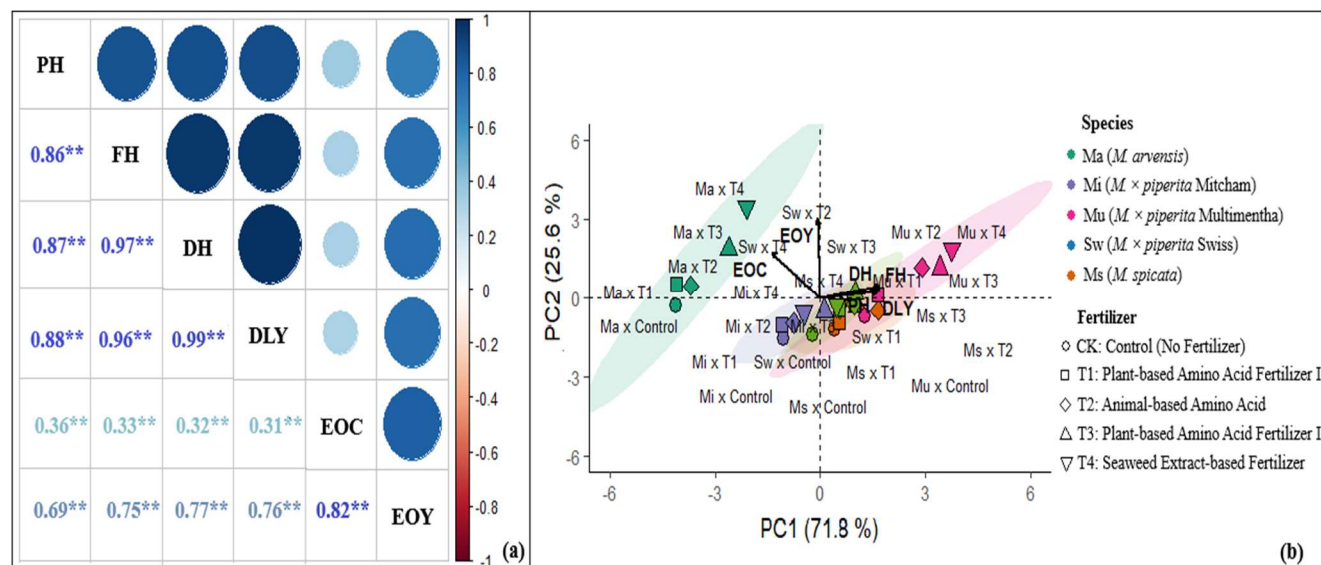


Figure 8. Pearson correlation analysis (a) and PCA of species × fertilizer interaction (b) based on agronomic performance and essential oil traits

DISCUSSION

Considering better soil health and environmental sustainability, it is imperative to use plant nutrients efficiently through the integrated application of inorganic fertilizers and organic sources such as bioformulations and biofertilizers (Bistgani *et al.*, 2018; Aswani *et al.*, 2020). Seaweed extract and amino acids are now widely employed in modern agricultural practices, effectively enhancing plant growth and productivity (Shehata *et al.*, 2011; Kumar and Aloke, 2020).

The current research focused on the effects of different foliar fertilizer sources including plant based amino acids, animal based amino acids and seaweed extract on growth parameters, yield and essential oil traits of mint species. Compared to the CK treatment, fertilizer applications caused increases in plant height (0.3 to 6.8%), fresh herb yield (14.1 to 33.3%), dried herb yield (10.8 to 32.4%), dried leaf yield (13.6 to 31.8%), essential oil content (8.1 to 11.4%) and essential oil yield (24.3 to 41.6%) (Table 3). Increasing the free amino acid content in amino acid based fertilizers improved fresh herb, dried herb and dried leaf yields. In addition, foliar application of the seaweed extract based fertilizer (T4) resulted in a clear increase in essential oil content.

The foliar application of amino acids enhances plant biochemical processes, nitrogen uptake, enzyme activity, ion transport, and stomatal regulation, thereby boosting photosynthetic rates and ultimately increasing plant growth and yield (Kowalczyk and Zielony 2008; Häusler *et al.*, 2014; du Jardin, 2015; Noroozlo *et al.*, 2019). Seaweed extract acts as an activator of cell division, enhances antioxidant levels, and stimulates the biosynthesis of tocopherol, ascorbic acid, and carotenoids

in chloroplasts. These compounds protect the photosynthetic apparatus and plant cells from stress-induced damage (Smirnov, 1995). Seaweeds are a rich resource of micro and macronutrients, amino acids, and vitamins, which can affect positively cellular metabolism, plant growth, and yield (Khan *et al.*, 2009; Abdel-Mawgoud *et al.*, 2010; Di Stasio *et al.*, 2018). Seaweed extracts were reported to enhance the growth and yield of crops by increasing nutrient availability and uptake (Battacharyya *et al.*, 2015; Van Oosten *et al.*, 2017; Shukla *et al.*, 2019). In previous studies, seaweed application was reported to increase yield in mint (Hakimzadeh *et al.*, 2022; Shalaby *et al.*, 2025). In similar studies, Hendawy *et al.* (2015) on mint, Aghaei *et al.* (2019) on hyssop, and Khan *et al.* (2019) on lettuce reported that biostimulants based on amino acids significantly improved plant dry matter yield. The present findings align with these results, as fertilizers containing amino acids and seaweed extract increased both agronomic performance and essential oil-related parameters in all mint species tested.

Essential oil content varied between 2.03% and 5.35% across species, and between 2.72% and 3.03% across fertilizer treatments (Table 3). In species × fertilizer combinations, the values ranged from 1.88% to 5.54% (Table 4). Differences in essential oil content were mainly associated with genetic factors, and all fertilizer applications resulted in higher values compared to the CK treatment. Asadi *et al.* (2023) reported a 15.75% increase in essential oil content of *Mentha × piperita* following amino acid fertilizer application. A similar increase was observed by Tursun (2022) in coriander plants treated with seaweed extract. Several other studies have also shown that organic fertilizers contribute to an increase in

essential oil concentration (Sheykhleslami and Almdari, 2019; Can and Katar, 2020; Tan *et al.*, 2023).

Mentha arvensis had the highest essential oil content among the different *Mentha* species evaluated (Table 3). T3 fertilizer treatment resulted in the highest dried leaf yield (Table 3), and the combination of *M. arvensis* × T3 produced the highest essential oil yield (150.31 L ha⁻¹) (Table 4). The increase in dried leaf yield contributed directly to the increase in essential oil yield. Correlation analysis confirmed strong positive relationships between essential oil yield, dried leaf yield, and essential oil content. These results are consistent with the findings of Asadi *et al.* (2023), who reported similar associations between dry matter and oil-related traits. Likewise, Soltanbeigi *et al.* (2021) highlighted that essential oil yield is closely related to both dried leaf yield and essential oil content.

Essential oil profiles differed greatly depending on *Mentha* species and the applied fertilizers, suggesting that each species reacts differently to foliar fertilizers. *Mentha arvensis* and *M. × piperita*, which contain menthol and menthone as their primary constituents, rank among the most produced and globally traded essential oils (Kalembe and Synowiec, 2019). Menthol content in *Mentha arvensis* ranged from 58.75% to 66.29%, with the highest values recorded under T4 and T1 applications. Menthone levels fluctuated between 9.61% and 21.02%, with the highest concentration observed under T3 treatment. *Mentha × piperita* Mitcham exhibited menthol concentrations ranging from 31.33% to 49.48%, peaking with T3, while menthone levels increased to 23.52% following T2 application. Fertilizer treatments reduced menthol content in the *M. × piperita* Multimentha from 52.23% (CK) to a range of 30.40–43.74%, whereas menthone increased significantly, reaching 32.59% with T4. The *M. × piperita* Swiss exhibited menthol concentrations between 35.50% and 45.07%, with the highest content observed under T4. Menthone levels varied from 14.41% to 25.40%, peaking with T3 treatment. These results suggest that T4 favored menthol accumulation, while T3 stimulated menthone biosynthesis in this cultivar. In *Mentha spicata*, the major essential oil constituents were carvone and carveol. Carvone content ranged from 31.70% to 44.12%, with the highest value detected under T3 treatment, whereas carveol varied between 6.29% and 16.75%, increasing substantially with T1 treatment. The results suggest that the influence of foliar fertilizers on essential oil components varies according to both species and the specific compound profile. The composition of essential oils varies based on genetic factors and environmental conditions like soil type, altitude, and water availability, while factors such as temperature, light, and diurnal variations also influence their accumulation (Telci *et al.*, 2010; Aali *et al.*, 2017; Soltanbeigi *et al.*, 2021).

Pearson's correlation is a common tool for evaluating trait relationships and improving trait prediction (Blondel *et al.*, 2015). Genetic correlations affect selection outcomes, making it crucial to identify high-yielding, stable *Mentha* varieties with quality oil (Gupta *et al.*, 2017). Pearson correlation analysis revealed strong associations among PH, FH, DH and DLY, while EOC showed weaker correlations with these traits. EOY correlated moderately to strongly with growth traits, highlighting the role of yield in oil productivity (Fig. 8a). These findings align with studies by Machiani *et al.* (2018), Gupta *et al.* (2017) and Joshi *et al.* (2024), highlighting the importance of biomass and oil content in breeding.

Principal component analysis (PCA) is a multivariate statistical method used to simplify complex, high-dimensional data sets by retaining most of the original data's variability, making it easier to visualize and interpret relationships among observations and variables (Abdi and Williams, 2010). Principal component analysis (PCA) was conducted to interpret the relationships among traits and species × fertilizer combinations. The first two components explained 97.4% of the total variation, with PC1 and PC2 accounting for 71.8% and 25.6%, respectively (Fig. 8b). The biplot distinguished species based on their performance under different fertilizer treatments. *Mentha arvensis* treated with T2, T3 and T4 was closely associated with higher essential oil yield (EOY) and essential oil content (EOC). In contrast, increased fresh, dried and dried leaf yield were associated with the combination of *M. × piperita* Multimentha and the fertilizers T2, T3, and T4. Additionally, *M. spicata*, *M. × piperita* Mitcham and *M. × piperita* Swiss grouped more closely with growth-related traits, indicating improved agronomic performance under certain fertilizers. These findings demonstrated the usefulness of PCA in visualizing how species respond differently to foliar treatments in terms of both agronomic performance and essential oil content and yield. Previous studies on mint have demonstrated the effectiveness of PCA in capturing genetic and phenotypic variation. For instance, Soilhi *et al.* (2020) reported that the first five principal components accounted for 88.1% of the variation in Tunisian mint genotypes, supporting the value of agronomic evaluation. Parrey *et al.* (2023) found that PC1 and PC2 explained 85.2% and 4.5% of the variance among peppermint cultivars and nitrogen treatments. Similarly, Joshi *et al.* (2024) showed that PCA explained 74.38% of the variation among 43 spearmint genotypes, providing insights into key trait associations essential for breeding and agronomic development.

The amino acid and seaweed-based foliar fertilizers applied in this study demonstrated significant potential to enhance both agronomic traits and essential oil profiles across various *Mentha* species. The PCA

biplot analysis clearly underscored the critical role of species-specific responses to different foliar fertilizers, indicating that species selection is fundamental for maximizing both mint agronomic performance and essential oil yield. Furthermore, Pearson correlation analysis revealed strong positive associations between essential oil yield and key agronomic parameters such as fresh herb yield, dried herb yield, and dried leaf yield. This suggests that improvements in yield traits directly influence essential oil productivity, highlighting the interconnected relationship between agronomic performance and essential oil profile.

Conclusions: This study evaluated the effects of foliar fertilizers containing amino acids and seaweed extracts on different *Mentha* species. Pearson correlation and PCA analyses demonstrated strong positive correlations among plant height, fresh herb, dried herb, and dried leaf yields, alongside a strong relationship between essential oil yield and content. Foliar applications significantly increased fresh herb, dried herb, and dried leaf yields, while essential oil yield and composition varied notably among species. Among the fertilizers compared, T3 (plant-based amino acids fertilizer II) and T4 (seaweed extract-based fertilizer) had the most favorable effects on yield. *Mentha* × *piperita* Multimentha exhibited superior herb yield, whereas *Mentha arvensis* showed higher essential oil content and yield. Notably, the combination of *M.* × *piperita* Multimentha X T4 (seaweed extract-based fertilizer) produced the highest dried leaf yield, while *M. arvensis* X T3 (plant-based amino acids fertilizer II) achieved the highest essential oil yield. These findings highlight the importance of species selection and foliar fertilizer management in mint cultivation to enhance yield and essential oil quality, promoting sustainable and efficient production practices.

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