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Research Article

Optimizing Peppermint (*Mentha piperita* L.) essential oil yield and quality: Roles of nitrogen, salicylic acid, and weed management

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Abstract

Peppermint has anti-inflammatory, antiseptic, and soothing properties due to its essential oil content. This plant is widely used in the pharmaceutical, cosmetic, and food industries and helps relieve stomachaches, headaches, and stress. The primary objective of this study was to evaluate the combined effects of nitrogen (N), salicylic acid (SA), and weed management on morphophysiological traits, essential oil content, and essential oil yield of peppermint. The present study was conducted under field conditions at Sari, Mazandaran Province, northern Iran, during the 2023–2024 growing season. In this research, a factorial arrangement within a randomized complete block design (RCBD) with three replications was conducted, which included three nitrogen (N) levels (0, 1.5, and 3 g L^{-1}), three salicylic acid (SA) levels (0, 1.5, and 3 mM), and two weed management practices (weeding vs. non-weeding). Results indicated that the increase in N and SA significantly enhanced plant height, chlorophyll content, biological yield, and essential oil content. Notably, the treatment with 3 g/L of N and 3 mM of SA without weed control showed the best results in increasing essential oil yield (169 kg ha^{-1}) and essential oil content (2.65%). This treatment also improved the absorption of nutrients such as N, phosphorus (P), and potassium (K), while increasing the plant's resistance to environmental stresses. These findings suggest that optimal management of N and SA, even under mild weed stress, can significantly enhance both the quality and quantity of peppermint yields. Therefore, this research can serve as a valuable reference for farmers and researchers aiming for sustainable production of this medicinal plant with reduced synthetic inputs.

Keywords: Essential oil, Nitrogen, Peppermint, Salicylic acid, Weed management

Introduction

Medicinal plants are recognized as one of the most important natural resources in the pharmaceutical, food, cosmetic, and hygiene industries (Camele *et al*., 2021; Hazrati *et al*., 2024). Among these plants, peppermint (*Mentha piperita* L.) holds a unique position in both traditional and modern medicine due to its bioactive compounds such as menthol, menthone, and volatile essential oils (Taylan *et al*., 2021; Tang *et al*., 2024). Peppermint essential oil is widely valued for its anti-inflammatory, antiseptic, and soothing properties, with applications ranging from treating digestive disorders and headaches to use in cosmetics and flavorings (Afkar, 2024).

However, the sustainable and optimal production of peppermint in field conditions is challenged by several interacting agronomic factors, including nutrient management, weed competition, and environmental stress responses (Dubey *et al*., 2022). Key among these factors are nitrogen (N) nutrition, the use of plant growth regulators like salicylic acid (SA), and weed control strategies, which can synergistically or antagonistically influence the plant's morphophysiological traits, biomass accumulation, and the biosynthesis of valuable secondary metabolites such

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as essential oils (Li *et al*., 2022; Sharaf-Eldin *et al*., 2022).

Nitrogen, as a primary macronutrient, is fundamental for synthesizing proteins, nucleic acids, and chlorophyll, thereby driving vegetative growth and photosynthetic capacity (Barlog *et al*., 2022). In aromatic plants like peppermint, N supply not only affects biomass but also modulates the metabolic pathways leading to essential oil production (Bghbani-Arani and Poureisa, 2024). However, the relationship is complex; while optimal N enhances growth, excessive or deficient levels can alter oil composition and yield, highlighting the need for precise, sustainable N management, such as foliar application to improve nitrogen use efficiency and reduce environmental leaching (Sharaf-Eldin *et al*., 2022; Peng and Ng, 2022).

Salicylic acid is a pivotal phenolic phytohormone involved in regulating plant defense responses and systemic acquired resistance (Ali, 2021; Khan *et al*., 2022). Exogenous SA application has been shown to enhance plant tolerance to various abiotic stresses, including nutrient deficiency and biotic interactions, by modulating antioxidant systems and upregulating defense-related genes (Lan *et al*., 2025). Moreover, SA acts as an elicitor, potentially stimulating the biosynthesis of secondary metabolites, including terpenoids (the primary constituents of mint essential oil) and phenolic compounds like anthocyanins (Li *et al*., 2022; Abdoli and Ghassemi-Golezani, 2025). This suggests that SA could be a valuable tool for improving both stress resilience and essential oil quality in peppermint cultivation.

Weed competition is a major constraint in medicinal plant production, directly reducing resource availability (light, water, nutrients) and thus impairing growth and yield (Walia and Kumar, 2021). Paradoxically, mild weed-induced stress can act as an elicitor, triggering defense mechanisms that increase the concentration of secondary metabolites like essential oils as a protective response (Khan *et al*., 2022). This stress response is often mediated through reactive oxygen species (ROS) signaling and the activation of phytohormone pathways such as jasmonic acid (JA) and SA, which upregulate key enzymes in terpenoid biosynthesis (De Mastro *et al*., 2021). Therefore, the decision to weed or not involves a critical trade-off: Removing weeds boosts biomass, while tolerating mild weed pressure may enhance oil concentration, with the net effect on essential oil yield per hectare depending on the balance between these two components (Jing *et al*., 2025).

Most previous studies have investigated the effects of N, SA, or weed management in isolation, despite their simultaneous and interactive occurrence under real-field conditions (Li *et al*., 2022). For instance, N availability may alter plant competitiveness under weed pressure, while SA could modulate the plant's physiological response to both N levels and weed stress. A factorial examination of these three factors is therefore essential to unravel their complex interactions and identify integrated management strategies that optimize both the quantity and quality of peppermint production (Dubey *et al*., 2022; Ostadi *et al*., 2022).

This study aimed to evaluate the individual and interactive effects of nitrogen fertilization (foliar application), salicylic acid (as an elicitor), and weed management (weeding vs. non-weeding) on the morphophysiological traits, essential oil content, and essential oil yield of peppermint (*Mentha piperita* L.) under field conditions. We hypothesized that (1) N and SA would synergistically improve plant growth and essential oil production, and (2) the presence of weeds would induce a stress response that increases oil concentration, potentially offsetting the reduction in biomass. The findings are expected to provide a practical, science-based framework for farmers to achieve sustainable peppermint production with optimized essential oil yield and reduced reliance on synthetic inputs.

Materials and methods

Experimental site and conditions: The field experiment was conducted during the 2023–2024 growing season at the research farm of Sari Agricultural Sciences and Natural Resources University, located in Sari, Mazandaran Province, northern Iran (36°33' N, 53°03' E; 15 m a.s.l.). The region has a humid subtropical climate with an average annual rainfall of 800 mm. The soil texture was silty loam, with initial physicochemical properties (0–30 cm depth) as follows: pH 7.2, EC 1.1 dS m^{-1}, organic matter 1.8%, total N 0.12%, available P 15.2 mg kg^{-1}, and exchangeable K 210 mg kg^{-1}.

Experimental design and treatments: A factorial arrangement based on a Randomized Complete Block Design (RCBD) with three replications was employed. The studied factors included nitrogen (N) fertilizer applied as foliar spray at three concentrations (0, 1.5, and 3 g L^{-1}) from a urea solution (17% N, Khazra Company), salicylic acid (SA) applied as foliar spray at three concentrations (0, 1.5, and 3 mM) from a commercial source (Merck Company), and weed management applied at two levels (weeding (manual removal four times during the season) and non-weeding). The dominant weed species in the experimental field were *Convolvulus arvensis* L., *Avena fatua* L., and *Chenopodium album* L., with an average density of 24 plants m^{-2} at the tillering stage. This resulted in a total of 18 treatment combinations (3 N × 3 SA × 2 weed management).

Agricultural practices: Land preparation included plowing, disking twice, and leveling in late January 2024. Peppermint rhizomes (15 cm long, each containing at least three buds) were planted at a depth of approximately 1 cm in late April 2024. Each experimental plot measured 2.5 m^2 (2 m × 1.25 m), with 0.5 m spacing between plots and 1 m spacing between blocks. Planting was done in five rows per plot with 25 cm between rows and 20 cm between plants within

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rows. Basal fertilization with phosphorus and potassium was applied based on soil test recommendations. Nitrogen treatments were applied as a foliar spray using a calibrated backpack sprayer at three stages: The four-leaf stage, followed by two more applications at 15-day intervals. A spray volume of 300 L ha^{-1} (equivalent to 300 mL m^{-2}) was used for all foliar applications, with 0.1% (v/v) Tween 20 added as a surfactant to improve adhesion. Applications were made early in the morning (6–8 AM), at least 4 hours before any irrigation or expected rainfall, to prevent wash-off. SA solutions were prepared and foliar-sprayed twice at 20 and 40 days after planting, using the same volume, surfactant, and timing precautions as for N application. In the weeding treatment, weeds were manually removed by hand four times during the growing season (at 3, 6, 9, and 12 weeks after planting). The crop was irrigated using a drip tape system every 6–8 days to maintain soil moisture at 80–85% of field capacity. Each irrigation event delivered approximately 45–50 m^3ha^{-1}, monitored by a time-domain reflectometry (TDR) probe at 30 cm depth.

Measured traits and methods, morphological and yield traits: Plant height (cm) was measured from the soil surface to the tip of the main stem on ten randomly selected plants per plot. Above-ground biological yield (kg ha^{-1}) was determined by harvesting all plants from a central area of 1 m^2 within each plot. Harvested samples were dried in a shaded environment.

Essential oil extraction and yield: Essential oil content (%) and yield (kg ha^{-1}) were determined by hydrodistillation of 50 g of dried leaves for 3 hours using a Clevenger-type apparatus, in accordance with ISO 9909 standard for *Mentha piperita* essential oil. The oil content was calculated as (volume of oil (mL) / weight of dry sample (g)) × 100. Essential oil yield per hectare was computed by multiplying the biological yield by the essential oil content.

Physiological and biochemical traits: During the flowering stage of the plant, chlorophyll *a*, chlorophyll *b*, total chlorophyll, and leaf carotenoid concentrations were measured to assess photosynthetic status and leaf health (Lichtenthaler and Buschmann, 2001). For this purpose, several plants were randomly selected, and six leaf discs were punched from fully expanded leaves and immersed in Falcon tubes containing 8 mL of methanol. The samples were transferred to the laboratory and kept in darkness at room temperature for 24 hours. Subsequently, the absorbance of the resulting extracts was measured spectrophotometrically at three wavelengths: 665.2, 652.4, and 470 nm. Finally, concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were calculated using Equations 1 to 4, respectively. Anthocyanin content (mg g^{-1} fresh weight) was measured according to the pH differential method described by Krizek *et al*. (1998).

Equation 1

$$Chl_a\ (\mu g/ml) = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

Equation 2

$$Chl_b(\mu g/ml) = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

Equation 3

$$Chl_{a+b}\ (\mu g/ml) = 1.44\ AA_{665.2} + 24.93\ A_{652.4}$$

Equation 4

$$\text{Carotenoid } (\mu g/ml) = (1000\ A_{470} - 1.63\ Chl_a - 104.96\ Chl_b)/221$$

Leaf nutrient analysis: Oven-dried leaf samples were ground and digested. Total nitrogen (N) was determined by the micro-Kjeldahl method (Chapman and Pratt, 1962). Phosphorus (P) concentration was measured by the vanadomolybdate yellow color method (Chapman and Pratt, 1962). Potassium (K) was determined using a flame photometer (Hamada and El-Enany, 1994).

Statistical analysis: All data were subjected to analysis of variance (ANOVA) appropriate for a factorial RCBD using SAS software (version 9.1). The significance of main effects (N, SA, and weed management) and their interactions was tested. Mean comparisons were performed using the Least Significant Difference (LSD) test at a 5% probability level ($P \leq 0.05$). Pearson correlation coefficients were calculated to examine relationships among the measured traits.

Results

Analysis of variance: The analysis of variance (ANOVA) revealed that nitrogen (N), salicylic acid (SA), and weed management had highly significant main effects on all measured morphophysiological, yield, and essential oil traits of peppermint (Tables 1 and 3). The interaction between N and SA was significant ($P < 0.05$) for essential oil yield (Table 1), underscoring their synergistic effect. However, other two-way and three-way interactions were generally non-significant for most traits.

Plant height: Plant height significantly changed under the influence of different levels of N, SA, and weed management (Table 1). Using an N treatment of 1.5 g L^{-1}, the plant height reached 35.26 cm, and in the 3 g L^{-1} treatment, it increased to 42.66 cm. These values represent increases of 14% and 38%, respectively, compared to the control treatment (30.83 cm). Additionally, in the 1.5 mM SA treatment, plant height increased to 35.69 cm, and in the 3 mM treatment, it rose to 44.77 cm, showing increases of 26% and 58% compared to the control treatment (28.3 cm). Under weeding conditions, plant height reached 38.57 cm, which is 14% more than in weedy conditions (33.93 cm) (Table 2).

Biological yield: Biological yield was also influenced by various treatments (Table 1). In the N treatment of 1.5 g L^{-1}, biological yield reached 1011.39 kg ha^{-1}, while in the 3 g L^{-1} treatment, it increased to 4812.55 kg ha^{-1}, indicating increases of 24% and 49%, respectively, compared to the control treatment (3226.02 kg ha^{-1}). In the 1.5 mM SA treatment, biological yield increased to 4003.01 kg ha^{-1}, and in the 3 mM treatment, it reached 4387.69 kg ha^{-1}, representing increases of 9% and 20% over the control

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Table 1. Results of analysis of variance related to plant height, biological yield, essential oil content, and yield traits in peppermint plants under N, SA, and weed management treatments

SOV	Plant height	Biological yield	Essential oil content	Essential oil yield
Block	145.77**	417630.36^{ns}	0.01^{ns}	434.92^{ns}
N (A)	642.38**	11327350.64**	2.86**	21885.38**
SA (B)	1225.10**	2390342.51**	1.37**	7585.35**
Weed management (C)	291.36**	1801694.03*	2.95**	2012.35*
A × B	5.08^{ns}	217996.35^{ns}	0.14^{ns}	1350.24*
A × C	0.80^{ns}	93732.58^{ns}	0.21^{ns}	910.17^{ns}
B × C	7.22^{ns}	4055.10^{ns}	0.16^{ns}	241.26^{ns}
A × B × C	5.62^{ns}	30603.56^{ns}	0.01^{ns}	16.92^{ns}
Error	19.02	320049.96	0.09	458.17
CV (%)	12.03	14.08	13.98	23.47

ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively

Table 2. Results of mean comparison related to plant height, biological yield, essential oil content, and yield traits in peppermint plants under N, SA, and weed management treatments

Treatments	Plant height (cm)	Biological yield (kg ha^{-1})	Essential oil content (%)	Essential oil yield (kg ha^{-1})
N (g L^{-1})				
0	30.83±1.85^{c}	3226.02±99.73^{c}	1.89±0.08^{b}	60.98±3.14^{c}
1.5	35.26±1.86^{b}	4011.39±141.68^{b}	2.07±0.09^{b}	83.20±5.00^{b}
3	42.66±2.22^{a}	4812.55±182.63^{a}	2.65±0.14^{a}	129.34±10.02^{a}
SA (mM)				
0	28.30±1.42^{c}	3659.25±153.57^{b}	1.94±0.09^{c}	71.68±5.21^{c}
1.5	35.69±1.67^{b}	4003.01±205.82^{b}	2.19±0.10^{b}	89.25±7.57^{b}
3	44.77±1.78^{a}	4387.69±236.29^{a}	2.49±0.16^{a}	112.60±12.01^{a}
Weed management				
non-weeding	33.93±1.71^{b}	3833.99±173.10^{b}	2.44±0.12^{a}	97.28±8.71^{a}
Weeding	38.57±1.91^{a}	4199.31±165.42^{a}	1.97±0.07^{b}	85.07±6.53^{b}

Means within each column followed by the same letter are not statistically different at ≤0.05 by least significant difference (LSD) test

Table 3. Results of analysis of variance related to leaf pigments and nutrients traits in peppermint plants under N, SA and weed management treatments

SOV	Chl a	Chl b	T Chl	a/b	Car	Anth	N	P	K
Block	0.21^{ns}	0.00^{ns}	0.20^{ns}	0.0011^{ns}	0.0921^{ns}	0.0285^{ns}	0.0458^{ns}	0.0000^{ns}	0.0071^{ns}
N (A)	44.72**	1.87**	64.45**	3.5140**	4.7853**	32.8041**	4.2528**	0.0399**	0.7746**
SA (B)	11.69**	1.60**	21.91**	0.0606^{ns}	0.4695**	3.7268**	1.0858**	0.0327**	0.6444**
Weed management (C)	6.32**	0.54**	10.54**	0.0234^{ns}	0.2015*	1.1441**	0.5017**	0.0207**	0.2061^{ns}
A × B	0.51^{ns}	0.05^{ns}	0.27^{ns}	0.7135^{ns}	0.0174^{ns}	0.1765^{ns}	0.0684^{ns}	0.0004^{ns}	0.0260^{ns}
A × C	0.19^{ns}	0.01^{ns}	0.18^{ns}	0.0743^{ns}	0.0178^{ns}	0.0749^{ns}	0.0075^{ns}	0.0003^{ns}	0.0035^{ns}
B × C	0.28^{ns}	0.02^{ns}	0.41^{ns}	0.0119^{ns}	0.0585^{ns}	0.1409^{ns}	0.0063^{ns}	0.0002^{ns}	0.0040^{ns}
A × B × C	0.15^{ns}	0.01^{ns}	0.10^{ns}	0.1562^{ns}	0.0288^{ns}	0.0232^{ns}	0.0037^{ns}	0.0002^{ns}	0.0045^{ns}
Error	0.43	0.07	0.41	0.4151	0.0619	0.1793	0.0565	0.0014	0.0634
CV (%)	11.76	14.89	8.72	20.06	13.08	14.11	13.51	12.44	12.46

ns, * and **: are non-significant and significant at 5 and 1% probability levels, respectively

(3659.25 kg ha^{-1}). Under weeding conditions, biological yield increased to 4199.31 kg ha^{-1}, which is 10% higher than in non-weeding conditions (3833.99 kg ha^{-1}) (Table 2).

Essential oil and yield content: Essential oil content also showed significant changes under different levels of N, SA, and weed management (Table 1). With an N treatment of 1.5 g L^{-1}, the essential oil content increased to 2.07%, and in the 3 g L^{-1} treatment, it reached 2.65%, corresponding to increases of 10% and 40% compared to the control (1.89%). Similarly, in the 1.5 mM SA treatment, essential oil content rose to 2.19%, and in the 3 mM treatment, it reached 2.49%, indicating increases of 13% and 28% over the control (1.94%). On the other hand, under non-weeding conditions, the essential oil content reached 2.44%, which is 19% higher than in weedy conditions (Table 2).

Essential oil yield was also significantly affected by various treatments (Table 1). In the N treatment of 1.5 g L^{-1}, essential oil yield reached 83.20 kg ha^{-1}, and in the 3 g L^{-1} treatment, it increased to 129.34 kg ha^{-1}, representing increases of 36% and 112%, respectively, compared to the control treatment (60.98 kg ha^{-1}). In the

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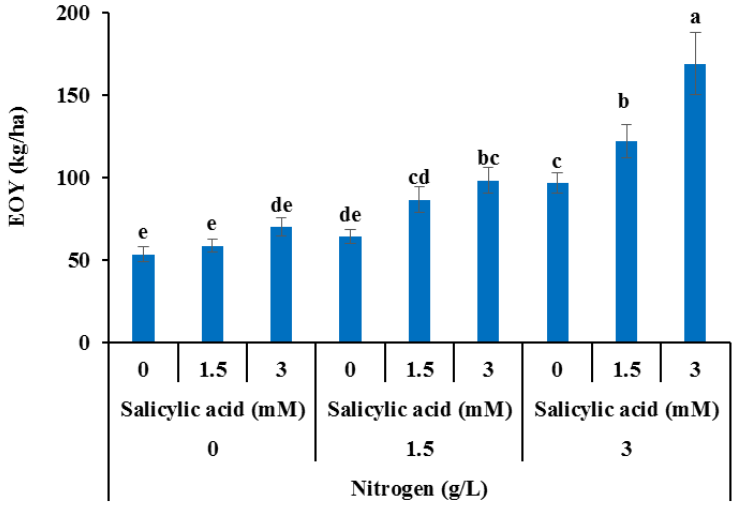


Figure 1. Results of mean comparison related to essential oil yield (EOY) in peppermint plants under N and SA treatments. Means within each column followed by the same letter are not statistically different at ≤0.05 by the least significant difference (LSD) test.

1.5 mM SA treatment, essential oil yield increased to 89.25 kg ha^{-1}, and in the 3 mM treatment, it reached 112.60 kg ha^{-1}, indicating increases of 24% and 57% compared to the control treatment (71.68 kg ha^{-1}). Under non-weeding conditions, the essential oil yield reached 97.28 kg ha^{-1}, which is 14% more than in weedy conditions (85.07 kg ha^{-1}) (Table 2). The interaction between N and SA notably impacted essential oil content. The results showed that increasing N levels combined with SA led to significant increases in essential oil content; at the highest N level (3 g L^{-1}) and the highest SA concentration (3 mM), the essential oil content reached 169 kg ha^{-1}, demonstrating the combined effect of these two factors on enhancing oil production (Figure 1).

A key trade-off was observed regarding essential oil content. While N and SA application increased both biomass and oil content, non-weeded conditions produced a 19% higher essential oil concentration (2.44%) compared to weeded plots (1.97%) (Table 2). This indicates that the mild stress from weed competition stimulated secondary metabolite production. Crucially, the synergistic effect of N and SA overcame the potential yield penalty from weeds. The combination of 3 g L^{-1} N and 3 mM SA under non-weeded conditions resulted in the highest essential oil yield of 169 kg ha^{-1} (Figure 1), demonstrating that optimal nutrient and elicitor management can maximize oil yield per hectare even in the presence of weeds.

Chlorophyll *a*: The content of chlorophyll *a* significantly changed under the influence of different levels of N, SA, and weed management (Table 3). In the treatment with 1.5 g L^{-1} N, the chlorophyll *a* content reached 5.95 µg mL^{-1}, and in the 3 g L^{-1} treatment, it increased to 6.97 µg mL^{-1}, showing increases of 54% and 80%, respectively, compared to the control (3.87 µg mL^{-1}). In the 1.5 mM SA treatment, chlorophyll *a* increased to 6.45 µg mL^{-1}, and in the 3 mM treatment, it reached 6.97 µg mL^{-1}, indicating increases of 33% and 44% compared to the control (4.85 µg mL^{-1}). Under weeding conditions, chlorophyll *a* reached 5.94 µg mL^{-1}, which is 13% more than in non-weeding conditions (5.25 µg mL^{-1}) (Table 4).

Chlorophyll *b*: The amount of chlorophyll *b* also increased with higher levels of N, SA, and weed management (Table 3). In the treatment with 1.5 g L^{-1} N, chlorophyll *b* reached 1.74 µg mL^{-1}, and in the 3 g L^{-1} treatment, it increased to 2.08 µg mL^{-1}, showing increases of 21% and 44%, respectively, compared to the control (1.44 µg mL^{-1}). In the 1.5 mM SA treatment, chlorophyll b increased to 1.85 µg mL^{-1}, and in the 3 mM treatment, it reached 2.06 µg mL^{-1}, indicating increases of 12% and 41% compared to the control (1.65 µg mL^{-1}). Under weeding conditions, chlorophyll *b* reached 1.85 µg mL^{-1}, which is 12% more than in non-weeding conditions (1.65 µg mL^{-1}). These findings indicate that N, SA, and weed removal play important roles in increasing chlorophyll b content (Table 4).

Total chlorophyll: Total chlorophyll showed a similar trend. In the treatment with 1.5 g L^{-1} N, total chlorophyll reached 7.69 µg mL^{-1}, and in the 3 g L^{-1} treatment, it increased to 9.05 µg mL^{-1}, showing increases of 45% and 70%, respectively, compared to the control (5.31 µg mL^{-1}). In the 1.5 mM SA treatment, total chlorophyll reached 7.79 µg mL^{-1}, and in the 3 mM treatment, it reached 8.51 µg mL^{-1}, showing increases of 13% and 35% compared to the control (6.91 µg mL^{-1}). Under weeding conditions, total chlorophyll reached 7.79 µg mL^{-1}, which is 13% more than in non-weeding conditions (6.91 µg mL^{-1}) (Table 4).

Chlorophyll *a* to *b* ratio: The ratio of chlorophyll *a* to *b* in the 1.5 g L^{-1} N and 3 g L^{-1} N treatments were 3.48 and 3.46, respectively, showing a significant increase compared to the control treatment (2.70). These increases were 29% and 28%. In the 1.5 mM SA

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Table 4. Results of mean comparison related to leaf pigments in peppermint plants under N, SA, and weed management treatments

Treatments	Chl a (µg mL^{-1})	Chl b (µg mL^{-1})	Total Chl (µg mL^{-1})	a/b	Car (µg mL^{-1})	Anthocyanins (mg g^{-1} FW)
N (g L^{-1})						
0	3.87±0.22 c	1.44±0.06 c	5.31±0.26 c	2.70±0.10 b	1.39±0.05 c	1.72±0.08 c
1.5	5.95±0.28 b	1.74±0.08 b	7.69±0.31 b	3.48±0.17 a	1.89±0.07 b	2.88±0.12 b
3	6.97±0.19 a	2.08±0.11 a	9.05±0.26 a	3.46±0.14 a	2.42±0.07 a	4.41±0.18 a
SA (mM)						
0	4.85±0.35 c	1.46±0.07 c	6.32±0.41 c	3.28±0.15 a	1.76±0.11 b	2.59±0.25 c
1.5	5.48±0.36 b	1.74±0.09 b	7.22±0.43 b	3.17±0.17 a	1.87±0.11 b	2.92±0.27 b
3	6.45±0.34 a	2.06±0.10 a	8.51±0.40 a	3.19±0.17 a	2.08±0.13 a	3.49±0.33 a
Weed management						
non-weeding	5.25±0.29 b	1.65±0.08 b	6.91±0.35 b	3.19±0.13 a	1.96±0.09 a	2.85±0.22 b
Weeding	5.94±0.32 a	1.85±0.09 a	7.79±0.38 a	3.23±0.14 a	1.84±0.10 a	3.15±0.25 a

Means within each column followed by the same letter are not statistically different at ≤0.05 by least significant difference (LSD) test

Table 5. Results of mean comparison related to leaf nutrients in peppermint plants under N, SA, and weed management treatments

Treatments	N (%)	P (%)	K (%)
N (g L^{-1})			
0	1.26±0.05 c	0.27±0.01 c	1.83±0.06 b
1.5	1.78±0.06 b	0.29±0.01 b	1.98±0.05 b
3	2.23±0.10 a	0.36±0.01 a	2.24±0.08 a
SA (mM)			
0	1.53±0.09 c	0.27±0.01 c	1.83±0.07 c
1.5	1.74±0.12 b	0.30±0.01 b	2.02±0.06 b
3	2.02±0.12 a	0.35±0.01 a	2.21±0.07 a
Weed management			
non-weeding	1.66±0.09 b	0.28±0.01 b	1.96±0.06 a
Weeding	1.86±0.10 a	0.32±0.01 a	2.08±0.06 a

Means within each column followed by the same letter are not statistically different at ≤0.05 by least significant difference (LSD) test

treatment, the chlorophyll *a* to *b* ratio was 3.17, and in the 3 mM treatment, it reached 3.19, which showed a slight decrease compared to the control. Under weeding conditions, the chlorophyll *a* to *b* ratio reached 3.23, which is 1% more than under non-weeding conditions (Table 4).

Carotenoid content: The carotenoid content also increased with higher levels of N and SA. In the treatment with 1.5 g L^{-1} N, the carotenoid level reached 1.89 µg mL^{-1}, and in the 3 g L^{-1} N treatment, it increased to 2.42 µg mL^{-1}, representing increases of 36% and 74%, respectively, compared to the control (1.39 µg mL^{-1}). In the 1.5 mM SA treatment, carotenoids reached 1.84 µg mL^{-1}, and in the 3 mM treatment, it increased to 2.08 µg mL^{-1}, showing a decrease of 6% and an increase of 18% compared to the control (1.96 µg mL^{-1}). Under weeding conditions, carotenoids reached 1.84 µg mL^{-1}, which is 6% lower than in non-weeding conditions (1.96 µg mL^{-1}) (Table 4).

Anthocyanins content: The content of anthocyanins also showed significant increases with higher levels of N, SA, and weed management. In the treatment with 1.5 g L^{-1} N, anthocyanin content reached 2.88 mg g^{-1} FW, and in the 3 g L^{-1} treatment, it increased to 4.41 mg g^{-1} FW, indicating increases of 67% and 156%, respectively, compared to the control (1.72 mg g^{-1} FW). In the 1.5 mM SA treatment, anthocyanins reached 3.15 mg g^{-1} FW, and in the 3 mM treatment, it increased to 3.49 mg g^{-1} FW, showing increases of 11% and 35% compared to the control (2.85 mg g^{-1} FW). Under weeding conditions, anthocyanins reached 3.15 mg g^{-1} FW, which is 11% more than in non-weeding conditions (2.85 mg g^{-1} FW) (Table 4).

Leaf nutrient concentrations: Leaf nutrient concentrations (N, P, K) were positively influenced by higher application rates of N and SA, and by weed removal (Table 5). This confirms improved nutrient uptake and assimilation under optimized management and reduced competition. Increasing N concentration from control to 1.5 g L^{-1} resulted in a 41.1% increase in leaf N. This increasing trend was reinforced with the application of 3 g L^{-1} N, raising the N content to 2.23% (a 76.2% increase compared to the control). The use of 1.5 mM SA increased leaf N by 13.7%. A concentration of 3 mM of this compound also led to a 31.9% increase. Weed removal increased leaf N by 12.1% compared to non-weeding treatments (Table 5).

A concentration of 1.5 g L^{-1} N increased leaf P by 7.4%. This amount was enhanced to 0.36% (a 33.3%

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Table 6. Results of Pearson correlation coefficients related to studied traits in peppermint plants under N, SA, and weed management treatments

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13
Plant height (1)	1												
Biological yield (2)	0.85**	1											
Essential oil content (3)	0.59**	0.66**	1										
Essential oil yield (4)	0.77**	0.87**	0.93**	1									
Chlorophyll *a* (5)	0.85**	0.94**	0.58*	0.77**	1								
Chlorophyll *b* (6)	0.96**	0.93**	0.62**	0.82**	0.89**	1							
Total chlorophyll (7)	0.88**	0.95**	0.60**	0.80**	0.99**	0.93**	1						
a/*b* (8)	0.29	0.53*	0.26	0.36	0.71**	0.33	0.64**	1					
Carotenoids (9)	0.71**	0.89**	0.80**	0.89**	0.88**	0.80**	0.88**	0.59**	1				
Anthocyanins (10)	0.80**	0.96**	0.69**	0.87**	0.92**	0.89**	0.93**	0.54*	0.95**	1			
N (11)	0.87**	0.98**	0.64**	0.85**	0.94**	0.94**	0.96**	0.52*	0.89**	0.97**	1		
P (12)	0.95**	0.91**	0.58*	0.78**	0.87**	0.95**	0.90**	0.34	0.75**	0.86**	0.91**	1	
K (13)	0.95**	0.92**	0.64**	0.82**	0.87**	0.97**	0.91**	0.32	0.78**	0.88**	0.93**	0.96**	1

*** and **: are significant at 5 and 1% probability levels, respectively**

increase over the control) at 3 g L^{-1} N. The 1.5 mM SA increased leaf P by 11.1%. The concentration of 3 mM also resulted in a 29.6% increase (to 0.35%). Weed removal increased leaf P by 14.3% (Table 5). Increasing N to 1.5 g L^{-1} raised leaf K by 8.2%. A concentration of 3 g L^{-1} also raised this amount by 22.4%. The use of 1.5 mM SA increased leaf K by 10.4% compared to the control. A concentration of 3 mM SA led to an increase of 20.8%. Weed removal increased leaf K by 6.1% (Table 5).

Correlation analysis: Correlation analysis showed strong positive relationships ($P < 0.01$) among most traits, particularly between essential oil yield and biological yield ($r = 0.87$), chlorophyll content, and leaf nutrients (Table 6). The exceedingly high correlations (e.g., $r = 0.97$ between anthocyanins and leaf N) are associative and likely reflect parallel responses to the applied treatments rather than direct causation.

Discussion

The results of this study clearly demonstrate that nitrogen (N) nutrition, salicylic acid (SA) application, and weed management play pivotal and interconnected roles in determining the growth, physiological performance, and essential oil productivity of peppermint. Overall, increasing N concentration and applying SA significantly enhanced plant height, biological yield, photosynthetic capacity, nutrient status, and essential oil yield, while weed competition imposed a measurable constraint on biomass production but simultaneously stimulated secondary metabolism. These findings align with existing knowledge on plant nutrition and stress physiology while also providing novel insight into how these factors can be strategically combined to overcome the classical trade-off between biomass accumulation and essential oil concentration in aromatic plants.

Plant height and biological yield responded strongly to increasing N concentration and SA application. The highest N level (3 g L^{-1}) increased plant height by 38% and biological yield by nearly 49% compared with the control, while SA at 3 mM increased plant height by 58% and biological yield by 20%. These improvements are consistent with the fundamental role of N in plant metabolism. Nitrogen is a structural component of amino acids, proteins, nucleic acids, and chlorophyll, and its availability directly stimulates cell division, leaf expansion, and canopy development, leading to enhanced photosynthetic capacity and biomass production (Barlog *et al*., 2022). The positive correlation observed between plant height and biological yield further confirms that longitudinal growth provides the structural basis for biomass accumulation (Peng and Ng, 2022).

Salicylic acid also contributed substantially to growth enhancement. As a signaling molecule, SA is known to regulate physiological processes including photosynthesis, stomatal conductance, antioxidant defense, and hormone balance. Its application likely reduced oxidative damage, stabilized cellular membranes, and improved metabolic efficiency, allowing plants to allocate more resources to growth (Ali, 2021; Lan *et al*., 2025). The synergistic effects of N and SA indicate that adequate nutrient supply combined with enhanced stress tolerance creates optimal conditions for vegetative development.

Weed management exerted a clear but comparatively smaller effect on growth traits. Weeding increased plant height and biological yield by 14% and 10%, respectively, primarily by reducing competition for light, water, and nutrients (Walia and Kumar, 2021; Jing *et al*., 2025). These findings are expected in peppermint, which has a relatively shallow root system and is particularly sensitive to early-season competition. However, while weed control improved biomass production, its influence on essential oil traits was more complex, reflecting a growth–stress trade-off. One of the most insightful outcomes of this study is the contrasting response of biological yield and essential oil content to weed competition. Non-weeded plots consistently exhibited higher essential oil content, approximately 19% greater than weeded plots, despite producing lower biomass. This phenomenon reflects a well-documented physiological strategy in medicinal

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and aromatic plants, where exposure to mild biotic stress activates secondary metabolic pathways responsible for the synthesis of defensive compounds, including essential oils (De Mastro *et al*., 2021).

Weed competition likely imposed moderate stress through reduced nutrient availability, altered light quality, and root interference. Such stress conditions can trigger signaling pathways mediated by jasmonic acid and salicylic acid, which in turn enhance the biosynthesis of terpenoid compounds such as menthol and menthone (Khan *et al*., 2025). In peppermint, essential oils serve not only as economically valuable metabolites but also as chemical defenses against herbivores and competitors (Devrnja *et al*., 2022; Kafle and Chung, 2025). Therefore, the increase in oil concentration under non-weeding conditions can be interpreted as an adaptive response to environmental pressure (Goncalves *et al*., 2023). However, while essential oil content increased under stress, essential oil yield (kg ha^{-1}) depended on both oil concentration and biomass production. As a result, treatments that strongly reduced biological yield did not necessarily maximize total oil yield. This highlights the importance of managing stress intensity; excessive stress can suppress growth, while mild stress can enhance secondary metabolism without severely limiting biomass (Walia and Kumar, 2021).

Beyond documenting this trade-off, the most important contribution of this study lies in demonstrating that it can be effectively mitigated through integrated management of N and SA. The combined application of high N (3 g L^{-1}) and high SA (3 mM) successfully balanced growth stimulation and metabolic activation, even under non-weeded conditions. This treatment produced both high biomass and high essential oil concentration, resulting in the maximum essential oil yield, which reached up to 169 kg ha^{-1} in some cases.

Mechanistically, this synergy can be explained by the complementary roles of N and SA (Moradi *et al*., 2023). Nitrogen enhances photosynthetic capacity and carbon assimilation, ensuring sufficient substrate availability for both primary growth and secondary metabolism (Sun *et al*., 2023; Amoah *et al*., 2025). Meanwhile, SA acts as an elicitor, priming defense-related pathways and amplifying the biosynthesis of secondary metabolites, particularly under mild stress conditions such as weed competition. Thus, instead of competing for resources, growth and secondary metabolism were simultaneously promoted (Song *et al*., 2023; Duhan *et al*., 2025). This finding is particularly important for peppermint cultivation systems where weed control is limited or costly. It suggests that rather than completely eliminating stress, growers can strategically manage nutrition and elicitors to exploit stress-induced increases in oil biosynthesis while maintaining acceptable biomass levels.

The observed increases in chlorophyll a, chlorophyll b, and total chlorophyll under N and SA treatments provide strong physiological support for the enhanced growth and yield. The highest chlorophyll a concentration (6.97 µg mL^{-1}) was recorded in both the 3 g L^{-1} N and 3 mM SA treatments. Nitrogen directly contributes to chlorophyll synthesis and the formation of photosynthetic proteins, including Rubisco and light-harvesting complexes (Li *et al*., 2022). Salicylic acid, on the other hand, is known to protect chlorophyll molecules from oxidative degradation and to stabilize thylakoid membranes under stress (Huang *et al*., 2023). The increase in the chlorophyll a/b ratio under higher N levels suggests improved light-use efficiency and adaptation of the photosynthetic apparatus to varying light conditions (Campos *et al*., 2023). Carotenoid content also increased with N application, reflecting enhanced photoprotection and antioxidant capacity. Interestingly, carotenoids decreased under weeding conditions, likely because reduced stress diminished the need for protective pigments (Necat Izgi *et al*., 2024).

Anthocyanin accumulation increased across treatments, with the highest levels observed at the highest N rate. Although anthocyanins are traditionally associated with stress responses, their increase in N- and SA-treated plants suggests a more nuanced role. Enhanced nutrient availability and SA signaling can stimulate phenylpropanoid metabolism, leading to greater synthesis of phenolic compounds, including anthocyanins (Aluko *et al*., 2025; Almeida *et al*., 2025). These pigments function as antioxidants, protecting cells from reactive oxygen species generated during rapid growth and high metabolic activity (Sadak *et al*., 2024). Therefore, anthocyanin accumulation in this study reflects not only stress mitigation but also improved physiological status and metabolic coordination (Abdoli and Ghassemi-Golezani, 2025). The simultaneous increase in chlorophylls and anthocyanins supports the concept of a coordinated response in which plants optimize both photosynthesis and defense (Shi *et al*., 2023).

Leaf N concentration increased significantly with rising N levels and SA application, indicating improved nutrient uptake and assimilation. This enhancement was closely linked to higher chlorophyll content and biological yield, underscoring the central role of N nutrition in peppermint productivity (Fan *et al*., 2021). Phosphorus and potassium concentrations also increased, particularly under weeding conditions, likely due to reduced competition and improved root access to soil nutrients (Al-Rishawi *et al*., 2023). Correlation analysis revealed strong positive relationships between leaf nutrient concentrations, plant height, chlorophyll content, biological yield, and essential oil traits. These associations suggest that improved nutritional status supports both primary growth processes and secondary metabolite production. However, while correlations indicate coordinated responses, causality should be interpreted cautiously (Jan *et al*., 2023).

From a practical perspective, the foliar N rates used in this study are substantially lower than conventional

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soil-applied N doses, reducing production costs and environmental risks such as nitrate leaching. Although SA application adds a minor input cost, the potential increase in essential oil yield (from approximately 60 to over 160 kg ha^{-1}) represents a highly favorable return on investment. This is especially relevant for low-input, organic, or weed-prone systems where herbicide use is restricted or undesirable (Esposito *et al*., 2025).

Conclusion

In conclusion, this study demonstrates that integrated management of nitrogen and salicylic acid can optimize peppermint production by simultaneously enhancing growth and essential oil biosynthesis. The combination of 3 g L^{-1} N and 3 mM SA effectively resolved the traditional trade-off between biomass and oil concentration, even under non-weeded conditions, resulting in the highest essential oil yield. This strategy offers a sustainable and economically viable model for peppermint cultivation by reducing reliance on high nitrogen inputs and herbicides while leveraging plant elicitors to improve both yield and quality.

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