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Original Article

Influence of Vermicompost and Nitrogen Treatments on the Enhancement of Morphophysiological Traits and Essential Oil Composition of Spearmint (*Mentha spicata* L.) for Double Harvesting Seasons

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ABSTRACT

This research investigates the impact of organic and chemical fertilizers on the total production and morphophysiological characteristics of *Mentha spicata* L., a medicinal plant, during two growing seasons in 2021. The study was conducted in a randomized complete block design at Zanjan University's research farm, employing treatments of vermicompost (at rates of 5, 10, and 15 tons per hectare), urea (at rates of 50, 100, and 150 kilograms per hectare), and a control treatment. Significant differences were observed in all measured indices between the fertilizer treatments and harvest times. Particularly, the application of vermicompost at a concentration of 10 tons per hectare significantly enhanced fresh and dry weights, stem height and diameter, number of branches, percentage and yield of essential oils, chlorophyll a, total chlorophyll, carotenoids, total phenols, flavonoids, and antioxidant capacity compared to other treatments. The urea treatment also significantly increased chlorophyll b, leaf width, and length compared to other treatments. Enzyme measurements conducted during one season revealed the highest levels of the APX enzyme in the vermicompost treatment (15 tons per hectare), the CAT enzyme in the vermicompost treatments (10 and 15 tons per hectare), and the SOD enzyme in the vermicompost treatment (10 tons per hectare) during the second harvest. Conversely, the control treatment exhibited the lowest levels across all measured traits. Overall, the results highlight the beneficial effects of using organic and environmentally friendly fertilizers in improving essential oils content, physiological traits, and the overall yield of *M. spicata* L., suggesting their potential for enhancing sustainable agriculture practices and medicinal plant productivity.

INTRODUCTION

Mentha spicata L., commonly known as spearmint, is a perennial and aromatic plant widely used in the food, pharmaceutical, and perfume industries worldwide [1]. Mint species, including spearmint, have long been recognized for their medicinal properties, such as antioxidant, anticancer, antimicrobial, antiviral, and antifungal effects [2]. The essential oils of spearmint contain various aromatic molecules, including carvone, carol, dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate, which contribute to its distinct aroma and therapeutic benefits [3].

In the context of increasing agricultural production and the need for sustainable practices, it is crucial to minimize the negative impact of chemical fertilizers and pesticides on the environment. This includes addressing the production of agricultural waste and wet garbage while maintaining soil health and nutrient availability for medicinal and aromatic plants [4]. Soil composition significantly affects the production of secondary metabolites in plants, as nutrients are absorbed in ionic form the soil. Therefore, it is important to adopt organic agricultural methods and reduce the harmful effects on soil health [5,6].

Organic fertilizers, such as vermicompost, offer a sustainable solution for improving soil texture, structure, aeration, and water retention. They also enhance the activity of beneficial microorganisms, resulting in improved plant growth and soil conditions. By minimizing the use of chemical fertilizers, organic fertilizers contribute to sustainable

food production while reducing environmental harm [7].

Vermicompost, a valuable organic component, has been extensively studied for its role in the agricultural ecosystem. It aids in the reuse and recovery of nutrients, reduces nitrogen losses, minimizes emissions of nitrogen gases and ammonia, and enhances soil fertility. Its application as a biological modifier has been investigated under different stress and non-stress conditions, revealing its positive impact on various agricultural parameters [8,9].

Nitrogen, an essential element for plant growth, promotes vegetative growth and influences the oil content in medicinal and aromatic plants [10]. Studies have shown that nitrogen deficiency can affect the production of essential oils in these plants, highlighting the importance of adequate nitrogen supply [11]. Additionally, high nitrogen input is crucial for achieving higher essential oil yields [12]. Considering nitrogen's significance for crop yield and food security, raising awareness among farmers regarding the importance of vermicompost and promoting the use of renewable and organic alternatives in sustainable agriculture becomes imperative [13].

Previous research on the effects of vermicompost fertilizer has demonstrated its superiority over other fertilizers, highlighting its crucial role in enhancing the growth and yield of various crops. For instance, vermicompost has been found to be superior in terms of nutrient content, agricultural sustainability, and food security when compared to other fertilizers in turnip production [14]. Similarly, its positive effects on maize yield, photosynthesis efficiency, root growth, and overall plant performance have been reported, emphasizing its significance in improving agricultural outcomes [15].

In light of these considerations, the present study aims to evaluate the effects of organic and chemical fertilizers on the morphophysiological characteristics and performance of spearmint, a valuable medicinal plant. By utilizing environmentally friendly fertilizers like vermicompost, we seek to minimize environmental impacts while optimizing plant growth and quality.

MATERIAL AND METHODS

An experiment was conducted to assess the impact of organic and chemical fertilizers on the performance and yield components of *M. spicata* L. The study followed a completely randomized complete block design with three replications across two production seasons: spring-summer and summer-autumn of 2021. The research was carried out at the Agricultural Faculty's research farm, at the University of Zanjan. The experimental treatments included vermicompost fertilizer at three different levels (5, 10 and 15 tons per hectare), urea fertilizer at three levels (50, 100 and 150 kilograms per hectare), and a control treatment without fertilizer. These treatments were randomly applied to plots with dimensions of 200 cm × 70 cm and 50 cm spacing between plots. Irrigation was performed regularly, every two days during summer and every three days during autumn, considering the ambient temperature, soil moisture, and regional rainfall. Fertilizers were applied after the initial plowing, and at the end of the growing season, sampling was conducted. The evaluated traits comprised morphophysiological characteristics, including fresh and dry weight, plant height, stem diameter, leaf length and width, percentage and yield of essential oils, number of branches per square meter, chlorophyll a, b, total chlorophyll, total phenol, flavonoid, antioxidant capacity, and antioxidant enzymes CAT, POD, and SOD activity. No additional additives were given to the plants before or after planting, and manual weed removal was performed. Harvesting was done after physiological maturity, and enzyme measurements were conducted in one season.

Morphological Traits

Immediately after harvesting, the fresh weight of the plants was measured using an electronic weighing scale. Five randomly selected plants from each treatment were used to measure leaf length, leaf width, plant height, stem diameter (measured using a Vernier caliper), and the number of branches, and the mean values were recorded.

Photosynthetic Pigments

Chlorophyll a, b, total chlorophyll, and carotenoids were measured following the method described by Arnon [16]. Fresh leaf tissue weighing 0.1 grams was crushed with 10 ml of 80% acetone. After centrifugation at 5000 rpm for ten minutes, the absorbance at 663, 645, 510, and 480 nm was measured using a spectrophotometer. The amounts of chlorophyll and carotenoids were calculated in milligrams per gram of fresh weight using the following formulas:

Total Chlorophylls = [20.2(A645) + 8.02(A663)] × V⁄(W× 1000)
Total carotenoids = [7.6(A480) −1.49(A510)] × V⁄(W× 1000)
Where V is the final volume of the extract, A is the absorbance at specific wavelengths, and W is the weight of the fresh sample.

Total Phenol and Flavonoids

To measure total phenol, one gram of dried plant sample was extracted with ten milliliters of methanol. The extract was then centrifuged at 5000 rpm for ten minutes, and the total phenol was measured using the Folin-Ciocalteu method. The clear extract was mixed with 300 microliters of 10% Folin-Ciocalteu reagent and rested for five minutes. Then, 300 microliters of 75 g/L sodium carbonate (Na2CO3) solution were added and kept in the dark for 30 minutes. The absorbance was measured at 765 nm using a spectrophotometer (Safas Monaco, RS 232), and the content of total phenol was determined by the calibration curve, expressed as milligrams of gallic acid equivalent per gram of fresh weight [17].

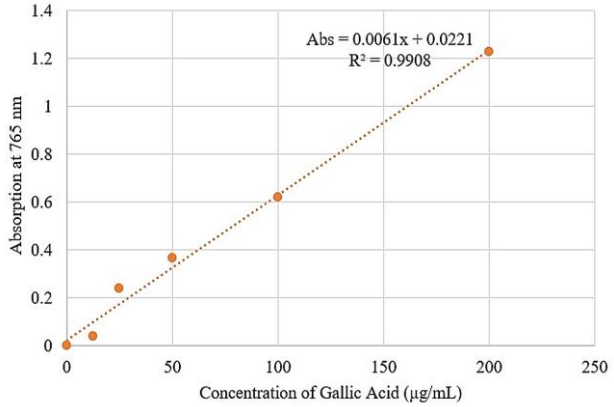

Fig. 1 Calibration curve of Gallic acid standards

To measure flavonoids, the aluminum chloride colorimetric method was used [18]. The clear extract was mixed with 60 microliters of 10% aluminum chloride, 60 microliters of 1 M potassium acetate, and 1650 microliters of distilled water. The mixture was kept at room temperature for 30 minutes, and the absorbance was measured at 415 nm using a spectrophotometer (Safas Monaco, RS 232). The content of flavonoids was determined by the calibration curve, expressed as milligrams of quercetin equivalent per gram of fresh weight.

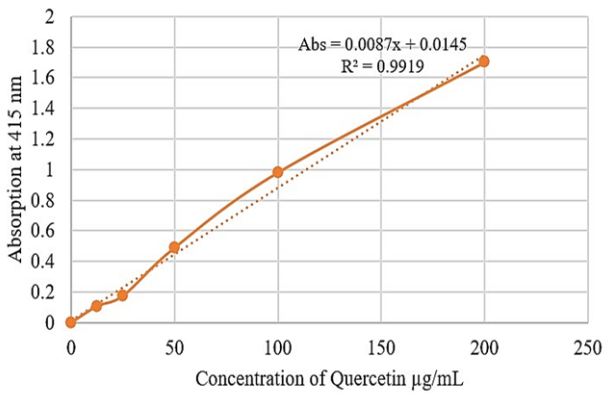

Fig. 2 Calibration curve of quercetin standards

Antioxidant Capacity

The measurement of antioxidant capacity was performed using the DPPH method according to the method described by Dehghan and Khoshkam [19]. Fifty microliters of plant extract were mixed with 1.0 mM DPPH (dissolved in methanol) and kept in the dark for 30 minutes. The absorbance of the solutions was measured at 517 nm using a spectrophotometer, and the antioxidant activity of the extracts was expressed as the percentage inhibition of DPPH radical using the following formula:
RSA% = 100 (AC-AS) / AC
Where AC is the absorbance of the control (empty DPPH solution), and AS is the absorbance of the sample.

Phytochemical Traits

To measure the percentage and yield of essential oils, collected plants were dried naturally in the shade for ten days under appropriate conditions. The leaves were then separated and ground. From each sample, 100 grams of dried powdered leaves were prepared using the steam distillation method with water and a Clevenger apparatus for essential oils extraction. The extraction time was consistent for all samples at two hours and thirty minutes. The composition analysis of the essential oils was performed using a GC-MS apparatus with gas chromatography (Hewlett Packard Hp-7890) and quadrupole mass spectrometry (Hewlett Packard, Agilent).

Evaluation of Antioxidant Enzyme Activities

To analyze the activities of antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX), 3 grams of frozen fresh leaf tissue were ground in 50 mM phosphate buffer (pH 7.8) containing 2.0 mM EDTA and 2% PVPP. The mixture was then centrifuged at 10000 rpm for 15 minutes at 4°C, and the resulting

supernatant was used for enzyme activity measurements. SOD activity was measured according to the method described by Nasr [20]. and the absorbance was recorded at 560 nm, with the results expressed as u/mg. CAT activity was measured at 240 nm according to the method described by Nasr [20]. with the results expressed as u/mg. APX activity was measured at 290 nm according to the method described by Nasr [20]. with the results expressed as u/mg.

Gas Chromatography (GC)

Hewlett Packard Hp-7890 gas chromatography with HP-5 column with an internal diameter of 0.5 micrometers and a length of 30 meters and a thickness of the stationary phase layer of 0.25 micrometers. The column temperature started at 50°C and finally reached 250°C. Helium gas was used as a carrier with a speed of 12 cm/s. The detector temperature was set at 280°C. The percentage of compounds that make up the essential oil was determined by calculating the surface area under each curve in the GC chromatogram whose detector was FID.

Gas Chromatography–Mass Spectrometry (GC–MS)

Hewlett Packard Agilent model chromatograph connected to a mass spectrometer was used to identify the compounds in the essential oil. Column with an inner diameter of 0.5 micrometers and a length of 30 meters, the thickness of the stationary phase is 0.25 micrometers. The temperature of the column started from 50 °C and finally reached 250 °C. The temperature of 280 °C was set as the detection temperature. Helium gas was used with a speed of 35 cm/s and an ionization energy of 70 electron volts. The thermal program was set in the range of 60-240 degrees with a speed of 3 degrees per minute and the temperature of the injection chamber was 220 degrees. Identification of the mass spectra of essential oil compounds was done using the inhibition time and the mass spectra of standard compounds.

Experimental Design and Data Analysis

The statistical analysis of the experimental data was performed using SAS software version 9.4. Mean values were compared using the Duncan test at a significance level of 1%.

RESULTS

The plants were harvested at two different time points, and the results revealed a significant interaction effect between the fertilizer and harvest time for stem diameter and dry weight traits at a significance level of 5%. Additionally, significant effects were observed for fresh weight, stem height, leaf length and width, and the number of branches per unit area at a significance level of 1%. Upon comparing the mean values (Table 2), the treatment with 10 tons of vermicompost at the first harvest time exhibited the highest fresh and dry weight, while the control treatment at the second harvest time showed the lowest values. The highest leaf length and width were observed in the urea treatment at the first harvest time. Conversely, the control treatment at the second harvest time displayed the lowest values. Stem diameter reached its highest size in the vermicompost treatments with 10 tons/ha and urea with 150 kg/ha at the second harvest time, while the control treatment at the first harvest time showed the smallest size. The treatment with 10 tons/ha of vermicompost at the first harvest time resulted in the highest stem height, whereas the lowest was observed in the vermicompost treatment with 5 tons/ha at the second harvest time. The number of branches per unit area was greatest in the vermicompost treatment with 10 tons/ha at the second harvest time, while the control treatment at the first harvest time exhibited the lowest value.

The interaction effect of fertilizer and harvest time was also significant for chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phenols, flavonoids, antioxidants, percentage and yield of essential oils, with significance levels of 1% for these traits. Upon examining the mean values (Table 4), the treatment with 10 tons of vermicompost at the first harvest time displayed the highest levels of chlorophyll a, total chlorophyll, and carotenoids, while the urea treatment with 100 kg/ha exhibited the highest level of chlorophyll b. The control treatment had the lowest levels of chlorophyll a, total chlorophyll, carotenoids, and chlorophyll b, with the treatment involving 5 tons of vermicompost at the second harvest time showing the lowest level of chlorophyll b. The treatment with 10 tons of vermicompost at the first harvest time resulted in the highest levels of total phenols (fig.1a), flavonoids (fig.2b), and antioxidant capacity (fig.3c), whereas the control treatment at the second harvest time displayed the lowest levels. The highest percentage (fig.5e) and yield of essential oils

(fig.4d) were observed in the treatment with 10 tons of vermicompost, while the control treatment at the second harvest time showed the lowest level. In the analysis of the essential oils in spearmint (*M. spicata* L.), in the first season, 38 components were identified from the dried aerial parts of the plants. The values of the components of the essential oils obtained from the leaves of the *M. spicata* L. species were determined in the specimens obtained in the first harvests. The values of the essential oils components of the *M. spicata* L. species are separately shown in (Table 7). The main components obtained from the dry leaves according to the average measures of the first harvest for vermicompost are as follows: Carvone 37.42%, D-Limonene 14.17%, Dihydrocarvone 10.65%. The activity of antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase, was measured at the second harvest time (Table 5).

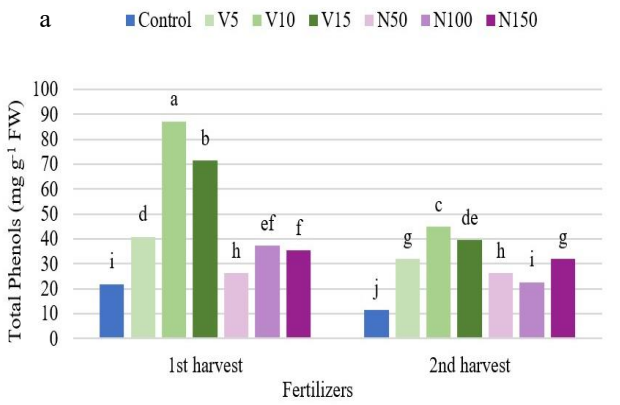

Fig. 3 A Effects of fertilizer type and harvest time on total phenol content in Spearmint

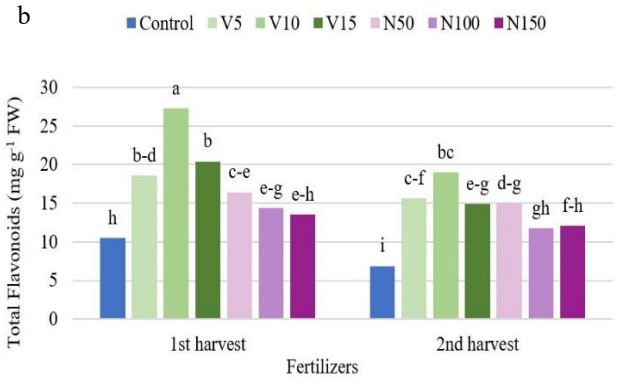

Fig. 4 B The effect of harvest time and fertilizer type on total flavonoids content in Spearmint

The ANOVA results indicated a significant effect of the treatment on all three antioxidant enzymes at a significant level of 1%, while the block effect was not significant. Upon comparing the mean values (Table 6), the treatment with 10 tons of vermicompost exhibited the highest level of superoxide dismutase, whereas the control treatment displayed the lowest level. The vermicompost treatments with 5 and 15 tons/ha, as well as the urea treatment with 50 kg/ha, showed the highest level of catalase, while the control treatment had the lowest level. The highest level of ascorbate peroxidase was observed in the urea treatment with 150 kg/ha, while the control treatment displayed the lowest level.

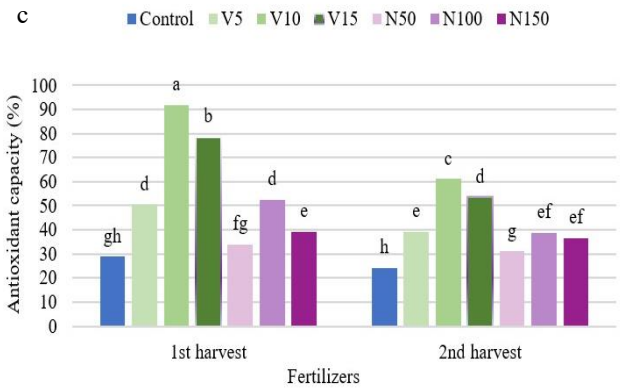

Fig. 5 C The effect of fertilizer type and harvest time on antioxidant capacity in Spearmint

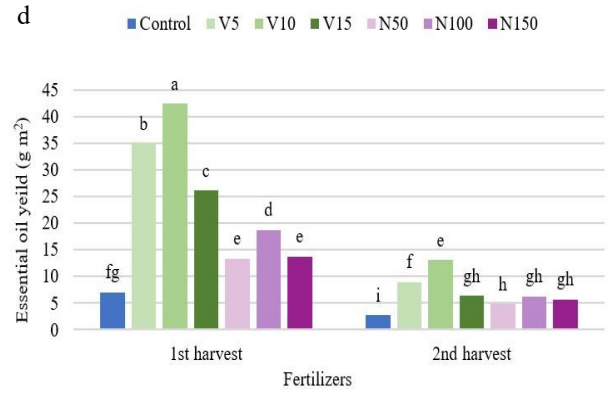

Fig. 6 D Effects of fertilizer type and harvest time on essential oils yield in Spearmint

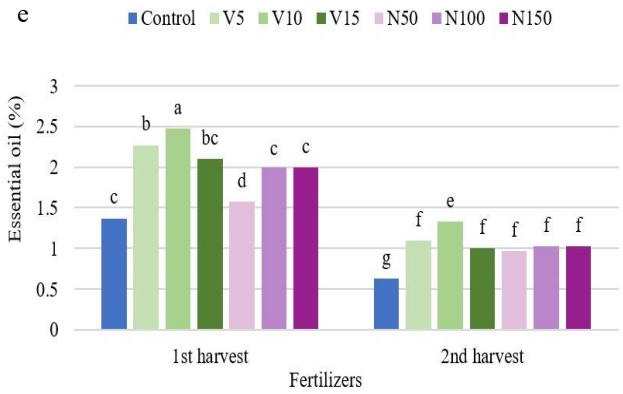

Fig. 7 E Effects of fertilizer type and harvest time on essential oils percent in Spearmint

Table 1 Combined analysis of variance on the traits studied Spearmint as affected by fertilizer treatments

Source of variation	Df	Fresh weight	Dry weight	Stem height	Stem diameter	leaf length	leaf width	Number of main branches
Time	1	24437426.9 **	1907466.6 **	3609.181 **	16.8593 **	2.4096 **	1.78148 **	16126.88 **
Treat	6	4905005.5 **	598776.07 **	525.4091 **	0.5964 **	2.2602 **	1.09332 **	71718.49 **
Time*Treat	2	1327072.63 **	108556.78 **	307.8645 **	0.09617 *	0.01928 *	0.02524 **	1432.04 **
Time*Error (a)	6	427.22 ns	298.448 ns	9.02094 ns	0.0057 *	0.0023 ns	0.00223 ns	200.023 ns
Error (b)	26	158.72	202.959	13.7778	0.0022	0.00368	0.00402	86.496
CV%		0.410863	1.660822	6.205489	3.236837	1.148198	2.783383	1.612973

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, NS: no significant
df degree of freedom

Table 2 Comparison of the average effect of fertilizers and harvest time on some morphological traits of Spearmint

Treatments		Physiological Traits						
	Time	Fresh weight g/m^2	Dry weight g/m^2	Stem height Cm	Stem diameter mm	leaf length cm	leaf width cm	Number of main branches m^2
Control	1st harvest	2596.71 i	514.40 h	76.57 b	0.50 i	4.26 g	1.50 f	412.67 h
V5		5597.53 b	1541.56 b	50.50 cde	0.81 fg	5.22 de	2.57 bc	649.33 d
V10		5855.14 a	1720.16 a	90.37 a	1.37 d	5.57 c	2.73 b	682.67 c
V15		3870.78 c	1242.80 c	78.40 ab	1.09 e	5.81 b	2.59 b	643.00 d
N50		2990.95 e	855.14 e	50.93cd	0.62 hi	6.32 a	2.34 d	491.33 f
N100		3255.14 d	935.80 d	59.80 c	0.68 gh	5.76 bc	2.71 b	514.67 f
N150		2637.86 h	686.42 f	77.03 b	0.84 f	5.73 bc	2.94 a	505.33 f
Control	2nd harvest	1748.15 n	431.28 i	55.77 cd	1.49 d	3.91 h	1.22 g	460.33 g
V5		2919.34 f	812.35 e	39.17 e	2.13 b	4.88 f	2.17 de	713.67 b
V10		2859.26 g	981.89 d	46.13 de	2.58 a	5.10 e	2.36 cd	768.67 a
V15		2386.01 j	637.86 g	53.13 cd	2.06 b	5.37 d	2.19 de	666.67 cd
N50		1866.67 m	500.41 h	52.65 cd	1.85 c	5.83 b	2.07 e	504.00 f
N100		2299.59 k	603.29 g	48.90 cde	2.16 b	5.12 e	2.17 de	510.67 f
N150	000	2046.09 l	545.68 h	58.07 cd	2.51 a	5.13 e	2.32 d	549.33 e

control: without biofertilizer, V5: with 5 kg/h vermicompost, V10: with 10 kg/h vermicompost, V15: with 15 kg/h vermicompost, N50: with 50 kg/h urea, N100: with 100 kg/h urea, N150: with 150 kh/h urea

Table 3 Combined analysis of variance on phytochemical traits of Spearmint as affected by fertilizer treatments

Source of variation	df	Chlorophyll A	Chlorophyll B	Total chlorophyll	Total carotenoid	Total phenols	Total flavonoids	Antioxidant capacity	Essential oil	Essential oil yield
Time	1	0.02525 **	1.2688 **	1.45228 **	0.10103 **	2639.4 **	142.416 **	1755.18 **	9.52381 **	2523.55 **
Treat	6	0.34276 **	0.19869 **	0.82088 **	0.11088 **	1772.7 **	124.9703 **	1951.46 **	0.50047**	382.859 **
Time*Treat	6	0.21040 **	0.07918 **	0.12962 **	0.00704 **	360.99 **	9.02322 **	184.148 **	0.06823 **	139.262 **
Time*Error (a)	4	0.00101 ns	0.00002 ns	0.00164 ns	0.00029 ns	0.4842 ns	1.4883 ns	2.1518 ns	0.00952 ns	1.85271 *
Error (b)	24	0.001132	0.000442	0.000403	0.000275	0.89368	1.243209	2.49272	0.00341	0.347059
CV%		2.523983	5.731875	1.190548	2.133145	2.495278	7.225916	3.346827	3.919439	4.039395

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, NS: no significant
df degree of freedom

Table 4 Comparison of the average effect of fertilizers and harvest time on some phytochemical traits of Spearmint

Treatments		Phytochemical Traits			
	Time	Chlorophyll-a (mg/g FW)	Chlorophyll-b (mg/g FW)	Total chlorophyll (mg/g FW)	Total carotenoid (mg/g FW)
Control	1st	1.34 defg	0.07 gh	1.41 g	0.68 fg
V5	Harvest	1.33 efg	0.42 c	1.74 d	1.04 a
V10		2.07 a	0.72 b	2.79 a	1.08 a
V15		1.16 h	0.35 cd	1.52 f	0.87 b
N50		1.17 h	0.68 b	1.85 c	0.70 ef
N100		1.02 i	0.83 a	1.84 c	0.74 de
N150		1.41 cde	0.73 b	1.97 b	0.84 bc
Control	2nd harvest	0.77 j	0.04 h	0.81 h	0.58 h
V5		1.44 cd	0.03 h	1.46 fg	0.88 b
V10		1.56 b	0.32 de	1.88 c	0.88 b
V15		1.24 fg	0.26 ef	1.50 f	0.77 d
N50		1.30 fg	0.22 f	1.52 f	0.63 gh
N100		1.49 bc	0.12 g	1.61 e	0.73 de
N150		1.36 def	0.37 cd	1.74 d	0.78 cd

control: without biofertilizer, V5: with 5 kg/h vermicompost, V10: with 10 kg/h vermicompost, V15: with 15 kg/h vermicompost, N50: with 50 kg/h urea, N100: with 100 kg/h urea, N150: with 150 kh/h urea

Table 5 Combined analysis of variance on some enzymatic activity of Spearmint as affected by fertilizer treatments

Source of variation	Df	SOD	APX	CAT
Treat	6	1233.1004 **	19.592609 **	0.316939 **
Block	2	5.933276 ns	0.020589 ns	0.000288 ns
Error	12	15.782398	0.016032	0.001488
CV%		5.197999	4.168715	11.58839

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, NS: no significant
df degree of freedom

Table 6 Comparison of the average effect of fertilizers on SOD, POD, CAT

Treatments	Enzymes		
	SOD (U/g)	APX (U/g)	CAT (U/g)
Control	39.78 e	0.41 f	0.02 d
V5	92.36 ab	2.64 d	0.44 b
V10	102.42 a	5.24 b	0.72 a
V15	80.08 bc	7.17 a	0.81 a
N50	63.31 d	0.72 ef	0.05 d
N100	78.41 c	1.07 e	0.08 d
N150	78.62 c	4.01 c	0.22 c

control: without biofertilizer, V5: with 5 kg/h vermicompost, V10: with 10 kg/h vermicompost, V15: with 15 kg/h vermicompost, N50: with 50 kg/h urea, N100: with 100 kg/h urea, N150: with 150 kh/h urea

Table 7 Chemical composition and characteristics of essential oils of *M. spicata* under Vermicompost, Urea and control treatment.

Components	RI	1st Harvest		
		Vermicompost %	Urea %	Control %
(2E)-Hexenal	846	0.56	0.54	0.42
α-Pinene	932	1.34	1.48	1.12
Sabinene	969	1.4	1.38	_
β-Pinene	974	1.84	1.79	1.6
β-Myrcene	988	1.12	1.46	0.99
3-Octanol	994	0.5	0.46	0.4
D-Limonene	1024	14.17	13.19	12.79
β-phellandrene	1025	_	_	1.06
1,8-cineole	1026	4.54	3.69	5.72
Isopulegol	1146	0.85	0.75	0.63
Menthone	1154	2.42	2.42	1.45
Borneol	1165	1.23	1.11	1.32
Cis-Dihydrocarvone	1191	10.65	10.96	0.44
neo-Dihydro carveol	1193	3.51	2.51	9.54
Pulegone	1237	_	_	19.35
Carvone	1242	37.42	35.37	22.19
trans-carvone oxide	1277	_	0.46	0.29
Dihydrocarveol acetate	1305	1.56	1.32	0.69
trans-Carvyl acetate	1337	0.46	_	0.43
Piperitenone	1342	1.37	2.11	1.59
Carveol acetate	1371	_	0.59	0.5
β-Cubebene	1387	1.27		
β-Bourbonene	1388	2.63	2.76	2.23
β-Copaene	1416	0.5	2.39	1.55
cis-Muurola-4(15),5-diene	1417	0.94	1.19	0.9
(E)-Caryophyllene	1418	4.31	5.49	5.99
Aromadendrene	1439	0.52	_	_
α-Humulene	1452	1.13	1.34	1.22
γ-Muurolene	1477	_	_	0.37

Germacrene D	1480	0.36	0.43	0.28
epi-Cubenol	1493	_	0.64	0.43
cis-Calamenene	1521	0.57	0.77	0.56
Caryophyllene oxide	1581	0.9	0.75	0.63
epi-α-Cadinol	1640	_	0.66	0.44
α-Cadinol	1653	_	0.37	0.36
Total	-	98.07	98.38	97.48

DISCUSSION

Our study aimed to investigate the impact of vermicompost and urea fertilizers on the performance and yield components of *M. spicata* L. (spearmint), a widely utilized medicinal and aromatic plant in the food, pharmaceutical, and perfume industries. Sustainable agricultural practices are of paramount importance to meet the growing demand for natural products while minimizing the environmental footprint of conventional farming methods. The results of our research shed light on the efficacy of vermicompost and urea as potential fertilization systems for improving the growth, biochemical content, and essential oils yield of spearmint.

The interaction effect of fertilizer and harvest time on various traits underscored the significance of understanding the temporal dynamics of plant responses to different fertilizers. Stem diameter, dry weight, fresh weight, stem height, leaf length, width, and the number of branches per unit exhibited significant interactions, suggesting that the effectiveness of fertilization strategies may vary depending on the growth stage of the plants. These findings highlight the importance of implementing precise fertilizer management practices to optimize the growth and development of spearmint at different stages of its life cycle.

Among the fertilizer treatments, the application of 10 tons of vermicompost per hectare consistently resulted in favorable outcomes. The vermicompost-treated plants exhibited the highest fresh and dry weight, which can be attributed to the nutrient-rich composition of vermicompost, including essential macro and micronutrients, as well as growth-promoting substances [21]. The observed increase in biomass could also be linked to improved soil structure and microbial activity facilitated by vermicompost, which enhances nutrient uptake and assimilation by spearmint plants [22]. Furthermore, the slow-release nature of vermicompost nutrients ensures sustained nourishment for the plants, contributing to better growth and performance over time [9].

Interestingly, urea fertilizer exhibited its highest impact on leaf traits, with the treatment of 50 kg/ha resulting in the tallest and widest leaves at the first harvest time. This aligns with previous studies that have demonstrated the role of nitrogen in promoting vegetative growth and leaf expansion in various plant species [10]. However, excessive nitrogen application can lead to adverse effects on plant growth and environmental pollution [12]. Therefore, optimizing nitrogen application rates to meet plant requirements without causing ecological harm remains a key consideration in sustainable agriculture [24].

The results pertaining to chlorophyll and carotenoid content further emphasize the benefits of vermicompost application. The vermicompost-treated plants demonstrated the highest levels of chlorophyll a, chlorophyll b, and total chlorophyll at the first harvest time, indicating enhanced photosynthetic efficiency. Chlorophylls are essential pigments involved in capturing light energy for photosynthesis, while carotenoids play a crucial role in protecting plants from photooxidative damage [25]. The higher levels of these pigments in the vermicompost-treated plants might be attributed to improved nutrient availability and physiological processes, leading to more efficient photosynthesis and overall plant health [26,27].

Moreover, the vermicompost-treated plants at the first harvest time exhibited the highest levels of total phenols, flavonoids, and antioxidant capacity. These findings are consistent with studies that have reported the positive influence of vermicompost on secondary metabolite synthesis in various plant species [27,28]. Phenolic compounds, including flavonoids, are potent antioxidants known for their beneficial effects on human health and plant defense mechanisms [25]. The higher content of these compounds in vermicompost-treated plants suggests that organic fertilization enhances the production of secondary metabolites in spearmint, potentially increasing its value as a medicinal plant.

Furthermore, the treatment with 10 tons of vermicompost per hectare yielded the highest percentage and yield of essential oils, underscoring the positive impact of vermicompost on the production of valuable aromatic compounds. Essential oils are sought-after ingredients in the food, pharmaceutical and perfume industries due to their diverse therapeutic and sensory properties [29]. The increased essential oil yield in vermicompost-treated plants could be attributed to the synergistic effects of improved vegetative growth, enhanced nutrient availability, and the presence of nitrogen, which is a vital element in essential oil synthesis [12,29].

The higher activity of antioxidant enzymes, such as superoxide dismutase, in the vermicompost-treated plants further supports the role of vermicompost in promoting plant defense mechanisms against oxidative stress [26]. Antioxidant enzymes play a critical role in detoxifying reactive oxygen species and protecting plants from various environmental stressors [26,30]. The enhanced antioxidant enzyme activity in vermicompost-treated plants indicates their ability to cope with oxidative damage and maintain cellular homeostasis under potentially stressful conditions.

CONCLUSION

In conclusion, our study highlights the effectiveness of vermicompost, particularly at the rate of 10 tons per hectare, as a sustainable and environmentally friendly fertilizer for enhancing the growth, biochemical content, and essential oils yield of *M. spicata* L. (spearmint). Vermicompost enriches the soil with essential nutrients and growth-promoting substances, leading to improved nutrient uptake and assimilation by the plants. The vermicompost-treated plants exhibited higher biomass accumulation, increased levels of chlorophylls, carotenoids, and secondary metabolites, as well as enhanced antioxidant enzyme activity, indicating improved photosynthetic efficiency and plant health. These findings underscore the potential of vermicompost as a key component in sustainable agricultural practices, promoting the cultivation of medicinal plants while minimizing environmental impacts and ensuring the production of high-quality, chemical-free products.

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Author Contribution

All authors contributed to the study conception and design. Experiments execution, data collection, and analysis were performed by M. Latifi, A. Kheiry, and F. Razavi and all authors revised it. All authors approved the manuscript submission.

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This manuscript has not been published or is being considered for publication anywhere.

Conflict of Interest

M. Latifi, A. Kheiry, F. Razavi and M. Sanikhani declare that they have no competing interests.

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