

Research article

The Effect of Nitrogen and Micronutrients on Growth and Essential Oil of *Dracocephalum moldavica* as a Medicinal Plant

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Abstract

The purpose of this research was to investigate the effects of nitrogen and micronutrient application on the yield and quality of *Dracocephalum moldavica* medicinal plant. The experiment was conducted as a factorial in the form of a completely randomized design with 4 replications. Experimental treatments included nitrogen at 4 levels: N₀ (no application), N₁ and N₂ (soil application of nitrogen from the source of ammonium nitrate of 100 and 150 mgkg⁻¹ pot soil, respectively), N₃ (foliar application of nitrate solution at a concentration of 1% + N₁) and micronutrients at 4 levels: M₀ (no application), M₁ (soil application of Fe, Zn and Mn each at the rate of 50 mgkg⁻¹ pot soil), M₂ (foliar application of Fe, Zn and Mn at a concentration of 0.3%) and M₃ (soil application and foliar application of Fe, Zn and Mn). The results showed that the use of nitrogen at the level of N₃ along with micronutrients at the level of M₂ significantly increased the yield of *Dracocephalum moldavica*. Moreover, this level can lead to the optimal use of agricultural inputs. Based on the results of this experiment, it can be concluded that foliar application of nitrogen and micronutrients had a positive effect on growth characters and can increase the yield of dry matter.

Keywords: *Dichocephalic moldavica* L.; micronutrients; nitrogen; essential oil

1. Introduction

The lemon balm plant, with the scientific name *Dracocephalum moldavica*, is an annual herbaceous plant belonging to the mint family. The genus *Dracocephalum* contains 45 herbaceous and shrub species in the world and 8 annual and perennial aromatic herbaceous species in Iran, some of which are unique to Iran (Yousefzadeh et al., 2013). Members of this genus are herbaceous and native to Central Asia but have also been domesticated in Central and Eastern Europe (Dastmalchi et al., 2007). One of the famous species of this genus, *Dracocephalum moldavica*, is widely cultivated in Iran and is generally used to prepare lemon balm as a heart tonic and sedative.

The effects of various amounts of nitrogen, phosphorus and micronutrients on yield and amount of essential oil of *Satureja hortensis* L. were investigated by Majidi Livarjani

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(2010). It was found that the low-use elements increased morphological traits of the plant, but this increase was not statistically significant, and the optimal yield of *Satureja hortensis* L. was obtained by consuming 200 kg of ammonium nitrate and 100 kg of ammonium superphosphate. Das et al. (2007) studied the effect of nitrogen fertilizer application on yield of summer savory (*Satureja hortensis* L.), a medicinal plant. It was found that the foliar application method, while increasing the dry matter, also increased the yield of essential oil.

Therefore, the purpose of this research was to investigate the effects of nitrogen and micronutrients on yield and morphological traits of *Dracocephalum moldavica*. Considering that one of the purposes of growing aromatic plants is to increase the yield of their dry matter and essential oil. In addition, there is a direct relationship between the performance of the vegetative body of the plant and the performance of the essential oil contained in it. Therefore, the ideal plant performance and improvement of essential oil yield could be achieved with nitrogen treatment and micronutrient's ability.

2. Materials and Methods

This research was carried out in order to investigate the effect of nitrogen levels and methods of micronutrient application on *Dracocephalum moldavica*. The pot experiment was performed in the research greenhouse of Islamic Azad University, Khoy Branch, using a factorial completely randomized design with 16 treatments and 4 replications. For this purpose, pots with a diameter of 30 cm and a height of 20 cm were selected. Prepared soil (10 kg) was filled into each of the pots. The analysis results of the studied soil sample are shown in Table 1.

The experimental treatments included nitrogen at 4 levels: N_0 (no application), N_1 and N_2 (soil application of nitrogen from the source of ammonium nitrate, 100 and 150 mg kg^{-1} potting soil, respectively), N_3 (foliar application of nitrate solution + N_1), and micronutrients at 4 levels: M_0 (no application), M_1 (soil application of Fe, Zn and Mn each at the rate of 50 mg kg^{-1} pot soil), M_2 (soil application and foliar application of micronutrients with a concentration of 0.3%) and M_3 (soil application and foliar application of Fe, Zn and Mn; $M_1 + M_2$). For planting, 15-20 seeds were first planted at a depth of 2.5 cm and the first irrigation of the pots was done immediately after planting. During the growth period, the humidity of the pots was kept at 70% field capacity (0.7 FC). Thinning was done three times at the two-leaf, four-leaf, and six-leaf stage to remove weak seedlings until the number of plants in each pot was reduced to four plants. In order to make the growing conditions of plants in pots equal to natural conditions, the pots were placed in the open space. No specific pests or diseases were observed in cultivated plants. The measured traits were the number of leaves, the diameter of the main stem, the fresh weight of the plant, the dry weight of flowers, stems and leaves, and the percentage of essential oil.

Statistical analysis of variance was done using SPSS software. Mean comparison was done using Duncan's multi-range test at the 5 percent significance level.

Table 1. Physicochemical properties of the experimental soil

Soil texture class	Sand	Clay	Silt	Mn	Zn	Fe	K	P	N	pH	EC (dS.m ⁻¹)
	(%)			mg.kg ⁻¹					(%)		
Sandy loam	40	30	30	8.6	0.71	4.7	275	10.2	0.06	7.5	1.29

3. Results and Discussion

In Table 2, the results of analysis of variance showed that the main effect of the factors studied on this trait was significant. In Table 3, among the levels of nitrogen used, the largest diameter of the main stem was treatment N₃ with an average of 1.39 cm, which was significantly higher than other levels of nitrogen. Regarding the levels of micronutrients, the greatest effect was from the M₂ treatment with an average of 1.20 cm; however, the result was not significantly different from the M₃ treatment (1.10 cm). Basically, the reason for the increase in the diameter of the main stem regarding the use of nitrogen fertilizer could be attributed to the effect of nitrogen intensification in vegetative growth, cell divisions in the plant organs, especially the stem. By producing photosynthetic material by the plant, these materials should provide the right conditions for the elongation of the stem. Increasing nitrogen increases plant height and increases absorption of nutrients from the soil, thus it may have a positive effect on plant growth. According to the studies of Faridvand et al. (2022) in the medicinal plant *Melissa officinalis*, the use of nitrogen fertilizer at different stages of growth led to an increase in the length of the flowering branch, which was significantly different from the control.

Table 2. Analysis of variance of traits

S.O. V	df	Dry Wight of Stem	Dry Wight of Flower	Fresh Wight of Plant	Number of Leaves	Diameter of Stem	Dry Wight of Leaves	Percentage of Essential Oil
nitrogen (A)	3	2.077**	0.329**	38.525**	415.22**	1.04**	0.153**	0.385**
Micro element (B)	3	0.029*	0.007**	1.109**	86.47**	0.261**	0.021**	0.041**
A*B	9	0.023*	0.008**	0.267**	4.11	0.022	0.004*	0.003
Error	32	0.01	0.002	6.52	12.54	0.053	0.0002	0.003
C.V %		13.04	8.28	6.53	9.04	21.96	10.94	1.17

*, ** and ns are respectively significant at the probability level of 0.05, 0.01 and non-significant

Table 3. Comparison of the average effects of the studied traits

	F Levels of Micronutrients*				N*			
	M ₃	M ₂	M ₁	M ₀	N ₃	N ₂	N ₁	N ₀
Diameter of stem	1.10 ^{ab}	1.20 ^a	0.95 ^b	0.93 ^b	1.39 ^a	1.06 ^b	0.96 ^b	0.78 ^c
Number of leaves	40.31 ^a	41.37 ^a	39.12 ^a	36.00 ^b	46.43 ^a	38.25 ^b	37.62 ^b	34.5 ^c
Percentage of essential oil	0.58 ^a	0.56 ^a	0.52 ^b	0.47 ^c	0.58 ^a	0.56 ^b	0.49 ^c	0.35 ^d

*Same superscript letters in the same row mean no statistically significant difference.

3.1 Number of leaves

The results of analysis of variance showed that the main effect of the factors studied on the number of leaves was significant (Table 2). Among the levels of nitrogen treatment, the highest number of leaves was found in the N₃ treatment (foliar application of ammonium nitrate at a concentration of 1% +N₁) with an average of 46.43. Among the levels of micronutrients, the greatest effect was found in the M₂ treatment (foliar application of Fe, Mn and Zn with a concentration of 0.3%) with an average of 41.37, which was similar to the M₁ treatment (soil application of micronutrients of Fe, Zn and Mn from each at the rate of 50 mgkg⁻¹ pot soil) and the M₃ treatment (soil application and foliar application of micronutrients Fe, Zn and Mn), which fell in the same statistical group (Table 3). The interaction effect of two factors on number of leaves was not significant. Karimzadeh et al. (2018) stated that the use of nitrogen fertilizer increased the number of leaves in the medicinal plant *Aloe Vera*. Mohammadi et al. (2016) reported that nitrogen fertilizer increased the number of leaves in the plant. Majidi Livarjani (2010) stated that the effects of nitrogen and micronutrients on the number of summer savory leaves (*Satureja hortensis* L.) were significant. Vafadar-Yengeje et al. (2019) also reported that different levels of nitrogen fertilizer had a significant effect at the 1% significance level on the number of leaves.

3.2 Fresh weight of the plant

Analysis of the variance of the traits showed that the levels of the studied factors and their interaction had a significant effect on the yield (Table 2). Comparison of the average interaction effect of nitrogen and micronutrients shows that the highest weight was obtained from the N₃M₃ treatment with an average of 7.2 g, which fell in the same statistical group as the N₃M₂ treatment (7.1 g). The lowest fresh weight of the plant was found in the N₀M₀ treatment with an average of 2.7 g (Table 4). This finding is consistent with the results found in marigold plant by Chelan et al. (2023). Appropriate plant nutrition with nitrogen significantly increases vegetative growth, durability of the leaf surface and production of photosynthetic materials, which ultimately increases the length of the vegetative growth period. Nitrogen deficiency has a negative effect on the growth processes, which leads to a decrease in the efficiency of photosynthesis and ultimately to a decrease in yield. Nitrogen was significant for many biological traits of calendula. In a study conducted by Kishimoto et al. (2005) on calendula (*Calendula officinalis* L.) with the application of 0, 20, 40, 60, 80 and 100 kg ha⁻¹ of nitrogen, it was found that the highest biological yield and the grain yield were obtained with the application of 80 kg ha⁻¹ of nitrogen. Similar results were obtained in the experiments of Nejatizadeh (2020) on summer savory and Wójtowicz et al. (2017) on lemon balm (*Melissa officinalis* L.). Based on the results obtained from this experiment on the application of nitrogen and micronutrients, it can be concluded that foliar application of nitrogen and micronutrients had a positive effect on quantitative yield (aerial organ function) and their application to this plant increased yield simultaneously. Considering that the purpose of the medicinal plant is to maximize the yield of the heads and branches in order to obtain the highest yield per hectare, the best treatment combination is ammonium nitrate foliar application in addition to soil application of nitrate. Moreover, the foliar application method is more economical and cost-effective compared to soil applications, and it is directly available to the plant, and waste and leaching are avoided.

Table 4. Comparison of the interaction effects of nitrogen and micronutrients on the traits studied in *Dracocephalum moldavica*

Treatment	Dry Weight of Leaves (g)	Dry Weight of Stem (g)	Dry Weight of Flower (g)	Fresh Weight of Plant (g)
N ₀ M ₀	0.23 ^h	0.41 ^{de}	0.34 ^e	2.7 ^g
N ₀ M ₁	0.24 ^{gh}	0.43 ^{de}	0.35 ^e	2.9 ^g
N ₀ M ₂	0.26 ^{fg}	0.49 ^d	0.36 ^e	3.2 ^g
N ₀ M ₃	0.25 ^{fg}	0.46 ^d	0.35 ^e	3.0 ^g
N ₁ M ₀	0.30 ^{ef}	0.48 ^d	0.47 ^d	3.7 ^f
N ₁ M ₁	0.32 ^{ef}	0.64 ^{bc}	0.45 ^d	4.0 ^{de}
N ₁ M ₂	0.32 ^{ef}	0.70 ^b	0.42 ^d	4.1 ^{de}
N ₁ M ₃	0.35 ^{de}	0.64 ^{bc}	0.44 ^d	3.9 ^{ef}
N ₂ M ₀	0.33 ^e	0.72 ^b	0.46 ^d	4.4 ^{cd}
N ₂ M ₁	0.37 ^{cd}	0.71 ^b	0.47 ^d	4.4 ^{cd}
N ₂ M ₂	0.45 ^b	0.66 ^{bc}	0.45 ^d	4.7 ^{cd}
N ₂ M ₃	0.43 ^{bc}	0.72 ^b	0.49 ^d	4.8 ^{cd}
N ₃ M ₀	0.42 ^{bc}	1.30 ^a	0.58 ^c	6.2 ^b
N ₃ M ₁	0.41 ^{bc}	1.30 ^a	0.68 ^b	6.1 ^b
N ₃ M ₂	0.52 ^a	1.30 ^a	0.79 ^a	7.1 ^a
N ₃ M ₃	0.54 ^a	1.30 ^a	0.72 ^a	7.2 ^a

*Similar letters in the same row mean no statistically significant difference.

3.3 Dry weight of flowers

Analysis of the variance of the traits showed that the levels of the studied factors and their interaction had a significant effect on the dry weight of the flower (Table 2). Comparison of the average interaction effects of nitrogen and micronutrients showed that the highest flower dry weight was obtained from the N₃M₂ treatment with an average of 0.79 g. The lowest dry weight of the flower was from the N₀M₀ treatment with an average of 0.34 g (Table 4).

Appropriate plant nutrition with nitrogen significantly increases vegetative growth, durability of the leaf surface and production of photosynthetic materials, which ultimately increase the length of the vegetative growth period. A lack of nitrogen has a negative effect on the processes, which leads to a decrease in the efficiency of photosynthesis and ultimately to a decrease in flower yield. The results obtained by Babalar et al., (2010) on summer savory and the findings of Moradbeygi et al., (2020) on the medicinal plant *Dracocephalum moldavica* are similar. Pirahmadi et al., (2025) reported on the medicinal plant (*Dracocephalum moldavica* L.) that the effect of nitrogen on the dry weight of the

flower was significant at 1% significance level, which was in line with the results of this research. In research on chamomile medicinal plant (Saeedi et al., 2015), it was shown that from the level of 75 kg ha⁻¹ and above, the use of nitrogen fertilizer was not significant in the number of flowers and subsequently the dry and wet weight.

3.4 Dry weight of stem

Analysis of the variance of the traits shows that the levels of the studied factors and their interaction had a significant effect on the dry weight of the stem (Table 2). Comparison of the average interaction effects of nitrogen and micronutrients shows that the highest dry weight of the stem was obtained from the N₃M₂ treatment with an average of 1.30 g, is similar to the N₃M₃, N₃M₀ and N₃M₁ treatments. In addition, the lowest dry weight of the stem was related to the N₀M₀ treatment with an average of 0.41 g (Table 4). The results of analysis of variance showed that the biofertilizers used had a significant effect on stem dry weight at the 1% level (Naseri et al., 2022).

3.5 Dry weight of leaves

Analysis of the variance of the traits showed that the levels of the studied factors and their interaction had a significant effect on the leaf dry weight (Table 2). Comparison of the average interaction effect of nitrogen and micronutrients shows that the highest amount of leaf dry weight was obtained from the N₃M₃ treatment with an average of 0.54 g, is similar to the N₃M₂ treatment (0.52 g) and the lowest dry weight of the leaf is related to the N₀M₀ treatment with an average of 0.23 g (Table 4). Nitrogen is one of the most important nutrients in increasing plant production by increasing dry matter yield, expanding leaf area, and improving photosynthesis (Said-Al Ahl et al. 2010).

3.6 Percentage of essential oil

The results of analysis of variance showed that the main effect of the studied factors on this trait was significant (Table 2). Among the levels of consumed nitrogen, the highest amount of essential oil is related to the N₃ treatment (spraying ammonium nitrate with a concentration of 1% + N₁) with an average of 0.58%, which had a statistically significant difference with other nitrogen levels. Among the levels of micronutrients, the greatest impact is related to treatment M₃ (soil application and spraying of micronutrients of Fe, Zn and Mn) with an average of 0.58 %, which was similar to M₂ (spraying of micronutrients of Fe, Zn and Mn with concentration of 0.3%) and fell in the same statistical group (Table 3).

The use of substances extracted from plants are among the alternatives that have recently attracted the attention of researchers. Such substances are of special importance due to the fact that they are non-toxic for plants and decompose naturally and also stimulate the natural metabolism of the host plant (Mohtashami et al, 2013). Essential oil yield is a function of essential oil percentage and dry matter yield. In the case of nitrogen, it was seen that its consumption increased the performance of essential oil because nitrogen has an essential role in the structure of chlorophyll, and on the other hand, it is the most important element in the synthesis of proteins, and its increase under favorable conditions leads to an increase in protein. With the increase of proteins, the plant develops the leaf surface, the number of sub-branches, the height and diameter of the stem, and the increase of these traits leads to the increase of photosynthetic substances. With the increase of photosynthetic substances, the number of flowers and petals increases and

finally the yield of essential oil increases. Our results were consistent with the results of Maham et al., (2013).

According to the research of Gholizadeh et al. (2010), the use of foliar spraying of 2% of nitrogen fertilizer on the aerial parts of *Dracocephalum moldavica* resulted in a significant increase in the percentage and yield of essential oil compared to the control and other treatments. Nitrogenous fertilizer at the rate of 80 kg ha⁻¹, applied to the soil, showed a significant difference with the control in terms of percentage and yield of essential oil. A number of researchers reported that high essential oil yield in peppermint was achieved under the conditions of high nitrogen application (Ahmadi and Hadipanah, 2014).

Although nitrogen is not present in the structure of the essential oil, its use leads to an increase in the secretory glands of the essential oil in peppermint leaves (Aćimović et al., 2022). The reason for the increase in essential oil secretory glands is the production and consumption of simple sugars, and as a result, the production of more primary compounds to produce essential oil. Furthermore, nitrogen causes the continuation of vegetative growth and as a result increases the production of essential oil (Hendawy & Khalid, 2011). In research on the medicinal plant *Dracocephalum moldavica*, it was shown that the proper application of nitrogen fertilizer can significantly increase the yield of essential oil in this plant (Aryafar et al., 2021).

In the study by Fallah et al., (2018), which was conducted to determine the effect of nitrogen fertilizer on the yield of peppermint essential oil, it was shown that the yield of essential oil per hectare increased with the consumption of 151 kg of nitrogen fertilizer per hectare. Rahimzadeh et al. (2011) reported that the yield of essential oil increased under the conditions of using nitrogen fertilizer. Nasiri (2016) stated that with the consumption of 100 kg ha⁻¹ of nitrogen fertilizer, the yield of basil essential oil increased due to the production of more leaves and branches per unit area. Also, the increase in essential oil yield under nitrogen application was due to its dependance on the essential oil percentage and the seed yield. Because nitrogen enhanced both parameters, it consequently raised the overall essential oil yield, which was in line with the results of our research study.

4. Conclusions

Based on the results of this experiment, it can be concluded that foliar application of nitrogen and micronutrients had a positive effect on yield and their use on *Dracocephalum moldavica* increased dry matter yield. In addition to soil application of ammonium nitrate, foliar application of the nutrients (iron, zinc, and manganese) had better results. The foliar application method is more economical and cost-effective, and the elements are directly available to the plant, therefore it would be desirable to use this method. Since the highest nitrogen application rate showed better performance in this study, it is recommended that studies be conducted with higher nitrogen rates. It is also suggested that different methods of applying other high-dose elements (soil application, seed priming, and foliar spraying) be used simultaneously.

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6. Authors' Contributions

Farzad Jalili: methodology, data analysis and drafting.

Mehdi Zohedi Aghdam: writing and editing.

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7. Conflicts of Interest

Authors do not have any conflict of interest to declare.

8. AI Declaration

This article is a research paper, and all steps were analyzed and written by me and my colleague, and no artificial intelligence was used.

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