

REPLACING NITROGEN CHEMICAL FERTILIZERS WITH VERMICOMPOST: A SUSTAINABLE STRATEGY TO ENHANCE ROSMARINUS OFFICINALIS L. PERFORMANCE UNDER WATER STRESS

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Received:15 September 2025

Revised:18 October 2025

Accepted: 13 November 2025

ABSTRACT:

A split-plot experiment using a randomized complete block design was conducted in Karaj, Iran (2023–2024) on *Melissa officinalis* (lemon balm), focusing on sustainable fertilization and irrigation. Three irrigation regimes were tested: optimal (I1), irrigation after 40% water depletion (I2), and after 55% depletion (I3). Six fertilizer treatments ranged from no fertilizer (F1) to varying mixes of urea and vermicompost (F2–F6). Results showed that higher water stress reduced seed yield, essential oil yield, chlorophyll content, and photosynthesis. Vermicompost improved soil quality and boosted plant biochemical and photosynthetic performance. This highlights the value of eco-friendly practices to enhance medicinal plant productivity while conserving resources.

Plain Language Summary

(lemon balm), a valuable medicinal plant. The research aimed to develop sustainable cultivation practices by testing various combinations of water and fertilizers to determine which conditions promote better growth and higher essential oil production. Results showed that increased water stress reduced seed yield, oil content, and chlorophyll levels. However, using vermicompost (worm-produced organic fertilizer) improved plant health, enhanced photosynthesis, and supported better growth even under mild stress conditions.

Keywords: *Rosmarinus officinalis*, essential oil, carotenoid, drought stress.

INTRODUCTION

As pharmaceutical science progresses, the value of medicinal plants in traditional medicine is being re-evaluated due to the limitations of synthetic drugs, such as side effects, environmental impact, and high costs. Medicinal plants provide bioactive compounds used in healthcare, cosmetics, and food industries (Heidarpour et al., 2024). Iran, with its rich biodiversity, is increasing the cultivation of these plants to meet growing demand (Ghadirnezhad Shiade et al., 2024).

Rosemary (*Rosmarinus officinalis* L.) is one such plant, known for its antimicrobial, antioxidant, and therapeutic properties (Omidbeigi, 2013; Dehghani Bidgoli et al., 2017). However, its cultivation in Iran faces challenges due to water scarcity, especially in arid and semi-arid regions affected by climate change and resource depletion (Hafeez et al., 2023; Ghadirnezhad Shiade et al., 2024).

While chemical fertilizers boost yields, they also cause pollution and raise costs (Hafeez et al., 2023). Sustainable alternatives like vermicompost a nutrient-rich organic fertilizer produced by earthworms can improve soil health, water retention, and plant resilience (Ibrahim et al., 2015). Considering Iran's low rainfall of about 250 mm, water stress is a major challenge, emphasizing the importance of effective fertilizer management to enhance plant resistance. This study explores replacing nitrogen fertilizer with vermicompost as a sustainable strategy to improve Rosemary growth under water stress conditions, contributing to more resilient agricultural practices.

MATERIALS AND METHODS

This experiment was carried out in the spring of 2022-2023 and 2023-2024 in the Karaj region with a geographic latitude of 35 degrees and '43' north and an altitude of 1215 meters above sea level with a long-term average rainfall of 244 mm, the highest amount of which is in December.

EXPERIMENTAL DESIGN

The experiment was conducted using a split-plot design in a randomized complete block with three replications. Main plots included three irrigation treatments: (I1) optimal irrigation to crop capacity; (I2) irrigation after soil moisture drops to 40% at root depth, then full irrigation; and (I3) irrigation after soil moisture drops to 55%, then full irrigation. Secondary plots involved six fertilizer treatments: (F1) control with no fertilizer; (F2) 100% nitrogen from urea; (F3) 75% urea + 25% vermicompost; (F4) 50% urea + 50% vermicompost; (F5) 25% urea + 75% vermicompost; and (F6) 100% vermicompost. The chemical properties and nitrogen content of the materials were analyzed and calculated according to a specified equation (equation 1).

Relationship 1: The amount of nitrogen required from organic fertilizer = dry weight of organic fertilizer x available nitrogen percentage x fertilizer nitrogen percentage

To supply 120 kg/ha nitrogen in lemongrass, vermicompost containing 2.2% nitrogen with a 35% release rate was used. Residual nitrogen (about 65%) was considered for year two, reducing vermicompost application accordingly (Hernández et al., 2016).

Site preparation, implementation and sampling

The land was prepared by plowing, disking, leveling, and forming ridges (Nedkov and Georgiev, 1991). Each plot had six rows, 35 cm apart, with a total width of 210 cm, length of 4 m, and 15 cm plant spacing. Vermicompost was mixed into the soil before planting. Badrashbo rhizomes were planted at the end of Farudin month, with distances of 1.5 m between blocks and 0.75 m between plots to prevent interference. Weed control was manual, and the same pattern was used for replanting in the second year.

Table 1- Vermicompost analysis analysis

Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	K (%)	P (%)	Total N	pH	EC (dS/m)	C/N	sample
56.1	151	39.89	7425	1.457	1.599	2.1	7.78	1.99	220.278	Vermicompost

Irrigation water was delivered via nozzles spaced 10 cm apart, each supplying 1.2 liters per hour under constant pressure. By knowing the number of nozzles per plot (covering 3 m²), the irrigation duration for each plot and regime was calculated to restore soil moisture levels to field capacity, based on the measured soil water content. The percentage of soil water discharge in the root zone was then evaluated using a specific formula (not provided here), to accurately schedule irrigation based on soil water status.

Relationship 2: Maximum Allowable Discharge (MDA)=FCi θi/ FC and PWP

MEASUREMENT OF TRAITS

The percentage essential oil

Essential oil was extracted by water distillation using a Clevenger apparatus. Fifty grams of dried plant material from each plot was boiled with distilled water for 40 minutes. Steam carried the oil to the condenser, where it separated and floated on water in a graduated tube. Oil yield was calculated as a percentage of dry weight. Samples were dried with sodium sulfate and refrigerated for chromatography.

Chlorophyll and carotenoids

Before harvesting, leaf greenness was measured using a SPAD-502 plus meter. Chlorophyll and carotenoid concentrations were determined by extracting 0.2 g of leaf tissue in 80% acetone (Arnon, 1949). The extract was

filtered, diluted to 25 ml with acetone, and absorbance measured at 645 and 663 nm. Total chlorophyll content (mg/g fresh leaf) was calculated using Porra's formula (Porra, 2002).

Relationship 4:

$$\frac{V}{1000W} [20.2 (A_{645}) + 8.02 (A_{663})] \times = \text{Total Chlorophyll}$$

Relationship 5:

$$\text{total chlorophyll} = [\text{Total Chlorophyll}] \times 0.895$$

Relationship 6:

$$\text{Carotenoid} = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645}) \times \frac{V}{1000W}$$

Measuring the amount of photosynthesis

Before harvesting, the plant's photosynthesis rate was measured using a Licor-6400 photosynthesis meter. Sampling occurred between 12:00 and 1:00 p.m., placing a mature leaf in the device's clamp, and after 60 seconds, the photosynthesis rate was recorded (Martins and Ruiz, 1992).

Statistical analysis

In this experiment, data on yield, morphological, physiological, and biochemical traits collected over two years were analyzed using SAS software. Means were compared using Duncan's multiple range test (DMRT) at a 5% significance level. Statistical groups were formed by comparing averages within their respective groups.

RESULTS AND DISCUSSION

Seed yield

The ANOVA results showed that irrigation and fertilizer treatments significantly influenced grain yield at the 1% level (Table 3). The highest yields were with 25% urea plus 75% vermicompost (F4), achieving 1,486.83 kg/ha in the first year and 1,605.72 kg/ha in the second, under all water conditions. Water stress reduced yields by affecting growth and photosynthesis, but vermicompost mitigates these effects by improving soil health, promoting root growth, and enhancing nutrient uptake. Over time, organic fertilization helps plants better withstand water stress by supporting key physiological processes, which minimizes oxidative damage and supports growth (Mohammadi et al., 2022).

The percentage of essential oil

The ANOVA results in Table 3 indicated that both irrigation and fertilizer treatments significantly affected essential oil percentage at the 1% level. As shown in Table 4, the highest essential oil percentages occurred under medium water stress, reaching 0.398% and 0.390% in the two years about 12% and 14% higher than under normal irrigation.

Regarding fertilizer, 100% vermicompost produced the highest essential oil content 0.40% in the first year and 0.392% in the second showing increases of roughly 18-19% over 100% urea. The combination of 25% urea and 75% vermicompost also performed well, ranking second in both years.

Furthermore, 100% vermicompost significantly increased essential oil content compared to other treatments at the 5% level. Organic fertilizers like vermicompost enhance secondary metabolite production by improving soil nutrients, producing plant hormones, and supporting nitrogen fixation (Mohammadi et al., 2022; Heidarpour et al., 2024).

Total chlorophyll

The ANOVA results in Table 3 showed that irrigation and fertilizer significantly affected chlorophyll a, b, and total chlorophyll at 1%. Normal irrigation resulted in the highest chlorophyll levels (around 1.66–1.69 mg/gFW for chlorophyll a, 0.60–0.62 mg/gFW for chlorophyll b, and 2.26–2.32 mg/gFW for total chlorophyll), which were about 65-70% higher than drought stress conditions. The combination of 25% urea and 75% vermicompost increased chlorophyll content by about 20-24% over urea alone. Water stress reduces chlorophyll as a protective

response, but vermicompost improves soil conditions, helping plants maintain chlorophyll levels and reduce drought effects (Pratyusha, 2022; Ahmad et al., 2024).

Carotenoid content

The ANOVA in Table 3 showed that both irrigation and fertilizer significantly influenced carotenoid content at 1%. Highest levels occurred under normal irrigation, with values of 2.23 mg/gFW in year one and 2.24 mg/gFW in year two around 23-26% higher than under drought. The best fertilizer treatment was 25% urea plus 75% vermicompost, increasing carotenoids by about 18-19% over urea alone. Water stress decreases chlorophyll but boosts carotenoids, which protect plants from oxidative damage (Khodabin et al., 2020; Ma et al., 2022). Vermicompost improves soil nutrients and water retention, helping plants cope with drought (Pratyusha, 2022).

The rate of photosynthesis

The ANOVA showed that irrigation, fertilizer, and their interaction significantly affected photosynthesis at 1%. The highest rates occurred with 25% urea plus 75% vermicompost (F4), reaching 10.92 and 11.56 $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in years one and two, across all irrigation levels. Water stress reduces photosynthesis by closing stomata and limiting CO_2 intake (Fathi et al., 2024). Vermicompost improves soil water retention and nutrients, helping plants maintain higher photosynthesis under drought (Ma et al., 2022; Mohammadi et al., 2022).

CONCLUSION

The results indicate that using vermicompost, either alone or combined with other fertilizers, effectively supplies nutrients for Badrashbo plants, significantly boosting performance and essential oil yield. Organic fertilization offers environmental and sustainability benefits over conventional methods and can mitigate drought stress effects. The study shows vermicompost can replace urea fertilizer, reducing chemical use without compromising biomass, which is environmentally and economically advantageous. It also improves soil quality, especially in degraded soils. Therefore, for optimal results, moderate to mild stress conditions combined with vermicompost fertilization are recommended for Badrashbo cultivation.

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Table 3- Variance analysis of investigated traits

		MS					
		seed yield	essential oil	essential oil yield	total chl	carotenoid	photosynthesis rate
Rep (r)	2	0.557 ^{ns}	0.000009 ^{ns}	0.138 ^{ns}	0.00004 ^{ns}	0.000002 ^{ns}	0.001 ^{ns}
Year (Y)	1	97630.6 ^{**}	0.001 [*]	3.78 ^{ns}	0.055 ^{**}	0.004 [*]	4.31 ^{**}
Year error (r x Y)	2	0.001	0.000002	0.009	0.00006	0.000001	0.001
Irrigation (I)	2	4298228.6 ^{**}	0.07 ^{**}	204.66 ^{**}	6.11 ^{**}	0.722 ^{**}	141.9 ^{**}
Main error	4	0.035	0.0000001	0.009	0.000001	0.0000001	0.00006
Fertilizer (F)	5	522713.1 ^{**}	0.022 ^{**}	28.66 ^{**}	0.313 ^{**}	0.005 ^{**}	38.51 ^{**}
I x F	10	34039.19 ^{**}	0.0001 ^{ns}	1.88 ^{ns}	0.004 ^{ns}	0.0001 ^{ns}	1.08 ^{**}
Y x F	5	770.77 ^{ns}	0.000004 ^{ns}	0.028 ^{ns}	0.00003 ^{ns}	0.000003 ^{ns}	0.030 ^{ns}
Y x I	2	6352.56 ^{ns}	0.000008 ^{ns}	0.244 ^{ns}	0.0008 ^{ns}	0.00007 ^{ns}	0.118 ^{ns}
Y x F x I	10	50.33 ^{ns}	0.000003 ^{ns}	0.001 ^{ns}	0.000003 ^{ns}	0.0000009 ^{ns}	0.0006 ^{ns}
Error	64	30591.95	0.0002	2.38	0.0046	0.0008	0.100
CV (%)	-	22.37	4.84	28.87	3.57	4.88	4.67

^{*}, ^{**} and ^{ns} respectively significant at the level of five, one percent and no significant difference

Table 4- Comparison of the average effect of irrigation and fertilizer on seed yield, total phenol and photosynthesis rate

		seed yield (first year)	seed yield (Second year)	photosynthesis rate (first year)	photosynthesis rate (Second year)
		kg.ha ⁻¹		μmol H ₂ O m ⁻² s ⁻¹	
Irrigation					
I ₁	F1	817.72b	883.14c	5.65f	6.00d
	F2	921.98b	995.74bc	7.60e	8.07c
	F3	1025.67ab	1107.69bc	8.16d	8.65c
	F4	1159.28ab	1252.02abc	9.78b	10.38b
	F5	1486.83a	1605.72a	10.92a	11.56a
	F6	1254.62ab	1354.92ab	9.23c	9.79b
I ₂	F1	506.98b	547.54c	4.30f	4.57d
	F2	571.62b	617.35bc	5.780e	6.13c
	F3	635.91ab	686.76bc	6.20d	6.58c
	F4	718.75ab	776.25abc	7.43b	7.89b
	F5	921.83a	995.55a	8.29a	8.77a
	F6	777.86ab	840.04ab	7.01c	7.44b
I ₃	F1	335.26b	362.08c	3.11e	3.29d
	F2	378.04b	408.37bc	4.18d	4.43c
	F3	420.52ab	454.15bc	4.48c	4.75c
	F4	475.30ab	513.33abc	5.37b	5.71b
	F5	609.59a	658.33a	6.01a	6.39a
	F6	514.39ab	555.51ab	5.08b	5.38b

The average of each given year related to each adjective that has common letters, their difference is not statistically significant at Duncan's five percent level.

Table 5- Comparison of the main effects of irrigation and fertilizer on some traits

seed yield (first year)	seed yield (Second year)	essential oil (first year)	essential oil (Second year)	T chl (first year)	T chl (Second year)	photosynthesis rate (first year)	photosynthesis rate (Second year)		
kg.ha ⁻¹			(%)	mg.g ⁻¹ FW		μmol H ₂ O m ⁻² s ¹			
Irrigation									
I ₁	1111.01a	1199.8a	0.346b	0.340b	2.261a	2.315a	9.07a	8.56a	9.07a
I ₂	688.83b	743.9b	0.398a	0.390a	1.932b	1.979b	6.90b	6.50b	6.90b
I ₃	455.52c	491.9c	0.308c	0.302c	1.451c	1.486c	4.99c	4.70c	4.99c
Fertilizer									
F1	553.32d	597.5d	0.299d	0.294d	1.723d	1.765e	4.62f	4.35f	4.62f
F2	623.88cd	673.8d	0.327c	0.320c	1.807c	1.851d	6.21e	5.85e	6.21e
F3	694.03bcd	749.5cd	0.339c	0.332c	1.829c	1.874cd	6.66d	6.28d	6.66d
F4	784.44bc	847.2bc	0.363b	0.357b	1.933b	1.980b	7.99b	7.53b	7.99b
F5	1006.08a	1086.5a	0.375b	0.368b	2.103a	2.153a	8.90a	8.41a	8.90a
F6	848.96ab	916.8ab	0.400a	0.392a	1.893b	1.940bc	7.54c	7.11c	7.54c

The average of each given year related to each adjective that has common letters, their difference is not statistically significant at Duncan's five percent level.