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RESEARCH ARTICLE

Quantitative and qualitative response of Dragon's head (*Lallemantia iberica* Benth) to different fertilizer sources and water deficit conditions

Mohammad Ali Fazli[1], Touraj Mir Mahmoodi[1]*, Amir Rahimi[2] & Saman Yazdanseta[1]

[1]Department of Agronomy, Mahabad Branch, Islamic Azad University, Mahabad 433-59135, Iran
[2]Department of Plant Production and Genetics, Faculty of agriculture, Urmia University, Urmia, Iran

*Email: toraj73@yahoo.com

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Abstract

Dragon's head plant is a vital source of antioxidants, antibacterials and pain relief. This research studied the effect of different fertilizer sources on some characteristics of Dragon's head at different water-deficit levels. The experimental design was a split plot based on a randomized complete block design with three replications. Experimental treatments included irrigation regimes at 3 levels (normal, mild and severe water deficit) and different fertilizer sources (control, NPK, humic acid, vermicompost and manure) conducted in 2 crop years. The results showed that severe water stress reduced the content of chlorophyll a (25.0 %), chlorophyll b (29.24 %), carotenoids (24.32 %), number of seeds per plant (60.08 %), biological yield (24.61 %), nitrogen (18.29 %) and potassium content (38.83 %) compared to the normal condition. In contrast, proline (18.81 %), carbohydrate (18.64 %), phenol (22.16 %), flavonoid (13.4 %), DPPH (24.63 %) and nitric acid radical scavenging (32.27 %) increased with severe water stress compared with the normal condition. Application of vermicompost increased the content of chlorophyll a (92.45 %), chlorophyll b (86.76 %), carotenoids (85.311 %), number of seeds per plant (87.81 %), biological yield (93.23 %), essential oil present (14.29 %), phenol (50.32 %), flavonoid (29.07 %), DPPH (15.83 %), nitric acid radical scavenging (42.47 %), chain-breaking capacity (6.18 %), nitrogen (19.49 %) and potassium content (26.62 %) over the control. The highest thousand kernel weight (32.5 g), seed yield (2343.6 kg ha^{-1}), essential oil yield (2.76 kg ha^{-1}), mucilage % (15.37 %) and mucilage yield (337.8 kg ha^{-1}) were recorded in the vermicompost under normal irrigation conditions. Additionally, vermicompost and animal manure treatment under mild stress conditions increased seed yield, essential oil yield, mucilage % and mucilage yield significantly compared to the corresponding control.

Keywords

chlorophyll a; humic acid; mucilage; organic fertilizer; vermicompost

Introduction

The cultivation of medicinal plants has increased (1). One of Iran's most important medicinal plant is the dragon's head (*Lallemantia iberica*, Lamiaceae family) (2). This plant possesses antioxidant, antibacterial and pain-relieving properties (2). Water scarcity poses a significant constraint for growing medicinal plants.

Water is one of the most important factors limiting agricultural productivity in arid and semi-arid regions (3). Dragon's head is primarily cultivated under rainfed conditions and can tolerate mild drought stress. However, severe drought stress significantly reduces the yield of this plant (4). Studies have shown that the water scarcity decreases the chlorophyll content, antioxidant enzyme activity (catalase, ascorbate peroxidase and superoxide dismutase), grain yield, biomass and seed oil content of flax compared to normal conditions (5). Supplemental irrigation in areas with limited water resources can enhance rainfall efficiency (6). Additionally, supplemental irrigation can mitigate the adverse effects of water deficit stress on growth, development and yield, while also improving water use efficiency, particularly in regions where irrigation supplements natural rainfall (7, 8). Research has documented that supplemental irrigation and biofertilizer treatments alleviate the negative impact of drought stress on cumin by increasing leaf water potential, enhancing carbon dioxide absorption efficiency, improving transpiration rate and supplying essential mineral elements (9).

Due to the detrimental effects of industrial fertilizers on the environment, there has been a growing emphasis on organic and sustainable agriculture (10). Animal manure can fulfil plants requirements for both macro and micronutrients, increase soil organic matter content, establish a balanced carbon: nitrogen (C: N) ratio, enhance nutrient uptake by plants and promote plant growth (11). Studies have indicated that the use of animal manure can mitigate the adverse effects of water deficit stress by adjusting osmotic pressure and regulating water absorption and cellular swelling (12).

Vermicompost, an organic fertilizer, enhances the soil's nutrient retention capacity, structure and moisture content. It also boosts microbial and antioxidant activity, thereby increasing tolerance to environmental stress (13). Consequently, organic fertilizers improve soil chemical properties such as Cations Exchange Capacity (CEC), pH and nutrient availability, leading to an increase in soil organic matter levels and land fertility (14). A research demonstrated that bio-organic fertilizers increased the phenol and flavonoid content of black cumin seeds (15). It was found that the application of animal manure (20 t ha^{-1}) alleviated the adverse effects of water deficit stress by supplying nutrients such as nitrogen (N), phosphorus (P) and potassium (K) and enhanced the quality of cumin essential oil (16). In a study on mint plants, it was observed that the application of vermicompost and chemical fertilizers significantly increased oil %, oil yield, essential oil % and plant essential oil yield compared to the control treatment (17).

Humic acid (HA) serves as an active ingredient in organic fertilizers, presenting a potential alternative to traditional soil fertilization methods, particularly in semi-arid regions, due to its ability to swiftly provide nitrogen (18). Research indicates that HA enhances nutrient transport and availability in soil. Moreover, humic compounds facilitate plant biochemical processes, thereby augmenting photosynthesis, respiration and the production of hormones and proteins (19).

Study on Dragon's Head, demonstrated that catalase, ascorbate peroxidase and superoxide dismutase levels decreased in rainfed plants, whereas application of organic fertilizer led to increased activity of antioxidant enzymes (20). Additionally, plants treated with vermicompost under supplemental irrigation exhibited the highest seed yield, biological yield, total flavonoids, total phenol, fixed oil yield and essential oil yield. The use of organic fertilizers has been associated with improved crop growth, increased plant dry weight and enhanced resistance to water scarcity, pests and diseases (16). Drought stress and environmental pollution from chemical fertilizers are 2 significant factors limiting the production of medicinal plants in Iran (21). Therefore, finding solutions to mitigate the adverse effects of these limitations can potentially increase medicinal plant production. One such solution involves using fertilizers to enhance soil and plants nutrition under adverse environmental conditions. Consequently, the present research aimed to investigate the impact of different fertilizer sources on the quantitative and qualitative characteristics as well as the essential oil content of dragon's head, under different moisture conditions.

Materials and Methods

Experimental Design

This experiment was carried out during the 2020 and 2021 growing seasons in a field in Naqdeh City, West Azerbaijan province, Iran. The field is situated at coordinates 36°57'N, 45°23'E and an elevation of 1299 m above sea level. The meteorological characteristics of the experimental site are illustrated in Fig. 1. Table 1 outlines some physical and chemical characteristics of the soil at the experimental site.

The experiment was conducted as a split plot design within a randomized complete block design, comprising 3 replications. The main plots were assigned to different irrigation treatments, which included complete irrigation (normal), supplemental irrigation (mild stress), and rainfed (severe stress). Within each main plots, sub plots were designated for various fertilizer treatments, namely control, NPK chemical fertilizer (comprising urea: 115 kg ha^{-1}+ triple superphosphate: 60 kg ha^{-1}+ potassium sulfate: 50 kg ha^{-1}+ micronutrients: 24 kg ha^{-1}), humic acid (420 kg ha^{-1}), vermicompost (15 t ha^{-1}) and animal manure (20 t ha^{-1}).

Plant Material

Before commencing the experiment in March, a composite soil sample was prepared from the experimental site and measurements of high- and low-consumption elements were conducted according to the established protocols. Additionally, some of the physical and chemical properties of the organic fertilizers used are shown in Table 2. The seeds were obtained from an indigenous variety of the Southern region of the Western Azerbaijan province. In this research, the plots were 3 m in length and 2 m in width, with each plot containing 6 rows of plants. The spacing between planting rows was set at 0.2 m, while the spacing

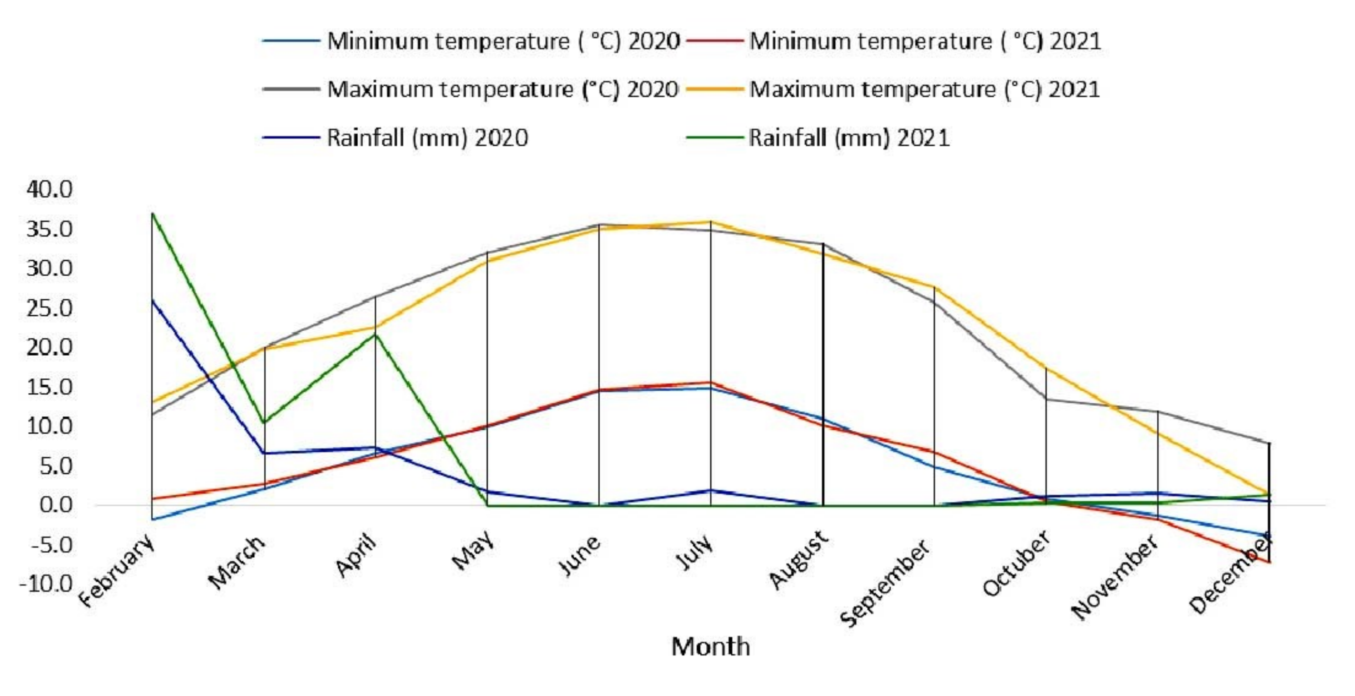

Fig. 1. Rainfall (mm), minimum and maximum temperature (°C) records of experimental site during 2020-2021.

Table 1. Some physical and chemical characteristics of the soil of the experimental site.

EC (ds m^{-1})	**pH**	**Texture**	**Clay**	**Silt**	**Sand**	**$CaCO_3$**	**Saturation percentage**
1.38	7.79	Clay loam	41 %	36 %	23 %	15.71 %	54 %
N	**Organic carbon**	**Mn**	**B**	**Zn**	**Fe**	**K**	**P**
%		**mg kg^{-1}**					
0.03	1.16	11.2	0.28	1.1	8.11	282	9.02

Table 2. Physical and chemical analysis of the used organic fertilizers.

Parameters	EC (dS m^{-1})	pH	N (%)	P (ppm)	K (ppm)	Organic matter (%)	pH
Vermicompost	4.19	8.06	56	43	53	8.10	8.65
Manure	3.80	8.2	42	32	17	16.00	7.50

between plants within rows was adjusted to achieve a density of 400 plants per square meter.

Before planting, vermicompost and animal manure were mixed in the soil using a heavy disc 25 to a depth of 25 to 30 cm. Humic acid was applied at three stages of growth: 30 days after greening, at the onset of flowering and during seed maturation, following the recommended dosage of 200 mg L^{-1}. The humic acid utilized, marketed under the trade name Humistar, contained 66 % humic acid and 15 % fulvic acid. Hinning and weeding operations were conducted throughout the growing season to manage plant density and weed infestations. The method described was used to determine the amount of water consumption for both supplemental and complete irrigation treatments (22).

Measurements

To determine the number of seeds, 10 randomly selected plants were sampled from each plot, and the number of seeds per plant was counted and recorded. The weight of seeds and the number of seeds from the 10 selected plants were used to calculate the thousand kernel weights. Grain yield and biomass at the physiological ripening stage were assessed by sampling a one square meter area from the 2 middle rows of the plots after removing any marginal effect. Leaf samples were collected at the initial stage of flowering. Essential oil was extracted through distillation with water using a Clevenger apparatus. The essential oil content was estimated using the weight method and the essential oil yield was calculated using the following equation:

Essential oil yield = Essential oil % × Seed yield…...(Eqn. 1)

The seed mucilage % was determined according to the method described (23). The mucilage yield per unit area, accounting for both mucilage content and seed yield was calculated accordingly:

Mucilage yield = Mucilage % × Seed yield …………(Eqn. 2)

Proline and carbohydrate concentrations were measured using the method (24), while chlorophyll a, b and carotenoid content were determined using the Lichtenthaler method (25) at the flowering stage.

A methanol extract was initially prepared from the samples to evaluate their antioxidant properties. Total phenolic and flavonoid content in the extracts was determined using the method described (26). Nitric oxide radical scavenging % and DPPH (2,2-diphenyl-1-picrylhydrazyl -2-diphenyl-1-picrylhydrazyl) radical scavenging percentage were measured according to the protocol (2). Chain-breaking activity was assessed using the method (27). Nutrient and potassium content in leaf samples were determined (26).

Data Analysis

The data collected in the present study were analyzed using the SAS 9.1 software. The effects of 2 independent factors, namely different fertilizer applications and irrigation conditions, as well as any potential interaction between them on the investigation traits, were analyzed using two-way ANOVA. Means were compared using Duncan's test at the $p < 0.05$ level. Graphs were generated using Excel.

Results

According to the results of the combined analysis of variance, there was a significant difference between the 2 years in terms of essential oil yield at the 5 % probability level. Among irrigation levels, significant differences were observed in chlorophyll b content, carotenoids, proline content, carbohydrates, seed number, thousand kernel weight, biological yield, seed yield, essential oil %, essential oil yield, mucilage %, mucilage yield, phenol content, DPPH radical scavenging and nitric acid radical scavenging at the 1 % probability level and in chlorophyll a, nitrogen and potassium content at the 5 % probability level. The difference between fertilizer treatments for all investigated traits was significant at the 1 % probability level. Significant differences were also observed between the interaction treatments in terms of thousand kernel weight and essential oil yield at the 1 % probability level, and seed yield, essential oil yield, mucilage % and mucilage yield at the 5 % probability level (Table 3).

Photosynthetic Pigments

The mean comparison results revealed that severe water deficit conditions led to a reduction in chlorophyll a, chlorophyll b and carotenoid content by 25.00, 24.99 and 24.32 % respectively compared to normal irrigation conditions (Table 4).

Among the fertilizer treatments, the application of

Table 3. Combine analysis of variance (mean of squares) for the effect of irrigation regime and manure on studied traits of Dragon's head.

	Year (Y)	(Y) Replication	Irrigation (I)	Y×I	Ea	Fertilizer (F)	Y×F	I×F	Y×I×F	Eb	CV %
Df	1	4	2	2	4	4	4	8	8	24	-
Chlorophyll a	0.003^{ns}	0.002	0.008*	0.001^{ns}	0.0015	0.033**	0.001^{ns}	0.001^{ns}	0.002^{ns}	0.001	25.26
Chlorophyll b	0.001^{ns}	0.004	0.04**	0.0004^{ns}	0.003	0.08**	0.001^{ns}	0.002^{ns}	0.0004^{ns}	0.003	18.25
Carotenoid	0.001^{ns}	0.004	0.04**	0.0004^{ns}	0.0004	0.04**	0.0006^{ns}	0.001n^{s}	0.0009^{ns}	0.002	25.02
Proline	0.00087^{ns}	0.00043	0.002**	0.00027^{ns}	0.0001	0.003**	0.0001^{ns}	0.000001^{ns}	0.0002^{ns}	0.0002	15.13
Carbohydrate	4.44^{ns}	8.93	45.56**	0.31^{ns}	5.23	56.75**	1.00^{ns}	0.32^{ns}	2.03^{ns}	4.91	15.31
Number of seeds	640.01^{ns}	229.6ns	77075.4**	173.3^{ns}	570.1	14676.0**	140.01^{ns}	1319.2^{ns}	256.7^{ns}	810	24.16
Thousand kernel weight	0.40^{ns}	0.10	10.93**	0.13^{ns}	0.17	7.18**	0.17^{ns}	0.56**	0.24^{ns}	0.16	10.97
Biological yield	17.78^{ns}	36.04	2051.43**	31.11^{ns}	25.5	3767.63**	6.67^{ns}	93.26^{ns}	36.67^{ns}	43.01	11.18
Grain yield	27.78^{ns}	12.97	1244.12**	20.28^{ns}	14.02	184. 90**	7.53^{ns}	18.89*	5.24^{ns}	7.74	17.56
Essential oil	0.0006^{ns}	0.0001	0.0012**	0.00007^{ns}	0.0001	0.0009**	0.0001^{ns}	0.0002^{ns}	0.0001^{ns}	0.0001	11.49
Essential oil yield	1.47*	0.18	20.68**	0.54^{ns}	0.32	4.38**	0.27^{ns}	0.59**	0.11^{ns}	0.19	23.24
Oil percentage	12.10^{ns}	3.19	34.60**	1.20^{ns}	8.7	69.13**	2.85^{ns}	5.74^{ns}	3.57^{ns}	4.77	10.58
Oil yield	99.26^{ns}	17.46	4239.34**	17.49^{ns}	48.01	842.33**	33.05^{ns}	89.79*	27.29^{ns}	43.83	19.78
Mucilage percentage	0.27^{ns}	1.01	23.14**	1.24^{ns}	1.12	14.57**	0.47^{ns}	3.27*	0.43^{ns}	1.12	8.39
Mucilage yield	2197.23^{ns}	793.11	202903.32**	1491.84^{ns}	920.01	25309.81**	667.14^{ns}	3303.51*	1377.55^{ns}	1507.89	18.87
Phenol	1.34^{ns}	0.29	17.61**	1.34^{ns}	1.05	51.17**	0.51^{ns}	1.83^{ns}	0.51^{ns}	1.21	14.63
Flavonoid	0.04^{ns}	0.01	0.14^{ns}	0.03^{ns}	0.04	0.82**	0.008^{ns}	0.17^{ns}	0.01^{ns}	0.01	11.44
DPPH	5.37^{ns}	1.71	41.44**	5.37^{ns}	3.01	17.06**	2.26^{ns}	2.84^{ns}	2.26^{ns}	1.79	12.49
Nitric acid	17.77^{ns}	4.51	199.24**	4.44^{ns}	26.43	476.69**	6.66^{ns}	30.39^{ns}	10.00^{ns}	16.02	15.6
Chain-breaking	0.044^{ns}	0.1	0.08^{ns}	0.04^{ns}	0.25	5.02**	0.04^{ns}	0.13^{ns}	0.04^{ns}	0.15	14.97
Nitrogen content	0.04^{ns}	0.15	0.55*	0.17^{ns}	0.25	0.63**	0.26^{ns}	0.008^{ns}	0.06^{ns}	0.13	16.46
Potassium content	0.01^{ns}	0.05	1.14*	0.02^{ns}	1.47	1.98**	0.01^{ns}	0.005^{ns}	0.004^{ns}	0.33	18.83

Df = Degrees of freedom , **Ea** = First error, **Eb** = Second error, **CV**= Coefficient of variation, ^{ns},** and * are no significant, significant at 1 and 5 % probability. levels respectively

Table 4. Mean comparison of irrigation treatments on studied traits of Dragon's head.

Irrigation	Chlorophyll a (mg g^{-1} DW)	Chlorophyll b (mg g^{-1} DW)	Carotenoid (mg g^{-1} DW)	Proline (mg g^{-1} DW)	Carbohydrate (mg g^{-1} DW)	Number of seeds /plant	Biological yield (Kg ha^{-1})	Essential oil present (%)
Normal	0.16a	0.107a	0.074a	0.101c	13.25c	168.72a	6712.10a	0.111b
Mild stress	0.14ab	0.094ab	0.064b	0.110b	14.47b	117.38b	5829.36b	0.123a
Severe stress	0.12b	0.081b	0.056c	0.120a	15.72a	67.34c	5059.63c	0.114b

DW = Dry Weight, Means followed by similar letters in columns are not significantly different at 5% probability level by LSR.

Table 4. Contd……

Irrigation	Oil present (%)	Phenol (mg gallic acid/g)	Flavonoid (mg quer-cetin/g DM)	DPPH radi-cal scav-enging (%)	Nitric acid radical scavenging (%)	Chain-breaking capacity (%)	Nitrogen content (mg g^{-1} DW)	Potassium content (mg g^{-1} DW)
Normal	19.49b	6.77b	0.97a	9.54b	22.16b	2.25a	2.46a	3.94a
Mild stress	21.60a	7.48ab	1.04a	10.71ab	25.51ab	2.60a	2.24ab	3.07b
Severe stress	20.89a	8.27a	1.10a	11.89a	29.31a	2.66a	2.01b	2.41c

Means followed by similar letters in columns are not significantly different at 5% probability level by LSR.

vermicompost not only resulted in the highest content of photosynthetic pigments but also significantly increased chlorophyll a, chlorophyll b and carotenoid content compared to the control treatment by 92.45, 86.76 and 85.10 % respectively (Table 5).

Proline Content

The results indicated that the proline content of the leaves increased with the intensification of the drought stress. The severe stress treatment exhibited the highest proline content (0.120 mg g^{-1} DW), while the normal irrigation treatment showed the lowest proline content (0.101 mg g^{-1} DW) (Table 4).

In the present study, the control treatment exhibited the highest proline content (0.133 mg g^{-1} DW) while the vermicompost treatment showed the lowest proline content (0.099 mg g^{-1} DW). The fertilizer treatments significantly reduced the proline content (Table 5).

Carbohydrate Content

Based on the results shown in Table 4, severe water deficit stress conditions increased leaf carbohydrate content by 18.64 % and 8.63 % respectively, compared to normal irrigation and mild stress conditions.

In the comparison of fertilizer treatments, the control treatment (12.93 %) and vermicompost (17.52 %) were associated with the minimum and maximum carbohydrate content in the leaves (Table 5).

Antioxidant Properties

Leaf Phenol

Among the irrigation levels, the severe stress and normal conditions exhibited the highest (8.27 mg g^{-1} FW) and lowest (6.77 mg g^{-1} FW) leaf phenolic content respectively (Table 4). The application of NPK and vermicompost increased the phenol content of leaves compared to the control treatment by 42.53 % and 50.31 % respectively (Table 5).

Table 5. Mean comparison of fertilizer treatments on studied traits of Dragon's head.

Fertilizer	Chlorophyll a (mg g^{-1} DW)	Chlorophyll b (mg g^{-1} DW)	Carotenoid (mg g^{-1} DW)	Proline (mg g^{-1} DW)	Carbohy-drate (mg g^{-1} DW)	Number of seeds /plant	Biological yield (Kg/ha)	Essential oil present (%)
Control	0.106d	0.068d	0.047c	0.133a	12.93c	84.16d	4173.21d	0.105c
NPK	0.136c	0.084c	0.057c	0.113b	14.46b	114.13bc	5408.51d	0.118a
Humic acid	0.138bc	0.087c	0.060bc	0.105bc	13.87bc	100.69cd	5360.91d	0.114b
Vermicompost	0.204a	0.127a	0.087a	0.099c	17.50a	158.06a	8063.32a	0.120ab
Manure	0.163b	0.103b	0.072b	0.102bc	13.57bc	132.03b	6329.32b	0.124a

Means followed by similar letters in columns are not significantly different at 5 % probability level by LSR.

Table 5. Contd…..

Fertilizer	Oil present (%)	Phenol (mg gallic acid/g)	Flavonoid (mg quer-cetin/g DM)	DPPH radical scavenging (%)	Nitric acid radical scav-enging (%)	Chain-breaking capacity (%)	Nitrogen content (mg g^{-1} DW)	Potassium content (mg g^{-1} DW)
Control	17.74d	6.30c	0.86b	10.42b	22.32bc	1.95b	2.59b	2.63b
NPK	20.56bc	8.98a	1.09a	11.37a	29.78a	2.22ab	3.16a	2.79ab
Humic acid	20.19c	5.60c	1.18a	9.70b	19.41c	2.22ab	1.74c	3.37a
Vermicompost	22.95a	9.47a	1.11a	12.07a	31.84a	2.33a	2.75b	3.33a
Manure	21.83ab	7.20b	1.14a	10.25b	24.94b	2.47a	2.77b	3.33a

Means followed by similar letters in columns are not significantly different at 5 % probability level by LSR, **NPK** = Nitrogen + phosphorus + potassium chemical fertilizer.

Flavonoid Content

In the present experiment, applying NPK, humic acid, vermicompost and animal manure increased the flavonoid content of leaves by 34.84 %, 48.84 %, 37.87 % and 42.42 % respectively compared to the control treatment (Table 4).

DPPH, Nitric Acid Radical Scavenging and Chain-Breaking Capacity

In the present study, severe stress conditions increased leaf DPPH content and nitric acid radical scavenging compared to normal irrigation conditions by 24.63 % and 32.26 % respectively (Table 4). Among the fertilizer treatments, applying vermicompost increased leaf DPPH and nitric acid radical scavenging by 15.83% and 42.65%, respectively, over the control treatment. It should be noted that there was no significant difference between vermicompost and NPK treatments in terms of the activity of these 2 enzymes (Table 5).

Among the fertilizer treatments, the highest number of seeds per plant (158.06 seeds) was recorded for the vermicompost application treatment. The lowest number of the mentioned trait (84.16 seeds) was assigned to the control treatment. However, no significant difference was detected between the control treatment and the application of humic acid (Table 5).

Thousand Kernel Weight

Mean comparisons revealed that the highest thousand kernel weight (5.32 g) was achieved with vermicompost application under normal irrigation conditions, while the lowest (2.23 g) was observed in the control treatment under severe stress conditions. Thousand kernel weight significantly increased across all five fertilizer treatments with increased water availability for the plant (Fig. 2).

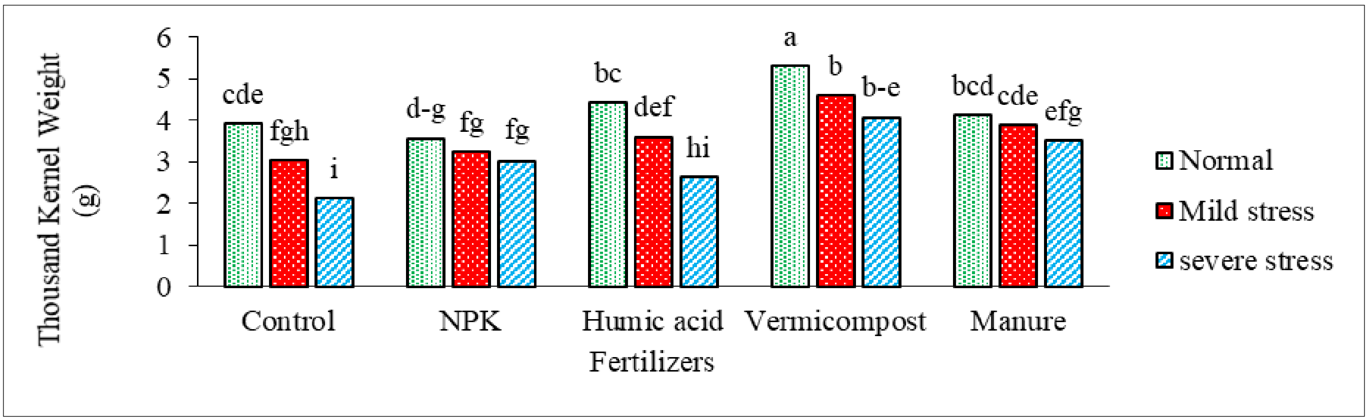

Fig. 2. Means comparison for the interactive effect of irrigation × fertilizer on thousand kernel weight (LSR, $p < 0.05$).

The results of the means comparison indicated that the plants treated with NPK had the highest (3.16 %) and the plants treated with humic acid (1.74 %) had the lowest chain-breaking capacity; the difference between the control treatment and vermicompost and manure treatments was not significant (Table 5).

Leaf Element Content

The present research results indicated that severe water stress conditions reduced the nitrogen and potassium content by 18.29 % and 38.83 % respectively, compared to normal irrigation conditions (Table 4).

According to the mean comparison results, the application of NPK increased the leaf nitrogen content by 19.69 % compared to the control treatment. Additionally, using vermicompost and animal manure increased the potassium content of leaves by 26.66 % compared to the control treatment. The control treatment in this study had the lowest nitrogen (1.95 mg g^{-1} DW) and potassium (2.63 mg g^{-1} DW) content (Table 5).

Agronomic Properties

Number of Seeds Per Plant

In this study, the normal irrigation treatment produced significantly the highest number of seeds per plant (168.72 seeds) compared to the mild stress (117.38 seeds) and severe stress (67.34 seeds) treatments (Table 4).

Biological Yield

The results indicated that the normal irrigation treatment (6712.10 kg ha^{-1}) yielded the highest biological yield, while the severe stress treatment (5059.63 kg ha^{-1}) resulted in the lowest.

Mean comparisons revealed that the vermicompost treatment (8063.32 kg ha^{-1}) exhibited the highest biological yield, followed by the manure treatment. Conversely, the control fertilizer treatment (4173.21 kg ha^{-1}) yielded the lowest biological yield in the study. Notably, there was no significant difference between the control and NPK and humic acid treatments.

Seed Yield

The means comparison results showed that the vermicompost application under normal irrigation conditions (2343.6 kg ha^{-1}) resulted in the highest seed yield, whereas the control treatment under severe stress conditions (1016.2 kg ha^{-1}) yielded the lowest. Under severe stress conditions, fertilizer treatments did not exhibit a significant advantage over the control treatment. However, under mild stress conditions, the application of vermicompost and animal manure, and under normal irrigation conditions, the use of all fertilizer sources significantly increased seed yield compared to the corresponding control treatment (Fig. 3).

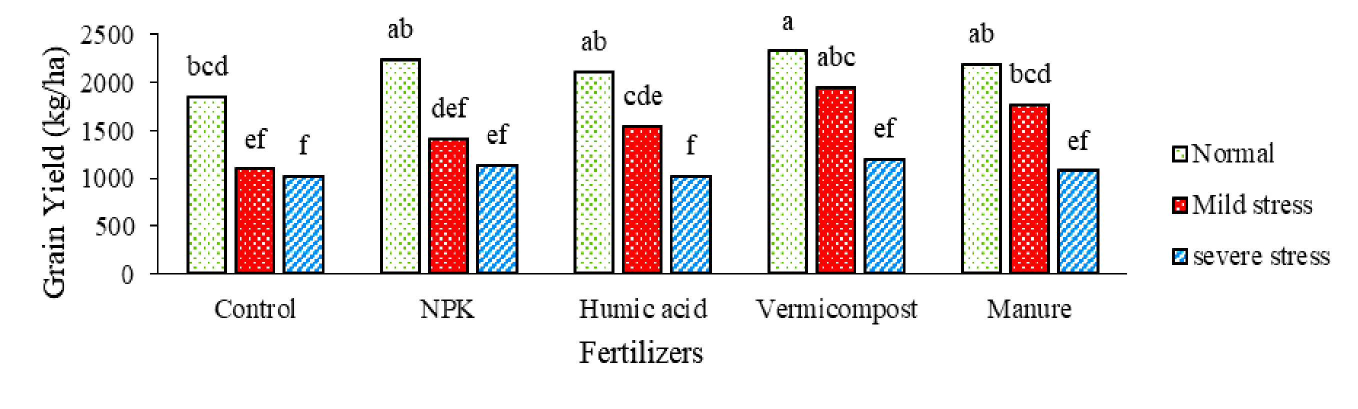

Fig. 3. Means comparison for the interactive effect of irrigation × fertilizer on grain yield (LSR, p < 0.05).

Essential Oil %

In this study, the highest percentage of essential oil was obtained from mild water deficit stress treatment (0.123 %), while the lowest % was recorded for normal irrigation (0.11 %) and severe stress conditions (0.114 %) (Table 4).

The means comparison showed that among the fertilizer treatments, the highest % of essential oil (0.124 %) was obtained with the manure treatment, while the lowest % (0.10 %) was recorded in the control treatment. Results revealed that, in addition to manure treatment, the use of NPK (12.38 %), humic acid (8.57 %) and vermicompost (14.28 %) increased the oil % compared to the control treatment (Table 4).

Essential Oil Yield

The result showed that plants treated with vermicompost under normal irrigation (2.76 kg ha^{-1}) had the highest essential oil yield. The difference between this treatment and the application of NPK, humic acid and manure under normal irrigation conditions as well as the application of vermicompost and manure under mild stress conditions, was not significant. The fertilizer control treatment recorded the lowest essential oil yield under severe stress conditions (1.07 kg ha^{-1}). However, the difference between this treatment and all 4 fertilizer treatments was insignificant under extreme water stress conditions. The results revealed that the essential oil yield decreases with increasing water stress, but using vermicompost and animal manure under mild stress conditions can moderate the adverse effects of water shortages on this treatment (Fig. 4).

Seed Mucilage %

Fig. 5 exhibits that the application of vermicompost under normal irrigation conditions (15.37 %) was associated with the highest seed mucilage %, while the no-fertilizer treatment (10.39 %) under mild water stress was related to the lowest %. It should be noted that the difference between

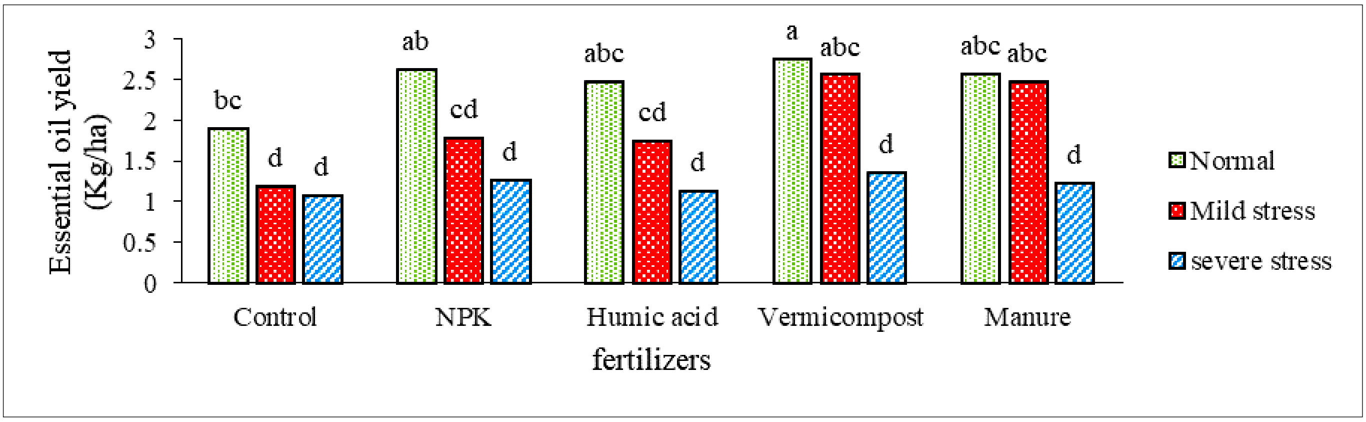

Fig. 4. Means comparison for the interactive effect of irrigation × fertilizer on essential oil yield (LSD, p < 0.05).

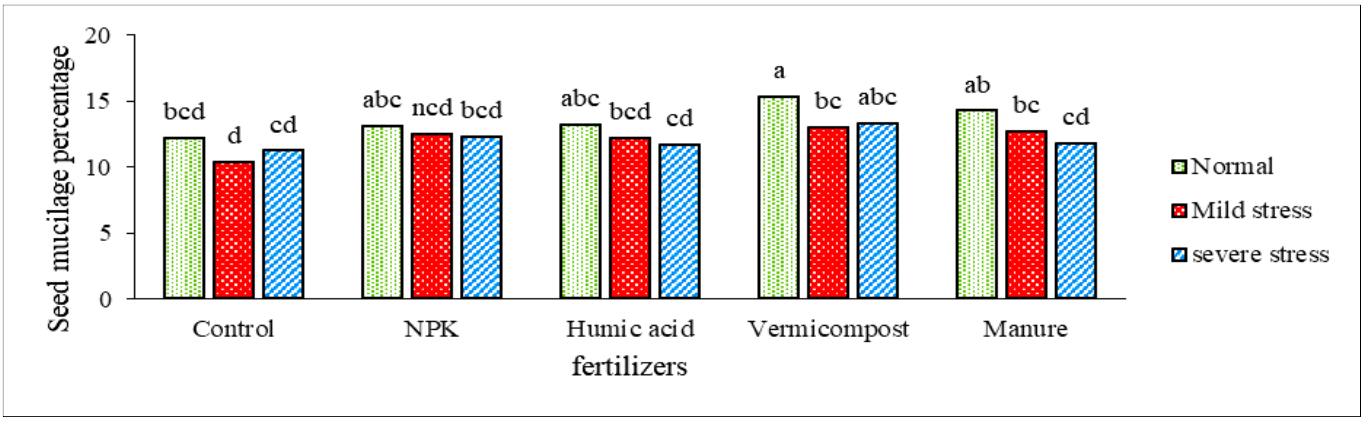

Fig. 5. Means comparison for the interactive effect of irrigation × fertilizer on seed mucilage percentage (LSR, p < 0.05).

vermicompost under normal irrigation treatment and the application of NPK, humic acid and manure under normal irrigation conditions was insignificant (Fig. 5).

Seed Mucilage Yield

The results indicated a reduction in seed mucilage yield due to decreased water availability. However, using vermicompost and animal manure under mild water deficit conditions improved the mucilage yield compared to the corresponding control treatment, thus mitigating the unfavorable effect of water stress on mucilage yield. Applying vermicompost under normal irrigation conditions (337.82 kg ha^{-1}) and the control treatment under severe irrigation stress conditions (113.68 kg ha^{-1}) resulted in the maximum and minimum seed mucilage yield, respectively (Fig. 6).

The results of this experiment demonstrated that the proline and soluble sugar content increased with reduced water availability. Overproduction of proline is a typical physiological response of plants to stressful environments. It has been reported that high levels of leaf proline can protect plants against severe drought stress and enhance drought stress tolerance (34). The breakdown of insoluble sugars can lead to the high accumulation of carbohydrates during stress, halt growth, or prompt the synthesis of these compounds from non-photosynthetic pathways (35).

The results indicate that the use of vermicompost decreased the proline content and increased the carbohydrate content compared to the control. Treated with vermicompost may enhance leaf water potential and leaf area while reducing chlorophyll photooxidation activity and carbohydrate content (36). Vermicompost has the potential to stimulate the synthesis of proline precursor amino acids (37, 38). Research on German chamomile (*Matricaria chamomilla* L.) also reported a decrease in proline content and an increase in carbohydrate content with the use of vermicompost (36).

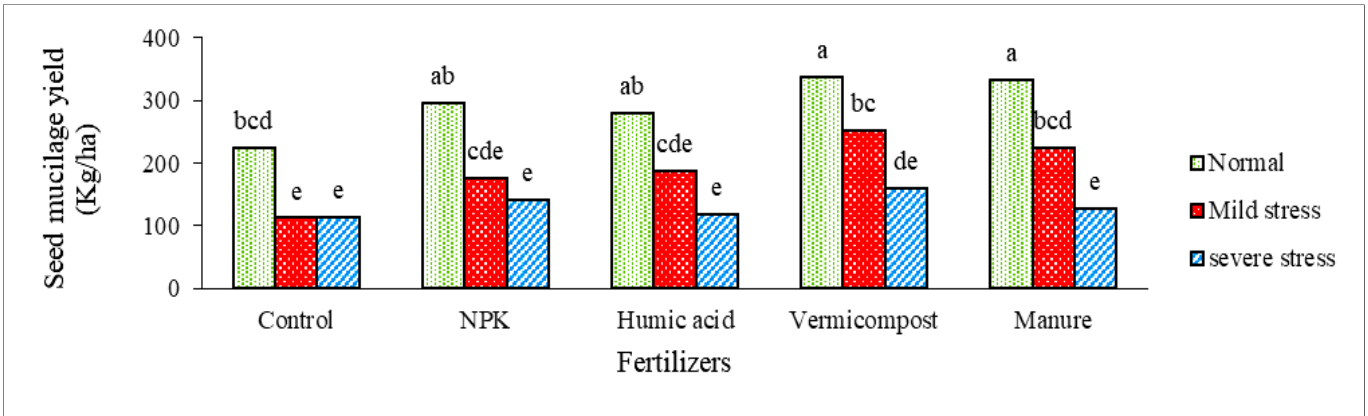

Fig. 6. Means comparison for the interactive effect of irrigation × fertilizer on seed mucilage yield (LSR, $p < 0.05$).

Discussion

Photosynthetic Pigments, Proline and Carbohydrates

Our findings demonstrate that water availability reduction in dragon's head plants led to decreased chlorophyll a, b and carotenoid content. Various factors contribute to this reduction, including the destruction of chlorophyll precursors, chloroplast peroxidation, chlorophyll photo-oxidation, imbalance of protein complexes, inhibition of chlorophyll biosynthesis and an increase in chlorophyllase enzyme activity, all exacerbated under water stress (28). In plants experiencing water stress conditions, closed stomata limit photosynthesis, resulting in lower carbon dioxide concentrations in chloroplasts and reduced internal CO_2 concentrations (29). Consequently, these processes collectively diminish the activity of RuBisCo enzyme, crucial in carbon fixation (30). Previous research on peppermint has also shown a decrease in leaf chlorophyll content under water deficit conditions (31). Similarly, a study demonstrated the reduction in photosynthetic pigment content in dragon's head under water stress (20).

Among the fertilizer treatments, the photosynthetic pigments in plants treated with vermicompost reached their maximum value. Due to chlorophyll and carotenoids being permanently bound to proteins, the application of vermicompost delivers the nitrogen needs of photosynthetic pigments and plant cell proteins, thereby enhancing the content of these pigments in crops (32, 33). A study emphasized that treating dragon plants with organic fertilizers, especially vermicompost, significantly increased photosynthetic pigments (20).

Elements Content

Water deficit stress led to a decrease in the nitrogen and potassium content in plant leaves compared to normal conditions. The reduced transpiration rate under water deficit conditions limited the flow of water mass into the soil, thereby restricting plant uptake of nutrient elements. It has been reported that as water stress intensifies in plants, the availability of nutrients decreases (36). Consistent with the findings of our experiment, a study demonstrated that the dissolution of elements decreases with decreasing soil moisture (39). Additionally, a study highlighted the reduction of nitrogen and phosphorus elements in the leaves of the dragon's head plants (20).

According to the results, applying NPK resulted in the highest leaf nitrogen content, while the use of vermicompost and animal manure led to the maximum leaf potassium content. It has been suggested that organic fertilizers can enhance nutrient absorption by the plant due to their positive impact on the physicochemical properties of the soil (40). Studies have reported enhanced absorption of elements such as nitrogen and potassium in treatments involving vermicompost (36, 41).

Antioxidants Characteristics

The results indicated a significant increase in the content of phenol, flavonoid, DPPH and nitric acid radical scavenging with decreased water availability for the plants. The antioxidant activity of compounds derived from dragon's heads has been well-established (42).

One of the biochemical changes that occur in plants under drought stress is the increase in reactive oxygen species (ROS) accumulation (43). Plant cells can counteract oxidative stress conditions by inducing the antioxidant system (44). Phenols play a vital role in plants due to their ability to scavenge radicals through their hydroxyl group (45). A study observed that exposuring dragon's head plants to water stress conditions increased the activity of antioxidant enzymes (20). In our study, enhancing the activity of the antioxidant system can mitigate the harmful effects of oxygen free radicals induced by water stress.

The highest levels of phenol, flavonoid, DPPH, nitric acid radical scavenging content and chain-breaking capacity were observed in plants treated with NPK and vermicompost. Phenol and flavonoids are believed to play a significant role in plant defense mechanisms against various ROS, thereby reducing the damage caused by free radicals (46). Organic fertilizers like vermicompost can alter metabolic compounds through gibberellin or cytokinin pathways, enhancing plant access to nutrients, improving their absorption and adjusting soil pH (47). Furthermore, it has been suggested that vermicompost treatment enhances soil physicochemical properties, facilitates the effective absorption of macro and microelements and increases the number of oil glands and sites of terpenoid synthesis. These processes stimulate the production of a high amount of secondary metabolites by inducing the expression of genes involved in phenol biosynthesis and other antioxidant compounds (48, 49).

In a study, treating eggplants (*Solanum melongena*) with organic fertilizers, especially vermicompost, enhanced the activity of antioxidant enzymes (50). Previous studies have also mentioned the increase in the activity of antioxidant enzymes in dragon heads (20) and peppermint (51) treated with vermicompost. It has been observed that fertilizer treatments were effective in suppressing DPPH radicals, with the highest DPPH antioxidant activity achieved using vermicompost (52).

Agronomic Characteristics

In this study, water deficit stress significantly decreased the number of seeds and plant biomass in the dragon's head. The reduction in these traits due to drought stress can be attributed to diminished cell division and growth, decreased photosynthesis, membrane degradation, incompatibility in water and nutrient uptake and their transmission (53).

Among the fertilizer treatments, the highest number of seeds and biological yield were observed in the plants treated with vermicompost.

Previously it has been observed that organic and biological fertilizers significantly improve plant development and growth, nutrient absorption and photosynthetic efficiency by enhancing the activity of alkaline phosphatase and acid phosphatase (54). The utilization of organic fertilizers impacts physiological processes by strengthening the enzyme system, facilitating the transfer of photosynthetic products, and stimulating cell division and elongation, ultimately resulting in increased growth and elemental content in the leaves (6).

In this study, although treatment with vermicompost under normal and mild water shortages produced the highest kernel weight and seed yield, the difference between vermicompost treatment and other sources of fertilizer under normal irrigation conditions in terms of seed yield was insignificant. Under water availability conditions, all four fertilizer treatments were able to increase grain yield significantly compared to the control treatment. However, under severe water stress conditions, none of the fertilizer treatments could significantly increase grain yield compared to the control treatment. In the present study, although water deficit stress reduced seed yield compared to normal conditions, treatment with vermicompost and animal manure significantly reduced the adverse effect of mild water deficit stress. The high grain yield in plants treated with vermicompost and animal manure under normal irrigation conditions and mild water stress may be attributed to the positive effects of these treatments on the content of photosynthetic pigments, nutrient absorption, antioxidant properties and the enhancement of seed yield components such as seed number and weight.

It has been reported that organic fertilizers improved photosynthesis efficiency and increased Syrian cephalaria's growth under rainfed conditions (14). Researchers attributed such results to the positive effects of organic fertilizers on vegetative growth, chlorophyll synthesis, and photosynthetic capacity, especially under water shortages (50). Animal manure and vermicompost directly affect the rate of photosynthesis and the plant's vegetative growth by increasing the soil's organic matter and the plant's access to nutrients (14). A study recorded the highest seed yield in the dragon's head plants with vermicompost treatment and winter-sown date (46).

A study demonstrated that applying different fertilizer sources enhanced dragon's head plants' biological and grain yields compared to control treatment under rainfed and complementary irrigations (20). In the study, treating peppermint with vermicompost increased the plant's growth and development under water-stress conditions (51). They stated that using vermicompost increases the plant's availability and absorption of nutrients. Another study documented that the application of manure increased grain yield and biological yield in purslane (*Portulaca oleracea*) (12).

Qualitative Characteristics

The current research results revealed that the highest essential oil percentage was obtained under mild water deficit stress conditions. A study on peppermint reported that the highest essential oil % was related to mild water deficit stress treatment and severe stress considerably reduced the % of this trait (49). Authors concluded that the maximum essential oil % of *Salvia officinalis* was obtained under mild deficit irrigation, with an average of 1.86 % (55). In the study, the oil content of the *Lallemantia iberica* plant decreased with the reduction of water available to the plant (4).

Among the fertilizer treatments, the use of vermicompost and animal manure significantly increased the essential oil content compared to the control. Under normal and mild water deficit condition, employing vermicompost and animal manure resulted in the highest essential oil yield. However, under severe water deficit conditions, the fertilizer application treatments did not exhibit significant superiority over the control treatment. An important point to mention is that treatment with vermicompost and animal manure under mild stress conditions yielded the same amount of essential oil as treatments using these 2 fertilizers under normal irrigation conditions. Additionally, it significantly increased the mentioned trait compared to the respective control treatments.

Essential oils are terpenoids, requiring ATP, acetyl-CoA and NADPH for synthesis (56). Another influential factor in essential oil biosynthesis is the provision of mineral nutrients for the plant (57). A study reported that treating plants with organic and biological fertilizers enriched the essential oil content and yield of purslane plants under water deficit conditions (12). Additionally, treatment with organic fertilizers can increase the essential oil-emitting glands in the flowers and leaves of the dragon's head plant under water deficit conditions.

A study demonstrated that the essential oil yield in peppermint plants significantly decreased under mild and severe water deficit stress compared to normal conditions (49). The increase in essential oil yield under normal irrigation conditions and mild stress in the present research is attributed to the rise of seed and essential oil yields respectively.

The highest mucilage % and seed mucilage yield were recorded under normal irrigation treatment and with the application of vermicompost and animal manure. Additionally, the use of these 2 treatments under mild stress conditions mitigated the adverse effects of water deficit on these traits and significantly increased the % and yield of mucilage compared to the corresponding control. Considering that essential and mucilage yield are products of essential and mucilage % as well as grain yield, any factors affecting these traits can affect essential and mucilage yield.

It has been documented that organic fertilizers increase the water potential of the leaf, the rate at which the plant utilizes transpiration, carbon dioxide levels and the production of plant growth stimulants. Furthermore, organic fertilizer treatments improve water absorption from the soil, the development of root growth and oil synthesis in Dragon's head plants (4, 15). Another study demonstrated that the treatment with vermicompost and animal manure increased the seed oil percentage and oil yield of Syrian cephalaria by increasing water absorption and nutrient uptake from the soil (14).

The positive effect of organic fertilizers on the production of photosynthetic products, especially carbohydrates, is undeniable. Considering that carbohydrates are precursors of fatty acid biosynthesis pathways, the transfer of carbohydrates from photosynthesizing leaves to seeds may also positively affect the oil percentage (15, 39). Previous studies have also highlighted the positive effects of bio-organic, chemical and vermicompost fertilizers in enhancing the quantity and quality of essential oil in the Moldavian balm plant (9, 57).

Authors observed the highest mucilage content in NPK fertilizer, vermicompost and manure (46). They also found that applying vermicompost during winter sowing resulted in the highest mucilage yield and essential oil yield. Furthermore, in their study, the application of manure, vermicompost and NPK fertilizer led to the highest essential oil content.

In the present study, under mild water stress conditions, treatment with vermicompost and animal manure improved the content of photosynthetic pigments and absorption of nutrients, consequently, enhancing seed yield, essential oil yield, oil yield and mucilage yield. It has been reported that absorption and transport of nitrogen in the form of NO^{-3} (Nitrate) are reduced during drought stress due to low turgor pressure and transpiration levels, leading to reduced amino acid synthesis and gene expression (58). Both organic and chemical fertilizers increase the production of medicinal plants by stimulating plant growth and metabolic processes. Nitrogen, a key element, has been found to accelerate cell growth and division; its presence in organic and chemical fertilizers can improve essential oil synthesis in medicinal plants (48).

Conclusion

The most important characteristics of the dragon's head plant are essential oil yield and mucilage yield .In the present study, all four fertilizer treatments under normal irrigation conditions increased both essential oil yield and mucilage yield compared to the control treatment, with no significant difference detected between them. Therefore, in the absence of water restrictions, all fertilizer treatments enhance the essential oil and mucilage yield, making all four fertilizer treatments recommended for increasing plant yield. However, under severe water stress conditions, no significant difference was observed between the control treatment and fertilizer sources. Thus, when water limitation is severe, using different fertilizer sources is not recommended. Under mild stress conditions, the application of vermicompost and manure mitigates the adverse effects of water scarcity stress, resulting in statistically similar essential oil and mucilage yields as applying these 2 fertilizer sources under normal irrigation conditions. It can be concluded that the use of vermicompost and animal manure, by improving the content of photosynthetic pigments, increasing nutrient absorption, enhancing antioxidant activity and promoting the synthesis of secondary metabolites, could increase essential oil and mucilage yield under mild stress conditions. Finally, using vermicompost and animal manure under mild stress conditions, especially in arid and semi-arid regions, can serve as a solution for developing dragon head production.

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Authors' contributions

MAF and TMM carried out the experiment and wrote the manuscript with support from SY and AR.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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