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ORIGINAL PAPER

Effects of ecological agriculture approaches on dragonhead (*Dracocephalum moldavica* L.) productivity and oil yield

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Abstract

The programmed application of organic fertilizers represents one relevant way to decrease the cost of nitrogen fertilizer, minimizing environmental pollution. We designed the field experiments as a factorial in a randomized complete block design with 20 treatments and three replications at two locations in Iran (Tehran and West Azerbaijan province). The tested treatments were as follows: two genotypes (Landrace and SZK-1 cultivar); seed inoculation treatments (with and without bacterial inoculation), and five fertilization regimes (FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 t ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 t ha^{-1}), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}). Application of azocompost enhanced total flavonoid content at a high level at Tehran and a medium level at Khoy. High doses of urea enhanced nitrogen accumulation in plants. The treatments with higher azocompost contents reflected the highest amounts of NPK. On the other hand, the Landrace cultivar evidenced higher chlorophyll concentration than the SZK-1 cultivar at Tehran. High doses of urea produced higher photosynthetic pigment in plants. Application of plant growth promoting regulation (at Tehran) and medium concentration of azocompost enhanced xylose concentration. In this context, the azocompost application improved mannose content. The essential oil yield in the Landrace cultivar was higher than that reflected by the SZK-1 cultivar. For both genotypes, results suggest that bacterial inoculation improves essential-oil production. Results showed that plant growth–promoting regulators and azocompost at medium and high levels improved most plant traits. Although the biological-based plant growth promoters exhibited positive effects in some experimental units, these effects were lower than that of a conventional fertilizer regime. Therefore, azocompost integrated with bacterial inoculation responded to be a suitable substitute for chemical fertilizer treatment.

Keywords Azocompost · Bio-fertilizers · Flavonoids · Medicinal plants · Organic fertilizer

Introduction

Increasingly, societies are interested in using medicinal plants instead of commercial drugs to deal with various health problems (Rahimzadeh et al., 2011). A key to producing medicinal plants is not just to get the greatest possible plant biomass (Mehalaine and Chenchouni, 2021a) but also the highest amounts of the active agent—for example, antioxidants, and ideally to do so with the lowest environmental impact. An approach that might achieve both objectives is ecological agriculture. Ecological agriculture uses organic amenders and biofertilizers to enhance crop production, reducing at the same time the environmental impacts (Boudjabi and Chenchouni, 2021). Such supplements can include things like Azolla, manure, and even adding specific microorganisms (Sabourifard et al., 2023). We asked whether we could use ecological agriculture approaches to enhance the overall productivity and the yield of essential oils in an important medicinal plant—the dragonhead (*Dracocephalum moldavica* L.). To attain this objective, we explored the effects of several ecological agriculture approaches on dragonhead productivity and oil yield.

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Recently, the interest in discoverying natural antioxidants has risen dramatically for three principal reasons: (1) Overwhelming epidemiological and clinical evidence that suggests an inverse relationship between the consumption of adequate amounts of fruit and vegetables and the risk of developing a chronic disease such as cancer (Rah Khosravani et al., 2017); (2) concerns regarding the safety of high consumption of synthetic compounds, traditionally used as preservatives in foods and beverages (Jahani et al., 2021); and (3) a general conception among the public that natural phytochemicals are inherently safer than synthetic chemicals (Keshavarz Mirzamohammadi et al., 2021a, b). The principal subjects of this research have been herbs, spices, and medicinal plants (Dastmalchi et al., 2007). However, a lack of proper germination and cultivation of medicinal plants means that research into cultivation techniques implies a risky process (Mehalaine et al., 2023). The long-term collateral effects derived from chemical drugs and the human tendency toward more natural products to maintain health have increased interest in using medicinal plants to treat health problems (Rahimzadeh et al., 2011). The above resulted in an increased demand for aromatic medicinal plants, especially for products cultivated in ecological conditions, and this trend is increasing worldwide (Berova et al., 2013; Mehalaine and Chenchouni, 2020, 2021b). Ecological cultivation of medicinal plants ensures their quality and reduces the potential for adverse effects on the quality of their products (Butar Butar et al., 2018). Previous investigations showed that both genotype and environmental factors influenced the active ingredients in medicinal plants (Camen et al., 2017; Mehalaine and Chenchouni, 2020, 2021b; Hosseini et al., 2023).

Proper management of manure amendments in the soil is one of the main issues affecting the successful cultivation of medicinal plants (Sabourifard et al., 2023). Increased use of chemical inputs (mineral fertilizers, fungicides, and pesticides) in conventional agriculture causes serious environmental problems, such as the pollution of soil and water resources. Poor soil quality and polluted surface water and groundwater resources affect the quality of the food produced under such conditions (Bouaroudj et al., 2019), contributing to air pollution, loss of biodiversity, and disturbed ecosystems (Keshavarz et al., 2018). A fundamental solution to these problems is to develop sustainable ecological agriculture using bio-fertilizers (Khalil and El-Noemani, 2015; Boudjabi and Chenchouni, 2021). The so-called 'ecological agriculture' is an integrated farming system based on principles in which the quality of agricultural produce is more important than yield quantity. Ecological agriculture eliminates the use of chemical fertilizers, pesticides, and growth regulators, by applying alternative strategies, including the application of crop residues, systems of crop rotation using legumes, organic fertilizers containing rock minerals, mechanical weed control, and bio-control of pests and diseases (Arrobas et al., 2018; Keshavarz and Sadegh Ghol Moghadam, 2017).

Using alternative plant nutrients such as bio-fertilizers presents an alternative strategy for supplying plant nutrition that reduces environmental impacts (Sun et al., 2020; Chenchouni et al., 2020). One such approach is by the use of biofertilizers, which are microorganisms that live in the rhizosphere and stimulate plant growth by some specific mechanisms, including fixing atmospheric nitrogen, producing siderophores, solubilizing minerals, synthesizing phytohormones, and increasing plant resistance to pathogens and pests (Raimi et al., 2021). Research has shown that plant growth–promoting rhizobacteria (PGPR) were affected by plant species and genotype (Egbuna et al., 2016), soil types, and agricultural practices, such as fertilizer application and type of fertilizer (Asadu et al., 2018a). Bacteria such as *Pseudomonas*, *Azospirillium*, and *Azotobacter* are commonly used in organic production systems to improve growth and yield (Asadu et al., 2018b; Dekak et al., 2020). Investigations about the effects of Azolla as a bio-fertilizer in peppermint (*Mentha piperita* L.) in Iran reported increases in growth associated with production (Ebrahimi Sborezi et al., 2021). According to Marashi et al. (2021), a fixed combination of organic and bio-fertilizers promoted increases in P-content in Psyllium (Marashi et al., 2021).

Azolla is superior among other floating plant species due to symbiotic nitrogen fixation with blue-green Anabaena algae. In Azolla, nutrient levels vary across time, and averages were 3.5% N, 3.8% K, and 0.6% Mg, and no Pb, Hg, or arsenic (Setiawati et al., 2018). Azolla species are considered aggressive weeds, but they have many advantages, such as N2-fixation and removal of heavy metals. Until now, most studies have focused on the Azolla–Anabaena association and nitrogen fixation in Azolla (Fernández-Zamudio et al., 2010), but very little is known about its habitat in terms of plant's needs as an invasive species in a new environment.

Moldavian balm syn. Moldavian dragonhead (*Dracocephalum moldavica* L.) is a perennial herb belonging to the Lamiaceae (Labiatae) family. Distribution in Iran is in the northern and northwestern parts of the country, especially in the western parts of Azerbaijan Province and the Albourz Mountains (Dmitruk and Weryszko-Chmielewska, 2010). Dragonhead has a sedative effect, commonly consumed as a tea; the therapeutic properties of its plant compounds are in the form of essential oils that include citronellol and geraniol (Kashchenko et al., 2022).

The objective of this study was to evaluate the effect of azocompost and plant growth–promoting regulation integration on Moldavian dragonhead among two genotypes and how these may influence by fertilizer treatments.

Materials and methods

The field experiments were carried out in 2009–2010 at two locations; Research Field at the Faculty of Agriculture, Tarbiat Modares University, Tehran (location 1) (35° 70′ N, 51° 40′ E and 1200 m a.s.l.) and Khoy Agricultural Research Center in West Azerbaijan province (location 2) (38° 35′ N, 44° 52′ E and 1040 m a.s.l.). The climate in Tehran was semi-arid, with long-term historical annual precipitation of 246 mm and an annual mean temperature of 17.69 °C. Other climate data for the growing season for both sites are in Table 1. On the other side, the climate in Khoy is semi-arid with warm and dry summers, mean annual rainfall, and temperature evaluations of 286.6 mm and 12°C, respectively. The soil characteristics of Tehran and Khoy are shown in Table 2 (Jahani et al., 2021; Keyvan Rad et al., 2022).

We designed the experiment as a factorial in a randomized complete block design with three replications. There were 20 treatments as follows: A factorial combination of two genotypes (G1, Landrace genotype, i.e., Orimieh; and G2, a selected cultivar SZK-1), two seed inoculation treatments (the bacterial inoculation with Azotobacter+Azospirillum +Pseudomonas, and without-inoculation), and five fertilization regimes; FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha-1), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}).

The physicochemical properties of the organic compost are in Table 2. We prepared the manure-based compost and half of the total urea fertilizer by hand and immediately incorporated it into the soil using a rototiller 3 days before planting. The experimental plots were top-dressed at the 6-leaf stage with the other half dose of urea, with dimensions of 3 m long, forming six rows 0.375 m apart (187.5 cm width), defining 2 m between plots to eliminate the influence of lateral water movement.

Seeds of SZK-1 (originally from Hungary) and Orimieh were obtained from the Zardband company and West Azerbaijan Agriculture and Natural Resource Research Center, respectively. Seeds were hand-planted on 9 April at Tehran and 10 May 2009 at Khoy at the rate of 1 g seeds m^{-1} of row. We thinned the established plants at the four-leaf stage to achieve a density of approximately 133,333 plants ha^{-1} at both locations. Weed control was done by hand, using a hoe or a rototiller whenever necessary; for opportune irrigation, we applied the required water volume during the plant growth stages. Plants were harvested at the full-flowering stage (Hussein et al., 2006) on 10 July 2009 in Tehran and 10 August 2009 at Khoy.

The determination of carbohydrate percentage of the herb solution was according to Dubois et al. (1956), and the photosynthetic pigments (chlorophyll a and b and total carotenoids mg g^{-1} FW) of leaves were according to Arnon (1949). Tests for flavonoids and anthocyanin contents were based on the method cited by Krizek et al. (1993).

Total N was determined in the dried samples of the first cut, using the modified Micro-Kjeldahl method according to AOAC (1990), determining macro elements after wet digestion. Total P was estimated using the vanadate-molybdate method described by Jackson (1973). Potassium was determined using a flame photometer described by Jackson (1965).

Table 2 Soil physical and chemical properties in two locations and azocompost

Soil properties	Location 1 (Tehran)	Location 2 (Khoy)	Azocompost
EC (ds m^{-1})	2.18	1.24	3.1
pH	7.4	7.7	5.7
Organic carbon (%)	1.25	0.81	31.4
Total N (%)	0.099	0.075	3
Available P (mg kg^{-1})	41	4	1.51
Available K (mg kg^{-1})	460	150	1.3
Fe (mg kg^{-1})	6	8.4	0.5
Zn (mg kg^{-1})	2.6	1.14	112
Cu (mg kg^{-1})	0.8	2.6	43
Mn (mg kg^{-1})	7	7.4	992

Table 1 Monthly temperature and rainfall during the growing season

Month	Temperature (°C)						Rainfall (mm)	
	Minimum		Maximum		Mean			
	Tehran	Khoy	Tehran	Khoy	Tehran	Khoy	Tehran	Khoy
April	0.0	3.58	21.8	15.25	11.8	9.41	41.7	34.63
May	5.2	8.32	29.8	23.16	18.4	15.74	31.9	9.55
June	13	12.93	33.6	27.35	24.8	20.14	2.8	52.93
July	18	17.12	41	33.58	29.8	25.35	0.0	11.01
August	18.2	16.52	38.6	32.31	29.2	24.41	0.0	4.03

We applied the analysis of variance (ANOVA) and comparison of means using the general linear model (GLM) procedure of the SAS Institute Inc (2002), testing the assumptions of variance analysis and verifying that residuals were random, homogenous, and with normal distribution determined by a mean of 0. The least significant difference procedure (LSD) at a probability level of 0.05 was applied to determine the statistically significant difference among treatment means.

Result

Different weather conditions occurred in each location during the growing season (April–August). The mean temperature at the first location (Tehran province) was higher than the second location (West Azerbaijan province). The distribution of precipitation was also slightly different in both of them. From the beginning of the flowering period until the full-flowering stage (June and July for Tehran and July and August for Khoy), the amount of precipitation at Khoy was higher than in Tehran. Results are presented separately for weather and soil conditions at each location. We explained the main effects for cases where the evaluation of two and three-way interactions was not statistically significant for each trait.

Nitrogen content in plant

Results showed that nitrogen content in plants was affected by the main effect of genotype and fertilization regime at Tehran and Khoy (Table 3). There was a significant difference between genotype and fertilizer regime (Table 3) and for the three-way interactions between the former mentioned treatments and seed inoculation for nitrogen content in plants at Khoy (Table 3). In Tehran, the G1 genotype produced a higher amount of nitrogen than reflected by the G2 genotype. Moreover, the highest and the lowest amounts for nitrogen percentage were determined as 0.91% and 7.0% for FR1 and FR5 treatments, respectively. We did not find a significant difference between treatments FR3, FR2, and FR1 (Table 4). At Khoy, interactions of fertilizer regime, bacteria, and genotype showed that in the G1 genotype (in both states, either with or without bacterial inoculation), the maximum amounts for plant nitrogen were estimated as 1.2% and 1.12%, respectively, recorded for FR2 treatment. Also, the lowest plant nitrogen content was reflected by the G1 genotype for FR5 treatment, at both locations and for treatments with and without bacterial inoculation, with 0.81% and 0.76%, respectively (Fig. 1). For the G1 genotype (without bacterial inoculation), there was no significant difference between treatments FR1–FR4. Results for the G2 genotype (with bacterial inoculation) were similar to those for the G1 genotype (with bacterial inoculation). The highest and the lowest amounts of nitrogen percentage were 1.19% and 0.84% for treatments FR2 and FR5, respectively. Also, in the G2 genotype (without bacterial inoculation), FR1 and FR4 fertilizer regimes produced the highest and the lowest amounts of nitrogen in plants, respectively (Fig. 1).

Phosphorus content in plant

The main effects of the fertilizer regime at both locations and the two-way interaction of these factors (genotype by fertilizer and bacteria by fertilizer) were significant for phosphorus content only at Khoy (Table 3). At Tehran, the highest and lowest percentages of phosphorus were recorded at 0.36 % and 0.31 % for fertilizer regimes FR5 and FR1, respectively (Table 4). The highest azocompost level (FR5) without urea significantly increased the percentage of phosphorus in plant organs.

Potassium content in plant

The main effects of the fertilizer regime at both locations and the genotypes factor were significant for the amount of potassium only at Tehran (Table 3). At Tehran, the G1 genotype showed a higher potassium content than reflected by the G2 genotype (Table 4). Moreover, the highest and lowest amounts of plant potassium percentage were determined at 2.79% and 2.51% in treatments FR5 and FR1, respectively (Table 4). At Khoy, results were the same as those for Tehran; the highest and the lowest amounts for potassium percentage were 1.86 % and 1.07 % from treatments FR5 and FR1, respectively (Table 4).

Chlorophyll

Analysis of variance showed that amounts of chlorophyll-a, b, and the total chlorophyll were affected by the factors genotype and fertilization regime and by the two-way interaction of these factors (genotype × fertilizer) for chlorophyll-a and total chlorophyll at location1 (Table 3). The assessed chlorophyll a, b, and the total were affected only by the fertilization regime at Khoy (some data not shown). At Tehran, higher amounts of chlorophyll a were recorded for both genotypes (G1 and G2) with an average of 1.155 and 0.9 mg g^{-1} FW at FR1 treatment, respectively (data not shown). In addition, the FR5 fertilizer regime produced the lowest amount of chlorophyll ‘a’, with an average of 0.619 and 0.692 mg g^{-1} FW in G1 and G2 genotypes, respectively (data not shown). Moreover, the whole fertilizer treatments (except FR5 in G2 genotype) increased the concentration of chlorophyll-a in the G1 genotype compared to results for the G2 genotype. In addition, a decrease in chemical nitrogen content reduced the

Table 3 Analysis of variance (mean squares) for the main and interaction effects of genotype, bacteria inoculation and fertilizer regimes on nutrient content, photosynthetic pigments, solution sugars and essential oil yield in dragonhead (*Dracocephalum moldavica* L.) tested at two locations

SOV	df	N	P	K	Tchl	CAR	FLA	ANT	Xylose	Glucose	Mannose	EOY
Tehran (Location 1)												
Replication	2	0.025ns	0.000ns	0.385**	0.24**	0.01**	780.1ns	1.35ns	0.35ns	1.55*	0.54ns	10.6ns
Genotype (G)	1	0.088*	0.000ns	0.237*	0.25**	0.000ns	457.0ns	0.24ns	0.63ns	1.55*	1.62**	75.1**
Bacterial inoculation (B)	1	0.008ns	0.000ns	0.030ns	0.000ns	0.003ns	271.8ns	0.005ns	1.09ns	1.96*	1.62ns	2.51ns
Fertilizer regimes (FR)	4	0.073**	0.0087**	0.13*	0.38**	0.007**	2949.2**	2.47**	1.3*	0.81 ns	0.81ns	307.9**
G×B	1	0.025ns	0.000ns	0.70ns	0.028ns	0.000ns	1172.2ns	0.035ns	3.66**	0.072ns	0.07ns	115.5**
G×FR	4	0.006ns	0.001ns	0.01ns	0.07*	0.003*	627.0ns	1.6*	0.53ns	1.28*	0.56ns	22.21**
B×FR	4	0.004ns	0.001ns	0.04ns	0.022ns	0.001ns	837.8ns	0.8ns	0.45ns	0.36ns	0.24ns	5.96ns
G×B×FR	4	0.003ns	0.001ns	0.002ns	0.012ns	0.002ns	588.4ns	1.05ns	1.18ns	0.1ns	0.89ns	11.27ns
Error	38	0.013	0.000	0.045	0.027	0.001	708.4ns	0.46	0.5	0.37	0.47	5.2
CV (%)		14.33	8.27	8.03	14.75	12.83	6.39	13.22	17.82	12.8	19.76	10.32
Khoy (Location 2)												
Replication	2	0.065*	0.00024**	0.15**	1.53**	0.039*	513.5ns	4.43ns	0.54ns	0.75*	1.24ns	16.93ns
Genotype (G)	1	0.104**	0.000ns	0.007ns	0.025ns	0.000ns	3829.01	0.24ns	0.000ns	0.96*	0.024ns	116.3*
bacterial inoculation (B)	1	0.006ns	0.000ns	0.000ns	0.018ns	0.005ns	16083**	0.099ns	0.11ns	1.49*	0.000ns	0.001ns
Fertilizer regimes (FR)	4	0.139**	0.00016**	0.059**	0.031**	0.029**	2859ns	0.54ns	0.52**	1.74**	0.94**	120.89*
G×B	1	0.023ns	0.000ns	0.002ns	0.17ns	0.001ns	6739*	1.29ns	0.76ns	0.017ns	0.53ns	27.98ns
G×FR	4	0.028ns	0.00079*	0.000ns	0.031ns	0.001ns	677.1ns	3.71*	2.38**	0.44ns	2.41**	30.16ns
B×FR	4	0.026ns	0.000084*	0.002ns	0.29ns	0.001ns	579.1ns	0.42ns	0.13ns	0.17ns	0.86ns	56.11*
G×B×FR	4	0.033*	0.000ns	0.004ns	0.2ns	0.000ns	2775.3ns	2.29ns	0.26ns	0.41ns	0.27ns	24.18ns
Error	38	0.012	0.000	0.006	0.11	0.002	1304.5	1.39	0.39	0.22	0.48	19.36
CV (%)		10.96	3.03	4.34	17.7	13.73	9.52	17.94	18.84	10.54	23.05	22.96

N, nitrogen content in plant; *P*, phosphorus content in plant; *K*, potassium content in plant; *Tchl*, total chlorophyll content; *CAR*, carotenoid content; *FLA*, total flavonoids content; *ANT*, anthocyanin content; *EOY*, essential oil yield

ns, not significant at the 0.05 probability level

*Significant at the 0.05 probability levels

**Significant at the 0.01 probability levels

Table 4 Mean comparison of genotype (G) and fertilizer regimes (FR) for nitrogen, phosphorus and potassium content of dragonhead plants in both locations

FR	G	Nitrogen (% DW)		Phosphorus (% DW)		Potassium (% DW)	
		Tehran	Khoy	Tehran	Khoy	Tehran	Khoy
G1		0.85^{a}	0.98 ^{b}	0.33^{a}	0.161 ^{a}	2.71^{a}	0.178 ^{a}
G2		0.78^{b}	1.06 ^{a}	0.32^{a}	0.162 ^{a}	2.58^{b}	0.180 ^{a}
	FR1	0.91^{a}	1.12^{a}	0.31^{b}	0.168 ^{b}	2.51^{b}	1.07 ^{b}
	FR2	0.85^{ab}	1.14^{a}	0.31^{b}	0. 170 ^{b}	2.57^{b}	1.74 ^{b}
	FR3	0.82^{ab}	1.004^{b}	0.32^{b}	0.172 ^{b}	2.69^{ab}	1.82 ^{a}
	FR4	0.68^{bc}	0.95^{bc}	0.35^{ba}	0.171 ^{b}	2.68^{ab}	1.84 ^{a}
	FR5	0.7^{c}	0.82^{c}	0.36^{a}	0.177 ^{a}	2.79^{a}	1.86 ^{a}

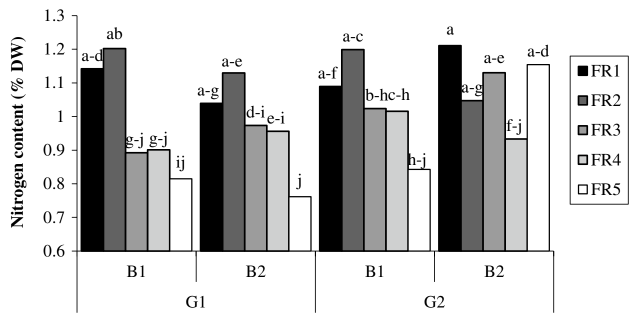

Fig. 1 Effects of genotype (G), bacterial inoculation (B) and fertilizer regimes (FR) treatments on the nitrogen content of dragonhead plants at location two. G1, Landrace genotype, i.e. Orimieh; and G2, a modern cultivar SZK-1; B1: bacterial inoculation with *Azotobacter*+ *Azospirillum* +*Pseudomonas*, B2: without inoculation; FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}). Means in each column followed by similar letter(s) are not significantly different at the 5% probability level-using Least Significant Difference (LSD)

content of chlorophyll-a in both genotypes. We observed similar results to chlorophyll a for the total chlorophyll, finding the highest and the lowest amount in both genotypes (G1 and G2) for treatments FR1 and FR5, respectively (Table 6). In addition, results showed that chlorophyll b concentration in the G1 genotype was higher than in the G2 genotype (data not shown). Results for Khoy showed that applying a higher dose of nitrogen fertilizer (FR1) produced high amounts of chlorophyll a and total. We also observed low amounts of chlorophyll types a, b, and the total one, in the FR5 treatment (Table 7, some data not shown). The application of the treatment FR3 produced the highest chlorophyll-b concentration in plant leaves (data not shown). Results showed that the G1 genotype reflected higher amounts of chlorophyll-a, b, and the total one at Tehran than the G2 genotype, demonstrating that genetic factors, climatic conditions, and soil type exert several effects on this process.

Total carotenoid

The main effects of the fertilizer regime at both locations and the two-way interaction between them (genotype × fertilizer regime) were significant for total carotenoid only at Tehran (Table 3). Results for Tehran showed that the highest and the lowest amounts of carotenoid in the G1 genotype were 0.30 and 0.19 mg g^{-1} FW at FR1 and FR5 treatments, respectively (Table 6). In the G2 genotype, there was no significant difference among fertilizer regimes, but the G1 genotype showed the highest and lowest amounts of total carotenoid with the FR1 and FR5 treatments (Table 6). At Khoy, the FR1 and FR5 treatments produced the maximum and minimum amounts of carotenoid (0.43 and 0.30 mg g^{-1} FW, respectively) (Table 7). According to these results, using nitrogen treatment at a high level (FR1) produced the maximum concentration of carotenoid in the leaves of plants at both locations.

Total flavonoids

The ANOVA showed that in Tehran, the fertilization regime influenced total flavonoid concentration in plants. The main effects of bacterial inoculation and the two-way interaction (genotype × bacterial inoculation) were significant for flavonoid content at Khoy (Table 4). At Tehran, applying 100% azocompost (FR5) produced the highest amount of total flavonoids, while the FR1 fertilizer regime evidenced the lowest values (Fig. 2). At Khoy, there was no significant difference in genotypes for the fertilizer regime, but FR3 promoted the highest content of total flavonoids and FR4 the lowest amount (data not shown).

Anthocyanin

The main effects of the fertilizer regime at Tehran and the two-way interaction between them (genotype × fertilizer) were significant for the amount of anthocyanin at both locations (Table 3). At the Tehran locality, the highest and the lowest amounts of anthocyanin were recorded for the G1 genotype for FR1 and FR2 treatments, respectively, while, in

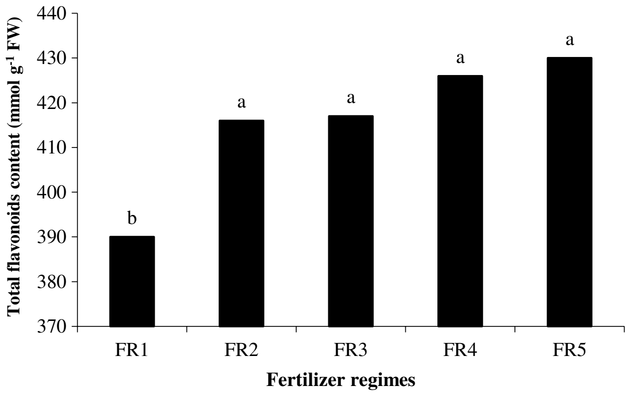

Fig. 2 Effects of fertilizer regimes (FR) treatments on total flavonoids content of dragonhead plants in locations one. FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}). Means, in each column, followed by similar letter(s) are not significantly different at the 5% probability level using Least Significant Difference (LSD)

the G2 genotype, there was no significant difference between fertilizer regime levels (Table 6). In the Khoy locality, the FR2 and FR5 treatments showed the highest and the lowest anthocyanin amounts in the G1 genotype, respectively. Besides, the FR2 treatment promoted a high anthocyanin content, followed by FR3, FR1, and FR4 treatments, and in the G1 genotype (Table 6). The G2 genotype reflected similar results at Tehran, where there was no significant difference between the fertilizer regimes treatments. According to the gained results, the fertilizer regime application reflected effects on the G2 genotype.

Xylose

The main effects of the fertilizer regime at both locations and the two-way interaction between them (genotype × bacterial inoculation) and (genotype × fertilizer regime) were significant for xylose at Tehran and Khoy, respectively (Table 3). At Khoy, the FR3 application reflected the highest xylose content in both genotypes. In the G1 genotype, all treatments with azocompost promoted significantly higher xylose content when compared with only nitrogen fertilizer (Table 6). In Tehran, the bacterial inoculation of the G1 genotype significantly increased the concentration of xylose, while for the G2 genotype, there was no significant difference between treatments with or without bacterial inoculation (Table 8). At Tehran, the FR3 treatment promoted the highest content of xylose (4.35 μg g^{-1} FW), showing that compost is reliable compared to the chemical nitrogen effects associated with increased xylose (Table 9).

Glucose

The main effects of genotype and bacterial inoculation and the two-way interaction (genotype × fertilizer) were significant for glucose at Tehran (Table 3). In Tehran, the FR4 fertilizer treatment produced the highest glucose content in the G1 genotype (Table 6). In addition, FR4 and FR5 treatments in both genotypes were at the same statistical levels. The FR5 and FR1 treatments produced the highest and lowest glucose contents for the G2 genotype, respectively (Table 6). It seems that the effect of azocompost on glucose amount was higher for the G2 genotype than for the G1, which produced a higher amount of glucose compared with the G2 genotype at Khoy (data not shown). In both localities, glucose content evidenced significant changes among the main factors (Table 9). At the Tehran site, the bacterial inoculation treatment significantly increased glucose content, improving it for both genotypes. According to the results, applying the FR3 and FR1 treatments produced the highest and the lowest amounts of glucose at Khoy. Hence, we suggest azocompost to enhance glucose at Khoy (Table 9).

Mannose

Results of the ANOVA showed that mannose concentration was affected by the genotype at Tehran (Table 3). The main effects of the fertilizer regime and two-way interaction (genotype × fertilizer regime) were significant for

Table 5 Mean comparison of bacterial inoculation and fertilizer regimes (FR) for phosphorus content and essential oil yield of dragonhead plants

Bacterial inoculation	Fertilizer regimes	Phosphorus (% DW)	Essential oil yield (kg ha^{-1})
		Khoy	
Azotobacter+ Azospirillum +Pseudomonas	FR1	0.167 ^{c}	21.7 ^{a}
	FR2	0.173 ^{b}	15.8 ^{bc}
	FR3	0.169 ^{bc}	23.4 ^{a}
	FR4	0.170 ^{bc}	20.8 ^{ab}
	FR5	0.180 ^{a}	16.03 ^{bc}
Without inoculation	FR1	0.169 ^{bc}	20.1 ^{ab}
	FR2	0.166 ^{c}	21.4 ^{a}
	FR3	0.174 ^{b}	23.3 ^{a}
	FR4	0.171 ^{bc}	14.8 ^{c}
	FR5	0.175 ^{ab}	16.03 ^{bc}

FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}). Means, in each column and genotype, followed by similar letter(s) are not significantly different at the 5% probability levelusing Least Significant Difference (LSD)

Table 6 Mean comparison of genotype (G) and fertilizer regimes (FR) for phosphorous content, photosynthetic pigments, solution sugars and essential oil yield of dragonhead plants

Genotype	Fertilizer regimes	P (% DW)	Tchl (mg g^{-1} FW)	CAR (mg g^{-1} FW)	ANT (mg g^{-1} FW)	ANT (mg g^{-1} FW)	Xylose (µg.g^{-1} FW)	Glucose (µg.g^{-1} FW)	Mannose (µg.g^{-1} FW)	EOY (kg ha^{-1})
		Khoy	Tehran	Tehran	Tehran	Khoy	Khoy	Tehran	Khoy	Tehran
G1	FR1	0.167 ^{de}	1.53 ^{a}	0.30 ^{a}	6.50 ^{a}	6.89 ^{ab}	2.68 ^{cde}	4.64 ^{bcd}	2.40 ^{de}	31.2 ^{a}
	FR2	0.174 ^{bc}	1.21 ^{b}	0.26 ^{b}	4.67 ^{c}	7.29 ^{a}	3.30 ^{bcd}	4.67 ^{bcd}	2.84 ^{bcde}	24.6 ^{bc}
	FR3	0.170 ^{bcde}	1.19 ^{b}	0.24 ^{b}	5.02 ^{bc}	7.15 ^{a}	3.70 ^{b}	4.41 ^{cd}	3.58 ^{bc}	24.5 ^{bc}
	FR4	0.169 ^{bcde}	1.14 ^{bc}	0.24 ^{b}	5.28 ^{bc}	6.31 ^{ab}	3.29 ^{bcd}	5.06 ^{abc}	2.75 ^{de}	18.2 ^{d}
	FR5	0.175 ^{ab}	0.82 ^{e}	0.19 ^{c}	4.69 ^{c}	5.53 ^{b}	3.57 ^{b}	4.38 ^{cd}	3.62 ^{ab}	17.3 ^{de}
G2	FR1	0.168 ^{cde}	1.21 ^{b}	0.27 ^{ab}	5.32 ^{bc}	6.68 ^{ab}	3.39 ^{bc}	4.26 ^{d}	3.18 ^{bcd}	24.8 ^{bc}
	FR2	0.166 ^{e}	1.09 ^{bcd}	0.25 ^{b}	5.17 ^{bc}	6.28 ^{ab}	3.13 ^{bcde}	4.60 ^{bcd}	2.78 ^{cde}	22.6 ^{c}
	FR3	0.173 ^{bcd}	1.03 ^{bcd}	0.26 ^{b}	5.52 ^{b}	5.98 ^{ab}	4.83 ^{a}	5.17 ^{ab}	4.43 ^{a}	25.7 ^{b}
	FR4	0.172 ^{bcd}	0.99 ^{cde}	0.25 ^{b}	4.72 ^{bc}	6.45 ^{ab}	2.56 ^{e}	5.14 ^{ab}	2.30 ^{e}	16.1 ^{de}
	FR5	0.180 ^{a}	0.92 ^{de}	0.24 ^{b}	4.80 ^{bc}	7.14 ^{a}	2.65 ^{de}	5.60 ^{a}	2.32 ^{e}	15.5 ^{e}

P, phosphorus content in plant; *Tchl*, total chlorophyll content; *CAR*, carotenoid content; *ANT*, anthocyanin content; *EOY*, essential oil yield

G1: Landrace genotype, i.e. Orimieh; G2: a modern cultivar SZK-1; FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}). Means, in each column followed by similar letter(s) are not significantly different at the 5% probability level-using Least Significant Difference (LSD)

mannose at Khoy (Table 3). At Tehran, the G2 genotype promoted a higher mannose content when compared to the G1 genotype (Table 9). We detected a significant difference between the fertilizer regimes. At Tehran, the highest and lowest mannose content were recorded with the FR5 and FR2 treatments, respectively (Table 9). In this context, we infer that applying azocompost integrated with urea enhanced the mannose content. At Khoy, the FR5 and FR1 treatments produced the highest and the lowest mannose contents in the G1 genotype (Table 6). In addition, the FR3 treatment reflected a high value, followed by FR2 in the G1 genotype. In the G2 genotype, the FR3 treatment produced the highest mannose content compared to the other treatments (Table 6). At Tehran, the treatments FR5 (3.74 µg g^{-1} FW) and FR3 (4.01 µg g^{-1} FW), and at Khoy FR5 (2.97 µg g^{-1} FW) produced higher mannose content compared to the chemical nitrogen fertilizer regime (Table 9). In addition, we observed similar results for mannose content as for glucose and xylose when using azocompost with improved concentrations. According to Kolton and Baran (2008), compost application increased the carbohydrate content in the extracted solution in Valerianella locustain compared to the chemical fertilizer treatment.

Essential oil yield

Analysis of variance showed that essential oil yield was affected by genotype and fertilization regime as well as the two-way interaction (genotype × bacterial inoculation) and (genotype × fertilization regime) at Tehran. At Khoy, besides the genotype and fertilization regime, the two-way interaction (genotype × fertilization regime) was significant for essential oil yield (Table 3). The FR3 treatment evidenced the highest essential-oil content in both bacterial inoculation

Table 7 Mean comparison of fertilizer regimes (FR) for photosynthesis pigments of dragonhead plants

Fertilizer regimes	Total chlorophyll (mg g^{-1} FW)	Total cartenoid (mg g^{-1} FW)
	Khoy	Khoy
FR1	2.143 ^{a}	0.438 ^{a}
FR2	2.084^{ab}	0.399^{ab}
FR3	2.130^{a}	0.378 ^{bc}
FR4	1.840 ^{b}	0.355 ^{c}
FR5	1.454 ^{c}	0.305^{d}

FR1: 100% urea (70 kg N ha^{-1}), FR2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), FR3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}), FR4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and FR5: 100% azocompost (15.55 ton ha^{-1}). Means, in each column, followed by similar letter(s) are not significantly different at the 5% probability level using Least Significant Difference (LSD)

Table 8 Mean comparison of genotype (G) and bacterial inoculation for total flavonoids content, xylose and essential oil yield of dragonhead plants

Genotype	Bacterial inoculation	Total flavonoids content (mmol g^{-1} FW)	Xylose (µg g^{-1} FW)	Essential oil yield (kg ha^{-1})
		Khoy	Tehran	Tehran
G1	*Azotobacter+ Azospirillum +Pseudomonas*	393.1 [a]	4.25 [a]	24.8 [a]
	Without inoculation	381.6 [a]	3.48 [b]	21.6 [b]
G2	*Azotobacter+ Azospirillum +Pseudomonas*	398.3 [a]	3.96 [ab]	19.8 [c]
	Without inoculation	344.4 [b]	4.18 [a]	22.1 [b]

G1: Landrace genotype, i.e. Orimieh; G2: a modern cultivar SZK-1. Means, in each column, followed by similar letter(s) are not significantly different at the 5% probability level using Least Significant Difference (LSD)

treatments (with or without bacterial inoculation) (Table 5). In addition, the lowest essential oil yield with or without bacterial inoculation was produced at FR2 (15.8 kg ha^{-1}) and FR4 (14.8 kg ha^{-1}) treatments, respectively (Table 5). Two-way interaction between genotypes and fertilizer regime showed that, in the G1 genotype, the FR1 treatment produced the highest essential oil yield and the FR5 treatment the lowest (Table 6). In addition, the FR3 treatment promoted in G2 a high value of essential-oil production, showing similar levels as those for the FR1 treatment. For the G2 genotype, the highest (25.7 kg ha^{-1}) and the lowest (15.5 kg ha^{-1}) essential oil yields were registered with FR3 and FR5 treatments, respectively (Table 6). At Tehran, considering an average of fertilizers treatments, essential-oil production for the G1 genotype was significantly higher than that for the G2 genotype (Table 6). Results at Tehran suggest that the G1 genotype (with bacterial inoculation) produced a higher yield for essential oil when compared to the G2 genotype, but we did not find a significant difference between results for G1 and G2 genotypes without bacterial inoculation (Table 8).

Discussion

The G1 genotype at Tehran and the G2 genotype at Khoy accumulated higher nitrogen amounts. It seems that difference in climatic and edaphic conditions between locations was one of the factors that affected nitrogen contents determined in plants. Results showed that treatments FR1 and FR2 produced higher percentages of nitrogen in plant organs in Tehran and Khoy, respectively. We infer that the nitrogen chemical fertilizer was more easily absorbed than the organic nitrogen amender. Moreover, available nitrogen in azocompost was released gradually into the soil, and this caused reduced nitrogen absorption in the FR5 treatment. The integrated treatment (FR3) reflected similar results as the FR1 treatment, suggesting that the amount of nitrogen in

Table 9 Mean comparison of genotype (G), bacterial inoculation (B) and fertilizer regimes (FR) for solution carbohydrate of dragonhead plants in both locations

G	B	FR	Xylose (µg g^{-1} FW)		Glucose (µg g^{-1} FW)		Mannose (µg g^{-1} FW)	
			Tehran	Khoy	Tehran	Khoy	Tehran	Khoy
G1			3.86 [a]	3.31 [a]	4.64 [b]	4.63 [a]	3.24 [b]	3.04 [a]
G2			4.07 [a]	3.32 [a]	4.96 [a]	4.38 [b]	3.73 [a]	3.005 [a]
	B1		4.10 [a]	3.36 [a]	4.98 [a]	4.66 [a]	3.65 [a]	3.03 [a]
	B2		3.83 [a]	3.27 [a]	4.62 [b]	4.35 [b]	3.32 [a]	3.02 [a]
		FR1	3.59 [b]	3.04 [b]	4.45 [b]	4.24 [b]	3.15 [b]	2.69 [b]
		FR2	3.65 [b]	3.21 [b]	3.64 [ab]	4.45 [b]	3.3[a b]	2.91 [b]
		FR3	4.35 [a]	4.26 [a]	4.79 [ab]	4.97 [a]	3.53 [ab]	4.01 [a]
		FR4	4.08 [ab]	2.93 [b]	5.10 [a]	4.43 [b]	3.72 [ab]	2.53 [b]
		FR5	4.15 [ab]	3.11 [b]	4.99 [a]	4.44 [b]	3.74 [a]	2.97[b]

G1: Landrace genotype, i.e. Orimieh; G2: a modern cultivar SZK-1; B1: bacterial inoculation with *Azotobacter+Azospirillum +Pseudomonas*, B2: without inoculation; F1: 100% urea (70 kg N ha^{-1}), F2: 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}), F3: 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}), F4: 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}) and F5: 100% azocompost (15.55 ton ha^{-1}). Means, in each column and treatment, followed by similar letter(s) are not significantly different at the 5% probability level-using Least Significant Difference (LSD) plant organs increased at Tehran. Treatment of integration of nitrogen fertilizer and azocompost, due to the addition of nitrogen to the soil, improved soil conditions, enhanced root growth, supplied moisture, and reduced nitrate leaching that caused an increase of nitrogen absorption by plants. The related literature showed that increasing compost and nitrogen levels or application-integrated fertilizers (a combination of organic and inorganic fertilizers) enhanced nitrogen content in medicinal plants (Keshavarz et al., 2018).

Results demonstrate that increasing the amount of phosphorus in plant organs contributed to a better P supply from organic fertilizer (azocompost). Similar previous findings were reported elsewhere in other research (Bagheri Novair et al., 2020). Due to higher P concentration in soil at Tehran, phosphate solubilizing bacteria did not evidence a remarkable effect on P absorption. Keshavarz (2020) reported that the main PGPR's influence on the supply of nutrients in poor soil was more efficient than in richer soil. Improvement of soil properties and nutrient availability by mycorrhiza, particularly in soils with low-P, is mainly attributed to extraradical hyphae making available the inaccessible water and nutrient to the root system. We found that improving the soil conditions might result in better biological and economic yield (Keshavarz and Sadegh Ghol Moghadam, 2017).

At Khoy, the G2 genotype accumulated more phosphorus in the aerial part of the plant (except FR2 treatment) compared to the G1 genotype (Table 6). It seems that the absorption ability of P in the G2 genotype was higher than in G1. Moreover, based on our results, the highest and the lowest amounts of P-percentage (in bacterial inoculation treatment) were obtained in FR5 (0.18%) and FR1 (0.16%) treatments, respectively (Table 5). On the other hand, in the treatment without inoculation, the highest amount of P-percentage was gained at 0.17% for FR5 (Table 5). We infer that a high level of azocompost had increased *Pseudomonas* bacterial activity and improved the supply of phosphorus to the soil. In addition, *Pseudomonas* bacteria, through converting phosphorus to its available form, increased absorption by the plant.

Bacterial inoculation showed that there was no significant effect on potassium content in plant organs at both locations of Tehran and Khoy. It appears that the type of bacteria was not viable for facilitating the release and uptake of P in soil. The authors reported that the effect of PGPR is affected by species and genotype (Keshavarz, 2020), soil type, and agronomic practices such as fertilizer application (Stoll et al., 2021). Organic fertilizers, as soil amenders, improve beneficial microorganisms' activities in the root zone, increase bacteria survival that stimulates plant growth and increase soil enzyme activities such as phosphatase and catalase (Keshavarz et al., 2018). Furthermore, using mycorrhiza is a proper method to increase soil fertility by providing the required mineral nutrients via two mechanisms: (1) Due to the extension of the hyphae network and root surface. (2) The increased total canopy transpiration accelerates the mass flow of these nutrients through the soil to the roots of inoculated plants (Keshavarz, 2020). We conclude that increasing the level of azocompost in integrated fertilizer improved the amounts of potassium in plant organs at both locations. In addition, azocompost, due to the improved condition of the rhizosphere, contributed to increased root growth and soil moisture content that enhanced potassium uptake (Vafadar-Yengeje et al., 2019).

Applying chemical N at a high level (FR1) compared to other fertilizers levels (especially FR5 treatment) increased the concentration of chlorophylls. The chlorophyll content diminished according to lower levels of urea application. In this context, an easy uptake of chemical N facilitated increased chlorophyll content in plant leaves. The authors reported that N is one of the most relevant factors in the structure of chlorophyll, amino acids, proteins, nucleic acids, and enzymes, in which reduction of N supply decreases the chlorophyll content in plant leaves. Based on our results, integrated fertilizer improved chlorophyll content in plants relative to sole azocompost treatment. Sotiropoulou and Karamanos (2010) observed higher and lower chlorophyll-a and b content in the chemical and organic fertilizer treatments.

Lower urea content in integrated fertilizer resulted in lower carotenoid concentration, while the integration of 50% azocompost + 50% nitrogen (FR3) compared with other fertilizer regimes (FR1 and FR5) could produce high total carotenoids (Table 7). Kopsell (2007) reported that the application of 105 mg L^{-1} nitrogen rather than other levels (6, 13, 26, and 52 mg L^{-1}) produced the highest amounts of lutein and -βcarotene in the Kale plant (*Brassica oleracea*). The related literature showed that increasing compost and nitrogen levels enhanced carotenoid in wavy-leaved saltbush (Li and Chang, 2021), pepper (*Capsicum annuum* L.) (Fiasconaro et al., 2019) and tomato (*Lycopersicon esculentum* L.) (Ahmadpour and Armand, 2020).

Flavonoids are one of the original components of dragonhead. The concentration of flavonoids in different plant species correlates to growth stage, variety, environmental conditions, soil properties, tillage, pest, disease, and fertilizers (Keshavarz et al., 2016). Keshavarz et al. (2021) explained the flavonoid synthesis associated with N deficiency in the Shikimate path. Higher N increased the amount of protein and amino acid and diminished the synthesis of flavonoids (Dahui et al., 2010; Miri et al., 2022). Keshavarz and Khodabin (2019) reported that limitations in the supply of nutrients and the high presence of pests and diseases in organic farming techniques compared to traditional agriculture are one of the main reasons for increased metabolite biosynthesis of flavonoids. Roteen concentration (one of the relevant flavonoids in Fagopirum) grown under organic conditions showed a significant increase in Fagopirum compared to results for conventional agriculture. Results of these tests showed that applying a high concentration of N decreased the content of flavonoids, while azocompost increased flavonoid content in plants. It seems that this increase in flavonoids was due to the condition of the rhizosphere that facilitated the supply of macro and micro elements by azocompost. According to Ebrahimian et al. (2021), applying nutrients, including K, Ca, Zn, and Cu, improved the flavonoid content in milk thistle (*Silybum marianum* L.). Nitrogen application at a high dose reduced the flavonoid content by 18–35% in *Chrysanthemum morifolium* (Bernard et al., 2020). However, the positive effects of organic fertilizers and organic and chemical fertilizer combinations on flavonoids were reported a time ago by Martinez-Blanco et al. (2011) in Cauliflower patchouli, Saikia and Upadhyaya (2011) in *A. racemosus*, Willd, and Shiow (2008) in strawberry.

About the total flavonoid content, it seems that the combination of 50% urea + 50% azocompost (FR3) was a suitable fertilizer regime at Khoy. Martinez-Blanco et al. (2011) reported that applying integrated compost and chemical fertilizer relative to chemical fertilizer enhanced total flavonoids in cauliflower. At Khoy, total flavonoids increased in the G1 genotype (with bacterial inoculation) but, for this case, we did not detect a significant difference between treatments with or without bacterial inoculation. In contrast, for the G2 genotype, the bacterial inoculation improved the total flavonoid content compared to the treatment without it (Table 8). We noticed that the bacterial inoculation enhanced the properties of the rhizosphere and facilitated the supply of nutrients that increased total flavonoids. According to Anwar AlyTaie et al. (2010), compost application and integration of compost with PGPR, due to N fixation and an ability to release nutrients, such as P and Fe, significantly increased total flavonoids compared with chemical fertilizer treatment in basil.

At Tehran, the application of chemical nitrogen improved total anthocyanin. According to Kawa-Miszczak et al. (2009), the application of a high level of nitrogen and low temperature increased anthocyanin concentration. In addition, Del Amor and Cuadra-Crespo (2008) reported that foliar application of urea to sweet pepper increased total anthocyanin compared with no application of urea. At Khoy, the application of integrated azocompost and urea relative to the sole application of azocompost improved total anthocyanin. We conclude that N application had a positive effect on the G1 genotype, and because of a reduction in urea percentage, the anthocyanin content decreased.

At Tehran, the FR3 treatment produced the highest amount of xylose (4.35 μg g^{-1} FW), showing that organic fertilizer combined with the chemical fertilizer increased the xylose concentration (Table 9). Therefore, FR3 was a suitable treatment for high xylose content production in plants at both locations. Azocompost, due to its optimum effect on plant growth, enzyme activity, and preparation of macro and microelements for plants, enhanced amounts of xylose. According to Hussein et al. (2006), higher content of organic fertilizer enhanced the total carbohydrate in dragonhead. The effect of the bacterial inoculation was positive on xylose (especially in the G1 genotype at Tehran). Keshavarz (2020) reported that compost and PGPR improved the root surface, water use efficiency, photosynthesis activity, and increased carbohydrate amount.

The effect of genotype was different in each location. Perhaps, this difference was related to differences in climatic and soil properties. The application of PGPR increased glucose content in both localities. Integrating the azocompost and N could enhance glucose content (except in the G1 genotype at Tehran) compared to the sole chemical nitrogen. It seems that the integration of both, azocompost with N, had a synergic effect on growth and photosynthesis activity, improving the soil condition, a primary issue to increase the supply of nutrients to obtain more glucose content. Kolton and Baran (2008) reported a positive effect of compost on carbohydrates.

For the G2 genotype, the FR3 treatment produced the highest amount of mannose compared to the other treatments. The FR5 treatment applied at Tehran locations, also FR3 and FR5, promoted higher mannose content compared to the solo chemical fertilizer at Khoy. The same results were achieved for mannose concentration as for glucose and xylose in that azocompost application. According to Kolton and Baran (2008), compost increased the carbohydrates solution in Valerianella locustain compared to the chemical fertilizer.

The G1 genotype reflected a higher essential-oil production than the G2 genotype at both locations. It seems that adaptation of the G1 genotype was high. Studies showed that PGPR could enhance the essential oil content in medicinal plants (Gharib et al., 2008). With due attention to the high dry weight yield and percentage of the essential-oil production (data not shown), it is reasonable to expect an increased essential oil yield in FR1 and FR3 treatments. Nitrogen deficiency in the FR5 treatment reduced the essential-oil content. A review of the related literature showed that nitrogen deficiency reduced essential oil yield because of reductions in photosynthesis, chlorophyll content and Rubisco activity, biomass yield, plant growth, and leaf area (Keshavarz Mirzamohammadi et al., 2021b). It seems that FR3 supplied the necessary micro and macro elements in the soil to increase organic carbon content, increase the rhizosphere dynamic and accelerate enzyme activity, processes that served to increase essential oil yield. Moreover, the positive effect of organic fertilizer and combined organic and chemical fertilizer application on essential-oil production have been reported by Keshavarz et al. (2018) in peppermint (*Mentha piperita* L.).

Conclusion

In summary, this study showed that combined azocompost and nitrogen fertilizer improved essential oil yield, total flavonoids, solution carbohydrates, and P and K contents of dragonhead. The application of PGPR reflected a positive effect on some traits, but those effects were weaker than that of the fertilizer regime. As expected, the application of chemical nitrogen increased total nitrogen and photosynthesis pigment rather than the organic treatment application in plants at both locations; nevertheless, we did not find a significant difference between the integrated fertilizer regime at the medium level and the control treatment on chlorophyll and carotenoid contents at Khoy. The application of Azolla and its compost (azocompost) is one of the best-known approaches for decreasing the cost of nitrogen fertilizer and minimizing environmental pollution. Integrated nitrogen fertilizer and Azolla compost improve the essential oil yield, flavonoids, and solution carbohydrate contents in the studied dragonhead plants.

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Author contribution Saeed Yousefzadeh: designed and performed the experiment. Hamed Keshavarz wrote the manuscript. Seyed Ali Mohammad Modares Sanavy: farm operator. All the authors have read and approved the final manuscript and agreed to the published version of the manuscript.

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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