



Intercropping and fertilizer type impact seed productivity and secondary metabolites of dragon's head and fenugreek

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ABSTRACT

Intercropping of medicinal plants is a relatively new practice to enhance sustainability in medicinal plant production. However, only a few studies paid attention to response of secondary metabolites of medicinal plants to organic fertilizers and bio-fertilizers in intercropping system. Here, we evaluated the effects of different fertilizer types (bacterial fertilizer, mycorrhizal fungi and vermicompost) plus an unfertilized control, in factorial combination with a monocrop or various intercropping ratios on seed and oil yields, root colonization, nutrient contents and secondary metabolites of dragon's head (DH) and fenugreek (F) in a two-year experiment. Organic and bio-fertilizers were effective in increasing the seed and fixed oil yields of dragon's head and fenugreek, essential oil of dragon's heads and the diosgenin and trigonelline content of fenugreek mainly due to enhanced nutrient uptake and root colonization. Across intercropping systems, bacterial and vermicompost fertilizers resulted in highest values for most parameters, followed by mycorrhizal and unfertilized control. Total land equivalent ratio (LER) for seed yield of DH and F was >1 in all comparisons, implying that intercropping always performed better than monocropping systems of both species. In the comparison among the intercropping systems, the highest seed, fixed and essential oil contents in dragon's head were attained with the 75DH:25F followed by the 60DH:40F intercropping ratios. The highest values of most secondary metabolites and oil compounds were obtained from the intercropping ratio of 60DH:40F with the bacterial biofertilizer. The main fatty acids in DH's oil were saturated palmitic acid and stearic acid and unsaturated oleic and linoleic acids. The main essential oil components of DH were thymol, carvacrol, spathulenol, and caryophyllene oxide. The major fatty acids of F were palmitic, linoleic, oleic, linolenic and stearic acids. The present research suggests that different intercropping system ratios with the application of organic fertilizers and biofertilizers represent an effective strategy to enhance the overall seed and oil yields and the secondary metabolites of dragon's heads and fenugreeks.

1. Introduction

Dragon's head (*Lallemantia iberica* L.) is an annual herb in the Lamaceae family (Khosravi Dehaghi et al., 2016) that is cultivated in different regions of Iran. The seeds of this species contain fixed oil, essential oil and mucilage. The plant has been traditionally used to improve the body immune system and for the treatment of dysentery, constipation, dry cough, promotion of good heart and brain functions,

and as a tranquilizer (Amanzadeh et al., 2011).

Fenugreek (*Trigonella foenum-graecum* L.) is an annual medicinal plant that belongs to the Fabaceae family (Hassanzadeh et al., 2011). The plant ability to fix atmospheric N, a main limiting nutrient in diverse production systems around the world (Adeyemi et al., 2020; Kumar et al., 2019; Diatta et al., 2020b), has been highlighted as a critical component for pursuing more sustainable agroecosystems (Bahmani et al., 2016). The seeds of fenugreek contain alkaloids like trigonelline

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and diosgenin, mucilage, proteins, and fixed oil (Wani and Kumar, 2018). Trigonelline is one of the main alkaloids in the plant and has been recognized for its positive properties against cancer, migraine, infections, high blood cholesterol, and diabetes (Khoja et al., 2011).

Intercropping, defined as the use of a field for growing two or more crops next to each other at the same time, can play an important role in achieving more diverse and sustainable systems (Rezaei-Chiyaneh et al., 2021b), and could be practiced in developing countries to increase the income of farmers per time and area unit (Lithourgidis et al., 2011). Current adoption of intercropping around the world is on the rise due to its diverse advantages, including better nutrient uptake (Alcon et al., 2020) and weed control (Weeraratne et al., 2017), enhancement of soil microbial activity (Gao et al., 2019), and soil fertility through N fixation (Kermah et al., 2018). Evidence shows that intercropping non N-fixing medicinal plants with legumes may improve the quantity and quality of their metabolites compared to monocropping, as a result of the N fixation from legumes and the concomitant increase in the resource use efficiency (Rezaei-Chiyaneh et al., 2020a; Jensen et al., 2020).

In high yielding conventional farming, farmers tend to apply fertilizers in excess to fulfill the high nutrient uptake of crops (Diatta et al., 2020a). However, this approach is expensive and has a detrimental impact on plant root expansion due to reducing soil permeability (Lin et al., 2019) and generates environmental concerns (Fathi et al., 2018; Czymmek et al., 2020; Battaglia et al., 2021). The mitigation of these environmental concerns, along with improvements in plant productivity and quality, requires the application of eco-friendly fertilizers in sustainable agricultural systems (Ostadi et al., 2020; Seleiman et al., 2021).

Biofertilizers are composed of beneficial bacteria or mycorrhizal fungi that improve plant nutrition by nitrogen fixation, phosphate dissolution, potassium ion release, sulfur oxidation, and ion uptake (Rashid et al., 2016). Currently, application of plant growth-promoting rhizobacteria (PGPR), phosphate solubilizing bacteria (PSB) and mycorrhizal fungi is increasing at a fast pace (Adnan et al., 2020). These bio-fertilizers can improve the quantitative and qualitative performances of medicinal and aromatic plants (Rezaei-Chiyaneh et al., 2020b).

The use of organic fertilizers such as vermicompost, timely supplies the crop with its nutrient requirements and increases the support and activity of beneficial soil microorganisms. As a result of these beneficial agronomic and environmental impacts, application of organic fertilizers in the cultivation of medicinal herbs has received increased attention in the last decade (Vafadar-Yengeje et al., 2019; Rezaei-Chiyaneh et al., 2021a).

Research on the effect of biofertilizers like bacterial fertilizer and mycorrhizal fungi, and organic fertilizers like vermicompost on seed yield and secondary metabolites production in dragon's head and fenugreek plants grown in intercropping systems has not drawn adequate interest up to date. Therefore, we conducted the present research to elucidate to which extent these practices can impact future choices of sustainable management in medicinal and aromatic plants systems. In particular, we assessed the interactions between different fertilizer types (bio or organic) and intercropping systems on seed yield, nutrient content, oil fatty acid composition, as well as concentration of bioactive compounds and land-use efficiency of dragon's head and fenugreek.

2. Materials and methods

2.1. Research site

The experiment was conducted in a farm located in Naqadeh, West Azerbaijan province, Iran in the 2017 and 2018 growing seasons. Based on physical and chemical properties determined from baseline soil samples (0–30-cm), soil texture was found to be silty clay with a pH of 7.8, electrical conductivity (EC) was 0.81 dS m^{-1} , organic matter content of 0.95%, and total N, P and K contents were 0.09%, 11.21 and

241 mg kg^{-1} , respectively. Weather data, including average precipitation, and air temperature of the research site from March to August in 2017 and 2018 was obtained from the Iran Meteorological Organization (<https://www.irimo.ir/eng/index.php>) (Table 1), while the chemical specification of the vermicompost is presented in Table 2.

2.2. Plant materials and seed yield of dragon's head and fenugreek

The experiment used a randomized complete block design (RCBD) with factorial arrangement of treatments and three replications. Two factors were investigated in this study. The first factor was cropping ratio between dragon's head (DH) and fenugreek (F), with 6 ratio-levels: 50DH:50F (1 row DH + 1 row F); 60DH:40F (3 rows DH + 2 rows F); 75DH:25F (3 rows DH + 1 row F); 25DH:75F (1 row DH + 3 rows F); 100DH:0F (dragon's head monocropping); and 0BC:100F (fenugreek monocropping). The second factor was fertilizer type, with 4 levels: bacterial (B) fertilizer [mix of two PSB (*Pantoea agglomerans* and *Pseudomonas putida*) and nitrogen-fixing bacteria (NFB) *Azotobacter vinelandii*]; mycorrhizal (M) fungi (mix of three mycorrhizal fungi, *Funneliformis mosseae*, *Rhizophagus irregularis* and *Claroideoglomus etunicatum*); vermicompost (V) (10 Mg ha^{-1}); and unfertilized control.

For bacterial fertilizers treatment, the seeds of both species were inoculated with the combination of PSB and NFB in the form of powder at a rate of 100 g ha^{-1} (Zist Fanavar Sabz Company, Iran). The bacterial population in the sample was 5×10^9 colony forming units (CFU) g^{-1} . Bacterial fertilizer powder was mixed with water and uniformly sprayed to cover the seed, and then seeds were air-dried. Prior to planting, vermicompost was hand applied at a rate of 10 Mg ha^{-1} . Each g of inoculum media contained 35 living spores of *F. mosseae*, 35 living spores of *R. irregularis* and 30 living spores of *C. etunicatum*; therefore, one g of inoculum contained 100 spores of the mycorrhizal fungi. At planting, 30 g of the inoculum, containing 3000 spores of *F. mosseae*, *R. irregularis* and *C. etunicatum*, was poured into each planting hole.

Seeds were planted on March 19, 2017 and March 20, 2018 at 40-cm row width for both species, with an on-row seed spacing of 2.5 cm for DH and 7.5 cm for F. Each plot was 3 m in length and 4 m wide. Final seed rate in the monocropping was 100 and 33 seeds m^{-2} for DH and F, respectively. Irrigation was applied every 10 d during the whole growing season. No herbicides were applied and weeds were removed by hand. Prior to harvesting, the external not harvestable rows at each experimental plot were cut at the ground level and biomass was discarded. At maturity, the four central rows (4 m^{-2}) at each plot were harvested for seed yield on August 11, 2017 and August 7, 2018.

2.3. Essential oil extraction

The essential oil of DH seeds was extracted from a 15 g seed sample with the water distillation method 3 h after the beginning of the distillation process. Extracted essential oils were dried using anhydrous sodium sulfate and then stored at 4°C in glass containers until further analysis (Morshedloo et al., 2017). The essential oil content and

Table 1

Weather data for the period from March through August in 2017 and 2018 in the study site at Naqadeh, Iran.

Year	March	April	May	June	July	August
Monthly average temperature ($^\circ\text{C}$)						
2017	8	14.5	20.8	25.6	28.8	28.6
2018	12.8	16.3	25.5	29.9	32.8	26.1
2-yr avg.	10.4	15.4	23.15	27.75	30.8	27.35
10-yr avg.	8.9	14.4	19.5	24.6	27.4	27.1
Monthly average precipitation (mm)						
2017	38.1	38.6	12.8	1.7	2.2	0.6
2018	78.6	55.5	20.5	0.0	0.2	0.0
2-yr avg.	58.35	47.05	33.3	0.85	1.2	0.3
10-yr avg.	55.6	43.2	28.4	3.4	2.9	2.5

Table 2

The chemical properties of vermicompost (average of two years).

pH	EC(dS.m ⁻¹)	Organic matter (%)	Total N(%)	Phosphorus (%)	Potassium (%)	Ca (%)	Mg (%)	Fe (%)	Cu (%)	Zn (%)	Mn (%)
7.2 ± 0.12	3.4 ± 0.08	8.5 ± 0.42	1.28±0.08	1.46±0.032	0.98±0.034	2.47±0.09	0.4 ± 0.023	0.6 ± 0.005	0.2 ± 0.001	0.9 ± 0.007	0.6 ± 0.003

essential oil yield were calculated as follows (Amani Machiani et al., 2019):

$$\text{Essential oil content (\%)} = \frac{\text{Extracted essential oil (g)}}{15 \text{ g of dragon's head ground sample}} \times 100$$

Then, the essential oil yield of DH (kg ha⁻¹) was calculated by multiplying the seed yield (kg ha⁻¹) with the essential oil content (%).

2.4. Essential oil analysis

Gas chromatography-mass spectrometry analysis was performed with an Agilent 7890/5975A GC/MSD. For separation of essential oil components, a HP-5 MS capillary column (5% phenyl methyl polysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used. The following oven temperature was applied: 3 min at 80 °C, subsequently 8 °C min⁻¹ to 180 °C, held for 10 min at 180 °C. Helium was used as carrier gas at a flow rate of 1 ml min⁻¹. The sample was injected (1 µL) in split mode (ratio, 1:50). Electron impact mode was 70 Ev. The components were recognized by comparing the calculated Kovats retention indices (RIs), calculated respect to a mixture of n-alkane series, and the mass spectra (Adams, 2007). A gas chromatography–flame ionization detection analysis was done with an Agilent 7890 A instrument. The separation was performed in an HP-5 capillary column. The analytical conditions were the same as above. Details regarding quantification methods used in this study can be found in Morshedloo et al. (2017) and Rezaei-Chiyaneh et al. (2021a).

2.5. Fixed oil isolation

To extract the fixed oils from DH and F 5 g of ground seed samples were placed in a Soxhlet apparatus and extracted in 300 mL of *n*-hexane. After 6 h, the used solvent was isolated from the oil by a rotary evaporator. The extracted oil was stored in amber glass bottles and refrigerated until analysis. Then, oil yield was calculated as follows:

$$\text{Oil yield} = \text{seed yield (kg ha}^{-1}\text{)} \times \text{fixed oil content (\%)}$$

2.6. Oil analysis

The oil of DH and F were analyzed using Gas Chromatography-Mass Spectrometry (GC–MS) following the method reported by Rezaei-Chiyaneh et al. (2020).

2.7. Trigonelline analysis

A 3 g sample of the dried seeds was extracted with 100 mL of water at 80 °C during 1 h and then the extract was filtrated and adjusted to 250 mL with distilled water. The homogenates were centrifuged at 2000 rpm for 15 min. The resulting solution was diluted at 1:5 v/v (water: methanol) and 30 µL of the diluted solution was injected into an HPLC device (Unicam –200) through a 0.45-µm filter. A C18 column (4.6 mm, 25 cm i.d.) was used for separation and identification of trigonelline. Following, 3 mM of hydrochloric acid per l of water solution was used as the mobile phase (pH=2) at a flow rate of 1 mL min⁻¹. For identification and quantification of compounds, the pure standard was injected using five different concentrations (0.1, 0.2, 0.5, 0.7 and 1 µg mL⁻¹). Trigonelline standard material was prepared with code T5509 from Sigma-

Aldrich. The used wavelength and retention time for the compound was 267 nm and 20 min, respectively. Using the area under the curve in terms of concentration, a calibration graph was drawn for each standard sample (Dadrasan et al., 2015).

2.8. Diosgenin analysis

Diosgenin content in the extracts was obtained by using the modified method of Oncina et al. (2000). Approximately 10 mg of each extract was analyzed with a Hewlett Packard liquid chromatograph (Unicam –200) along with a diode-array detector in a wavelength range of 190–500 nm. Separation was performed by reverse phase chromatography using a Bondapak C18 (4.6 mm, 25 cm i.d.) column. Mobile phase was programmed using an isocratic system, with a mixture of acetonitrile: water (90:10 v/v) at 1 ml min⁻¹ flow at 35 °C. Alterations in absorbance at 214 nm were quantified by comparison with an external standard. To quantify diosgenin an external standard comparison was utilized.

2.9. Seeds nutrient analysis

Seed nutrient concentration was calculated by grinding 1 g of seeds (Weidhuner et al., 2019) to obtain a fine powder and then heating the powder at 600 °C for 5 h. The obtained ash was used to determine the absorption of nutrients. Nitrogen (N) content was determined by the Kjeldahl method and phosphorus (P) by the yellow method (Tandon et al., 1968).

2.10. Mycorrhizal root colonization

Root colonization percentage was determined using 10 plants per experimental plot. To determine root colonization for both plant species, 1 cm of the root bits were placed into a formalin-acetone alcohol (FAA) solution (13 ml formalin, 5 ml glacial acetic acid, 200 ml 50% ethanol) for 24 h. Samples were rinsed with distilled water and cleared in 10% KOH for 1 h at 90 °C. Following, roots were placed in 1% hydrochloric acid for 3 min. The samples were then stained with 0.05% trypan blue, boiled for 45 min, and placed in lactoglycerol for 24 h. The root colonization percentage was measured as the ratio between the number of root segments containing vesicles, arbuscules or hyphae and the total number of root segments sampled (Phillips and Hayman, 1970; Wang et al., 2019).

2.11. Land equivalent ratio (LER)

For DH and F intercropping, partial (LER_{DH} and LER_F) and total LER (LER_T) was calculated according to the following equations (Ofori and Stern 1987):

$$\text{LER}_{\text{DH}} = (\text{Y}_{\text{DHI}} / \text{Y}_{\text{DHS}}) \quad (1)$$

$$\text{LER}_{\text{F}} = (\text{Y}_{\text{FI}} / \text{Y}_{\text{FS}}) \quad (2)$$

$$\text{LER}_{\text{T}} = \text{LER}_{\text{DH}} + \text{LER}_{\text{F}} \quad (3)$$

Where, Y_{DHI} and Y_{DHS} are DH seed yield in intercropping and sole cropping, respectively. Y_{FI} and Y_{FS} represent F seed yield in intercropping and monocropping, respectively.

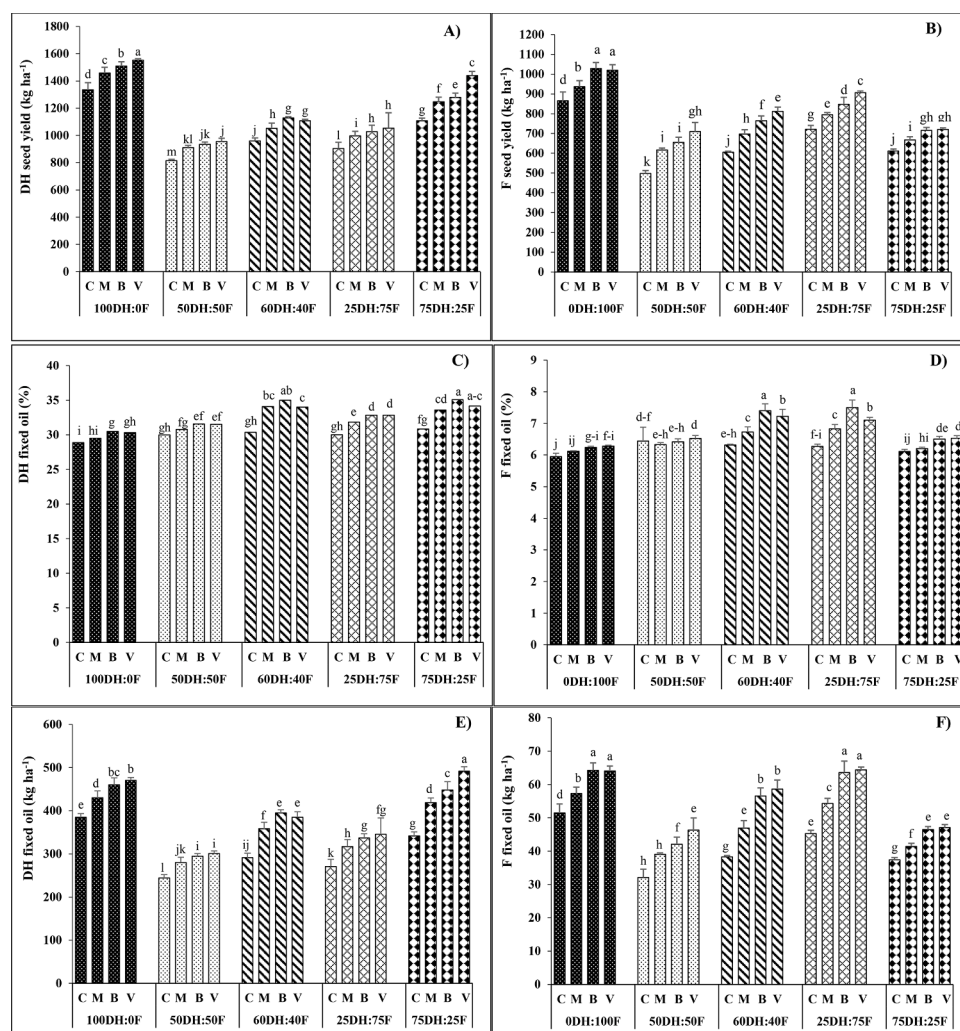


Fig. 1. Dragon's head and Fenugreek seed yield (A and B), fixed oil (C and D), and fixed oil yield (E and F), as influenced by different cropping patterns (100DH:0F; 0DH:100F; 50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F; where DH and F indicate the ratios of dragon's head and fenugreek in intercropping pattern) and fertilizer type [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. Different lower-case letters above bars indicate significant difference at the $P \leq 0.05$ level, according to Duncan's multiple range test.

2.12. Statistical analyses

Analysis of variance was performed using PROC Mixed procedures of SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Fertilizer type, intercropping ratio and year were considered as fixed effects, and blocks were considered random. Mean comparisons were performed with the Duncan's multiple range test at the $p < 0.05$ cut-off level.

3. Results

3.1. Seed yield

The main effects of cropping ratio and fertilizer type were significant ($p < 0.01$) on seed yield. In addition, the interaction between cropping ratio and fertilizer type was significant on seed yield of dragon's head (DH) and fenugreek (F) (Supplemental Table 1 and 2). Seed yield of

Table 3

Proportion of dragon's head oil constituents across treatments resulting from different intercropping systems and fertilizer types (average of two years). [†]

Component	100DH:0F				50DH:50F				60DH:40F	
	C	M	B	V	C	M	B	V	C	
Myristic acid	0.06±0.001	0.04±0.002	0.12±0.05	0.05±0.003	0.08±0.007	0.05±0.003	0.07±0.005	0.06±0.001	0.12±0.02	
Palmitic acid	6.33±0.14	6.63±0.17	8.96±0.21	7.3 ± 0.11	5.62±0.09	6.57±0.17	6.07±0.16	6.38±0.14	5.01±0.10	
Stearic acid	15.02±0.33	16.84±0.44	14.4 ± 0.46	15.37±0.42	11.06±0.26	13.96±0.55	11.85±0.36	11.32±0.22	12.77±0.30	
Oleic acid	14.65±0.38	15.76±0.34	15.02±0.44	15.68±0.47	12.04±0.24	13.68±0.56	12.35±0.30	12.13±0.29	14.07±0.56	
Linoleic acid	53.07±1.87	54±1.96	54.87±1.67	54.42±1.50	57.94±1.88	58.6 ± 2.01	59.74±1.82	60.8 ± 2.11	59.38±1.34	
Linolenic acid	0.8 ± 0.02	0.7 ± 0.04	1.03±0.09	0.68±0.02	0.67±0.07	0.76±0.01	0.62±0.02	0.72±0.01	0.74±0.04	
Arachidic acid	0.07±0.003	0.08±0.005	0.16±0.04	0.07±0.01	0.07±0.003	0.07±0.02	0.06±0.003	0.06±0.02	0.35±0.09	
Methyl linolenate acid	0.45±0.01	0.15±0.02	0.13±0.07	0.15±0.08	0.22±0.06	0.06±0.004	0.06±0.002	0.13±0.05	0.27±0.04	
Stearidonic acid	0.04±0.005	0.05±0.003	0.08±0.005	0.06±0.002	0.06±0.06	0.06±0.001	0.06±0.002	0.06±0.007	0.08±0.005	
Total identified (%)	90.49	94.25	94.77	93.78	87.76	93.81	90.88	91.66	92.79	

[†]C (Control), M (Mycorrhiza), B (Bacteria), V (Vermicompost), 100DH:0F (Dragon's head mono cropping), 50DH:50F, 60DH:40F, 75DH:25F and 25DH:75F indicate the ratios of Dragon's head and fenugreek in cropping patterns; data are mean ± SE ($n = 3$); the main components are showed by bold values.

dragon's head was also affected by the year (Supplemental Table 3). The overall trends show higher seed yields with monocropping (100DH:0F) for both crops, followed by the 75DH:25F in DH (Fig. 1A) and the 25DH:75F intercropping in F (Fig. 1B). Within each intercropping system, both DH and F showed highest seed yields under vermicompost (V) application, followed by or not significantly different than bacterial (B) fertilizer type, and then mycorrhizal (M) and unfertilized control. The overall highest DH seed yield across comparisons was attained with the monocrop fertilized with vermicompost (100DH:0F + V), but this treatment was not significantly different than the bacterial biofertilizer (0DH: 100F + B) and vermicompost (0DH: 100F + V) in F, whereas the lowest seed yields for both plant species were obtained with the unfertilized treatment in the 50DH:50F intercropping system (Fig. 1A and B). Compared to unfertilized control, application of V, M, and B increased seed yield by 10.6%, 19.8% and 14.8% in DH, and by 21.5%, 12.4% and 26.3% in F, respectively (Fig. 1A and B).

3.2. Fixed oil content and oil yield

The analysis of variance indicated that the main effects of cropping ratio and fertilizer type were significant on fixed oil content and oil yield of dragon's head (DH) and fenugreek (F), and the interaction effect of cropping ratio and fertilizer type was significant on the fixed oil content and oil yield of both species (Supplemental Table 1 and 2). Fixed oil content for DH and F was higher in intercropping compared to the monocropping system (Fig. 1). For instance, averaged across all fertilizer types in the four intercropping systems, fixed oil content in DH was 8.7% greater than that of monocropping system (i.e., first four columns in the left; Fig. 1C). In DH the 60DH:40F + B and 75DH:25F + B treatments produced the overall highest fixed oil contents (~35%), which were about 15% greater than the unfertilized control within each system, and 21.5% greater than the fixed oil content in the unfertilized monocropping system. In F, the 60DH:40F + B and 25DH:75F + B systems resulted in the overall highest fixed oil content (~7.5%), whereas the lowest fixed oil content (6.0%) occurred with the unfertilized monocropping system (Fig. 1D).

Across intercropping systems, highest fixed oil yields were found in the monocropping system both for DH (Fig. 1E) and F (Fig. 1F), as a result of high seed yield with moderate fixed oil concentration, and in the 75DH:25F system for DH (Fig. 1E) and 25DH:75F for F (Fig. 1F), as a result of moderate seed yield and high fixed oil contents. Within each intercropping system, higher fixed oil yields corresponded to V and B, followed by M and then unfertilized control. In DH, fixed oil yield in 75DH:25F + V (492 kg ha⁻¹) doubled the overall lowest fixed oil yield found in the unfertilized 50DH:50F system (244 kg ha⁻¹) (Fig. 1E). In F, fixed oil yields in the monocropping and the 25DH:75F systems with V and B fertilizers (about 64 kg ha⁻¹) doubled the overall lowest oil yield produced in the unfertilized monocropping (32 kg ha⁻¹) (Fig. 1F).

3.3. Oil composition

Main oil fatty acids in DH were linoleic (range: 54.1–63.0%; average 58.5%), stearic (range: 11.1–16.8%; avg. 13.1%), and unsaturated oleic (range: 11.2–16.7%; avg. 12.9%). Main fatty acids in F were linoleic (36.0–39.2%; avg. 37.6%), oleic (15.2–26.3%; avg. 20.8%), and linolenic (13.0–29.1%; avg. 21.1%) (Table 3). The 60DH:40F + B treatment in DH resulted in the highest contents of oleic acid and linoleic acid in DH (Table 2). In F, the 25DH:75F + B had the highest content of oleic acid, while the 25DH:75F + V resulted in the highest linolenic acid content (Table 4). The monocropping + M produced the highest content of stearic acid in DH and F (Tables 3 and 4). Regardless of the intercropping ratio, however, both bacterial and organic fertilization increased most oil compounds in DH compared with unfertilized control (Table 3).

3.4. Essential oil content and yield of dragon's head

The main effects of cropping ratio and fertilizer type were significant on essential oil content and yield (Supplemental Table 1). However, the interaction between cropping ratio and fertilizer type was recorded on essential oil content and yield, while the growing year was significant on essential oil yield (Supplemental Table 2 and 3). Essential oil content across intercropping and fertilizations was 5% greater than that of monocropping. Higher essential oil contents were observed with the 60DH:40F followed by the 75DH:25F intercropping ratios. Highest overall essential oil content corresponded to 60DH:40F + V, whereas the lowest content was found in the unfertilized monocrop (Fig. 2A).

Monoculture produced higher oil yields for each fertilizer, followed by the 75DH:25F. The overall highest essential oil yield (2.02 kg ha⁻¹) was obtained when vermicompost was applied in the monocropping and the 75DH:25F intercropping (Fig. 2B). Similar to seed yield (Fig. 1A), fixed oil content (Fig. 1C) and yield (Fig. 1E), the highest essential oil content (Fig. 2A) and yield (Fig. 2B) corresponded to the V, followed by B, and M fertilizers. Across all comparisons, V, B, and M increased the average essential oil yield by 33%, 25% and 18%, respectively, compared to the unfertilized control (Fig. 2B).

3.5. Chemical composition of dragon's head essential oil

Across all comparisons, the main essential oil components in DH were caryophyllene oxide (range: 12.6–28.9%; avg. 22.1%), spathulenol (range: 10.6–18.5%; avg. 15.6%), carvacrol (range: 7.7–13.4%; avg. 10.3%), and thymol (range: 7.0–15.9%; avg. 10.2%) (Table 5). The highest content of caryophyllene oxide (28.9%) was observed with the 60DH:40F + V treatment. The highest values of spathulenol (21.3%) and thymol (15.9%) occurred with the 25DH:75F + B, whereas highest carvacrol (13.4%) content was found in the 60DH:40F + B (Table 5). On

60DH:40F			25DH:75F			75DH:25F					
M	B	V	C	M	B	V	C	M	B	V	
0.06±0.004	0.08±0.002	0.12±0.1	0.05±0.02	0.04±0.002	0.06±0.002	0.24±0.03	0.07±0.02	0.15±0.01	0.3 ± 0.03	0.04±0.001	
5.65±0.17	5.22±0.13	5.24±0.14	6.14±0.17	5.85±0.11	6.88±0.10	6.58±0.13	5.97±0.20	6.04±0.23	7.35±0.24	6.24±0.27	
11.89±0.35	12.99±0.52	12.84±0.40	11.85±0.20	13.22±0.37	13.23±0.48	12.38±0.60	12.69±0.51	13.56±0.41	12.74±0.39	12.81±0.52	
16.28±0.41	16.69±0.44	15.3 ± 0.57	11.15±0.33	11.69±0.24	12.72±0.28	11.47±0.35	12.14±0.52	14.59±0.62	12.96±0.45	12.36±0.3	
61±1.64	62.98±1.21	62.12±1.72	58.12±2.22	60.05±2.32	59.27±1.90	58.87±2.07	59.34±1.50	60.15±2.27	60.27±1.95	59.72±1.72	
0.7 ± 0.03	0.71±0.06	0.97±.04	0.65±0.08	0.7 ± 0.02	0.83±0.05	0.51±0.01	0.82±0.01	0.7 ± 0.04	0.5 ± 0.02	0.62±0.06	
0.13±0.34	0.08±0.001	0.23±0.6	0.05±0.004	0.08±0.002	0.06±0.004	0.22±0.03	0.08±0.002	0.2 ± 0.06	0.11±0.01	0.06±0.004	
0.1 ± 0.03	0.07±0.008	0.14±.04	0.04±0.001	0.08±0.001	0.02±0.006	0.24±0.02	0.08±0.002	0.28±0.06	0.1 ± 0.09	0.09±0.005	
0.1 ± 0.04	0.05±0.006	0.05±0.08	0.05±0.003	0.1 ± 0.03	0.05±0.005	0.15±0.01	0.1 ± 0.04	0.2 ± 0.01	0.09±0.006	0.06±0.001	
95.91	98.87	97.01	88.1	91.81	93.12	90.66	91.29	95.87	94.42	92	

Table 4Proportion of fenugreek oil constituents across treatments resulting from different intercropping systems and fertilizer types (average of two years).[†]

Component	100F:0DH				50DH:50F				60DH:40F	
	C	M	B	V	C	M	B	V	C	
Myristic acid	0.21±0.01	0.17±0.05	0.16±0.02	0.1 ± 0.01	0.2 ± 0.04	0.12±0.06	0.1 ± 0.01	0.13±0.03	0.12±0.09	
Palmitic acid	10.16±0.33	10.73±0.33	10.37±0.21	11.95±0.24	11.33±0.27	12.25±0.19	12.05±0.18	11.7 ± 0.28	12.08±0.34	
Margaric acid	0.26±0.024	0.24±0.012	0.19±0.034	0.11±0.014	0.17±0.015	0.15±0.027	0.17±0.003	0.14±0.009	0.13±0.03	
Linoleic acid	36.03±1.41	37.16±1.31	36.32±1.42	36.48±1.65	36.05±1.12	36.97±1.76	36.93±1.24	38.75±1.41	35.06±1.64	
Oleic acid	20.01±0.78	22.29±0.78	21.5 ± 0.80	23.05±0.48	20.51±0.65	20.78±0.98	20.91±0.64	20.97±0.53	17.46±0.34	
Linolenic acid	16.03±0.24	16.45±0.54	17.05±0.50	21.96±0.32	19.4 ± 0.98	21.05±0.14	22.38±0.08	21.75±0.48	22.47±0.81	
Stearic acid	6.56±0.14	6.68±0.11	4.81±0.16	4.93±0.08	4.01±0.13	4.65±0.18	4.41±0.06	4.18±0.16	3.15±0.16	
Arachidic acid	0.41±0.01	0.43±0.01	0.29±0.04	0.09±0.02	0.09±0.01	0.12±0.05	0.08±0.03	–	–	
Total identified (%)	89.67	94.15	90.69	98.67	91.76	96.09	97.03	97.62	90.47	

[†]C (Control), M (Mycorrhiza), B (Bacteria), V (Vermicompost), 100F:0DH (fenugreek mono cropping), 50DH:50F, 60DH:40F, 75DH:25F and 25DH:75F indicate the ratios of Dragon's head and fenugreek in cropping patterns; data are mean ± SE (n = 3); the main components are showed by bold values.

average, the three fertilized treatments under intercropping increased the thymol, spathulenol, and caryophyllene oxide content by 17%, 4%, and 13%, respectively, when compared with fertilized treatments in monocropping system. On the other hand, the average carvacrol in monocropping was 8% greater than that in the intercroppings (Table 5).

3.6. Trigonelline in fenugreek

The main effects of cropping ratio and fertilizer type were significant on trigonelline. However, the interaction between cropping ratio and fertilizer type was significant on trigonelline (Supplemental Table 1 and 2). Trigonelline uptake was also significantly affected by the year (Supplemental Table 3). The highest trigonelline production was recorded in the 25DH:75F system (Fig. 3A), and within each intercropping system, under the B, by V, M and unfertilized control. Across all comparisons, 25DH:75F + B system had the highest trigonelline content (7.15 mg g⁻¹), whereas the unfertilized monocropping system

had the lowest trigonelline content (4 mg g⁻¹) (Fig. 3A). Averaged across fertilizers, the four intercropping systems increased trigonelline production by almost 39% when compared with the monocropping system. Furthermore, the application of mycorrhizal fungi, bacteria fertilization, and vermicompost increased trigonelline production by 6%, 17%, and 11%, respectively, versus unfertilized control (Fig. 3A).

3.7. Diosgenin in fenugreek

The main effects of cropping ratio and fertilizer type on diosgenin was significant ($p \leq 0.01$) (Supplemental Table 1). Also, the interaction between cropping ratio and fertilizer type was significant on diosgenin ($p \leq 0.01$) (Supplemental Table 2). The highest diosgenin content (0.75 mg g⁻¹) corresponded to the 60DH:40F + B treatment. On average, and compared to monocropping, intercropping increased diosgenin production by 16%. Moreover, the application of M, B, and V increased diosgenin by 5%, 13%, and 10%, respectively, over the unfertilized

Table 5Proportion of dragon's head essential oil constituents across treatments resulting from different intercropping systems and fertilizer types (average of two years).[†]

Component [‡]	RI	100DH:0F				50DH:50F				60DH:40F	
		C	M	B	V	C	M	B	V	C	
α-pinene	934	2.1 ± 0.01	2.88±0.02	3 ± 0.09	2.5 ± 0.01	1.94±0.06	1.21±0.01	1.92±0.02	1.44±0.04	1.01±0.03	
β-myrcene	990	1.1 ± 0.02	4.4 ± 0.11	1.5 ± 0.01	2.5 ± 0.03	1.35±0.02	2.3 ± 0.07	2 ± 0.03	1.9 ± 0.05	3.99±0.04	
3-carene	1011	1.98±0.06	3.22±0.09	–	–	1.11±0.01	1.1 ± 0.04	2.09±0.04	1.11±0.07	1.23±0.02	
Limonene	1030	1.88±0.09	1.66±0.07	0.5 ± 0.02	0.48±0.001	–	1.11±0.03	2.22±0.01	0.99±0.03	2.98±0.04	
Linalool	1099	1.61±0.02	1.2 ± 0.03	3.67±0.04	1.84±0.04	2.24±0.01	4.81±0.12	2.42±0.05	2.21±0.02	1.14±0.07	
(Z)-p-Menth-2-en-1-ol, cis-trans-p-Menth-2-en-1-ol	1123	0.22±0.01	0.44±0.01	0.78±0.05	1.01±0.02	–	0.99±0.02	–	0.81±0.04	2.03±0.01	
Geraniol	1141	3.89±0.05	0.99±0.02	0.54±0.06	2.55±0.03	1.88±0.07	–	–	1.21±0.01	–	
Thymol	1253	5.3 ± 0.03	1.09±0.03	0.58±0.01	1.03±0.02	2.85±0.03	2.49±0.06	5.7 ± 0.09	1.62±0.001	1.47±0.009	
Carvacrol	1291	7.87±0.11	8.8 ± 0.24	10±0.14	8.01±0.12	7.28±0.16	10.32±0.97	13.61±0.12	8.1 ± 0.22	7.08±0.09	
Trans-caryophyllene	1299	8.85±0.14	10.8 ± 0.33	11.55±0.43	13.15±0.89	9.18±0.59	10.51±0.29	12.26±0.21	11.57±0.70	10.09±0.08	
Germacrene-D	1425	0.94±0.03	2.11±0.07	3.55±0.08	1.08±0.07	4.64±0.15	2.08±0.02	1.37±0.04	3.44±0.02	1.02±0.06	
β-Bisabolene	1487	0.35±0.02	1.4 ± 0.07	0.4 ± 0.01	1.19±0.07	2.11±0.01	1.9 ± 0.07	0.96±0.04	3.84±0.09	1.03±0.02	
γ-cadinene	1510	1.99±0.01	2.66±0.03	0.5 ± 0.02	5.88±0.11	2.56±0.07	2.5 ± 0.04	–	–	–	
β-Calacorene	1527	1.02±0.02	0.99±0.04	0.67±0.03	–	2.68±0.02	1.83±0.05	–	–	2.3 ± 0.01	
Germacrene-d-4-ol	1559	4.9 ± 0.09	2.21±0.01	4.71±0.08	2.85±0.02	2.99±0.01	6.15±0.01	0.95±0.02	1.61±0.02	0.56±0.02	
Spathulenol	1574	5.88±0.01	–	0.46±0.01	0.51±0.03	1.77±0.04	1.88±0.06	1.01±0.001	1.86±0.07	0.9 ± 0.06	
Caryophyllene oxide	1586	15±0.88	12.4 ± 0.44	17.14±0.22	15.68±0.32	17.71±0.78	15.39±0.82	17.61±0.68	11.36±0.72	15.51±0.66	
Di-epi-1,10-cubenol	1592	12.6 ± 0.19	17.9 ± 0.55	24.83±0.87	22.82±0.98	22.47±0.82	22.32±0.76	15.4 ± 0.60	26.99±1.21	27.9 ± 0.102	
epi-α-Cadinol	1616	3.11±0.08	–	–	3 ± 0.01	2.99±0.06	2.68±0.11	3.31±0.03	1.78±0.09	–	
α-Cadinol	1645	2.8 ± 0.09	3.2 ± 0.03	1.5 ± 0.01	1.22±0.03	1.21±0.01	1.11±0.04	1.44±0.02	1.34±0.05	1.67±0.07	
Caryophyllenol II	1658	0.24±0.01	3.7 ± 0.03	1.57±0.01	1.17±0.06	1.55±0.02	–	1.81±0.04	5.05±0.16	1.33±0.06	
α-Bisabolol	1676	0.29±0.05	2.2 ± 0.02	2.44±0.04	1.87±0.01	1.98±0.07	–	2.21±0.02	–	–	
Hexadecanal	1687	0.33±0.03	2.21±0.01	2.83±0.07	1.2 ± 0.02	1.36±0.01	3.16±0.02	3.33±0.04	2.37±0.08	1.69±0.02	
Phytol	1847	3.2 ± 0.07	2.8 ± 0.05	2.5 ± 0.08	1.9 ± 0.02	1.11±0.07	1.92±0.06	2.55±0.08	0.5 ± 0.001	1.1 ± 0.05	
Total identified (%)	2110	3.5 ± 0.09	3.2 ± 0.09	0.55±0.06	0.45±0.03	1.2 ± 0.04	1.01±0.02	1.89±0.006	3.68±0.04	1.07±0.04	
		90.95	92.46	95.77	93.89	96.16	98.77	96.06	94.78	90.1	98.54

[†]C (Control), M (Mycorrhiza), B (Bacteria), V (Vermicompost), 100DH:0F (Dragon's head mono cropping), 50DH:50F, 60DH:40F, 75DH:25F and 25DH:75F indicate the ratios of Dragon's head and fenugreek in cropping patterns; data are mean ± SE (n = 3); the main components are showed by bold values.

[‡]Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams and NIST 08; RI, by comparison of retention index with those reported in Adams and NIST 08; Std, by comparison of the retention time and mass spectrum of available authentic standard.

60DH:40F			25DH:75F			75DH:25F					
M	B	V	C	M	B	V	C	M	B	V	
0.11±0.06	0.11±0.05	0.09±0.004	0.1 ± 0.01	0.12±0.03	0.18±0.01	0.2 ± 0.03	0.19±0.05	0.24±0.02	0.14±0.01	0.18±0.06	
12.3 ± 0.25	12.41±0.20	12.25±0.31	10.24±0.14	11.25±0.23	10.99±0.30	10.87±0.28	10.44±0.33	11.49±0.18	10.8 ± 0.21	10.78±0.32	
0.11±0.021	0.16±0.017	0.1 ± 0.02	0.14±0.01	0.24±0.04	0.27±0.02	0.34±0.01	0.31±0.02	0.26±0.08	0.17±0.04	0.26±0.025	
37.13±1.87	39.18±1.90	39.16±1.40	37.09±1.41	37.59±1.77	37.81±1.31	36.32±1.81	36.34±1.90	37.05±1.51	37.56±1.65	36.91±1.51	
18.25±0.70	18.94±0.76	19.53±0.70	15.16±0.93	16.04±0.78	24.33±0.98	18.42±0.82	20.09±0.77	20.83±0.48	22.37±0.90	23.87±0.72	
22.56±0.90	23.01±0.54	23.9 ± 0.74	24.19±0.99	25.37±0.41	24.21±0.54	29.08±0.59	19.02±0.64	19.66±0.20	19.72±0.71	20.29±0.92	
3.61±0.22	4.53±0.26	4.56±0.10	4.38±0.09	4.81±0.14	2.03±0.19	3.76±0.23	6 ± 0.21	5.78±0.12	5.51±0.19	5.08±0.15	
–	0.11±0.01	0.08±0.06	–	–	–	0.36±0.07	0.35±0.02	0.45±0.05	0.34±0.03	–	
94.07	98.45	99.67	91.3	95.42	99.82	99.35	92.74	95.76	96.61	97.37	

control (Fig. 3B).

3.8. Nutrient content of fenugreek and dragon's head

The N and P content in both species were influenced by the main effects of cropping ratio and fertilizer type (Supplemental Table 2), as well as by their interaction (Supplemental Table 2). N content of dragon's head was also significantly affected by the year (Supplemental Table 3). The N content in the seed of F and DH was 3.8% and 8.5% greater in 2017 than in 2018, respectively. However, year did not have a significant effect on several variables under study in both species (Supplemental Table 1 and 2). The 60DH:40F + B treatment had the highest contents of N and P both in DH (26.1 g N kg⁻¹ and 2.58 g P kg⁻¹; Fig. 4A and C) and in F (40.4 g N kg⁻¹ and 3.1 g P kg⁻¹; Fig. 4B and D). In DH, this treatment was not different than 75DH:25F + B. The lowest content of N and P in both species occurred with the unfertilized monocropping systems (Fig. 4A, B, C and D).

3.9. Mycorrhizal root colonization

The main effects of cropping ratio and fertilizer type were significant ($p \leq 0.01$) on the Mycorrhizal root colonization of the DH and the F roots (Supplemental Table 1). In addition, the interaction between the two factors on the root colonization of both crops was significant ($p \leq 0.01$) (Supplemental Table 2). The root colonization of dragon's head was also affected by the year (Supplemental Table 3). The highest root colonization of DH (48%) and F (63%) were observed in the 60DH:40F + M and the 25DH:75F + M systems, whereas the lowest occurred with the unfertilized monocropping system for both species (Fig. 5A and B). Averaged across fertilizer treatments, the intercropping systems increased root colonization by 16% in DH and by 32% in F, compared to the monocropping.

60DH:40F			25DH:75F			75DH:25F					
M	B	V	C	M	B	V	C	M	B	V	
–	1.5 ± 0.01	1.15±0.03	–	7.13±0.01	0.97±0.08	2.86±0.01	1.5 ± 0.08	1.6 ± 0.01	–	1.12±0.04	
2.5 ± 0.06	0.5 ± 0.02	1.33±0.01	–	3.16±0.03	0.31±0.01	1.65±0.02	0.5 ± 0.01	1.4 ± 0.02	–	–	
1.22±0.05	–	1.11±0.04	1.44±0.01	2.06±0.02	1.44±0.02	1.66±0.01	2.9 ± 0.05	–	1.66±0.02	–	
–	1.81±0.05	1.09±0.01	–	4.52±0.03	1.21±0.01	2 ± 0.03	3.11±0.01	1.77±0.01	1.76±0.02	–	
2.32±0.02	3.44±0.09	1.9 ± 0.05	2.55±0.01	1.97±0.02	–	4.61±0.08	3.88±0.03	2.18±0.06	3.21±0.05	4.12±0.1	
–	–	0.77±0.01	–	1.75±0.01	–	1.03±0.01	1.73±0.04	–	1.63±0.01	1.88±0.02	
–	–	0.58±0.05	1.05±0.03	–	0.9 ± 0.04	1.22±0.001	1.74±0.02	2.21±0.04	2.11±0.02	2.66±0.03	
1.2 ± 0.01	1.22±0.03	1.55±0.06	2.21±0.04	–	1.11±0.01	–	1.1 ± 0.01	1.78±0.02	1.58±0.01	1.7 ± 0.01	
12.8 ± 0.07	10.17±0.21	7.5 ± 0.09	7 ± 0.01	11.52±0.05	15.89±0.22	15.76±0.31	8 ± 0.04	8.96±0.07	12.77±0.21	12±0.28	
12.86±0.06	13.36±0.12	9 ± 0.08	8.1 ± 0.05	7.89±0.08	11.37±0.12	8.87±0.11	7.73±0.08	9.5 ± 0.07	8.99±0.12	9.29±0.34	
4.41±0.01	3.63±0.05	2.26±0.01	3.06±0.06	–	2.8 ± 0.01	4.7 ± 0.00	3.22±0.07	2.9 ± 0.01	1.8 ± 0.02	2.31±0.05	
1.05±0.04	1.75±0.03	1.99±0.09	3.4 ± 0.05	–	–	5.47±0.01	3.97±0.09	2.08±0.01	1.63±0.02	1.88±0.04	
1.66±0.01	2.55±0.07	1.22±0.05	1.93±0.02	1.88±0.01	2.38±0.03	1.47±0.008	4.9 ± 0.05	–	3.19±0.08	3.38±0.01	
2.9 ± 0.08	1.32±0.05	1.2 ± 0.04	1.12±0.01	–	–	2.44±0.05	1.88±0.04	–	–	–	
1.18±0.05	1.29±0.02	1.95±0.03	3.03±0.05	1 ± 0.01	3 ± 0.02	1.99±0.01	4.14±0.02	2.73±0.03	2.72±0.04	3.24±0.06	
0.92±0.04	0.28±0.05	2.21±0.02	–	6.66±0.09	5.92±0.07	0.9 ± 0.06	0.9 ± 0.03	1.03±0.01	–	–	
17.68±0.96	17.72±0.59	18.52±0.81	17.26±0.23	13.31±0.41	21.33±0.52	16.61±0.48	10.64±0.20	16.28±0.76	13.44±0.54	15.39±0.89	
19.37±0.88	26.41.87	28.9 ± 0.21	25.87±0.19	15.52±0.75	15.89±0.66	16.05±0.33	22.97±0.94	23.53±1.08	28.02±0.43	25.91±1.25	
1.11±0.06	–	–	–	–	–	1.67±0.21	1.8 ± 0.01	1.55±0.01	–	1.64±0.01	
1.12±0.03	0.21±0.02	2.11±0.04	7.69±0.09	2.96±0.01	0.69±0.43	0.44±0.21	1.78±0.01	7.21±0.09	1.22±0.01	–	
3.01±0.08	–	1.1 ± 0.01	1.07±0.03	–	2.21±0.01	–	2.59±0.03	0.88±0.001	–	4.72±0.09	
2.22±0.05	–	1.43±0.01	3.15±0.02	4.15±0.01	3.8 ± 0.04	3.54±0.02	2.22±0.05	1.01±0.01	5.13±0.08	2.21±0.06	
5.73±0.07	5.82±0.05	3.61±0.09	–	5.16±0.02	0.48±0.01	0.67±0.06	–	–	–	–	
1.14±0.03	2 ± 0.01	1.56±0.05	1.81±0.01	1.21±0.01	1.33±0.02	3.12±0.07	2.22±0.02	1.89±0.01	1.86±0.01	1.9 ± 0.01	
2.14±0.08	1.05±0.002	4.98±0.02	5.29±0.08	4.91±0.05	1.62±0.02	1 ± 0.08	2.43±0.06	5.91±0.07	4.64±0.09	3.64±0.06	
96.03	99.02	97.03	96.76	94.65	99.73	97.85	96.4	97.36	98.99	90.95	

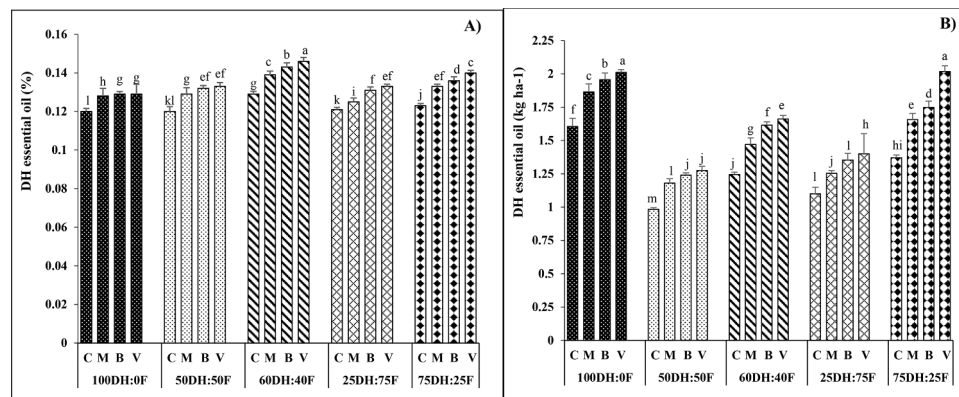


Fig. 2. Dragon's head essential oil concentration (A) and yield (B), as influenced by different cropping patterns (100DH:0F; 50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F; where DH and F indicate the ratios of dragon's head and fenugreek in intercropping pattern) and fertilizer type [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. Different lower-case letters above bars indicate significant difference at the $P \leq 0.05$ level, according to Duncan's multiple range test.

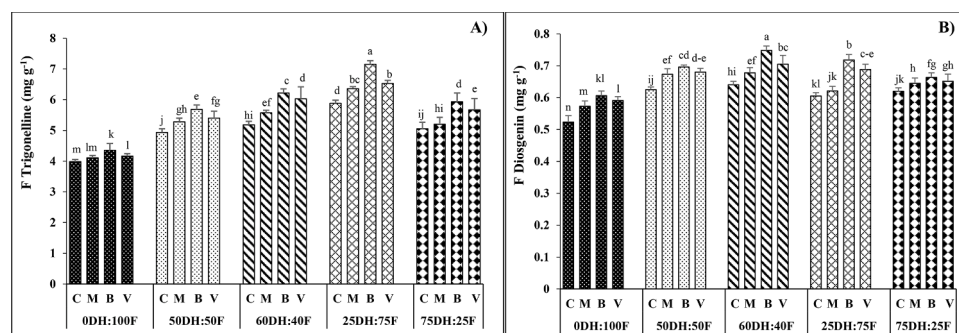


Fig. 3. Fenugreek Trigonelline (A) and Diosgenin (B) concentration, as influenced by different cropping patterns (0DH:100F; 50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F; where DH and F indicate the ratios of dragon's head and fenugreek in intercropping pattern) and fertilizer type [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. Different lower-case letters above bars indicate significant difference at the $P \leq 0.05$ level, according to Duncan's multiple range test.

3.10. Land equivalent ratio (LER)

For each fertilizer type, higher partial LERs of DH and F were calculated in the 75DH:25F intercropping system, followed by the 60DH:40F, 25DH:75F and the 50DH:50F (Fig. 6). The highest partial, both in DH (0.92) and F (0.88), and total LER (1.80) were calculated for the 75DH:25F + V treatment. The lowest partial, both in DH (0.60) and F (0.57), and total LER (1.17) corresponded to the unfertilized 50DH:50F treatment.

4. Discussion

All parameters analyzed in this study were improved in response to mycorrhizal fungi, bacterial and vermicompost application. The maximum seed yields with vermicompost and bacteria application across most comparisons can be attributed to an increased availability and uptake of nutrients and water following fertilizer addition. Previous research found that the plant growth-promoting rhizobacteria (PGPRs) and AMF increase the yields of plants such as potato (*Solanum tuberosum* L.), tomato, basil, garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) through enhancing root growth and plant access to nutrients and water (Adavi and Tadayoun, 2014; Caradonia et al., 2019; Mahdavi et al., 2019; Golubkina et al., 2020).

As expected, maximum seed yields of both plant species occurred under monocropping, considering that intercropping treatments were replacement series and lower yields were attributed to a lower number of plants ha⁻¹. The use of the LER concept provides with a better indicator of productivity when comparing intercropping and monocropping systems. As shown in Fig. 6, total LER was >1 in all the combinations of

intercropping x fertilizer treatments, implying the superiority of the intercroppings over the monoculture comparisons studied here. This can be related to the formation of different niches and optimal exploitation of resources, N credit from fixation by legumes, and the improvement in growing conditions for the non-legume component of the intercropping system (Amani Machiani et al., 2019). These results agree with the findings of Koocheki et al. (2019) in a saffron (*Crocus sativus* L.)-pumpkin (*Cucurbita pepo* L.)-watermelon (*Citrullus lanatus* L.) and Rezaei-Chiyaneh et al. (2020b) in a fennel-common bean intercropping system. When this occurs, and if a suitable planting ratio and plant density are selected for an intercropping system, the economic yield of the system can be increased due to the enhancement in the plant's root systems and the optimal exploitation of environmental conditions in intercropping systems (Raza et al., 2019).

Fixed oil content and oil constituents also increased in response to the higher availability and nutrient uptake resulting from organic and biofertilizers application. Similarly, the application of plant growth-promoting bacteria (PGPR) enhanced the fixed oil content and oil constituents of safflower (*Carthamus tinctorius* L.) in intercropping with faba bean (*Vicia faba* L.) (Saeidi et al., 2018), and fennel in intercropping with common bean (Rezaei-Chiyaneh et al., 2020b). Research shows that micronutrients act as the cofactor of the enzymes responsible for the biosynthesis of fatty acids in fixed oil and improve their qualitative traits by enhancing their oil content (Rezaei-Chiyaneh et al., 2016). Moreover, enhancement in the availability of nutrients increases the plant photosynthesis rate (Chen et al., 2017), leading to an increased assimilation of primary and secondary metabolites such as fixed oil compounds (Thoma et al., 2020).

The increase in essential oil content and yield in dragon's head

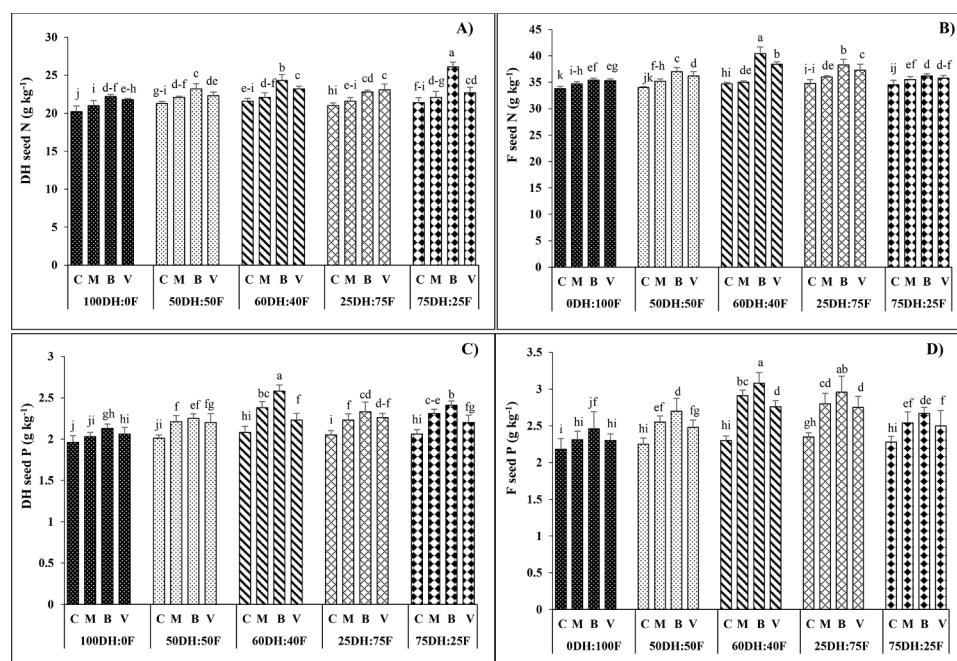


Fig. 4. Dragon's head and Fenugreek seed N (A and B), and P content (C and D), as influenced by different cropping patterns (100DH:0F; 0DH:100F; 50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F; where DH and F indicate the ratios of dragon's head and fenugreek in intercropping pattern) and fertilizer type [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. Different lower-case letters above bars indicate significant difference at the $P \leq 0.05$ level, according to Duncan's multiple range test.

following application of fertilizers may be associated with the major role of nutrients, especially N, in development and division of new essential oil-containing cells, essential oil canals, exudation pathways, and glandular trichomes that are responsible for the exudation and biosynthesis of monoterpenes (Amani Machiani et al., 2019; Schuurink and Tissier et al., 2020). Organic and biofertilizers applied in this study positively impacted the phosphate-solubilizing, N-fixing and other beneficial microorganisms in the soil by providing optimal accessibility to the nutrients which, in turn, likely influenced and increased the production of essential oil constituents and oil yields (Rezaei-Chiyaneh et al., 2020a; Ostadi et al., 2020). Compounds like terpenoids, a main component of essential oil that need N and P for their synthesis, were likely benefitted with the addition of readily available nutrients provided by the fertilizers used in this study. Similarly, Rezaei-Chiyaneh et al. (2021a) concluded that dragonhead (*Dracocephalum moldavica* L.) intercropped with common bean with organic fertilizer application increased the Moldavian balm's essential oil productivity by increasing the amount of geraniol and geranyl acetate compared with monocropping system.

Trigonelline and Diosgenin content increased when intercropping systems including more than 40% of Fenugreek in their ratios were fertilized with bacterial biofertilizer. Previous results were reported by Salehi et al. (2018) in a fenugreek-buckwheat (*Fagopyrum esculentum* Moench) intercropping system fertilized with organic manure, and by

Abdelkader and Hamad (2015) (safflower-fenugreek intercropping) and Baghbani-Arani et al. (2017) (fenugreek monocrop) fertilized with vermicompost.

A higher content of nutrients in the intercropping system under vermicompost application can be ascribed to the increased activity of microorganisms, water retention capacity, and gradual release of nutrients across the plant growth cycle. As a result, improvements in soil structure with vermicompost application have the potential to enhance plant nutrient uptake (Goswami et al., 2017; Rezaei-Chiyaneh et al., 2021a). In addition, the application of bacterial fertilizer presumably increased the plant nutrient uptake due to a concomitant increase in the root surface area. Several studies have demonstrated the positive effects of bio and organic fertilizers in intercropping systems on nutrient uptake of medicinal plants, especially when soil P is limited (Weisany et al., 2016; Amani Machiani et al., 2019).

Although maximum overall root colonization was related to the application of mycorrhizal fungi in both plant species, the root colonization in intercropping systems across the three fertilizer treatments considered in this study consistently outyielded the root colonization of the monocropping system in most comparisons. Previous research suggests that this may be attributable to the greater proportion of green cover and soil coverage, enhanced soil moisture content and better nutrient balances across the plant cycle under intercropping conditions,

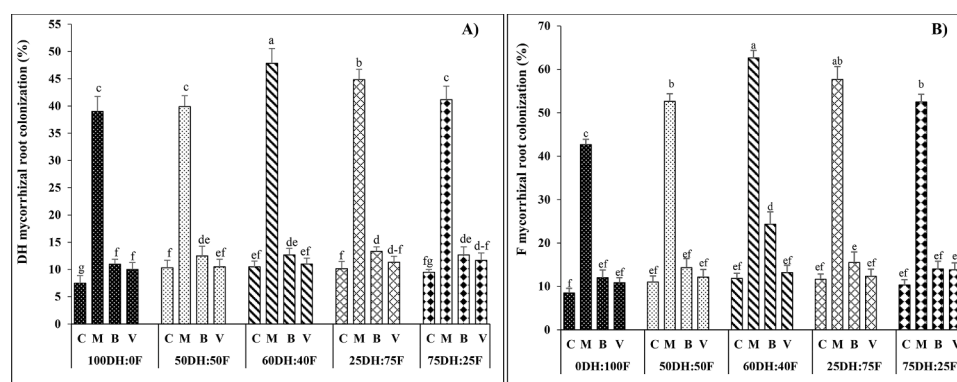


Fig. 5. Dragon's head and Fenugreek root mycorrhizal root colonization (A and B), as influenced by different cropping patterns (100DH:0F; 0DH:100F; 50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F; where DH and F indicate the ratios of dragon's head and fenugreek in intercropping pattern) and fertilizer type [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. Different lower-case letters above bars indicate significant difference at the $P \leq 0.05$ level, according to Duncan's multiple range test.

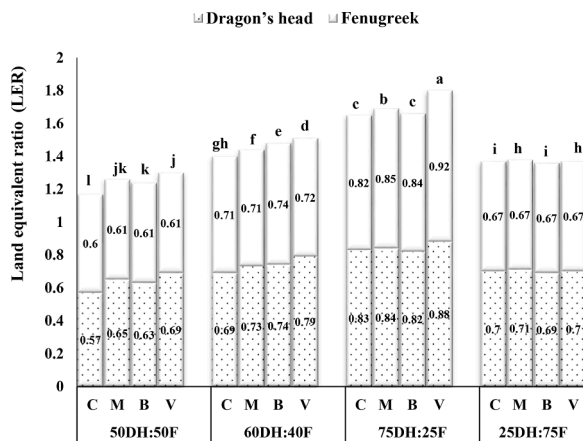


Fig. 6. Partial and total land equivalent ratio (LER) for seed yields of dragon's head (DH) and Fenugreek (F) in different intercropping patterns (50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F) and fertilizer types [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. **Fig. 6.** Partial and total land equivalent ratio (LER) for seed yields of Dragon's head (DH) and Fenugreek (F) in different intercropping patterns (50DH:50F; 60DH:40F; 25DH:75F; 75DH:25F) and fertilizers sources [C (Control); M (mycorrhizal); B (bacterial); V (vermicompost)]. Different lower-case letters above bars indicate significant difference at the $P \leq 0.05$ level, according to Duncan's multiple range test.

all favorable conditions for increased root colonization and expansion across the soil profile (Lekberg et al., 2008; Shukla et al., 2013; Rezaei-Chiyaneh et al., 2021b). Consistent with this, Hage-Ahmed et al. (2013) and Wahbi et al. (2016) reported that AMF inoculation increased root colonization, biomass, length, density, and influenced nutrient uptake in wheat and tomato (*Solanum lycopersicum* L.), when these crops were intercropped with leek and faba bean. Higher N and P plant uptake in intercropping systems has been associated to increases in the production of root exudates and H^+ and the activity of acid phosphatase that resulted in enhanced availability and absorbability of nutrients, especially immobile elements such as P, when compared with monocropping (Li et al., 2010, 2016). This enhanced nutrient uptake under different intercropping systems was further increased and reached the highest N and P uptake for both plant species following addition of a bacterial fertilizer mix consisting of two phosphate solubilizing bacteria and one nitrogen-fixing bacteria in our study. This practice can play an important role in the nutrients availability to crops by fixing N and dissolving P that is otherwise unavailable for plant uptake, thereby improving root growth by synthesizing growth promoters (Bindraban et al., 2020).

5. Conclusion

Our results show that application of organic fertilizers and bio-fertilizers in intercropping systems positively impacted the soil micro-organisms and the colonization of plant roots in the soil profile, which in turn increased the plant nutrient uptake for both plant species. Altogether, these responses led to increases in seed yield, fixed oil of both dragon's head and fenugreek, essential oil of dragon's head and in the diosgenin and trigonelline content of fenugreek. Across the different intercropping ratios, addition of bacterial and vermicompost fertilizers proved to boost plant metabolism, nutrient uptake and yield components of both plant species more effectively than mycorrhizal fungi and the untreated control. Across fertilizer treatments, all intercropping ratios reported here showed better results for all the parameters under study when compared to the monocropping systems of each plant species. Utilization of 75DH:25F in dragon's head, and 25DH:75F in Fenugreek with bacterial and vermicompost addition maximized the outcomes for most parameters across most comparisons. Thus, these

intercropping ratios and fertilizer types combinations can be regarded as the most overall sustainable and economically sound options for the production of medicinal plants. However, 60DH:40F with bacterial biofertilizer application may also represent an interesting choice, as this treatment combination resulted in the maximum synthesis of most secondary metabolites and oil compounds in both plant species.

Credit author statement

Conceptualization: E.R.-C., H.M.; Investigation: E.R.-C., M.L.B., W.E.T.; Methodology: E.R.-C., W.E.T., G.C.; Supervision: E.R.-C., H.M., M.L.B., G.C.; Roles/Writing – original draft: E.R.-C., M.L.B., W.E.T., G.C.; Writing - review & editing: E.R.-C., M.L.B., W.E.T., G.C.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.scienta.2021.110277](https://doi.org/10.1016/j.scienta.2021.110277).

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