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Intercropping fennel (*Foeniculum vulgare* L.) with common bean (*Phaseolus vulgaris* L.) as affected by PGPR inoculation: A strategy for improving yield, essential oil and fatty acid composition

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ABSTRACT

In order to evaluate the quali-quantitative traits of fennel (Foeniculum vulgare L.) when intercropped with common bean (Phaseolus vulgaris L.) in different cropping patterns and under PGPR application, a two-years field experiment (2015 and 2016) was arranged as factorial design based on randomized complete blocks (RCBD) with three replications. The first factor consisted of six cropping patterns including sole cropping of fennel, sole cropping of common bean, and different intercropping ratios of fennel to common bean (1:1, 2:2, 3:2, 4:2), whereas the second factor included the application and non-application of PGPR. The results showed that the highest seed yields of common bean (2474.83 kg ha⁻¹) and fennel (2730.08 kg ha⁻¹) were produced with sole cropping combined under PGPR application. The fennel essential oil (EO) and seed oil (fixed oil) content in all intercropping patterns were higher than those in sole cropping. Furthermore, under PGPR application, the seed yield, EO content, EO yield, fixed oil content and oil yield of fennel increased by 20.9, 16.4, 39.3, 10.3 and 33.3 %, respectively, compared with control. Based on the chemical analysis of fennel EO, the main constituents were (E)-anethole (73.71-81.10%), fenchone (3.44-6.18%), limonene (3.49-5.82%) and methyl chavicol (4.06-7.22%). The major fatty acids in fennel fixed oil were oleic (77.17-82.90%), linoleic (6.50-8.97%) and palmitic acids (3.25-6.80%). The highest content of unsaturated fatty acids (oleic and linoleic acids) and (E)anethole were obtained with intercropping ratios (fennel to common bean) of 2:2 and 3:2 under PGPR application, respectively. Furthermore, the highest land equivalent ratio (1.32) was obtained with intercropping ratio of 3:2 under PGPR application. Our findings showed that the intercropping ratio of 2:2 and 3:2 upon PGPR biofertilization may be suggested to farmers instead of sole cropping for enhancing the fennel EO and fixed oil quali-quantitative composition.

1. Introduction

Intercropping has been defined as the simultaneous planting of more than one species in the same place of land in which the plants usually spend a great part of their growth periods simultaneously (Ofori and Stern, 1987). Among different cropping systems, the intercropping of legumes with other plants is the most prevailing type in traditional farming of developing countries (Amani Machiani et al., 2019). The results of previous studies demonstrated that intercropping systems improved quali-quantitatively the yield per unit area (Salehi et al., 2018), increased the resource use efficiency such as water, nutrients

and radiation (Wang et al., 2017), and enhanced the soil fertility and plant nutrient supply (Chen et al., 2019). This is beneficial for reducing the soil erosion and amount of chemical fertilizers, having positive effects on the environment (Sharma et al., 2017). Intercropping may also be beneficial to alleviate pests and diseases, for weed control, and to reduce the input and compensation of the growing costs of production (Bedoussac et al., 2015). Besides, intercropping has been shown to improve the EO quality and quantity in medicinal and aromatic plants (Amani Machiani et al., 2019; Fallah et al., 2018).

Legumes, as the major protein-rich plant sources, are the second main source of food for human after cereals (Bedoussac et al., 2015).

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Common bean (*Phaseolus vulgaris* L.) is an annual plant species from the Fabaceae family that contains 19.7–24.3% protein so that it is the most economically important edible legume in the world (Peoples et al., 2009). Due to its capability of fixing nitrogen (0–160 Kg N ha⁻¹), common bean can improve soil fertility in rotations with many crops (Celmeli et al., 2018). Indeed, each individual plant of legumes can be regarded as a small-scale factory of N chemical fertilizer that not only meets the N requirement of the plant itself, but it is also beneficial to the nearby crops (Chekanai et al., 2018). Therefore, legumes can display a substantial role in the sustainability of the agricultural systems (Celmeli et al., 2018). Common bean can be subjected to sole cropping or intercropping with other plant species. Intercropping systems that contain a legume like common bean can enhance N storage and uptake by plants and can help the management of N fertilization (Latati et al., 2016).

Fennel (*Foeniculum vulgare* Mill., Apiaceae) is an annual, biennial or perennial medicinal and aromatic plant species widely grown in Iran (Mozaffarian, 2013). Fennel is classified as an appetizer, expectorant, galactogogue, stomachic, eupeptic, carminative, sex hormone stimulator, anti-inflammatory and sedative agent (Telci et al., 2019; Oktay et al., 2003). The major constituents of fennel essential oil (EO) are (*E*)-anethole, methyl chavicol, fenchone and limonene, making it exploitable in medicinal, food and cosmetic industries (Telci et al., 2019). The type and quantity of components in fennel EO vary with plant cultivar, geographic origin, climate, growth, development stage and genetics (Telci et al., 2009). The highest EO content is found in seeds (2–6%), whereas roots contain a lower amount (0.6-0.7 %). In addition, seeds contain macronutrients like proteins (18–20%) and fixed oil (12–18%) (Omidbaigi, 2008).

Nowadays, demand for medicinal and aromatic plants has increased in many countries as awareness that natural products are no-toxic and without side effects and can be acquired at affordable prices (Jamshidi-Kia et al., 2018). On the other hand, given the likely negative impacts of the excessive use of herbicides and chemical fertilizers on the profile of active ingredients in medicinal and aromatic plants, most of food, pharmaceutical and cosmeceutical companies prefer materials derived from sustainable and organic systems (Fonseca-Santos et al., 2015). The long-term application of chemical fertilizers damages the soil structure, causes environmental pollution and threats the safety of plants, animals and humans (Amani Machiani et al., 2018a). In this respect, the application of biofertilizers is an innovative approach in organic farming (Patel et al., 2016). Application of biofertilizers such as plant growthpromoting rhizobacteria (PGPR) can be successfully exploited as an ecological and environmentally friendly method for enhancing the productivity of crops including medicinal and aromatic plants (Ahemad and Kibret, 2014). PGPR are bacterial colonization rhizospheres of plant that improve the plant growth and development via different mechanisms such as N2 fixation and solubilization of phosphate, potassium, essential minerals and producing siderophores that chelate iron and make it available to the plant root (Grobelak et al., 2015). The PGPR enhance the root surface area for the uptake of nutrients and water, inducing the synthesis of plant hormones like auxins, cytokinins and gibberellins, thereby promoting plant growth and resistance to environmental stress, pests and diseases (Zaidi et al., 2015). Ghorbanpour and Hatami (2014) indicated that PGPR could play a significant role in improving the yields of sage (Salvia officinalis L.). On the other hand, the productivity of medicinal and aromatic herbs in low-input conditions, e.g. intercropping, without using chemical fertilizers, is a good strategy to achieve optimal yields with minimum external input application. This can reduce the need of these inputs in agronomic systems in the long term (Timsina, 2018). It seems that the application of biofertilizers like PGPR in intercropping systems can partially satisfy chemical fertilizer requirements of the systems.

Nowadays, based on the industrial importance of medicinal and aromatic plants such as fennel, different strategies should be applied to improve the quality and quantity of these herbs. To the best of our knowledge there are a few studies on the improvement of crop quality by intercropping systems. Moreover, there are no reports on the effects of PGPR application in fennel and common bean intercropping systems. Thus, it is necessary to explore the integrated impact of PGPR on quantity and quality of EO and fixed oil constituents of fennel in intercropping systems with common bean. Given the importance of this issue, the present study was aimed to: (i) evaluate the EO productivity and oil content and fatty acid composition of fennel as affected by intercropping systems under PGPR application; (ii) maintain higher productivity of both plants and clean products provision towards elimination of chemical fertilizers (iii); evaluate the efficiency of resource utilization by determining advantageous indices using the land equivalent ratio (LER) index; (iv) and determine the optimum combination treatments.

2. Materials and methods

2.1. Site description

A two-year field experiment was conducted (March–September 2015 and March–September 2016) at a research farm located in Naqadeh, West Azerbaijan, Iran (long. 45°24′, lat. 36°57′, 1320 m a.s.l.). The soil texture was given by silty clay with a pH of 7.7. The soil contained 0.92 % organic carbon, 0.28 % total N, 12.80 mg kg $^{-1}$ available P and 252 mg kg $^{-1}$ available K (depth of 0–30 cm). The weather conditions during the experiments are reported in Fig. 1.

2.2. Plant materials and cultivation

This research was carried out with a factorial design based on randomized complete block design (RCBD) with 12 treatments and three replications. The first factor included six cropping patterns consisting of one row of fennel plus one row of common bean (1:1), two rows of fennel plus two rows of common bean (2:2), three rows of fennel plus two rows of common bean (3:2), four rows of fennel plus two rows of common bean (4:2) and sole cropping of both fennel and common bean. The second factor included treatment with or without PGPR [i.e., nitrogen-fixing soil bacteria (Azotobacter vinelandii + Rhizobium phaseoli), P-solubilizing bacteria (the combination of Pseudomonas putida and Pantoea agglomerans) and K-solubilizing bacteria (the combination of Pseudomonas koreensis and P.vancouverensis)]. The seeds of common bean were inoculated with R.phaseoli in addition to the other bacteria before sowing. It should be noted that R.phaseoli was used only for common bean inoculation.

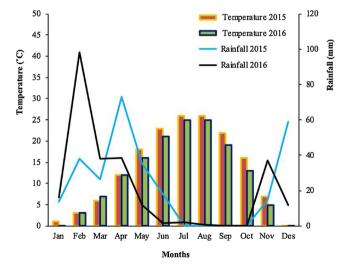


Fig. 1. Monthly average temperature and precipitation in 2015 and 2016 growing seasons.

Common bean seeds were obtained from the Agricultural and Natural Resources Organization of Khomein, Arak, Iran. Also, fennel seeds were obtained from Pakan Seeds Company, Isfahan, Iran. The bacteria (*R.phaseoli*) were provided from the Soil and Water Research Institute, Iranian Ministry of Agriculture, whereas the other bacteria were provided from Zist Fanavar Sabz company manufacturing, Qom, Iran. Before planting, the seeds of the two species were inoculated with PGPR (consumption of 5×10^8 colony forming units (CFU) per g biofertilizer plus 2 L water ha $^{-1}$), and a sugar cube (20 %) was completely sprayed to cover all the seeds surface and dried in the shade to be ready for planting.

Also, consumption of sulfur supplier bacteria (containing *Thiobacillus* at the rate of 10^8 active bacteria per g biofertilizer) in powdered form was applied at the rate of $7 \, \mathrm{kg} \, \mathrm{ha}^{-1}$. To ensure their effectiveness, the treatments containing sulfur supplier bacteria were added for three weeks before sowing to activate bacteria, and then incorporated into the soil. For better bacterial activity, cattle manure was uniformly distributed on the experimental plots at the rate of $5 \, \mathrm{tha}^{-1}$ and then incorporated into the soil at a depth of $20 \, \mathrm{cm}$ by a shovel.

In order to keep low-input conditions and focus the impact of PGPR, no chemical fertilizers, herbicides and pesticides were applied to the treatments during land preparation and growth period.

Fennel and common bean under intercropping were arranged in replacement series. The inter-row spacing was 40 cm for both species, but the on-row spacing was set to 10 cm for common bean, and 25 cm for fennel; the length of the rows was 3 m.

The number of rows for fennel sole cropping, common bean sole cropping, 1:1, 2:2, 3:2 and 4:2 cropping patterns were 6, 6, 6, 8, 10 and 12, respectively. The area of cropping plots was 7.2, 7.2, 7.2, 9.6, 12 and $14.4\,\mathrm{m}^{-2}$ for the fennel sole cropping, common bean sole cropping, 1:1, 2:2, 3:2 and 4:2 cropping partners, respectively. The treatments were separated 100 cm from each other and 2 m from the plot border.

The final optimum plant density in sole cropping was 10 plants $\rm m^{-2}$ for fennel and 25 plants $\rm m^{-2}$ for common bean. The fennel and common bean seeds were sown on 25th March and 2th May 2015 and 2016, respectively.

Chisel plow, disk harrowing and leveler were used for the final seedbed preparation of experimental field before planting each year. Also, after sowing of both plants the furrow irrigation was performed. The field was in a fallow condition last year.

The first irrigation was performed immediately after seed sowing. The subsequent irrigations were performed at 6-8-day intervals depending on climatic conditions and plant requirements. Also, the weeding operation was performed regularly by hand as required.

2.3. Plants harvesting and measurements

In order to count the number of *Rhizobium* nodules at the flowering stage of common beans, five plants were completely harvested with their root system by a hoe at a soil depth of 50 cm. Then they were washed with distilled water and placed under a microscope to count active (pink) and inactive (gray) nodules.

Common bean was harvested before full maturation of seeds on 11th August 2015 and 18th August 2016. Also, fennel was harvested before full maturation of seeds on 6th September 2015 and 11th September 2016. The final seed yields were determined by harvesting all plants from 3.6 ${\rm m}^{-2}$ for each plot. After harvesting, the samples were dried at room temperature under darkness for 10–14 days. Once no variations were observed in their weights for 24 h, the seed yield were recorded. The seed yield of common bean and fennel was measured at 13–14% moisture content.

The common bean seed protein content was calculated based on simple N content using the Kjeldahl method. After the N content was measured, the protein content was obtained multiplying the N percentage by 6.25 (Jones, 1941).

2.4. Essential oil isolation

The fennel EO was obtained by a Clevenger apparatus using the hydro-distillation method. Thirty g of seeds were ground and inserted into 1 L glass flasks filled with 300 mL of distilled water. Then, they were boiled for 3 h to exhaust the whole plant material. The EO was collected in specific glass containers after adding sodium sulfate and kept at 4 $^{\circ}$ C in the darkness until GC-FID and GC-MS analyses. After measuring the EO content, the fennel essential oil yield was calculated as seed yield \times EO content (%) (Amani Machiani et al., 2018a).

2.5. Analysis of essential oil

For GC–MS analysis an Agilent 7890A-5975C (USA) gas chromatograph equipped with a HP-5MS capillary column (5 % phenyl methylpolysiloxane, 30 m l., 0.25 mm i.d., 0.25 µm f.t.) was used. The following oven temperature was used: 3 min at 80 °C, subsequently 8 °C/min to 180 °C, held for 10 min. Transfer line temperature was 240 °C. The flow rate of carrier gas (helium) was 1 mL/min. The injector split ratio was 1:50 and mass range acquisition was from 40 to 500 m/z in electron impact (EI) mode at 70 eV. The EO constituents were identified using the procedure reported by Morshedloo et al. (2018). Briefly, the retention index (RI) of components was calculated by comparison with a standard mixture of n-alkanes (C_7 - C_{28}) (Sigma-Aldrich, USA) and the obtained RI_S along with the mass spectra were compared with those reported in commercial libraries and literature (Adams, 2007; NIST 08, 2008). The available authentic standards (Sigma-Aldrich, USA) were also co-injected for identifying the major components.

For GC-FID analysis, an Agilent 7890 A (Agilent technology, USA) instrument, coupled with a FID detector and a HP-5 capillary column (The same as above) was used. The similar oven temprature as above was used. Injector and detector temperatures were 230 and 240 °C, respectively. The quantification (relative percentages) was performed according to the presedure reported by Morshedloo et al. (2017).

2.6. Extraction of fixed oil

In order to extract the fennel fixed oil, the dried seeds were reduced into a powder. The seed oil was extracted according to the AOCS (1993) method; briefly seeds (5 g) were extracted using 300 mL of *n*-hexane in a Soxhlet extractor. After 6 h, the solvent was removed from the oil with a rotavapor (Heidolph, Schwabach, Germany). Then, the oil was collected in a specific glass container to isolate and identify the compounds.

Fatty acids were converted to fatty acid methyl esters (FAMEs) to make them volatiles; they were analyzed by GC-FID. For this purpose, 0.1 g of oil was mixed with 1.5 mL of hexane and 0.2 mL of 2 N methanolic KOH, then vortexed for 5 s and centrifuged at 2500 rpm for 1 min. The upper layer was taken and kept at 4° C for analysis.

An Agilent 6890 N, GC apparatus (Wilmington, DE, USA) equipped with a FID detector was used for analysis. For FAME separations an HP-88 capillary column (88 % - Cyanopropy) aryl-polysiloxane, 100 m l., 0.25 mm i.d., 0.2 μ m f.t.) (Agilent) was used. The oven temperature was programmed as follows: 5 min at 140 °C, subsequently 4 °C/min to 240 °C, held for 15 min at 240 °C. The carrier gas was nitrogen, and flow rates were 1.0 mL/min and 45 mL/min, respectively. Temperatures of the injection port and detector were set to 260 °C and 280 °C, respectively. The injector was set in a split mode (split ratio of 1:30). The ChemStation software was used to acquire and process data. For the identification of fatty acids a FAME mixture (Supelco 37 Component FAME Mix Bellefonte, PA, USA) was used.

2.7. Land equivalent ratio (LER)

For the evaluation of advantage or disadvantage of intercropping patterns of fennel with common bean the land equivalent ratio (LER)

Table 1The number of nodule, nodule dry weight, seed protein and seed yield of common bean in different cropping patterns and PGPR application.

		· ·		
Treatments	Number of nodule	Nodule dry weight (g plant ⁻¹)	Seed protein (%)	Seed yield (kg ha ⁻¹)
PGPR (P)	38.40	0.39	19.12	1740.57
Control	34.00	0.33	18.80	1398.17
LSD (P = 0.05)	0.7	0.007	0.06	48.01
Cropping pattern	s (C)			
Fs	32.25	0.31	18.70	2362.42
1:1	36.16	0.35	18.97	1176.25
2:2	38.91	0.41	19.10	1563.08
3:2	37.75	0.36	19.24	1488.25
4:2	35.91	0.34	18.78	1256.83
LSD (P = 0.05)	1.10	0.01	0.10	75.9
Cropping pattern	$s \times PGPR$			
Cs (control)	30.66	0.29	20.60	2250.00
1:1 (control)	35.07	0.33	20.83	1018.01
2:2 (control)	36.00	0.36	20.95	1333.00
3:2 (control)	35.80	0.33	20.94	1255.33
4:2 (control)	34.33	0.33	20.63	1134.50
Cs + P	33.84	0.32	20.78	2474.83
1:1 + P	37.33	0.36	21.10	1334.50
2:2 + P	42.83	0.46	21.55	1793.17
3:2 + P	40.50	0.40	21.25	1721.16
4:2 + P	37.50	0.36	20.92	1379.33
$LSD \ (P = 0.05)$	1.55	0.01	0.15	107.36
Year (Y)	NS	NS	**	**
P	**	**	**	**
I	**	**	**	**
$P \times I$	**	**	**	**
$Y \times P$	NS	NS	NS	NS
$Y \times I$	NS	NS	NS	NS
$Y \times P \times I$	NS	NS	NS	NS

Cs (common bean sole cropping), 1:1, 2:2, 3:2 and 4:2 indicate the ratios of fennel and common bean in cropping patterns.

LSD: Least significant difference.

NS ** indicated non significant and significant difference at 1 % probability level, respectively.

was calculated using the following formula (Ofori and Stern, 1987):

$$LER = \frac{Y_1}{F_1} + \frac{Y_2}{C_2} \tag{1}$$

in which Y_1 and Y_2 represent the yield of the first and second species in the intercropping system, respectively, and F_1 and C_2 are their yield in sole cropping.

2.8. Statistical analysis

Data were statistically analyzed with the SAS 9.4 software package. The means were compared to the least significant difference (LSD) test at the p<0.05 level.

3. Results

3.1. Common bean

The results presented in Table 1 showed that the main effects of PGPR and cropping pattern and the interaction effect of cropping pattern \times PGPR was significant on the root nodule number, nodule dry weight, seed protein content and seed yield of common bean. Also, the year effects were significant on seed yield and seed protein content. On the other hand, the effects of year \times PGPR, year \times cropping pattern and year \times PGPR \times cropping pattern on these traits were not significant (Table 1).

3.1.1. Number of rhizobium nodule and nodule dry weight

The highest number of nodule (42.83) and nodule dry weight

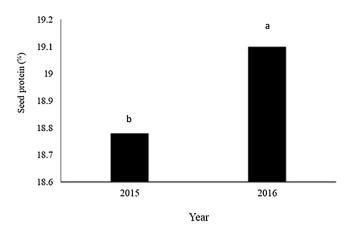


Fig. 2. The protein content of common bean seeds in 2015 and 2016; values are means of two determinations. The same letters in each shape show non-significant difference at $P \leq 0.05$ by LSD test.

(0.46 g) were observed in the intercropping ratio of 2:2 (fennel: common bean) under PGPR application, while the lowest values (30.66 number and 0.29 g, respectively) were recorded in common bean sole cropping without using PGPR (Table 1). Also, the average of intercropping patterns gave an increase of 16.0 and 19.4 % of the nodule number and dry weight, respectively, compared with common bean sole cropping. Furthermore, the application of PGPR increased these traits by 12.9 and 18.2 %, respectively, compared with control (Table 1).

3.1.2. Seed protein content

Mean comparisons revealed that the highest seed protein content (21.55 %) was achieved in the intercropping ratio of 2:2 under PGPR application. On the other hand, the minimum content of seed protein (20.60 %) was obtained in common bean sole cropping without using PGPR. The results demonstrated that the seed protein content in the intercropping ratios of 1:1, 2:2, 3:2 and 4:2 was 1.3, 2.7, 1.9 and 0.4 % higher than that in common bean sole cropping, respectively. Furthermore, the seed protein content increased by 1.8 % under PGPR application (Table 1). Finally, the seed protein content in the second year was about 1.7 % higher than that obtained in the first year (Fig. 2).

3.1.3. Seed yield

The results showed that the highest seed yield of common bean $(2474.83\,\mathrm{kg\ ha}^{-1})$ was obtained in the sole cropping under PGPR application whereas the lowest one $(1018.01\,\mathrm{kg\ ha}^{-1})$ was observed in the intercropping ratio of 1:1 without PGPR application. The seed yields of common bean in the cropping ratios of 1:1, 2:2, 3:2 and 4:2 decreased by 50.2, 33.8, 37.0, and 46.8 % compared with the sole cropping, respectively. Moreover, PGPR application increased the seed yield by 24.5 % compared with control. Finally, the seed yield in the second year was 5.0 % higher than that of the first year (Fig. 3).

3.2. Fennel

The results of variance analysis indicated that the effects of cropping pattern and application of PGPR were significant on seed yield, EO content, EO yield, fixed oil content and oil yield. Furthermore, the interaction between cropping patterns and PGPR was significant on all studied traits. Seed yield, EO yield and fixed oil yield were affected by the year. On the other hand, the effects of year \times PGPR, year \times cropping pattern and year \times PGPR \times cropping pattern on these traits were not significant (Table 2).

3.2.1. Seed yield

Mean comparisons revealed that the highest seed yield of fennel

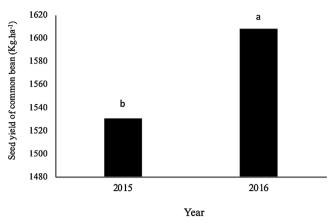


Fig. 3. The seed yield of common bean in 2015 and 2016. The same letters in each shape show non-significant difference at $P \le 0.05$ by LSD test.

Table 2The seed yield, EO content, EO yield, fixed oil content and oil yield of fennel in different cropping patterns and PGPR application.

Treatments	Seed yield (kg ha ⁻¹)	Essential oil content (%)	Essential oil yield (kg ha ⁻¹)	Fixed oil content (%)	Oil yield (kg ha ⁻¹)
PGPR (P)	1871.87	3.69	67.43	15.30	282.06
Control	1548.80	3.17	48.42	13.87	211.57
LSD (P = 0.05)	38.29	0.058	1.77	0.19	6.40
Cropping patterns (C)					
Fs	2597.00	2.88	75.05	12.94	336.84
1	1262.42	3.33	42.24	14.50	183.18
2:2	1348.25	3.86	52.75	15.71	213.12
3:2	1629.00	3.70	60.96	15.70	258.52
4:2	1715.00	3.38	58.60	14.07	242.42
LSD (P = 0.05)	60.55	0.09	2.80	0.30	10.12
Cropping patterns \times PGPR					
Fs (control)	2464.00	2.80	69.06	12.50	308.24
1:1 (control)	1165.00	3.18	37.19	14.30	166.32
2:2 (control)	1250.00	3.31	41.48	14.44	180.57
3:2 (control)	1416.67	3.39	48.02	14.42	204.38
4:2 (control)	1448.33	3.19	46.34	13.70	198.37
Fs + P	2730.08	2.96	81.04	13.39	365.44
1:1 + P	1359.83	3.47	47.30	14.71	200.04
2:2 + P	1446.50	4.42	64.02	16.85	245.66
3:2 + P	1841.33	4.01	73.91	16.98	312.67
4:2 + P	1981.67	3.57	70.87	14.45	286.48
$LSD \ (P = 0.05)$	85.63	0.13	3.97	0.43	14.31
Year (Y)	**	NS	*	NS	*
P	**	**	**	**	**
I	**	**	**	**	**
$P \times I$	**	**	**	**	**
$Y \times P$	NS	NS	NS	NS	NS
$Y \times I$	NS	NS	NS	NS	NS
$Y \times P \times I$	NS	NS	NS	NS	NS

Fs (fennel sole cropping), 1:1, 2:2, 3:2 and 4:2 indicate the ratios of fennel and common bean in cropping patterns.

LSD: Least significant difference.

NS, *, ** indicated non significant, significant difference at 5 % and significant difference at 1 % probability level, respectively.

(2730.08 kg ha⁻¹) was attained in the sole cropping after PGPR application. Otherwise, the lowest seed yield (1165 kg ha⁻¹) was recorded in the intercropping ratio of 1:1 without PGPR application. However, there were no significant differences in terms of seed yield between the intercropping ratio of 2:2 and 1:1 without PGPR application. Also, the seed yields of fennel in intercropping pattern ratios of 1:1, 2:2, 3:2 and 4:2 decreased by 51.40, 48.08, 37.27 and 33.96 % compared with fennel sole cropping, respectively. In addition, PGPR application increased the seed yield by 20.86 % compared with control

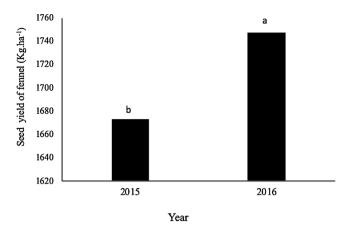


Fig. 4. The seed yield of fennel in 2015 and 2016. The same letters in each shape show non-significant difference at $P \le .05$ by LSD test.

(Table 2). The seed yield in the second year was about 4.42 % higher than the first year (Fig. 4).

3.2.2. EO content, EO yield and compositions

Mean comparisons showed that the EO content of fennel in all the intercropping patterns was higher than that in sole cropping. The highest EO content (4.42 %) was obtained in the intercropping ratio of 2:2 under PGPR application. On the other hand, the lowest EO content (2.80 %) was obtained in the fennel sole cropping without PGPR application. Generally, the average EO content in intercropping patterns was 24.0 % higher than that in sole cropping. Furthermore, the application of PGPR enhanced the EO content by 16.4 % compared with control (Table 2). In addition, the results demonstrated that the highest EO yield of fennel (81.04 kg ha⁻¹) was reached in sole cropping after PGPR application while the lowest EO yields (37.19 kg ha⁻¹) was achieved in the ratio of 1:1 without PGPR application. Interestingly, the application of PGPR increased the EO yield by 39.3 % compared with control (Table 2). Furthermore, the EO yield in the second year was 3.7 % higher than that in the first year (Fig. 5).

Based on the GC-FID and GC-MS analyses, a total of 17 compounds were identified in the fennel EO, accounting for 94.61–98.99% of the total compositions (Table 3). The main constituents were (*E*)-anethole (73.71–81.10%), fenchone (3.44–6.18%), limonene (3.49–5.82%) and methyl chavicol (4.06–7.22%). The highest amounts of (*E*)-anethole and fenchone were obtained in the intercropping ratio of 3:2 under PGPR application (Table 3). The maximum content of limonene was recorded in the intercropping ratio of 4:2 under PGPR application. On the other hand, the highest and lowest content of methyl chavicol was achieved in the sole cropping without PGPR followed by the

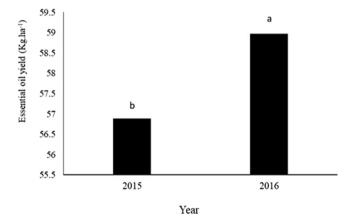


Fig. 5. The essential oil yield of fennel in 2015 and 2016. The same letters in each shape show non-significant difference at $P \le 0.05$ by LSD test.

Table 3

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Proportion

1 a-Pinene 2 Camphene 3 Sabinene 4 Myrcene 5 a-Phellan	a-Pinene Camphene Sabinene										ı				
	ene phene aene				Fs	Fs+ P	1:1	1:1 + P	2:2	2:2 + P	3:2	3:2+ P	4:2	4:2 + P	Average composition
	ohene nene	934	932	5.28	0.56 ± 0.04	0.49 ± 0.00	0.5 ± 0.08	0.57 ± 0.11	054 ± 0.24	0.66 ± 0.91	0.64 ± 0.02	0.81 ± 0.11	0.89 ± 0.34	0.73 ± 0.21	0.63
	nene	949	946	5.57	0.07 ± 0.00	ı	0.16 ± 0.04	0.15 ± 0.09	ı	0.07 ± 0.00	0.09 ± 0.08	0.11 ± 0.16	0.12 ± 0.12	0.1 ± 0.10	0.08
		973	696	6.05	0.16 ± 0.09	0.12 ± 0.54	0.22 ± 0.13	0.27 ± 0.00	0.22 ± 0.29	0.16 ± 0.04	0.19 ± 0.11	0.15 ± 0.21	0.22 ± 0.07	0.18 ± 0.09	0.19
	ene	066	886	6.38	0.34 ± 0.08	0.29 ± 0.004	0.56 ± 0.32	0.57 ± 0.11	0.34 ± 0.00	0.34 ± 0.07	0.39 ± 0.04	0.48 ± 0.14	0.42 ± 0.11	0.4 ± 0.67	0.41
	α-Phellandrene	1005	1004	69.9	0.11 ± 0.01	0.12 ± 0.08	0.16 ± 0.00	0.19 ± 0.77	0.1 ± 0.11	0.09 ± 0.21	0.11 ± 0.19	0.16 ± 0.64	0.08 ± 0.09	0.15 ± 0.08	0.12
	Limonene	1030	1024	7.24	4.09 ± 0.19	$4.25~\pm~0.11$	$3.49~\pm~0.18$	4.31 ± 0.08	5.16 ± 0.19	5.44 ± 0.99	4.92 ± 0.11	4.10 ± 1.11	5.75 ± 0.09	5.82 ± 0.90	4.87
, 1,8-C	1,8-Cineole	1032	1026	7.3	0.14 ± 0.04	0.09 ± 0.01	0.18 ± 0.09	0.21 ± 0.06	0.17 ± 0.11	0.14 ± 0.01	0.16 ± 0.03	0.12 ± 0.04	0.19 ± 0.09	0.00 ± 0.00	0.15
$-\theta$ -(Z) 8	(Z)-β-Ocimene	1036	1032	7.38	0.8 ± 0.01	0.59 ± 0.08	1.2 ± 0.32	1.16 ± 0.12	0.76 ± 0.004	0.8 ± 0.23	0.85 ± 0.76	0.89 ± 0.09	0.93 ± 0.01	0.85 ± 0.15	0.88
9 y-Teri	y-Terpinene	1059	1054	7.89	0.04 ± 0.07	0.21 ± 0.12	0.15 ± 0.14	0.08 ± 0.004	0.09 ± 0.09	0.04 ± 0.00	0.08 ± 0.01	0.10 ± 0.14	I	ı	0.08
10 Fenchone	hone	1091	1082	8.62	3.44 ± 1.08	4.93 ± 0.11	5.73 ± 0.19	6.16 ± 0.01	4.94 ± 0.23	$4.4~\pm~0.32$	5.39 ± 1.11	6.18 ± 0.92	5.57 ± 1.53	5.94 ± 0.84	5.54
1 Camphor	hor	1142	1138	9.92	0.18 ± 0.03	0.2 ± 0.01	0.27 ± 0.14	0.25 ± 0.05	0.15 ± 0.00	0.18 ± 0.05	0.18 ± 0.05	0.23 ± 0.047	0.21 ± 0.11	0.21 ± 0.19	0.20
12 Methy	Methyl chavicol	1200	1199	11.14	$7.22~\pm~0.12$	6.50 ± 0.19	$4.5~\pm~1.11$	4.66 ± 0.98	4.79 ± 1.21	$4.22\ \pm\ 1.08$	4.54 ± 1.32	4.06 ± 0.08	4.63 ± 0.90	4.86 ± 0.08	4.5
13 Fench	Fenchyl acetate	1236	1232	11.97	0.06 ± 0.02	ı	0.09 ± 0.00	0.08 ± 0.01	ı	0.06 ± 0.004	ı	ı	ı	0.01 ± 0.008	0.03
14 p-Anis	p-Anisaldehyde	1250	1247	12.44	0.24 ± 0.04	0.29 ± 0.01	0.17 ± 0.03	0.15 ± 0.09	0.32 ± 0.24	0.34 ± 0.11	0.29 ± 0.16	0.22 ± 0.09	0.4 ± 0.09	0.14 ± 0.008	0.25
15 (Z)-Aı	(Z)-Anethole	1255	1249	12.39	0.32 ± 0.13	0.14 ± 0.07	0.21 ± 0.01	0.21 ± 0.08	0.1 ± 0.00	0.32 ± 0.16	0.16 ± 0.00	0.12 ± 1.04	0.14 ± 0.00	0.10 ± 0.94	0.18
16 (E)-Aı	(E)-Anethole	1293	1282	13.27	73.71 ± 3.10	74.26 ± 2.58	77.33 ± 1.70	77.76 ± 2.19	79.86 ± 3.78	80.73 ± 2.90	79.55 ± 3.24	81.10 ± 3.67	77.18 ± 1.99	79.16 ± 2.28	79.07
17 Germ	Germacrene D	1487	1476	17.44	0.15 ± 0.01	0.18 ± 0.06	0.16 ± 0.00	0.21 ± 0.14	0.14 ± 0.32	0.15 ± 0.004	0.2 ± 0.12	0.16 ± 0.05	0.14 ± 0.12	0.17 ± 0.005	0.16
18 Total	Total identified				94.61	94.66	80.96	6.97	97.68	98.14	97.74	66.86	96.95	98.1	97.34

RI, linear retention indices on DB-5 MS column, experimentally determined using homologue series of n-alkanes. P(PGPR), Fs (fennel sole cropping), 1:1, 2:2, 3:2 and 4:2 indicate the ratios of fennel and common bean in cropping patterns; data are mean \pm SE (n = 3).

intercropping ratio of 3:2 under PGPR application, respectively. Overall, the average contents of (*E*)-anethole, fenchone and limonene in the intercropping patterns were 6.87, 32.22 and 16.79 % higher than those in sole cropping, respectively. Furthermore, PGPR application enhanced the content of these constituents by 1.2, 10.2 and 2.1 % when compared with control (Table 3).

3.2.3. Seed oil content, Oil yield and compositions

The results showed that the fennel fixed oil content ranged from 12.50 to 16.98 %. The minimum oil content was observed in fennel sole cropping without using PGPR, while the highest one was produced in the intercropping ratio of 3:2 under PGPR application. Generally, the average oil content in intercropping patterns was about 15.7 % higher than that in fennel sole cropping. Noteworthy, the oil content increased by 10.3 % after PGPR application when compared with control (Table 2).

The maximum oil yield (365.44 kg ha⁻¹) was observed in fennel sole cropping after PGPR application whereas the minimum oil yield (166.32 kg ha⁻¹) was obtained in the intercropping ratio of 1:1 without PGPR. Overall, the average oil yield in intercropping patterns was about 50.2 % lower than that in sole cropping. Interestingly, application of PGPR enhanced the fixed oil yield by 33.3 % when compared with control (Table 2). The yield oil in the second year was about 3.2 % higher than that of the first year (Fig. 6).

According to GC–MS analyses, a total of 8 fatty acids were detected in the fennel seed fixed oils. Among them, the monounsaturated oleic acid (77.17–82.90%) was the major component followed by the polyunsaturated linoleic acid (6.50–8.97%) and saturated palmitic acid (3.25–6.80%). The highest amount of mono- and polyunsaturated fatty acids (oleic and linoleic acids) was achieved in the intercropping ratio of 2:2 under PGPR application. Overall, the intercropping patterns increased the content of oleic acid and linoleic acid by 4.8 and 27.7 %, respectively, when compared with sole cropping. On the other hand, the highest amount of palmitic acid was obtained in the sole cropping without PGPR. The application of PGPR enhanced the content unsaturated fatty acids by 1.1 and 6.4 %, respectively (Table 4).

3.2.4. Land equivalent ratio (LER)

The highest partial LER of fennel (0.73) and common bean (0.72) was obtained in the ratio of 3:2 and 2:2 after PGPR application, respectively, whereas the lowest values (0.47 and 0.45, respectively) were achieved in the intercropping ratio of 1:1 without PGPR (Fig. 7). In other intercropping patterns, the partial LER of fennel was higher than that of common bean. It can be assumed that fennel is the dominant plant and is positively influenced by the intercropping with common bean. The lowest total LER (0.92) was obtained from the 1:1 intercropping ratio without PGPR whereas the highest one (1.32) was

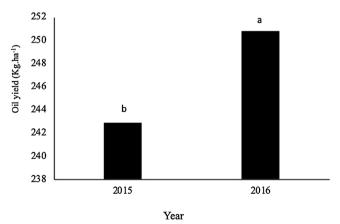
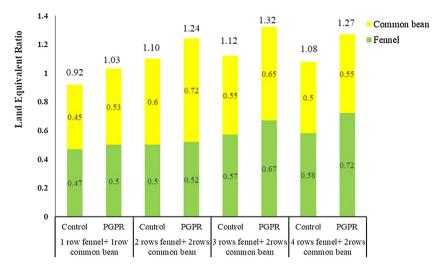


Fig. 6. The oil yield of fennel in 2015 and 2016. The same letters in each shape show non-significant difference at $P \le 0.05$ by LSD test.

Proportion of fennel oil constituents under different cropping patterns and PGPR application (average of two years).

No	No Component								Planting patterns			
		Fs	Fs+ P	1:1	1:1+ P	2:2	2:2 + P	3:2	3:2+ P	4:2	4:2 + P	Average composition
1	Myristic acid	0.30 ± 0.04	0.30 ± 0.04 0.32 ± 0.01	0.15 ± 0.00	0.18 ± 0.09	0.11 ± 0.04	0.14 ± 0.12	0.06 ± 0.11	0.08 ± 0.07	0.19 ± 0.14	0.11 ± 0.003	0.16
2	Palmitic acid	6.80 ± 0.11	6.16 ± 0.19	4.54 ± 0.23	4.58 ± 0.65	$5.14~\pm~0.12$	3.84 ± 0.07	5.20 ± 0.54	3.25 ± 0.76	4.11 ± 0.04	4.05 ± 0.45	4.76
3	Palmitoleic acid	1.61 ± 0.4	1.52 ± 0.09	0.92 ± 0.11	0.89 ± 0.08	1.08 ± 0.04	0.86 ± 0.2	0.78 ± 0.09	1.08 ± 0.04	0.98 ± 0.12	1.10 ± 0.02	1.08
4	Stearic acid	1.14 ± 0.09	1.22 ± 0.10	1.06 ± 0.3	1.51 ± 0.1	0.95 ± 0.08	0.90 ± 0.17	0.89 ± 0.05	1.21 ± 0.11	1.20 ± 0.7	1.14 ± 0.01	1.12
2	Oleic acid	77.17 ± 3.10	78.94 ± 2.19	81.54 ± 4.55	81.88 ± 3.5	82.80 ± 4.10	82.90 ± 1.98	80.50 ± 2.55	82.20 ± 4.77	81.10 ± 3.45	81.58 ± 2.37	81.06
9	Linoleic acid	6.50 ± 0.25	6.80 ± 0.56	8.10 ± 0.11	8.77 ± 0.05	8.81 ± 1.54	8.91 ± 0.97	8.16 ± 1.15	8.41 ± 1.11	7.80 ± 1.04	8.97 ± 0.66	7.24
7	Linolenic acid	0.10 ± 0.04	0.12 ± 0.14	0.15 ± 0.08	0.11 ± 0.00	0.15 ± 0.09	0.12 ± 0.18	0.11 ± 0.09	0.08 ± 0.1	0.06 ± 0.004	0.05 ± 0.01	0.10
8	Arachidonic acid	0.10 ± 0.001	0.11 ± 0.01	0.09 ± 0.08	0.13 ± 0.1	0.14 ± 0.08	0.11 ± 0.09	0.09 ± 0.00	0.05 ± 0.06	0.10 ± 0.03	0.11 ± 0.14	0.10
	Total identified compounds (%)	93.62	96.59	96.55	98.05	99.18	98.78	92.76	98.36	96.71	97.11	95.62

SE P(PGPR), Fs (fennel sole cropping), 1:1, 2:2, 3:2 and 4:2 indicate the ratios of fennel and common bean in cropping patterns; data are mean ±



Different intercropping patterns under PGPR application

Fig. 7. Land equivalent ratios (LER) values in different intercropping patterns and PGPR application.

observed in the intercropping pattern of 3:2 under PGPR application (Fig. 7). Thus, the intercropping ratio of 3:2 improved the land use efficiency by 32 % compared with sole cropping.

4. Discussion

Our results revealed that intercropping improved the nodule number and dry weight compared with common bean sole cropping. The highest nodules number and dry weight were achieved in the intercropping ratios of 2:2 and 3:2 under PGPR application, respectively. The increase of number of nodules in the roots of the intercropped common bean could be due to the better root growth and favorable conditions available for inoculation of bacteria into nodules under intercropping (Liu et al., 2017). Some researches have shown that when legumes are intercropped with non-legumes species, the nodule formation of legume in intercropping system increased as a consequence of the stimulation of nitrogen fixation and dissolution of P and other nutrients, which acidify the rhizosphere (Liu et al., 2017). Alike, Liu et al. (2019) indicated that the nodule number and dry weight of faba bean (Vicia faba L.) were increased under intercropping with wheat (Triticum aestivum L.) when compared with sole cropping. Similarly, the increasing number of nodules and nodule dry weight of legumes in intercropping patterns was previously shown in several studies (Liu et al., 2017; Bargaz et al., 2015). On the other hand, the application of PGPR increased the nodule numbers and dry weight compared with control. In this regard, previous studies demonstrated that access to nutrients, especially phosphorus and micronutrients, plays an important role on the biological fixation of nitrogen (Weisany et al., 2013). Application of PGPR increased the production of phytohormones that stimulate root growth, thus enhancing the root area available for nutrient uptake (Boon Kuan et al., 2016). It seems that the use of phosphate solubilizing bacteria (Pseudomonas putida and Pantoea agglomerans), with timely delivery of phosphorus and micronutrients, has a significant role in increasing the formation of nodules. On the other hand, the application of Thiobacillus bacteria along with other growth stimulating bacteria enhanced the productivity by reducing the soil pH and providing better conditions for the uptake of nutrients, especially P and micronutrients (Fe, Mn, Zn, and Cu) (Paul and Lade, 2014). This explains the role of PGPR in the process of nodule formation and N fixation in the root zone. Afzal et al. (2010) indicated that the combined inoculations of Bradyrhizobium sp. with Pseudomonas enhanced the root nodulation in soybean (Glycine max L.) resulting from the synergistic effect of the two bacterial species. Also, Htwe et al. (2019) noted that the co-inoculation of *Bradyrhizobium* strains and *Streptomyces griseoflavus* increased significantly the nodule formation and nitrogen fixation in soybeans, mung beans (*Vigna radiate* L.) and cowpeas (*Vigna unguiculata* L).

Our results showed that the highest seed yield was obtained in the sole cropping of common bean and fennel. The reduction of the seed yield of the two species in intercropping systems may be due to the maximum interspecific competition level occurring in intercropping systems compared to sole cropping. Besides, the higher production (higher yield per unit area) in common bean and fennel sole cropping can be attributed to the homogeneous environment under sole cropping systems (Amani Machiani et al., 2018a). On the other hand, the meaningful decrease of fennel and common bean seed yield in the intercropping ratio of 1:1 may be attributed to the higher competitive ability of fennel with common bean due to the reduced complementary and the facilitative interaction of the two plants species. The higher seed yields of other intercropping patterns compared to the intercropping ratio of 1:1 were probably due to the better use of environmental resources by balancing inter-specific and intra-specific interactions (Bedoussac and Justes, 2011). In a study on soybean (Glycine max L.) and dill (Anethum graveolens L.) intercropping system, Rostaei et al. (2018) reported that the highest seed yield of soybean and dill was achieved in both plants sole cropping. A higher productivity in sole cropping has also been reported by Fallah et al (2018) in dragonhead soybean systems, Amani Machiani et al. (2018a) in peppermint intercropped with faba bean, Liu et al. (2017) in faba bean intercropped with wheat, Salehi et al. (2017) in buckwheat intercropped with fenugreek, and Lai et al. (2016) in mustard intercropped with chickpea. In this studies, it was found that the increase of seed yield in both plants under PGPR application could be due to the increase of the synthesis of plant hormones and mobilization of accessible nutrients (Egamberdieva et al., 2015). Seved Sharifi et al. (2017) reported that PGPR application in intercropping systems can provide a sufficient amount of nutrients leading to a decrease in competition for nutrients. Also, the improvement of the plant growth after the establishment of symbiotic relationship with bacteria is related to the higher level of nitrogen fixation (Amirnia et al., 2019), addition or transfer of nitrogen from legumes to the non-legume plant, and better growth and nutrient uptake from the soil (Dakora and Phillips, 2002). Similarly, Saeidi et al. (2018) reported that the seed yield of safflower in intercropping with faba bean was significantly increased after application of biofertilizers. Studies of Jalilian et al. (2017) and Singh et al (2013) on safflowerbitter vetch and pigeon pea (Cajanus cajan L.)/mung bean (Phaseolus radiatus) intercropping systems showed that application of biofertilizers

increased the yield of the main components in both plants.

Our results demonstrated that the intercropping system increased the seed protein content compared with sole cropping. The highest seed protein content was measured in the intercropping ratio of 2:2 and 3:2 under PGPR application. The maximum seed protein content in intercropped common bean was attributed to the higher nitrogen fixation which lead to the improvement of the nitrogenase activity and root nodulation (Fallah et al., 2018; Banik et al., 2006). Basaran et al. (2017) noted that intercropping sorghum with cowpea improved the seed protein content in cowpea compared with sole cropping. The higher seed protein content in intercropping systems has been previously reported by Vrignon-Brenas et al. (2018) in wheat-white clover intercropping. In addition, the seed protein content of common bean increased under PGPR application. PGPR supplies both macro- and micronutrients which are essential for the growth of plants (Egamberdieva et al., 2015). In the present research, the increment in the overall seed protein content was probably due to the increase of nitrogen-fixing symbiotic Rhizobium that allows nitrogen accessibility over a longer time during the plant growth, and increases the absorption of nutrients, especially nitrogen (Rostaei et al., 2018). Similarly, Saeidi et al. (2018) reported that the seed protein content of faba bean (Vicia faba L.) intercropped with safflower (Carthamus tinctorius L.) increased under PGPR application.

The results showed that the fennel fixed oil content in all the intercropping patterns was higher than that in sole cropping. The maximum fixed oil content was recorded in the intercropping ratios of 2:2 and 3:2 under PGPR application. This may be due to the more light, water and nutrients received by plants in the intercropping systems compared with those grown in sole cropping. This improves growth, photosynthesis and dry matter allocation to the seed and consequently also its oil quality (Gitari et al., 2018). In addition, the rate of fixed oil accumulation may be influenced by other factors including genotype, sowing date, ecological conditions, soil fertility, planting density and cropping pattern (Sabzalian et al., 2008). In the present study, PGPR application was effective in increasing the fixed oil content in the fennel seeds by providing conditions for the uptake of macro- and micronutrients and satisfying plant requirements in a timely manner. Thiobacillus bacteria also help in solubilizing inaccessible forms of soil nutrition elements and facilitating their transport in plants. Among them, sulfur is important for the metabolism of fatty acids (Behal et al., 2002). In addition, potassium increases metabolism and transformation of carbohydrates and affects the contents of fixed oil in seeds. Therefore, the use of potassium solubilizing bacteria (through the combination of Pseudomonas koreensis and P.vancouverensis) and sulfur supplier bacteria (Thiobacillusspp.) plays an important role in increasing the production of fennel oil. Noteworthy, the oil yield showed a significant positive correlation with the seed yield and fixed oil content. As a consequence, any factor that increases these indices may increase the oil yield as well. Saeidi et al. (2018) reported that PGPR application resulted in the improvement of the oil content in safflower seed. Similar results have been reported by Mirzaei and Vazan in safflower (2013) and Akbari et al. (2011) in sunflower.

Oleic and linoleic acids have beneficial effects on the human health. In this study, the fixed oil from fennel seeds proved to be a rich source of these fatty acids. On the above, the fennel fixed oil can be considered healthy just like other oilseeds including canola and sunflower. The content of these unsaturated fatty acids increased in different intercropping patterns. The highest amount of mono- and polyunsaturated fatty acids was observed in the intercropping ratio of 2:2 under PGPR application. This increase was attributed to the better utilization of resources for photosynthesis, particularly light and CO₂ (Wang et al., 2017). In addition, the application of PGPR improved the quality of fennel oil constituents compared with untreated plants. This might be due to the good utilization of nutrients supplied for the oil metabolism (Schroder and Kopke, 2012). Shu-tian et al. (2018) suggested that the availability of nutrients promotes the oil metabolism and transformation

of carbohydrates and affects the oil contents. Similarly, Seyed Sharifi et al. (2017) reported that the inoculation with PGPR improved the safflower oil quality by enhancing the content of unsaturated fatty acids and reducing that of saturated fatty acids. The findings of this study were similar to the results previously reported by Chehaba et al., 2019 in olive trees intercropped with legumes, Saeidi et al. (2018) in safflower and Luis et al. (2013) in soybean seeds.

In our study, the fennel EO content and yield improved in different intercropping patterns under PGPR application. The EO of medicinal and aromatic plants are mostly composed of terpenoid compounds. The biosynthetic units (isoprenoids) making up these metabolites need Acetyl-CoA, NADPH and ATP, whose synthesis depends on the availability of elements such as N and P (Morshedloo et al., 2017; Ormeño and Fernandez, 2012). On the other hand, the increase of EO content in some aromatic plants is correlated to a greater density of trichomes, the main structure for EOs biosynthesis (Harrewijn et al., 2000). In addition, the increase of EO content in fennel intercropping systems may be due to the N availability through nitrogen fixation by common bean, use of soil nutrients, availability of nutrients after PGPR application, better distribution of light by the mixed canopy of the two species, and facilitative and complementary effects causing a more efficient use of available resources (Streit et al., 2019; Wang et al., 2017; Egamberdieva et al., 2015). Here, the higher EO content in inoculated crops was attributed to the increased bacterial colonization and improved nutrient status of the host crop. EOs are regarded as mixtures of plant secondary metabolites whose quantity depends significantly on primary metabolites (Amani Machiani et al., 2019). Thus, any factor that increases plant photosynthesis can enhance the production of secondary metabolites including essential oils. Fallah et al. (2018) reported that the EO biosynthesis is influenced by distribution, size and number of oil gland cells, and availability of nutrients such as N and P. In the present research, application of phosphate and potassium-solubilizing bacteria, N-fixing bacteria, and S-oxidizing bacteria improved the EO productivity by reducing the soil pH and providing appropriate conditions for the uptake of nutrients like N, P and microelements (Fe, Mn, Zn, and Cu) (Paul and Lade, 2014). The increase of EO yield in response to PGPR application was due to the increase of seed yield and biosynthesis of terpenes. The EO yield showed a positive correlation with the seed yield and EO content. Consequently, any factor that improves these indices could increase the EO yield. These results were in agreement with those reported by Ghorbanpour and Hatami (2014), who noted that PGPR application improved the EO content, yield, and EO constituents of Salvia officinalis L. Similar results were also reported by Banchio et al. (2008) in Origanum majorana L. The quality of fennel EOs improved in different intercropping patterns under PGPR application. The main EO components were (E)-anethole, fenchone, limonene and methyl chavicol. The content of these constituents, except methyl chavicol, in intercropping patterns was higher than those in sole cropping. The improvement of EO quality of medicinal plants in the intercropping systems may be explained by the more efficient use of available resources such as water, solar radiation and nutrients compared with sole cropping (Weisany et al., 2016 and Amani Machiani et al., 2018a). Also, the application of PGPR in different intercropping patterns provided the appropriate conditions for activity of beneficial microbes in the soil and enhanced the EO quality by supplying optimal amounts of macro- and micronutrients (Liu et al., 2019; Grobelak et al., 2015). It seems that PGPR application in intercropping systems increased the main EO constituents such as (E)-anethole by affecting nutrient uptake and optimal use of plant growth factors. The improvement of the EO quality was also reported by Amani Machiani et al. (2018a); Rostaei et al. (2018); Weisany et al. (2016) and Verma et al. (2013) in peppermint-faba bean, soybean-dill, common bean-dill and peppermint-geranium intercropping systems, respectively.

Our results indicated that the partial LER of fennel was higher than that of common bean. It can be assumed that fennel was the dominant plant and was positively influenced by intercropping with common

bean. In addition, the calculated LER in all the intercropping patterns (except 1:1 ratio without PGPR) was greater than 1, indicating that application of PGPR can amplify the benefits of legume-based intercropping. The LER > 1 in intercropping systems may be attributed to the better use of nutrients, water, radiation and space and better crop architecture and distribution (Amani Machiani et al., 2019; Rezaei-Chianeh et al., 2011). In addition, the application of PGPR in the intercropping system improved the plant growth and provided nutrients to the plants. In this respect, the complementary and facilitative effect on the exploitation of resources by the root system and phenology as well as resource use efficiency can be stated as a practical justification for the efficacy of intercropping systems. Higher LER values have also been reported by Amani Machiani et al. (2018b); Salehi et al. (2018); Moghbeli et al. (2018) and Koocheki et al. (2018) for peppermint-faba bean, buckwheat-fenugreek, onion-fenugreek and saffron-pumpkinwatermelon intercropping systems, respectively.

5. Conclusions

In the present study, we found out that the yields of fennel and common bean were affected by different intercropping patterns. The highest seed yield of both plants was obtained in the sole cropping systems. However, fennel EO and fixed oil contents were higher in all the intercropping patterns compared with sole cropping. Furthermore, application of PGPR improved the productivity and qualitative characteristics of both plants. PGPR application increased the seed yield of common bean and fennel up to 20 and 24 %, respectively. The highest amount of (E)-anethole, fenchone, and unsaturated oleic and linoleic acids were obtained in the intercropping ratios of 3:2 and 2:2 under PGPR application, respectively. This may be due to the better root growth and favorable conditions available for inoculation of bacteria into nodules under intercropping and by supplying optimal amounts of macro- and micronutrients. The highest LER (1.32) was obtained in the ratio of 3:2 after PGPR application giving a 32 % higher productivity in land use when compared with the sole cropping of the two species. Based on the obtained results, the intercropping ratio of 2:2 and 3:2 (fennel: common bean) under PGPR application may be suggested to farmers as a useful treatment enhancing productivity and quality of fennel EO compared with sole cropping. Finally, the usage of PGPR in intercropping systems can be useful from an economic, social and environmental perspective allowing the elimination or reduction of the chemical inputs.

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