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Improving essential oil yield and compositions of basil (Ocimum basilicum L.) through mycorrhiza fungi and bacteria in intercropping system

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Abstract

A two-year study was conducted to investigate the effects of fertilizer treatments on growth characteristics, essential oil (EO) yield and compositions of basil (*Ocimum basilicum* L.) in intercropping with fenugreek (*Trigonella foenum-graecum* L.). A factorial experiment was arranged in a randomized complete block design with three replicates in 2021 and 2022. The first factor was cropping pattern including sole cropping of basil and fenugreek, strip intercropping of basil (B)-fenugreek (F) with replacement method in the ratios of 2B:2F, 4B:2F, 5B:3F and an additive intercropping of 100% basil + 50% of fenugreek (100B:50F). The second factor was fertilizer at three levels including application of 100% chemical fertilizer (100% CF), 50% chemical fertilizer + biofertilizer (50% CF + Bio) and biofertilizer (Bio). The greatest basil height was observed in 2B:2F cropping pattern and 50% CF + Bio treatment (50.39 cm). The greatest yield of dry herbage was observed in 5B:3B cropping pattern with 100% CF and 50% CF + Bio treatments. The greatest yields of EO were observed in sole basil and 2B:2F intercropping (0.87 g m^{-2}), in 50% CF + Bio treatment. Methyl chavicol, methyl eugenol, β-caryophyllene and 1,8-cineole, were the main chemical constitutes of EO. The contents of major components of EO were affected differently by cropping pattern and fertilizer. The greatest content of methyl chavicol (53.7%), was observed in 2B:2F and Bio fertilizer treatment. At all treatments, the land equivalence ratios (LER) were more than 1.0 and the highest value (1.99) was obtained in 2B:2F intercropping with 50% CF + Bio treatment. Application of 50% CF + Bio fertilizer treatment in 2B:2F intercropping, improved the productivity of basil-fenugreek intercropping, enhanced the EO compositions and could be used in sustainable production of basil.

Introduction

Basil (*Ocimum basilicum* L.) is an essential oil-containing medicinal plant in family of Lamiaceae in sub-tropical and tropical climates[1]. The EO of basil is a component of dental products, oral health, and food industry[2]. Due to its antimicrobial, antioxidant, analgesic, anti-ulcerogenic and anti-inflammatory properties, it is frequently used in traditional medicine[3]. It is also could be used as industrial purposes (pharmaceuticals, cosmetics and perfumery) and additive species, which could be related to its richness in flavonoids, phenols and essential oils[4].

Modern agriculture is based on monocropping that lead to unsustainability, causes increases in pests and diseases pressure and reduction in nutrients availability in the soil[5,6]. Intercropping is a strategy that improve the productivity of the field by growing the different crops in a same time through increasing the resources use efficiency[7]. When the medicinal plants and spices, intercropped with main crops, they can reduce the pest density and population, because of aromatic and volatile materials which released in the field[8,9,10]. The demand for medicinal and aromatic plants is increasing all over the world, because they play an important role in the economic and ecological ambits of rural communities[11]. Intercropping is a strategy to improve crop productivity against time and place, by increasing the resources use efficiency, managing weeds, diseases and pests, and reduction in soil erosion[12]. Using legume crops in intercropping is an effective strategy to supply nitrogen requirement for companion crop[10,13] in intercropping system of medicinal plants, improve the yield and quality in cropping system[14,15,16]. In previous studies observed that medicinal plants intercropping with legume crops, improved the EO yield and compositions[7,9,10,17,18].

In sustainable agriculture, using micro-organisms as an inoculum has an important role in nutrients cycle and decrease in application of chemical fertilizers because of their negative effects on agroecosystems[7,19]. One of the most prevalent biofertilizers is arbuscular mycorrhizal fungi (AMF), which improve the root distribution in soil and increase total effective surface of the roots[7,20]. In cereals, sesame and sunflower, *Azotobacter* has been used as a biofertilizer. It has been reported that *Azotobacter* produces indole acetic acid and gibberellins and also increases N_2 fixation[10,21]. Increasing the plant development, growth and yield, also have been observed in inoculation of *Azospirillum* by improving N_2 status of the plant through N_2 fixation and synthesis of auxin[22]. Using biofertilizers cause to achieve safe and high-quality products without detrimental effects on environment and human health[16,23]. In Moldavian balm, using biofertilizer increased the EO and yield[24,25], and also improved the geranyl acetate and geraniol contents[24].

Long-term application of chemical fertilizers increases crop yield, but disturbs the ecological balance of soil, increases water pollution and decreases the population of soil micro-organism[7,26] . Also, in medicinal plants, demand for organic and safe products has increased because of health consideration[7]. Among the biofertilizers available in Iran, Biopharm biofertilizer is noteworthy, which contains both *Azospirillum* and *Azotobacter* [7]. Combined application of biofertilizers, especially these bacteria along with mycorrhizal fungi species, could effectively increase the growth characteristics and EO yield of basil. Since there is limited information about the intercropping of basil with fenugreek (*Trigonella foenum-graecum* L.) in Iran, so this study was conducted to evaluate the effects of intercropping and biofertilizers on EO yield and composition of basil and advantage of intercropping by land equivalent ratio (LER).

Results

Plant height. The basil height was affected significantly ($p \leq 0.01$) by year, cropping pattern, fertilizer and interaction of year × cropping pattern, year × fertilizer and cropping pattern × fertilizer (Table 1). The mean comparison for interaction of year × fertilizer (Table 2) showed that in both years the greatest plant heights were observed in 50% CF+ Bio fertilizer. In 2021, the plant height in 50% CF + Bio treatment, enhanced by 17.7% in comparison with 100% CF, while this increase was 11.8% in 2022. At all fertilizer treatments, the plant heights in 2022 decreased compared with those in 2021 (Table 2). The results for effect of year × cropping pattern (Table 3) showed that in both years the greatest plant heights were observed in 2B:2F cropping pattern. In 2021, the plant height in 2B:2F cropping pattern, increased by 23.4% compared with basil sole cropping, while in 2022, this increase was 17.6%. At sole basil and intercrops, the plant heights in 2022 decreased compared with those in 2021 (Table 3). The interaction effect of cropping pattern × fertilizer on plant height (Fig. 1) indicated that at 2B:2F, 4B:2F and 5B:3F intercropping pattern, the plant heights increased compared with sole basil and the highest plants were obtained in 2B:2F intercropping. At all cropping patterns the 50% CF+ Bio treatment had the greatest plant highest.

Table 1. Analysis of variance for effect of cropping pattern and fertilizer treatment on growth traits, EO content and yield of basil.

Source of variation	df	Plant height	Dry herbage yield	EO content	EO yield
Year (Y)	1	**	**	**	**
Block × Y	4	**	**	**	**
Cropping pattern (C)	4	**	**	**	**
Y × C	4	**	**	**	ns
Fertilizer (F)	2	**	**	**	**
Y × F	2	**	**	ns	ns
C × F	8	**	**	**	**
Y × C × F	8	ns	ns	ns	ns
CV (%)		13.2	16.7	1.65	11. 6

ns, * and **: non -significant and significant at P 0.05 and P 0.01, respectively

Table 2. Mean comparison for interaction of year × fertilizer treatment on plant height and dry herbage yield of basil. Different letters indicate significant differences at $p \leq 0.05$.

Year	Fertilizer treatment	Plant height (cm)	Dry herbage yield (g m^{-2})
	100% CF	40.41 c	100.2 b
2021	50% CF + Bio	47.55 a	104.1 a
	Bio	42.32 b	104.9 a
	100% CF	34.35 e	96.13 d
2022	50% CF + Bio	38.42 c	99.62 bc
	Bio	35.97 d	98.87 c

Table 3. Mean comparison for interaction of year × cropping pattern on plant height, dry herbage yield and EO content of basil. Different letters indicate significant differences at $p \leq 0.05$.

Year	Cropping pattern	Plant height (cm)	Dry herbage yield (g m^{-2})	EO content
	Sole cropping	40.32 e	136.1 a	0.60 f
	2B:2F	49.75 a	83.77 g	0.95 a
2021	4B:2F	45.61 b	96.71 e	0.78 c
	5B:3F	41.83 d	102.8 c	0.67 e
	Additive (100B:50F)	39.62 f	95.96 e	0.78 c
	Sole cropping	34.27 i	127.7 b	0.56 h
	2B:2F	40.29 c	80.27 h	0.86 b
	4B:2F	38.77 g	92.79 f	0.70 d
2022	5B:3F	35.56 h	98.91 d	0.58 g
	Additive (100B:50F)	33.68 j	91.38 f	0.70 d

Dry herbage yield. Dry herbage yield of basil was affected significantly ($p \leq 0.01$) by year, cropping pattern, fertilizer and interaction of year × cropping pattern, year × fertilizer and cropping pattern × fertilizer treatment (Table 1). The mean comparison for interaction of year × fertilizer treatment (Table 2) showed that the greatest herbage yields were obtained in 2021, 50% CF+ Bio and Bio fertilizer treatments and the lowest one in 2022, 100% CF treatment. The dry herbage

yields in 2022 decreased compared with those in 2021. The results for interaction of year × cropping pattern (Table 3) showed that in both years the greatest dry herbage yields were observed sole cropping pattern and decreased in all intercropping patterns. The highest values for dry herbage yield were obtained in 5B:3F cropping pattern and decreased by 24.5 and 22.5 % compared with basil sole cropping, in 2021 and 2022, respectively. The interaction of fertilizer treatment × cropping pattern on herbage yield (Fig. 2) indicated that the dry herbage yields at all intercropping patterns, reduced in comparison with sole basil and the highest values were obtained in 5B:3F intercropping. In sole cropping, 2B:2F and 5B:3F cropping patterns, the dry herbage yields were not significantly different in 100% CF and 50% CF+ Bio fertilizer treatments, while in 4B:2F and 100B: 50F cropping patterns, the dry herbage yields were not significantly different in all fertilizer treatments (Fig. 2).

Table 4. Mean comparison for effect of year on EO content (%) yield of basil. Different letters indicate significant differences at $p \leq 0.05$.

Year	EO content (%)	EO yield (g m^{-2})
2021	0.75 a	0.76 a
2022	0.68 b	0.65 b

EO content (%). The results of ANOVA indicated that the EO content was affected significantly by year, cropping pattern, fertilizer and interaction of cropping pattern × fertilizer (Table 1). The means comparison indicated that the greatest EO content (1.06%) was obtained in 2B:2F with application of 50% CF+Bio fertilizer treatment (Fig. 3). At 2B:2F cropping pattern, in all fertilizer treatments, the EO contents increased compared with those in sole cropping. Also, at 4B:2F and 5b:3F cropping patterns, the highest EO contents were observed in 50% CF+Bio fertilizer treatment. Results for interaction effect of year × cropping pattern on basil EO content (Table 3) indicated that in both years, the greatest EO contents were obtained in 2B:2F intercropping pattern. Also, at all intercrops and sole basil, the EO contents in 2021 were higher than those in 2022.

EO yield. The results of ANOVA indicated that the EO yield was affected significantly by year, cropping pattern, fertilizer treatment and interaction of cropping pattern × fertilizer treatment (Table 1). The comparison of means (Fig. 4) indicated that the highest EO yield of bail (0.871 g m^{-2}) was observed in sole basil. 2B:2F and 5B:3F had the highest (0.867 g m^{-2}) and lowest (0.811 g m^{-2}) EO yield, respectively, among the intercropping patterns (Table 6). In 50%CF+Bio, the EO yield significantly enhanced (0.871 g m^{-2}) compared with those of 100% CF (0.779 g m^{-2}) and Bio (0.657 g m^{-2}) treatments (Fig 4). The basil EO yield of in 2021 (0.76 gm^{-2}) was greater than that in 2022 (0.65 gm^{-2}) (Table 4).

EO composition. The compositions of basil EO was affected differently by intercropping pattern and fertilizer treatment. The EO of basil was consisted of 24 constitutes accounting for 97.4 - 98.4% of the total compositions (Table 5). Based on GC-MS analysis, the main components of the EO were methyl chavicol (31.5–53.7 %), methyl eugenol (19.8 - 30.4%), 1,8 cineole (4.2 – 10.3%), β-caryophyllene (3.5 - 4.8%), β-citral (2.2 - 6.9%), cis-α-bisabolene (2.1 – 4.9%) and caryophyllene oxide (0.9 – 3.8%). In sole basil, the highest content of methyl chavicol (50.6%) was obtained in 100% CF treatment, while, in in all intercropping patterns, the highest contents of methyl chavicol were obtained in 100% CF Bio treatment. In 2B:2F, 4B:2F, 5B:3F and 100b:50F intercropping patterns, the contents of methyl chavicol in Bio treatments increased by 12.1, 40.6, 27.1 and 23.4%, respectively, compared with those in 100% CF. The greatest content of methyl chavicol (53.7%) was observed in 2F:2B intercropping patterns and Bio fertilizer (Table 5). In sole cropping, 4B:2F and 5B:3F intercropping patterns, the highest contents of methyl eugenol were observed in 50%CF+Bio treatment that increased by 42.0, 7.1, and 4.7 %, respectively, compared with 100% CF treatment. At 2B:2F and 100B:50F intercropping patterns, the highest contents of methyl eugenol were obtained in 100% CF treatment. In sole cropping, the highest content of 1,8-cineol (6.9%) was observed in 100%CF treatment, while in all intercropping patterns the 50%CF+Bio treatment had the highest contents. In sole cropping, 4B:2F and 100B:50F intercropping patterns, the greatest contents of β-caryophyllene were observed in 50%CF+Bio treatment, while in 2B:2F and 5B:3F patterns, the greatest contents of β-caryophyllene were obtained in Bio treatment (Table 5).

Land Equivalent Ratio (LER). In both years, all fertilizer treatments and intercropping patterns, the values of LER_T were higher than 1 (Table 6), which reveals the relative advantage of intercropping over sole basil. The highest values of LER_T were obtained in 50% CF+Bio fertilizer treatment and 2B:2F intercropping pattern (1.99 and 1.93, for 2021 and 2022, respectively). Evaluating the partial LERs (Table 6) in both years for 2B:2F intercropping pattern indicated that increasing the LER_B and LER_F led to enhance in LER_T. The lowest LER_T values were obtained in additive (100B:50F) intercropping pattern. For all fertilizer treatments, the LER_T values in 2B:2F and 4B:2F intercropping patterns in 2021 were higher than those in 2022, while the LER_T values in 5B:3F and 100B:50F intercropping patterns in 2021 were lower.

Compounds	RI[a]	Sole cropping			2B:2F			4B:2F			5B:3F		
		100%CF	50%CF +Bio	Bio	100%CF	50%CF +Bio	Bio	100%CF	50%CF +Bio	Bio	100%CF	50%CF +Bio	Bio
β-Pinene	919	0.8	-	-	-	-	-	1.3	1.2	1.1	1.3	2.9	0.6
1,8-Cineole	953	**6.9**	**6.8**	**5.5**	**4.5**	**4.7**	**4.3**	**8.1**	**9.4**	**4.2**	**9.2**	**10. 3**	**5.4**
cis-Anethole	1106	0.2	0.9	0.9	0.8	0.8	0.8	1.1	1.2	0.7	1.5	1.6	0.7
Fenchol	1112	1.7	0.6	1.8	1.2	1.5	1.6	0.8	1.4	0.9	1.1	0.9	1.7
trans-Chrysanthemal	1124	0.4	0.8	0.9	-	-	-	0.9	0.7	0.8	0.9	1.8	0.9
Rosefuran epoxide	1161	0.3	0.9	0.8	-	-	-	0.7	0.7	0.6	0.8	0.9	0.9
Methyl chavicol	1195	**50.6**	**44.5**	**41.8**	**47.9**	**46.9**	**53.7**	**35.2**	**31.5**	**49.5**	**35.7**	**36.4**	**45.4**
β -Citral	1241	**2.2**	**2.8**	**2.7**	**3.4**	**2.9**	**2.8**	**3.9**	**5.2**	**5**	**3.4**	**3.4**	**3.5**
Geraniol	1252	0.8	-	0.7	-	-	-	0.9	0.7	0.7	0.6	0.9	0.7
α-Cubebene	1344	0.2	0.1	0.9	0.7	0.8	0.9	0.4	0.1	0.8	0.5	0.8	0.7
β -Cubebene	1347	0.5	0.4	0.7	0.9	0.9	1.1	1.6	0.8	1.2	0.9	0.7	0.6
Methyl eugenol	1361	**20.5**	**29.1**	**26.7**	**23.2**	**20.4**	**19.8**	**28.4**	**30.4**	**21.3**	**27.8**	**29.1**	**23.5**
α -Copaene	1376	0.1	0.1	0.6	0.6	0.8	-	-	-	0.2	0.6	1.1	0.7
δ-Cadinene	1403	0.3	-	0.8	0.5	0.9	-	0.7	0.6	0.2	0.7	0.9	0.6
β-Caryophyllene	1419	**3.7**	**4.2**	**3.5**	**3.7**	**3.7**	**3.9**	**4.3**	**4.8**	**3.7**	**3.9**	**3.6**	**4.3**
β -Farnesene	1433	0.1	0.4	0.3	0.8	1.2	-	0.5	0.3	0.4	0.9	0.7	1.1
trans-β -Farnesene	1448	0.4	0.3	0.8	0.6	0.9	0.8	0.6	0.4	0.6	1.1	1.5	1.1
Germacrene D	1475	0.5	0.2	0.5	0.8	1.2	0.8	0.6	0.3	0.5	0.5	1.7	0.5
Nerolidol	1517	-	0.2	0.2	0.8	0.9	-	0.6	-	0.8	0.7	0.9	0.5
***Cis*-α-Bisabolene**	1532	**4.4**	**4.1**	**3.5**	**4.1**	**4.7**	**3.9**	**4.1**	**4.9**	**2.1**	**3.1**	**3.7**	**2.5**
Caryophyllene oxide	1578	**1.5**	**1.2**	**1.8**	**1.4**	**1.4**	**1.3**	**2.1**	**1.4**	**1.5**	**1.8**	**2.1**	**0.9**
α-Bisabolol	1674	0.4	0.3	0.9	0.8	1	-	0.8	0.3	0.5	0.5	1.2	0.8
Eicosane	2063	0.6	0.2	0.5	0.7	0.9	1.1	-	0.5	0.3	0.6	0.8	0.5
Octadecanoic acid	2164	0.8	0.1	0.7	0.8	0.7	1.3	0.3	0.6	0.5	-	0.8	0.2
Total identified (%)		97.9	98.2	97.5	98.2	97.2	98.1	97.9	97.4	98.1	98.1	98.4	98.3

Table 5. Proportion of basil EO compositions under different cropping patterns and fertilizer treatments.

Table 6. Land equivalent ratio (LER_T) values at different intercropping patterns and fertilizer treatments in 2021 and 2022. LER_F: partial LER of fenugreek, LER_B: partial LER of basil, LER_T: total LER.

		2021			2022		
Fertilizer	Intercropping pattern	LER_F	LER_B	LER_T	LER_F	LER_B	LER_T
	2B:2F	0.89	0.93	1.83	0.90	0.91	1.81
	4B:2F	0.52	0.88	1.40	0.47	0.84	1.31
Bio	5B:3F	0.35	0.79	1.14	0.52	0.75	1.27
	Additive (100B:50F)	0.26	0.88	1.14	0.36	0.86	1.22
	2B:2F	**1.01**	**0.98**	**1.99**	**0.93**	**1.00**	**1.93**
	4B:2F	0.56	0.96	1.52	0.51	0.96	1.47
50% CF+Bio	5B:3F	0.41	0.93	1.34	0.66	0.93	1.59
	Additive (100B:50F)	0.27	0.95	1.22	0.35	0.95	1.30
	2B:2F	0.84	0.96	**1.80**	0.79	0.96	**1.75**
	4B:2F	0.47	0.90	1.37	0.47	0.88	1.35
100% CF	5B:3F	0.28	0.76	1.04	0.51	0.71	1.21
	Additive (100B:50F)	0.20	0.88	1.08	0.29	0.86	1.15

Discussion

Plant height. The plant height as a growth index, could be affected by the growth conditions, and increasing the basil height in 2021 could be due to higher precipitation than 2022. In 2B:2F intercropping, the bail height was higher than sole cropping, that may be attributed to higher light competition between plants in intercropping than to sole basil. The concentration of auxin may increase by shading obtained by fenugreek which in turn enhanced the basil height[7,10,27]. In additive (50:100) intercropping, the competition between component crops for main resources such as light, nutrients and water, reduced the plant growth and height. The fertilizer treatment of 50%CF + Bio had the greatest plant height at all cropping patterns. Increasing the length of internodes, leaf area and photosynthetic rate by improving the nitrogen availability, could increase the plant height[27]. Also, more competition for light by increasing the total density in additive intercropping (100B:50F), might be the reason for increasing the basil height. obtained[28] the similar results as the tomato plant height increased in intercropping with common bean. Also reported that the plant height of fenugreek (in intercropping with Moldavian balm) increased in 100% chemical fertilizer compared with biofertilizer and biofertilizer +50% chemical fertilizer treatments[7].

Dry herbage yield. In replacement intercropping patterns (2B:2F, 4B:2F and 5B:3F), the dry herbage yield of basil reduced in comparison with sole basil that could be explained by reduction in proportion of basil in intercropping patterns. In application of chemical fertilizer in intercropping of soybean-Moldavian balm[29], observed that the Moldavian balm herbage yield decreased in intercropping patterns in comparison with sole crop. Also, in faba bean-Moldavian balm[9] and Moldavian balm-fenugreek[7,10] intercrops, observed that the herbage yield in intercropping patterns, reduced in comparison with sole medicinal crops. The basil dry herbage yield increased in 5B:3F intercropping compared with 2B:2F and 4B:2F patterns. Intercropping with fenugreek as a legume crop, could increase the nitrogen content in the soil through N_2 fixation mechanism which lead to improve in growth and yield of component crop[10,27]. The competition between basil and fenugreek for N acquisition, is anticipated to mitigate through mentioned nitrogen resource[30]. 100%CF and 50%CF + Bio treatments, had greater dry herbage yield than Bio treatment in sole cropping, 2B:2F and 5B:3F intercropping patterns, while the dry herbage yields in 100%CF and Bio treatments were not significantly different in 4B:2F and 100B:50F patterns. These findings are in contrast to those of soybean-dill[31], soybean-black cumin[32] and Moldavian balm[29] intercropping which observed that the yields of medicinal crops in organic fertilizer treatment were higher than those in chemical fertilizer in sole and intercropping patterns. Also reported[10] that in 1:1 intercropping pattern of Moldavian balm-fenugreek, the dry herbage yield of Moldavian balm in biofertilizer treatment (arbuscular mycorrhiza fungi + bacteria), increased compared with chemical fertilizer treatment. Chemical properties, cation exchange and water holding capacity of the soil improve with application of organic fertilizers which increase the basil (*Ocimum basilicum* L.) herbage yield[33]. Also, previous studies[24,25] indicated that the basil herbage yield increased with application of biofertilizer and organic manure. Using biofertilizers and organic manure, improved the herbage yield in basil (*Ocimum basilicum* L.)[34].

EO content (%) and yield. In medicinal plants, the EO content could be affected by environmental parameters such as light intensity and quality and water availability[35,36] the main limited resources that may cause competition between components crops in intercropping[7,10]. In faba bean-peppermint and soybean-peppermint intercropping, the 40:60 and 3:2 intercropping patterns had the highest EO contents of peppermint, respectively[37,38]. The basil EO content in 50% CF+ Bio treatment was higher than 100%CF and Bio treatments, while reported that the EO content of Moldavian balm in arbuscular mycorrhiza fungi+ bacteria treatment was higher than chemical fertilizer treatment[10]. In 50%CF+Bio treatment, improving the EO yield of basil may be attributed to increase in nutrient's availability and acquisition. The previous studies on *Salvia officinalis* L. and *Thymus vulgaris* L. revealed that EO content was not affected by biofertilizer treatment[39]. In medicinal plants, the EOs are produced as secondary metabolites and their production is correlated with higher amounts of macro- and micronutrients, and photosynthetic efficiency[40]. The 50%CF+Bio treatment had higher EO yield than 100%CF and Bio treatments which may be due to improve in macronutrients availability such as nitrogen and phosphorus[10], which in turn increased the basil EO yield. The highest EO yield of fennel (*Foeniculum vulgare* Mill.) was obtained in a combined treatment of biofertilizer and 50% nitrogen and phosphorus chemical fertilizers[41]. In intercropping with faba bean, the EO yield of Moldavian balm in chemical fertilizer and biofertilizer treatments were greater than

vermicompost treatment[9]. In this study, unsuitable soil conditions for applied mycorrhiza and bacteria (Myco-Root and Biofarm), could be the reason for reduction in basil EO yield in Bio and 50%CF+Bio treatments compared with 100%CF. Comparing the intercropping patterns revealed that the highest EO yield was obtained in 2B:2F that could be attributed to increase in basil EO content in this intercropping pattern compared to others. reported that in Moldavian balm-fenugreek intercropping, the greatest EO yield of Moldavian balm was obtained in 4:2 intercropping pattern[10]. In previous studies on legume-medicinal plant intercropping, the highest EO yields were obtained in 2:1 soybean-dill intercropping[31], 2:1 soybean-black cumin intercropping[32] and 1:2 soybean-Moldavian balm intercropping[29].

EO composition. In essential oil-bearing plants, the quality of EO in terms of type and number of constituents is so important and impact its characteristics, overall effectiveness and therapeutic benefits[10]. The GC-MS analysis of EO obtained from dry herbage of basil indicated that it contains 24 constituents (Table 7). The main constituents of EO were methyl chavicol (31.5–53.7%), methyl eugenol (19.8–30.4%), 1,8-cineole (4.2-10.3%), β-caryophyllene (3.5–4.8%), β-citral (2.2–6.9%), cis-α-bisabolene (2.1–4.9) and caryophylene oxide (0.9–3.8%). The greatest content of methyl chavicol was achieved in 2B:2F with Bio fertilizer treatment (Table 7), while the lowest content was obtained in 4B:2F whit 50%CF+Bio. observed[42] that in sole cropping of basil with chemical fertilizer of nitrogen the greatest methyl chavicol content was obtained. They stated that decrease in competition for nutrients acquisition in basil sole cropping and application of nitrogen chemical fertilizer, caused increase in content of methyl chavicol. The contents of bornil acetate, eucalyptol, linalool and eugenol in EO of basil affected by nitrogen fertilizer treatment[43]. Also, they stated that more nitrogen application, decreased the linalool content in EO and increased methyl chavicol content. The greatest contents of caryophyllene, geranial and citral were obtained in maize + 25% basil intercropping with no fertilizer[42]. It could be concluded that some factors such as competition for nutrient and light under intercropping system and nutrient deficiency may be underlined as factors that increase the synthesis of these compounds. Environmental factors such as drought and salinity stresses, and deficiencies of nutrients could affect the main compounds in EO[10,44]. Different amounts of nitrogen was found to affect significantly the main components of sweet basil EO, as the greatest content of bergamotene was obtained when the lowest amount of nitrogen was applied[45]. reported[9] that in intercropping patterns of faba bean-Moldavian balm, the geraniol contents in EO increased in biofertilizer and vermicompost treatments but the geranyl acetate content, increased in chemical fertilizer application. The greatest and lowest caryophyllene, menthol and germacrene-D were obtained in peppermint-soybean intercropping of and sole peppermint, respectively[37]. Also, in Moldavian balm-fenugreek intercropping[10], observed that in application of nitrogen fixing bacteria + mycorrhiza fungi, the contents of geranial in EO of Moldavian balm in intercrops of 100:50, 1:1 and 2:2, enhanced by 34.6, 25.1, and 15.5 % than sole crop. In medicinal plants, growth-promoting bacteria, are able to improve growth parameters, affect EO compositions through mechanisms of improving the nitrogen fixation, increase in available nitrogen, synthesis of growth stimulants such as gibberellin and auxin, and increasing the development and growth of root as well as improve in nutrients and water uptake[7,10.46,47].

Land Equivalent Ratio (LER). The advantage of the intercropping patterns was assessed by LER_T valuesthatare in agreement with[48], which stated that the interspecific facilitation is due to the supplementary use of limited resources by intercropping components. also reported[49] that a slight increase in LER greater than 0.5 depends on the degree of complementarity of intercropping components. reported that in grain legume-cereal intercrop, LER_T demonstrated that in intercropping patterns, the growth resources were used more efficiently from 27 to 31%, than sole cropping[30]. The highest LER_T (1.99) was obtained in 2B:2F intercropping with 50%CF+Bio that is in consistent with findings on intercropping of Moldavian balm-faba bean[9]. The lowest LER_T (1.08) was observed in additive 100B:50F intercropping with 100%FC, while[7,10] reported that in Moldavian balm-fenugreek intercropping, the highest LER value was obtained in additive 100:50 intercropping with application of chemical fertilizer. Probably, the reason for decrease in LER in additive 100B:50F intercropping is the lack of optimal density because it is an increase in interspecific competition between basil and fenugreek. In maize - common bean intercropping, the of LER_T values with application of nitrogen fertilizer showed a variable result including increase or decrease[50].

Conclusions. The plant height, dry herbage yield, EO content and yield of basil were significantly influenced by interaction of cropping pattern × fertilizer treatment. The greatest plant height, EO content and yield were obtained in 2B:2F intercropping with application of 50%CF+Bio treatment. The greatest dry herbage yield of basil was observed in 5B:3F intercropping pattern. The major chemical constitutes of basil EO (methyl chavicol, methyl eugenol, β-caryophyllene and 1,8-cineole) were influenced differently by cropping pattern and fertilizer treatment. The highest content of methyl chavicol (53.7%), was observed in 2B:2F and Bio fertilizer treatment, while the greatest content of methyl eugenol (30.4%) was obtained in 4B:2F intercropping pattern. In all fertilizer treatments and cropping patterns, the land equivalence ratios (LER_T) were obtained more than one that indicates balm-fenugreek intercropping has increased the production efficacy. The highest LER_T value (1.99) was obtained in 2B:2F intercropping with 50% CF + Bio treatment. It means that this intercropping patterns with application of reduced rate of chemical fertilizer in combination with mycorrhiza fungi and growth promoting rhizobacteria, improved the yield of both crops in intercropping. On the other hand, the application of chemical fertilizer could be reduced by 50% through application of biofertilizers and intercropping. Using such environmentally-friendly strategies will improve the yield and quality of basil, reduces the harmful effects of chemical fertilizers in agroecosystems and could be recommended to the growers of medicinal plant in a sustainable production system.

Materials and Methods

Experimental design and field operation. A two-season (2021-2022) experiment was conducted at the research field in west Azarbaijan, Iran (latitude 3901_N, longitude 4438_E, altitude 1850 m above sea level). The total precipitation and mean temperature for months of growth season in experimental site are presented in Table 7. The soil physicochemical properties at depth of 0-30 cm in experimental field is shown in Table 8. The experiments were arranged as factorial based on randomized complete block design with three replicates. The first factor was cropping pattern including sole cropping of basil (B), sole cropping of fenugreek (F), intercropping patterns based on replacement method at ratios of 2 row of basil + 2 row of fenugreek (2B:2F), 4 rows of basil + 2 rows of fenugreek (4B:2F), and 5 rows of basil + 3 rows of fenugreek (5B:3F) and additive intercropping of basil + fenugreek (100B: 50F) (100% density of basil + 50% density of fenugreek planted between basil rows). The basil is the main crop, and the fenugreek is dominating crop. The second factor was

fertilizer treatment at three levels including application of 100% chemical fertilizer (100% CF), 50% chemical fertilizer + biofertilizer (50% CF + Bio) and biofertilizer (Bio). 50 kg ha^{-1} urea and 80 kg ha^{-1} triple superphosphate (based on the soil test), applied at planting time as 100% CF. For biofertilizer (Bio) treatment, the combination of Myco-Root + Biofarm was used.

Biofertilizer of Myco-Root contains 10^7 to 10^8 CFU/g arbuscular mycorrhiza fungi (AMF) of *Glomus etunicatum*, *Glomus intraradices*, and *Glomus mosseae* produced by Zist Fanavar Pishtaz Varian Company, Karaj, Iran. For bacteria inoculation, the Biofarm was used which contains 2×10^7 CFU/g *Azotobacter chroococcum* and *Azospirillum brasilense* by Nature Biotechnology Company (Biorun) Karaj, Iran. 1 kg of basil and fenugreek seeds were placed on a clean surface in the shade, and after spraying some water on them, 40 g of Myco-Root (AMF) and 40 mL of Biofarm (bacteria) biofertilizers were added and mixed thoroughly, so that a layer of biofertilizer covered the all seeds[7]. Also, the *Sinorhizobium meliloti* was used forinoculation of fenugreek seeds for nitrogen fixing through symbiosis.

For field preparation, in the spring the moldboard ploughing (25–30 cm) was used, followed by two times disking. The fenugreek and basil seeds were planted at densities of 50 and 32 plants m^{-2}, respectively. They were planted with row space of 25 cm on 4 May 2021 and 15 May 2022, in intercropping patterns and sole cropping. In sole basil, sloe fenugreek, 2B:2F and B100: F50 intercropping patterns, the size of the experimental plots were 3 m × 3 m (12 rows wide × 3 m long). In 4B:2F and 5B:3F intercropping patterns, the size of plots was 4 m × 3 m (16 rows wide ×3 m long). Immediately after planting of crops, the furrow irrigation was done with intervals of 5 days till maturity of the plants. At all experimental plots the weeds were removed weekly by hand-weeding. No pesticide application was done during growth season in experimental field.

Ethical approval. This experimental research upon plants complies with relevant institutional, national, and international guidelines and legislation. Fenugreek and basil seeds used in experiment, were purchased from Tabriz, Iran.

Growth traits and dry herbage yield. 10 plants were randomly selected in each plot (after removing side rows and 0.5 m from the sides of the middle rows). The basil height was measured with a steel rule at maturity stage on 8 August 2021 (96 days after sowing) and 22 August 2022 (99 days after sowing). At flowering stage of basil (49 and 51 days after in 2021 and 2022, respectively) the plants in 1 m^{-2} of all plots were harvested and dried for 7 days in the shade at room temperature to determine the dry herbage yield of basil as g m^{-2}. In order to the determine of fenugreek grain yield, the plants in 1.5 m^2 of all plots were harvested from middle rows on 5 September 2021 and 2022. Harvested plants were dried at room temperature for 14 days and the grain yield per m^{-2} was measured after threshing.

	Year	May	June	July	August	September
Total precipitation (mm)	2021	13.45	2.59	3.82	0.0	3.62
	2022	10.57	0.87	0.0	0.0	0.0
Mean temperature (°C)	2021	16.23	19.65	25.0	24.0	18.52
	2022	20.04	23.12	29.1	29.0	20.4

Table 7. Monthly mean temperature and total precipitation in 2021 and 2022 growing seasons in experimental area

Table 8. Physicochemical properties of the soil of experimental area in depth of 0–30 cm.

al	Soil texture			pH	Organic matter (%)	EC (dS m^{-1})	Total N (%)	P (mg kg^{-1})	K (mg kg^{-1})
	Silty loam								
Value	Clay (%)	Silt (%)	Sand (%)	7.9	0.83	0.78	0.10	9.8	225
	26.94	43.06	30						

EO content and yield. After harvesting the dry herbage of basil, it was powdered and then 50 g of that was hydro-distilled for 3.0 h by a clevenger device[51] (British Pharmacopoeia, 1988). For dehydration of EO samples, the anhydrous sodium sulphate was used and after that the samples were stored at 4 °C until GC-MS analysis. The content (%) and yield (g m^{-2}) of EO were calculated as follows[10]:

$$\text{EO content (\%)} = \frac{\text{EO (g)}}{\text{50 g basil herbage}} \times 100$$

$$\text{EO yield (g m}^{-2}\text{)} = \text{Herbage yield (g m}^{-2}\text{)} \times \text{EO content (\%)}$$

GC-MS analysis. Gas chromatograph (Agilent 6890N) with a 5973 mass-selective detector (Agilent Technologies, CA, USA) was used for gas chromatography mass spectrometry (GC-MS) analysis. The method was used as priviously described[52]. Comparison of the mass spectra of each component either with those stored in the Adams mass spectral-retention index libraries[53] and Wiley 7.0 was employed for identification of EO constituents.

Land equivalent ratio (LER). The land equivalent ratio (LER) was employed to evaluate the advantage of intercropping (basil-fenugreek) patterns[9]:

$LER_B = Y_{BI} / Y_B$ $LER_F = Y_{FI} / Y_F$

$LER_T = LER_F + LER_B$

Where Y_{BI} and Y_B are the basil dry herbage yields in intercrop and sole cropping, respectively. Y_{FI} and Y_F are the fenugreek grain yields in intercrop and sole cropping, respectively. When the LER value is higher than 1.0, indicates that intercropping has increased the production efficiency in comparison with sole crops and the LER value of 1.0 indicates that components of intercropping have the same competitive ability or no competition was occurred between them. When the inter- specific competition is higher than interspecific facilitation, the value of LER is lower than 1.0, reveals that intercropping patterns has no advantage over sole crops[10].

Statistical analysis. The SAS version 9.0.3 was employed for ANOVA (analysis of variance). The combined ANOVA was done based on RCBD (complete randomized block design) with 15 treatments and three replications for two years (2021 and 2022). Analysis of variance did not perform for LER_B, LER_F, and LER_T data. The assumptions of variance homogeneity and normality were tested for experimental data, and there was no need for data transformation. Duncan's multiple range test was used for mean comparison at $p \leq 0.05$.

Data availability

The necessary information is available from the corresponding author on reasonable request.

Declarations

Ethical approval.

This experimental research upon plants complies with relevant institutional, national, and international guidelines and legislation. Fenugreek and basil seeds used in experiment, were purchased from Tabriz, Iran.

Competing interests

The authors declare no competing interests.

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Author Contribution

A.E.: conducted the experiment, collected the data, analyzed the data, and wrote the manuscript. R.A.: reviewed the final data, edited and reviewed the manuscript. A.D.M.N.: edited the manuscript. All authors reviewed the manuscript.

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Figures

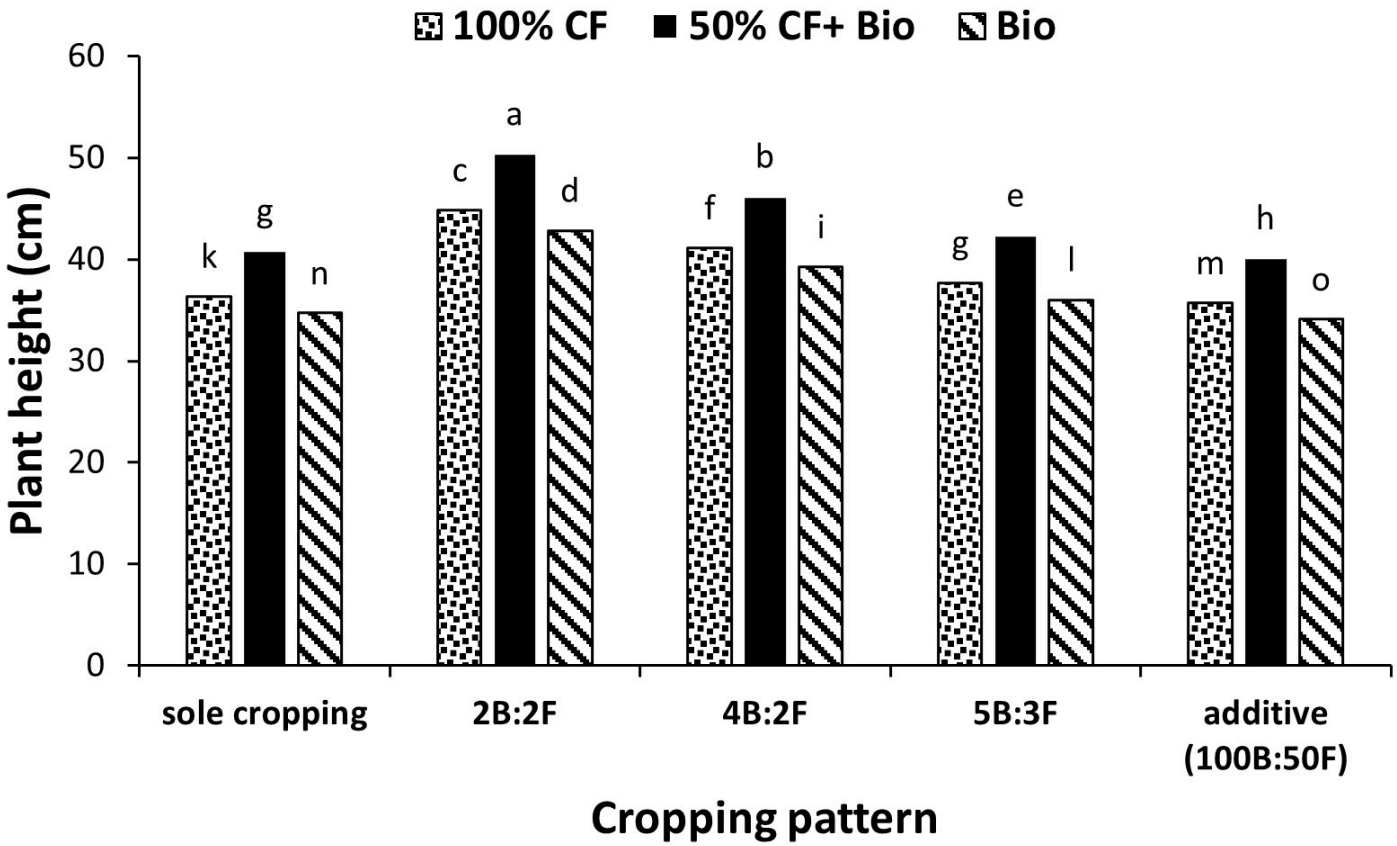


Figure 1

Mean comparison for interaction of cropping pattern × fertilizer treatment on plant height of basil. Different letters indicate significant differences at $p \leq 0.05$.

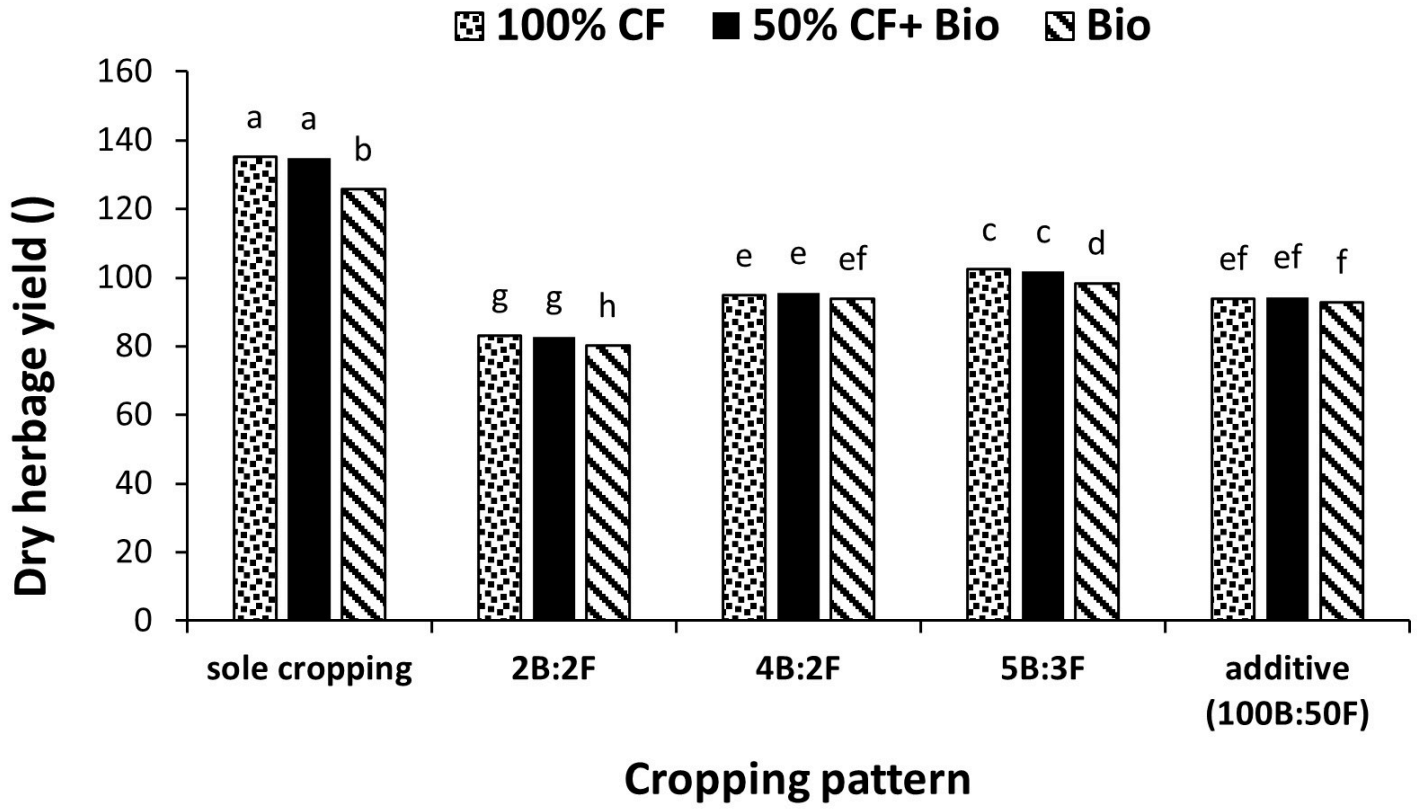


Figure 2

Mean comparison for interaction of cropping pattern × fertilizer treatment on dry herbage yield of basil. Different letters indicate significant differences at $p \leq 0.05$.

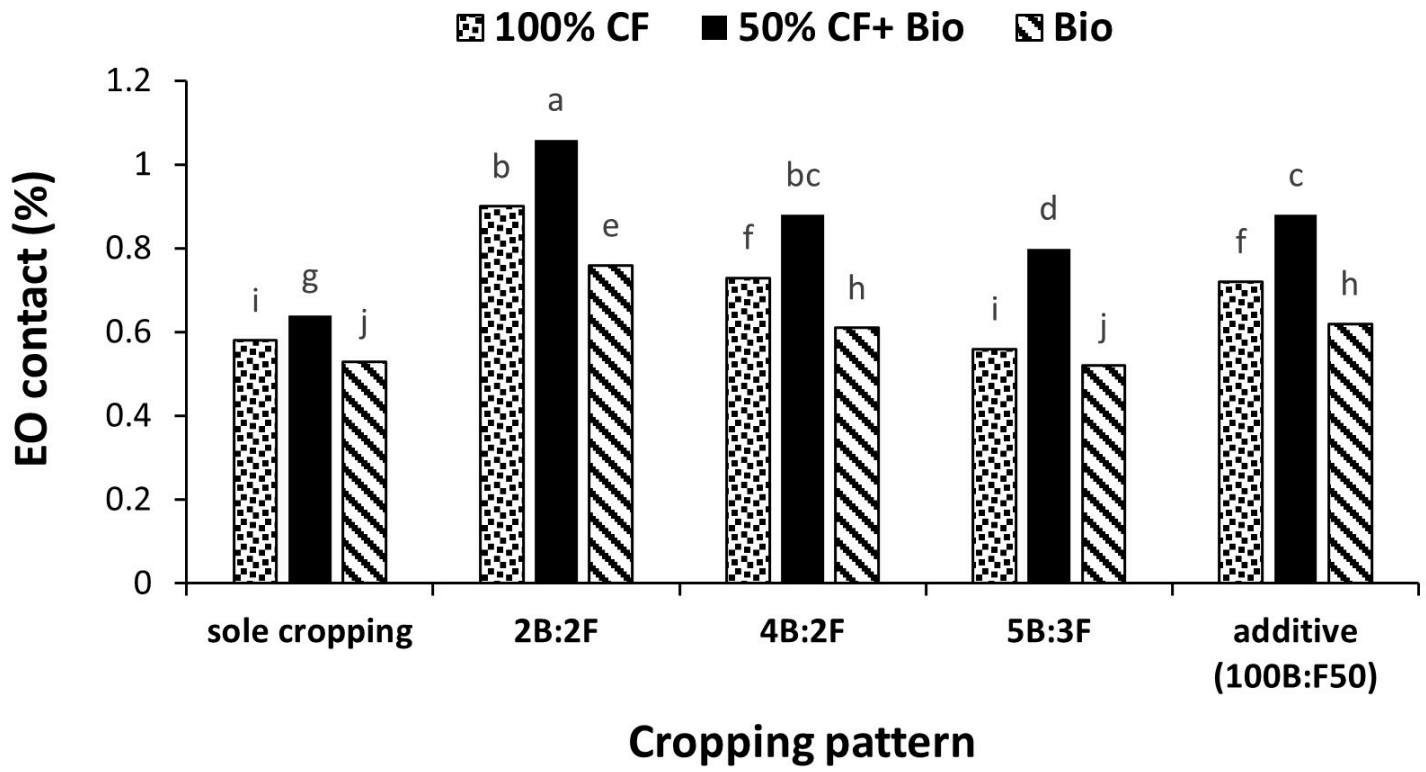


Figure 3

EO content of basil as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

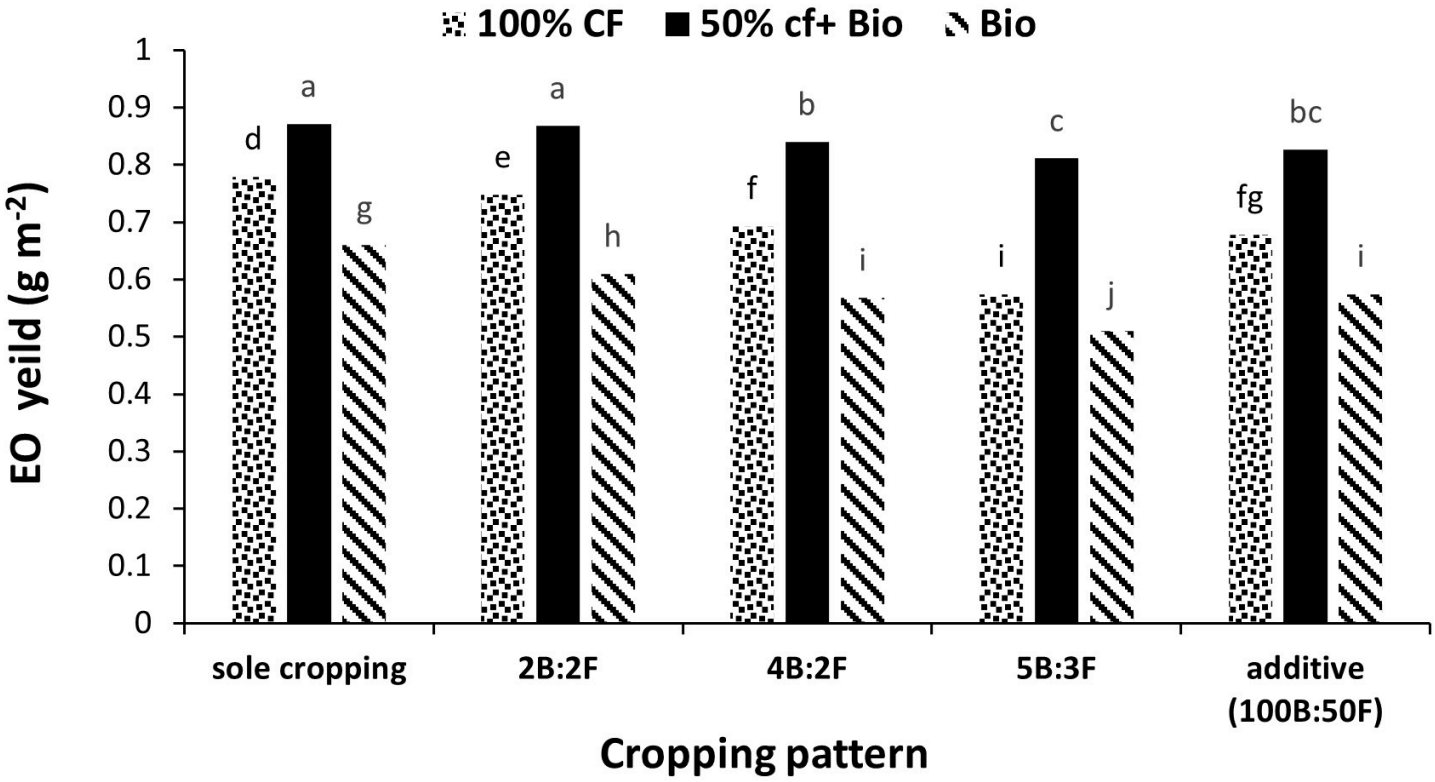


Figure 4

EO yield of bail as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.