

Essential oil yield and compositions of *Dracocephalum moldavica* L. in intercropping with fenugreek and inoculation with mycorrhizal fungi and bacteria

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Abstract

Intercropping is one of the most important components of sustainable production of agricultural products. The effects of chemical fertilizer (CF), arbuscular mycorrhizal fungi (AMF) and the combination of AMF and bacteria (AMF + B) in sole Moldavian balm (Mb) (*Dracocephalum moldavica* L.) and intercropping with fenugreek (F) (*Trigonella foenum-graecum* L.), was studied on growth characteristics, essential oil yield and compositions of Moldavian balm. The highest dry herbage yield (6132 kg ha^{-1}) was obtained in Mb:F(4:2) cropping pattern and CF treatment. The highest values of essential oil content were observed in the Mb:F (2:2) cropping pattern (0.261%) and AMF + B treatment (0.259%). After sole-Moldavian balm, the highest essential oil yield (15.28 kg ha^{-1}) was obtained in Mb:F (4:2) cropping pattern and AMF + B treatment. Geranial, geranyl acetate, geraniol, neral, and nerol were the main chemical constituents of essential oil. In CF and AMF + B treatments the geranial contents in intercropping patterns of Mb:F (1:1), (2:2) and (100:50), were greater than that of sole-Moldavian balm. The highest LER_T values were observed in Mb:F (100:50) cropping pattern in 2021 (1.70 and 1.63 for CF and AMF + B treatments). Generally, it can be concluded that Mb:F (100:50) intercropping and use of AMF + B bio-fertilizer could be recommended to medicinal plant growers in sustainable production systems.

Introduction

Conventional agricultural systems are designed in such a way which have reduced the existing plant diversity to a minimum and have caused instability of economic performance, increased damage of pests and diseases, reduced quality of crops, severe soil erosion and loss of natural resources¹. Sustainable agriculture is a combination of agricultural management knowledge that can bring good biological, environmental and economic value in the long run. Intercropping of different species, cultivars or different isolates is one of the main components of sustainable agriculture². In this cropping system, the goal is to increase the yield in terms of time and place and the plants use environmental resources with the highest efficiency^{3,4}. The calculation of intercropping evaluation indices enables more accurate and precise decisions about the choice of superior treatment and makes it possible to interpret the results of competition between plants, indicate the relative advantage of intercropping to sole crop⁵.

Economic and environmental problems caused by the wasted of nitrogen fertilizers as a result of processes such as denitrification and nitrate leaching have led to the replacement of chemical fertilizers in recent years by biological nitrogen stabilization systems as part of sustainable agricultural programs⁶. In sustainable production bio-fertilizers and organic fertilizers could be used to reduce application of chemical fertilizers, support soil microbial activities and health, improve the biosynthesis of secondary metabolites and phytochemicals and affect crop quality and yield^{2,6}. It seems that the combined using of bio-fertilizers and intercropping could improve the production stability in arable lands.

Moldavian balm (*Dracocephalum moldavica* L.) is domesticated in Central and Eastern Europe and native to Central Asia^{4,7}. According to Amini et al.^{8,9} in Moldavian balm the geranial, geraniol, neral, and geranyl acetate are the main components of essential oil. Karimzadeh Asl et al.¹⁰ reported that use of bio-fertilizer in Moldavian balm increased the essential oil yield, geraniol and geranyl acetate contents. Vafadar-Yengeje et al.² found that intercropping and bio-fertilizers application increased the content and yield of essential oil in Moldavian balm. Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest known medicinal plants in the world, in Fabaceae family¹¹. It is cultivated mostly in cold regions with moderate or low rainfall¹². This medicinal plant has therapeutic properties and could be used as treatment of indigestion and diabetes, and as well as lowers blood pressure and cholesterol¹³. Seeds of fenugreek contain trigonelline and diosgenin, fixed oil, mucilage and proteins¹⁴.

One of the most successful intercropping systems is the cultivation of legumes with non-legume crops, which in most cases causes the superiority of intercropping over sole crop⁵. This is due to the ability of bio-stabilization of atmospheric nitrogen by legumes and has many ecological advantages¹⁵. In this cropping system, nitrogen stabilized by legumes is transferred to the accompanying crops and can contribute to yield stability in low-input agriculture¹⁶. The use of fenugreek in intercropping in addition to the optimal use of land, causes biological stabilization of nitrogen and also prevents environmental pollution by improving soil fertility and reducing the use of nitrogen fertilizer¹³. Intercropping of Moldavian balm with faba bean (*Vicia faba* L.)² and peppermint (*Mentha × piperita* L.) with soybean (*Glycine max* L.)¹⁷ affected the composition and yield of essential oil. Also the composition and yield of essential oil in coriander (*Coriandrum sativum* L.) and black cumin (*Nigella sativa* L.) were improved in intercropping with soybean¹⁸.

The purpose of this study was investigating the effect of bio-fertilizer treatments on essential oil yield and compositions of Moldavian balm at intercropping patterns with fenugreek and to determine the best intercropping pattern and fertilizer treatment based on land equivalent ratio (LER) index.

Results

Plant Height

Combined ANOVA indicated that the Moldavian balm height was affected significantly by year, cropping pattern, fertilizer treatment ($p \leq 0.01$) and interaction effect of year × cropping pattern ($p \leq 0.01$) and cropping pattern × fertilizer treatment ($p \leq 0.05$) (Table 3). The results of interaction effect of year × cropping patterns showed that at all cropping patterns the plant heights in 2020 were greater than those in 2021 (Fig. 1). In both years the plant height in Mb:F(100:50) cropping pattern decreased compared with those in sole Moldavian balm and other intercropping patterns (Fig. 1). The interaction of cropping pattern × fertilizer treatment indicated that in Mb:F(1:1), Mb:F(2:2) and Mb:F(4:2) cropping patterns and sole cropping the plant heights were not significantly different in CF and AMF + B treatments but in Mb:F(100:50) cropping pattern the plant height in AMF + B fertilizer treatment (83.3 cm) was greater than those in CF and AMF treatments (Fig. 2).

Leaf Area Index (Lai)

The results of the combined ANOVA (Table 3) indicated that the LAI was affected significantly by cropping pattern, fertilizer treatment and the interaction of cropping pattern × fertilizer treatment. The mean comparison of LAI in two years showed a higher LAI in 2020 (7.87) compared to that in 2021 (6.57) (Table 4). In intercropping pattern of Mb:F(1:1) the LAIs in AMF and AMF + B fertilizer treatments were higher than those in sole Moldavian balm and the highest LAI (9.56) was observed in AMF + B fertilizer treatment. The lowest LAI was obtained in intercropping pattern of Mb:F(100:50) and the LAIs at all fertilizer treatments were not significantly different (Fig. 3).

Table 1
The combined analysis of variance for effects of cropping pattern and fertilizer treatment on growth traits, essential oil content and yield of Moldavian balm. ns, * and **: non -significant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Source of variation	df	Plant height	Leaf area index	Dry herbage yield	Essential oil content	Essential oil yield
Year (Y)	1	**	**	**	**	**
Block × Y	4	n.s	n.s	n.s	n.s	n.s
Cropping pattern (C)	4	**	**	**	**	**
Y × C	4	**	n.s	*	n.s	**
Fertilizer (F)	2	**	**	**	**	**
Y × F	2	n.s	n.s	*	n.s	**
C × F	8	*	*	**	n.s	*
Y × C × F	8	n.s	n.s	n.s	n.s	n.s
CV (%)	-	5.52	11.62	9.28	8.22	13.67

ns, * and **: non -significant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2

The effect of year, cropping pattern and fertilizer treatment on Moldavian balm height, leaf area index, dry herbage yield, essential oil content and yield. Mb: Moldavian balm, F: fenugreek. CF, AMF and AMF + B are 100% chemical fertilizer, arbuscular mycorrhizal fungi bio- fertilizer and arbuscular mycorrhizal fungi + bacteria bio-fertilizers, respectively. Means within each column with similar letter are not significantly different at $p \leq 0.05$.

Effect	Levels	Plant height (cm)	Leaf area index	Dry herbage yield (kg ha ⁻¹)	Essential oil content (%)	Essential oil yield (kg ha ⁻¹)
Year	2020	95.88 ± 0.71 a	7.87 ± 0.125 a	5467 ± 68.75 a	0.261 ± 0.003 a	14.27 ± 0.248 a
	2021	84.17 ± 0.71 b	6.57 ± 0.125 b	4470 ± 68.75 b	0.226 ± 0.003 b	10.06 ± 0.248 b
Cropping pattern	Sole Moldavian balm	93.13 ± 1.17 b	7.17 ± 0.198 b	6819 ± 108.71 a	0.232 ± 0.005 cd	16.06 ± 0.392 a
	Mb:F (1:1)	96.66 ± 1.17 a	8.41 ± 0.198 a	3938 ± 108.71 d	0.252 ± 0.005 ab	10.11 ± 0.392 c
	Mb:F (2:2)	93.75 ± 1.17 ab	7.55 ± 0.198 b	3817 ± 108.71 e	0.261 ± 0.005 a	10.04 ± 0.392 c
	Mb:F (4:2)	89.06 ± 1.17 c	7.55 ± 0.198 b	5538 ± 108.71 b	0.245 ± 0.005 bc	13.72 ± 0.392 b
	Mb:F (100:50)	77.56 ± 1.17 d	5.41 ± 0.198 c	4744 ± 108.71 c	0.228 ± 0.005 d	10.89 ± 0.392 c
Fertilizer treatment	CF	91.67 ± 0.91 a	7.60 ± 0.153 a	5198 ± 84.21 b	0.237 ± 0.004 b	12.45 ± 0.304 b
	AMF	86.86 ± 0.91 b	6.42 ± 0.153 b	4343 ± 84.21 c	0.235 ± 0.004 b	10.13 ± 0.304 c
	AMF + B	91.56 ± 0.91 a	7.64 ± 0.153 a	5366 ± 84.21 a	0.259 ± 0.004 a	13.91 ± 0.304 a

Dry Herbage Yield

The results of ANOVA showed that the dry herbage yield of Moldavian balm affected significantly by year, cropping pattern, fertilizer treatment, interaction effects of year × cropping pattern, year × fertilizer treatment and cropping pattern × fertilizer treatment (Table 3). Moldavian balm dry herbage yield in 2020 (5467 kg ha⁻¹) was significantly higher than that in 2021 (4480 kg ha⁻¹) (Table 4). The interaction effect of year × cropping pattern (Fig. 4) indicated that at all cropping patterns, the dry herbage yields in 2020 were greater than those in 2021. Among the intercropping patterns the highest dry herbage yield was

obtained in Mb:F(4:2) cropping pattern. The interaction effect of year $\times$ fertilizer treatment (Fig. 5) indicated that dry herbage yield in AMF + B treatment was not significantly different with CF treatment. The interaction effect of cropping pattern $\times$ fertilizer treatment showed that at all intercropping patterns the dry herbage yield decreased compared with that in sole Moldavian balm (Fig. 6). The highest dry herbage yields were observed in Mb:F(4:2) intercropping pattern. In Mb:F(100:50) intercropping pattern the highest dry herbage yield (5322 kg ha^{-1}) was observed in AMF + B treatment, while in Mb:F(4:2) intercropping pattern the highest dry herbage yield (6132 kg ha^{-1}) was observed in CF treatment.

Essential Oil Content (%)

Moldavian balm essential oil content affected significantly by year, cropping pattern and fertilizer treatment. The interaction effect of cropping pattern $\times$ fertilizer treatment was not significant on essential oil content (Table 3). The mean comparison indicated that the Moldavian balm essential oil content in 2020 (0.261%) was higher than that in 2021 (0.226%) (Table 4). The highest essential oil content (0.261%) was obtained in Mb:F(2:2) intercropping pattern that was not significantly different with Mb:F(1:1). The lowest essential oil contents (0.228 and 0.232%) were obtained in Mb:F(100:50) and sole Moldavian balm. The essential oil content in AMF + B fertilizer treatment (0.259%) was significantly higher than those in CF (0.237%) and AMF (0.225%) fertilizer treatments (Table 4).

Essential Oil Yield

The essential oil yield affected significantly by year, cropping pattern, fertilizer treatment, interaction effects of year $\times$ cropping pattern, year $\times$ fertilizer treatment and cropping pattern $\times$ fertilizer treatment (Table 3). The mean comparison (Fig. 7) indicated that at all cropping patterns the essential oil yield in 2020 were higher than those in 2021. The greatest essential oil yield was observed in Mb:F(4:2) intercropping pattern. The interaction effect of year $\times$ fertilizer treatment (Fig. 8) indicated that in 2020 the essential oil yield in AMF + B treatment was not significantly different with CF treatment and in 2021 the essential oil yield in AMF + B treatment was higher than that in CF treatment. The interaction effect of cropping pattern $\times$ fertilizer treatment (Fig. 9) indicated that the essential oil yield decreased at all intercropping patterns compared with that in sole Moldavian balm. In Mb:F(2:2), Mb:F(4:2) and Mb:F(100:50) intercropping patterns the essential oil yield in AMF + B and CF treatment, were not significantly different but in Mb:F(1:1) intercropping, the highest essential oil yield was obtained in AMF + B treatment (Fig. 9).

Essential Oil Composition (Gc-ms)

Moldavian balm essential oil compositions were assessed through GC-MS analysis and it was found that the essential oil was consisted of 19 components, accounting for 98.3–99.2% of the total compounds (Table 5). Results of the GC-MS analysis indicated that the main essential oil constituents were geranial (6.3–61.4%), geranyl acetate (2.4–47.1%), geraniol (3.4–25.9%), neral (0.9–20.5%) and nerol (0.1–13.5%). The essential oil constituents affected differently by cropping pattern and fertilizer treatment. In sole Moldavian balm the highest geranial content (61.4%) was obtained in AMF bio-fertilizer treatment, by 46.9% increase compared with that in CF fertilizer (Table 5). In Mb:F(1:1) intercropping pattern the contents of geranial in AMF and AMF + B treatments increased by 9.0 and 12.8%, respectively, compared with CF fertilizer treatment, while in Mb:F(2:2) intercropping pattern the contents of geranial in AMF and AMF + B treatments decreased. In sole Moldavian balm the geraniol contents in AMF and AMF + B fertilizer treatments decreased by 79.8 and 42.6% compared with that in CF fertilizer treatment (Table 5). In Mb:F(1:1) and (2:2) intercropping patterns the geraniol contents in AMF and AMF + B fertilizer treatments increased. The highest content of geranyl acetate (47.1%) was observed in Mb:F(2:2) intercropping pattern and AMF + B fertilizer treatment which increased by 276.8% compared with that in sole Moldavian balm. In sole Moldavian balm and Mb:F(4:2) intercropping pattern the contents of geranyl acetate in AMF and AMF + B fertilizer treatments increased compared with those in CF fertilizer treatments; while in intercropping patterns of Mb:F(1:1) and (2:2) the contents of geranyl acetate in AMF and AMF + B fertilizer treatments decreased. In all intercropping patterns and sole Moldavian balm the contents of neral in AMF and AMF + B fertilizer treatments improved in comparison with those in CF fertilizer treatments. The highest content of nerol (13.5%) was observed in sole Moldavian balm and CF fertilizer treatment. In sole Moldavian balm and intercropping patterns of Mb:F(2:2) and (4:2) the nerol contents in AMF and AMF + B fertilizer treatments decreased compared with those in CF fertilizer treatments.

Table 3

Proportion of Moldavian balm essential oil constituents under different cropping patterns and fertilizer treatments (an average over the two years). MB: Moldavian fenugreek. CF, AMF and AMF + B are 100% chemical fertilizer, arbuscular mycorrhizal fungi bio-fertilizer and arbuscular mycorrhizal fungi + bacteria bio-fertilizer respectively.

Compounds	RI ^a	Sole Moldavian balm			Mb:F (1:1)			Mb:F (2:2)			Mb:F (4:2)			Mb:F (100:50)	
		CF	AMF	AMF + B	CF	AMF	AMF + B	CF	AMF	AMF + B	CF	AMF	AMF + B	CF	AMF
4-Hexen-1-ol,	879	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.5
1-Octen-3-ol	975	-	-	-	0.5	-	-	-	-	0.8	-	-	-	-	-
6-Methyl-5-hepten-2-one	983	-	-	-	1.7	-	-	0.4	1.2	1.2	1.1	-	-	-	0.7
Benzaldehyde	998	4.8	-	0.3	-	-	-	-	-	0.5	-	-	-	-	-
Linalool oxide	1087	-	-	-	0.7	-	-	-	-	1.2	-	-	-	-	-
Linalool	1104	-	-	-	1.5	-	-	0.9	0.1	2.9	0.4	1.5	-	-	1.1
Nerol	1235	13.5	0.6	3.4	2.5	2.8	6.7	3.7	3.2	0.1	3.1	2.2	0.5	2.9	11
Neral	1242	0.9	8.9	6.4	2.2	4.6	3.3	3.2	16.2	4.4	8.1	20.5	18.1	6.0	14
Linalyl formate	1258	5.2	-	5.4	-	0.7	5.9	-	5.8	-	-	-	-	-	-
Geraniol	1263	25.8	5.2	14.8	8.1	26.2	25.9	13.5	21.4	14.2	20.5	9.2	25.8	21.7	25
Geranial	1275	41.8	61.4	43.1	47.8	52.1	53.9	51.7	26.3	50.2	37	43.1	6.3	46.8	15
Geranyl formate	1305	-	12.2	11.5	0.1	-	-	-	-	0.4	20.9	-	-	10.4	-
Neryl acetate	1367	3.5	-	0.8	0.5	-	-	-	-	4.0	-	-	-	-	2.1
Geranyl acetate	1386	3.1	10.2	12.5	31.2	12.3	2.4	25.3	23.5	17.4	7.7	21.8	47.1	9.1	26
Farnesol	1722	-	-	0.2	0.5	-	1.1	-	0.2	0.2	-	-	0.8	1.0	-
Hexadecanoic acid (CAS)	1947	-	-	-	0.2	-	-	-	-	0.5	-	-	-	-	0.5
Di-(2-ethylhexyl)phthalate	2480	-	-	-	1.3	-	-	-	1	0.3	-	-	-	-	-
Total identified (%)		98.6	98.5	98.4	98.8	98.7	99.2	98.7	98.9	98.3	98.8	98.3	98.6	98.4	98

^aRI: Retention Indices on the HP-5 MS column.

LER

In both years the LER_T indices of all intercropping patterns were higher than 1.0 (Table 6). The highest LER_T values were obtained in Mb:F(100:50) intercropping patterns and CF treatments (1.58 and 1.70 in 2020 and 2021, respectively) and the lowest values in Mb:F(4:2) and AMF treatment (1.03 and 1.04 in 2020 and 2021, respectively). Among the replacement intercropping patterns the LER_T values in intercropping patterns of Mb:F(1:1) and (2:2) were higher than Mb:F(4:2). In Mb:F(1:1) and (2:2) intercropping patterns the highest LER_T values were observed in AMF + B fertilizer treatment and in Mb:F(100:50) intercropping patterns the highest LER_T values were observed in CF treatment. Comparison of LER_{Mb} and LER_F (partial LERs) in Mb:F(1:1) and (2:2) intercropping patterns showed that in most of the treatments the LER_F values were higher than LER_{Mb} values, which indicates that intercropping had more positive effect on fenugreek. In Mb:F(4:2) intercropping pattern the LER_{Mb} values were higher than those of LER_F; while in Mb:F(100:50) intercropping pattern the LER_{Mb} values were lower than LER_F.

Table 4

Land equivalent ratio (LER) values at different intercropping patterns and fertilizer treatments in 2020 and 2021. Mb: Moldavian balm, F: fenugreek. LER_{Mb}: LER of Moldavian balm, LER_F: LER of fenugreek, LER_T: total LER. CF, AMF and AMF + B are 100% chemical fertilizer, arbuscular mycorrhizal fungi bio-fertilizer and arbuscular mycorrhizal fungi + bacteria bio-fertilizers, respectively.

Intercropping pattern	Fertilizer treatment	2020			2021		
		LER _{Mb}	LER _F	LER _T	LER _{Mb}	LER _F	LER _T
Mb:F (1:1)	CF	0.54	0.58	1.12	0.44	0.75	1.19
	AMF	0.65	0.58	1.23	0.67	0.58	1.24
	AMF + B	0.56	0.68	1.24	0.66	0.71	1.37
Mb:F (2:2)	CF	0.56	0.72	1.28	0.59	0.66	1.25
	AMF	0.51	0.65	1.16	0.58	0.55	1.13
	AMF + B	0.56	0.75	1.31	0.58	0.67	1.26
Mb:F (4:2)	CF	0.87	0.22	1.09	0.85	0.26	1.11
	AMF	0.82	0.21	1.03	0.78	0.26	1.04
	AMF + B	0.79	0.25	1.04	0.81	0.24	1.05
Mb:F (100:50)	CF	0.65	0.93	1.58	0.72	0.98	1.70
	AMF	0.74	0.75	1.49	0.63	0.69	1.32
	AMF + B	0.72	0.83	1.55	0.76	0.87	1.63

Discussion

The Moldavian balm height in 2020 was higher than that in 2021, which can be attributed to more precipitation in 2020 than 2021 (Table 1). It was found that the Moldavian balm height increased in Mb:F(1:1) and (2:2) intercropping patterns with application of CF and AMF + B fertilizers (Fig. 2). According to Yang et al.¹⁹ in shade conditions caused by intercropping, increasing plant height is expected by reducing the ratio of red to far red light (FR/R) and reducing the amount of photosynthetic active radiation. Increasing the plant height in intercropping conditions can be attributed to competition for more light interception and lack of light penetration in the vegetation and non-decomposition of auxin hormone in these conditions²⁰. Also, Ebwongu et al.²¹ in corn (*Zea mays* L.)-potato (*Solanum tuberosum* L.) intercropping observed that the height of potato increased in intercropping compared to sole cropping. Also, increasing plant height in intercropping with legumes can be associated with high nitrogen availability²². Considering that Moldavian balm height decreased in Mb:F(100:50) additive intercropping pattern compared to sole crop, the reason for this decrease in height can be attributed to competition between crops for water, nutrients and biospace²², which reduced the growth and height of Moldavian balm in intercropping. Agegnehu et al.²³ also reported that the faba bean height reduced significantly in intercropping with barley (*Hordeum vulgare* L.) due to interspecific competition. In Mb:F(100:50) intercropping pattern the greatest plant height was observed in AMF + B bio-fertilizer treatment. The reason for increasing the plant height with the use of bio-fertilizer may be explained by better absorption of phosphorus, nitrogen and potassium, which ultimately leads to improved growth traits such as plant height²⁴.

The highest LAI was recorded in Mb:F(1:1) cropping pattern with application of AMF + B bio-fertilizer. The reason for the increase in Moldavian balm LAI in this treatment can be explained by increasing the nutrients (N + P) availability through bio-fertilizer (AMF + B bio-fertilizer) treatment and biological nitrogen fixation of by fenugreek. Nitrogen is one of the most important nutrients which affects crops vegetative growth, especially the development of leaf area by affecting the size and longevity of each leaf²⁵. Our results are consistent with Yavas and Unay²⁶ on intercropping of corn and legumes. Reedy & Willey²⁷ also observed that in intercropping of pearl millet (*Pennisetum glaucum* L.) and peanut (*Arachis hypogaea*) the millet LAI increased significantly compared to sole crop. They concluded that the most important factor of this superiority was the positive role of peanut in increasing the soil fertility for millet. Decrease in LAI of Moldavian balm in Mb:F(100:50) intercropping compared with other cropping patterns could be attributed to interspecific competition occurred between Moldavian balm and fenugreek.

At all cropping patterns the Moldavian balm dry herbage yields in 2020 were greater than those in 2021 which may be related to higher precipitation in 2020 that lead to increase in growth and yield of Moldavian balm. After sole cropping, among the intercropping patterns the highest dry herbage yield was related to the Mb:F(4:2) cropping pattern, in which the proportion of Moldavian balm was higher than other cropping patterns. Vafadar-Yengeje et al.² reported that in intercropping of faba bean-Moldavian balm, the herbage yield of Moldavian balm in sole cropping was higher than intercropping patterns. Also in Moldavian balm-soybean²⁸ and black cumin-soybean¹⁸ intercropping observed that crop yields decreased in replacement intercropping patterns compared with those in sole crops. In 2020 the dry herbage yield in AMF + B fertilizer treatment was not significantly different with CF treatment and in 2021 increased compared with that in CF treatment. Also, the increase in dry herbage yield of Moldavian balm as a result of bio-fertilizers application could be attributed to facilitative effect

of arbuscular mycorrhizal fungi on fenugreek N-fixation and N transfer to Moldavian balm as reported by Li et al.²⁹. In arbuscular mycorrhizal inoculation treatment, the faba bean N fixation and transferring to the intercropped wheat (*Triticum turgidum* L.) increased by 20%³⁰.

The essential oil contents in Mb:F(1:1) and (2:2) intercropping patterns (0.252 and 0.261%) increased compared with those in Mb:F(100:50) and sole Moldavian balm (0.228 and 0.232%). Rezaei-Chiyaneh et al.³¹ reported that the essential oil contents in all intercropping patterns of fennel (*Foeniculum vulgare*, Mill.)-common bean, were higher than that of sole fennel. The essential oil content in AMF + B fertilizer treatment was higher than those of CF and AMF fertilizer treatments. Since essential oil is one of the secondary metabolites, its production is influenced by nutrients availability and photosynthesis rate³². Increasing the content of essential oil with the use of AMF + B bio-fertilizer treatment could be attributed to improvement of plant growth characteristics and essential oil-forming glands due to the increase in the level of absorption and access to nutrients³³. It seems that the intercropping of Moldavian balm with fenugreek has increased the essential oil content by providing the main essential nutrients such as nitrogen which is affecting the activity of photosynthetic enzymes³⁴ and as a result the content of essential oil. On the other hand, use of bio-fertilizers provides the possibility of better access to macro and micro nutrients by the plant, as a result, they are effective in improving the amount of essential oil³⁵. Also, Kapoor et al.³⁶ concluded that the use of bio-fertilizers caused changes in concentration of plant phytohormones which increase the formation of essential oil secreting glands and ultimately lead to the production of more secondary metabolites. Increasing the content of essential oil due to use of bio-fertilizers in medicinal plants such as fennel³⁴ and Moldavian balm^{2,9} has been specified.

Interaction effect of cropping pattern × fertilizer treatment (Fig. 9) on essential oil yield showed that the greatest values were observed in Mb:F(4:2) intercropping pattern. At all intercropping patterns except the Mb:F(1:1), the essential oil yield in AMF + B and CF treatments were not significantly different. Weisany et al.³⁷ also found that in coriander-soybean intercropping, application of arbuscular mycorrhizal fungi (*Glomus intraradices*) significantly increased the essential oil yield of coriander. In 1:1 faba bean- Moldavian balm intercropping pattern, the essential oil yield of Moldavian balm in chemical fertilizer and bio-fertilizer application was not significantly different². Also in previous studies increasing the essential oil yield of Moldavian balm^{9,10} and basil (*Ocimum basilicum* L.)³⁸ as a result of bio-fertilizers application are specified. At all cropping patterns and fertilizer treatments the essential oil yield in 2020 were higher than those in 2021 that can be explained by higher precipitation in 2020 growth season which in turn increased the dry herbage yield, essential oil content and yield of Moldavian balm in this year (Table 4, Figs. 7 and 8).

Based on the results of GC-MS analysis, it was found that geranial, geranyl acetate, geraniol, neral and nerol were the main constituents of essential oil. In previous studies, it has been found that geraniol, geranial, geranyl acetate, neral and neryl acetate were the main constituents of essential oil in Moldavian balm^{2,9,28} Hussein et al.³⁹ reported that in Moldavian balm the geranial and linalool were the main constituents of essential oil. The effects of fertilizer treatment and cropping pattern on major constituents of essential oil were different. The highest amount of geranial (61.4%) was observed in AMF treatment in sole cropping, which increased by 18.3 and 19.6% compared with that in AMF + B and CF treatments, respectively. Our results did not match with those of Vafadar-Yengeje et al.² in intercropping of faba bean-Moldavian balm which reported that the highest geranial content was observed in chemical fertilizer treatment. The highest content of geraniol (26.2%) was related to the AMF treatment in the Mb:F(1:1) cropping pattern. Vafadar-Yengeje et al.² found that in 1:1 faba bean-Moldavian balm intercropping the geraniol content was higher in bio-fertilizer and vermicompost treatments compared with chemical fertilizer. Also it has been found that the bio-fertilizers improved the content of geraniol in essential oil of Moldavian balm in comparison with control treatment¹⁰.

The AMF + B fertilizer treatment in Mb:F(4:2) cropping pattern showed the highest content of geranyl acetate (47.1%). Karimzadeh Asl et al.¹⁰ also found that the greatest geranyl acetate content of Moldavian balm essential oil was obtained in bio-fertilizer treatment. On the other hand, the geranyl acetate content in CF fertilizer treatment of Mb:F(1:1) cropping pattern (31.2%) was higher than other cropping patterns. Also, the lowest contents of geranyl acetate in CF and AMF treatments were related to sole Moldavian balm (3.1 and 10.2%, respectively). In this regard, in faba bean-Moldavian balm intercropping, the content of geranyl acetate in chemical fertilizer treatment increased in all intercropping patterns compared to sole Moldavian balm². Ganjewala and Luthra⁴⁰ reported that a negative correlation was observed between the amount of geraniol and geranyl acetate in lemongrass (*Cymbopogon flexuosus* L.); as geraniol increased, the content of geranyl acetate decreased. They also reported that this correlation could be related to conversion of geranyl acetate to geraniol during the leaf growth period.

It has been found that differences in ecological factors, genetic origin, and cropping patterns cause quantitative and qualitative differences in essential oil compounds⁴¹. In previous studies, the positive effect of intercropping and use of bio-fertilizers on improving the essential oil composition of medicinal plants has been reported^{2,42,43}. Even though many studies indicated the positive effect of bio-fertilizer on essential oil constituents of medicinal plants such as white horehound (*Marrubium vulgare* L.)⁴⁴ and Moldavian Balm^{9,10}, on the other hand, a number of studies show the advantageous effect of chemical fertilizers on the essential oil compositions²⁸. Different biosynthesis pathways of these components may be responsible for different responses to fertilizer application⁴⁵. The biosynthesis of many organic compounds is influenced by the amount of nutrients in the soil, including nitrogen, due to the high impact of these compounds on the biosynthesis of essential oils⁴⁶. It can be expected that the use of bio-fertilizers through the provision of more nutrients and the creation and division of new essential oil-containing cells, glandular trichomes and essential oil channels increase essential oil in medicinal plants¹⁷. Also accumulation of mono or diterpenes could be increased by Mycorrhizal fungi through enzymatic activity that starts from geranyl diphosphate (GPP), their common precursor⁴⁷. According to the results of the present study, bio-fertilizers and different cropping patterns can be used to some extent in order to improve the yield and quality of Moldavian balm essential oil.

The LER is one of the intercropping evaluation indices that show the intercropping advantage in terms of land use. The evaluation of LER values (Table 6) showed that the highest LER_{Mb} was obtained in the Mb:F(4:2) cropping pattern and CF fertilizer (0.87 and 0.85 in 2020 and 2021, respectively). Also, the highest LER_F values were obtained in Mb:F(100:50) cropping pattern and CF fertilizer treatment (0.93 and 0.98 in 2020 and 2021, respectively). In general, the partial LERs for fenugreek were higher than those of Moldavian balm (except for Mb:F(4:2) cropping pattern), that indicated the intercropping had more

positive effect on fenugreek. Monti et al.⁴⁸ concluded that the increase in partial LER higher than 0.5 depends on the complementary degree of the intercropping components. Also, LER_T values higher than 1.0 indicate the superiority of intercropping over sole crop. Previous researches have shown that superiority in intercropping is due to different morphological properties and growth and the tendency of intercropping components to make optimum use of sources such as soil, moisture, light and nutrient elements²⁹. Also there is a difference in the root structure, distribution of the canopy cover and the nutritional needs of the plants in intercropping^{4,49}.

The highest LER_T (1.70) was obtained in Mb:F(100:50) cropping pattern and CF treatment in 2021, accounted for which was equivalent to an increase of 70% in land productivity compared to sole cropping of two crops. The results of previous studies have also shown that when the legume species beside the other species are planted as a intercropping, due to the complementary effect, nitrogen fixation is stimulated, which increases the growth and yield of the legume species due to the increase in the number of active nodes²⁵. Although the presence of species together increases competitiveness to absorb environmental resources, if one species has nitrogen fixation ability, competitive pressure will be reduced because the legume species will have less competition with other species in nitrogen absorption⁵⁰. More distribution of nitrogen in the soil through nitrogen fixation and lower leaves fall of legumes improve soil fertility and adjacent plant growth in intercropping⁵¹. Fenugreek also appears to acidify the rhizosphere through nitrogen stabilization, thereby increasing the solubility and absorption of phosphorus by Moldavian balm⁵².

Conclusions

The Moldavian balm height, LAI, dry herbage and essential oil yield were affected significantly by interaction effect of cropping pattern × fertilizer treatment. At all cropping patterns except the Mb:F(1:1) intercropping, the LAIs in AMF + B fertilizer treatments were not significantly different with those in CF treatments. The highest dry herbage yield was obtained in Mb:F(4:2) intercropping pattern. In Mb:F(1:1) and (100:50) intercropping patterns the highest dry herbage yields were observed in AMF + B fertilizer treatment. Among the fertilizer treatments the highest essential oil content (0.259%) was obtained in AMF + B treatment. At all cropping patterns except the Mb:F(1:1), the essential oil yields in AMF + B fertilizer treatments were not significantly different with those in CF treatments. Geranial (6.3–61.4%), geranyl acetate (2.4–47.1%), geraniol (3.4–25.9%), neral (0.9–20.5%) and nerol (0.1–13.5%) were the main chemical constituents of Moldavian balm essential oil and affected differently by cropping patterns and fertilizer treatments. The LER_T indices in all intercropping patterns were greater than 1.0 in both years, which indicates more efficient use of land and resources in intercropping patterns than sole crop. In both years the LER_T values in Mb:F(100:50) intercropping patterns and AMF + B fertilizer treatments (1.55 and 1.63) were higher than those in other intercropping patterns and fertilizer treatments. Finally, according to the obtained results, additive intercropping of Moldavian balm with fenugreek (Mb:F(100:50)) and the combined use of arbuscular mycorrhizal fungi and bacteria (AMF + B) bio-fertilizers could be introduced as a eco-friendly and alternative methods to sole cropping and chemical fertilizer application in line with the goals of sustainable and cleaner production of Moldavian balm.

Material And Methods

Experimental Site

This research was conducted in Maragheh city in East Azarbaijan province, Iran (latitude 37°4 N, longitude 46°26 E, altitude 1478 m above sea level) in 2020 and 2021 growth seasons. The climatic data of monthly total precipitation and mean temperature of the experimental site during the growth seasons of 2020 and 2021 are presented in Table 1. The soil physicochemical characteristics of the experimental field at a depth of 0–30 cm are presented in Table 2.

Table 5
Monthly total precipitation and mean temperature in 2020 and 2021 growing seasons in the experimental area.

	Year	May	June	July	August	September
Total precipitation (mm)	2020	14.23	2.03	3.30	1.27	0.0
	2021	12.71	0.0	1.2	0.0	0.0
Mean temperature (°C)	2020	20.2	25.5	29	26	24.8
	2021	22.5	28.7	29.5	29.1	24.3

Table 6
Physicochemical properties of the soil of experimental area in depth of 0–30 cm.

Parameter	Soil texture			pH	Organic matter (%)	EC	Total N	P	K
	Sandy loam					(dS m ⁻¹)	(%)	(mg kg ⁻¹)	(mg kg ⁻¹)
Value	Clay (%)	Silt (%)	Sand (%)	7.28	1.0	1.76	0.033	16.5	620
	9	27	64						

Experimental Procedure

The factorial experiments were carried out based on randomized complete block design with three replications in 2020 and 2021. The cropping pattern (first factor) including Moldavian balm sole cropping (Mb), fenugreek sole cropping (F) and replacement intercropping ratios including 1 row of Moldavian balm + 1

row of fenugreek (Mb:F(1:1)), 2 rows of Moldavian balm + 2 rows of fenugreek (Mb:F(2:2)), and 4 rows of Moldavian balm + 2 rows of fenugreek (Mb:F(4:2)) and additive intercropping of Moldavian balm + fenugreek Mb:F(100: 50) (100% optimum density of Moldavian balm + 50% optimum density of fenugreek planted between Moldavian balm rows). The fertilizer treatment (second factor) was consisted of 100% chemical fertilizer (CF), application of arbuscular mycorrhizal fungi (AMF) and combined application of AMF and bacteria (AMF + B).

CF treatment was 50 kg ha⁻¹ urea and 80 kg ha⁻¹ triple superphosphate (according to soil test results); which were used at Moldavian balm planting time. Myco-Root bio-fertilizer contains arbuscular mycorrhiza fungi (AMF) of *Glomus mosseae*, *Glomus intraradices* and *Glomus etunicatum* with count 10⁷ to 10⁸ CFU/gr is provided by Zist Fanavar Pishtaz Varian Company, Karaj, Iran. This bio-fertilizer is an easy-to-use powder form that is used for crops as seed inoculation. According to the manufacturer's instructions, the seeds required for the experimental area were placed in the shade on nylon or a clean surface, and after spraying a small amount of water on them, the required amount of AMF bio-fertilizer was added and mixed thoroughly; so that all the seeds were covered with a uniform layer of bio-fertilizer and after drying the seeds in shade, the planting was done. In order to inoculation of bacteria (B), Biofarm bio-fertilizer contains *Azospirillum*, *Azotobacter* and *Pseudomonas* bacteria with a population of 2 × 10⁷ CFU/gr is provided by Nature Biotechnology Company (Biorun) Karaj, Iran. Similar to the Myco-Root, the seeds of the Moldavian balm and fenugreek were inoculated with Biofarm and then planted.

The Moldavian balm and fenugreek were planted with densities of 32 and 50 plants m⁻², respectively. Both crops were planted on 04 May 2020 and 15 May 2021. For intercropping patterns the experimental plots had different rows number with 25 cm row space and 3 m long. The furrow irrigation was done after planting of both crops with 5-day intervals. The weeds in plots were removed by hand weeding.

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

Plant height

In both years in order to measure the height of Moldavian balm, at maturity stage 10 plants from each plot were randomly selected after removing the marginal effects (side rows and half a meter from the sides of the middle rows) and plant height was measured.

Leaf area index (LAI)

In both years the LAI was determined at flowering stage (on 29 July 2020 and 9 August 2021). The Delta-T leaf area meter (Delta-T Devices, Cambridge, England) was used for measuring the leaf areas. By dividing the leaf area to the ground area the LAI was obtained⁵³.

Dry herbage yield

Moldavian balm plants were harvested manually from each plot at the flowering stage to determine dry herbage yield on August 5, 2020 and August 12, 2021, and after drying in shade and room temperature (for 7 days), the herbage yield was measured as kg ha⁻¹ ².

The content and yield of essential oil

In flowering stage the Moldavian balm herbage was harvested, dried and powdered. Then 100 g from powder was hydrodistilled for 3.0 h by a Clevenger device⁵⁴. In order to dehydrate the obtained essential oil, the anhydrous sodium sulphate was used; and by measuring the amount of essential oil, it was expressed as a percentage (EO content). The samples were stored at 4°C until GC-MS analysis. Also, the yield of essential oil (kg ha⁻¹) was calculated as follows².

Essential oil yield (kg ha⁻¹) = Essential oil content (%) × Herbage yield (kg ha⁻¹)

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was done on an Agilent 6890N gas chromatograph with a 5973 mass-selective detector (Agilent Technologies, CA, USA) as described with Vafadar-Yengeje et al.². Identification of compounds was carried out by comparison of the mass spectra of each component either with those stored in the Wiley 7.0 and Adams mass spectral-retention index libraries⁵⁵.

LER assessment

In Moldavian balm-fenugreek intercropping patterns the LER values were assessed as follows² as:

$$LER_{Mb} = Y_{Mbi} / Y_{Mb} \quad \text{and} \quad LER_F = Y_{Fi} / Y_F \quad (1)$$

$$LER_T = LER_{Mb} + LER_F \quad (2)$$

Where Y_{Mb} and Y_{Mbi} are the Moldavian balm dry herbage yields in sole cropping and intercropping, respectively, and Y_F and Y_{Fi} are the fenugreek grain yields in sole cropping and intercropping patterns, respectively. Also, LER_{Mb} and LER_F represent the partial LER of Moldavian balm and fenugreek, respectively, and LER_T is the total LER. When LER_T is equal to 1.0, indicates the same competitive ability of both crops in intercropping. This situation occurs if the inter-specific competition is equal to the intra-species competition in the intercropping components. In addition, if the increase in the yield of one crop is accompanied by a decrease in the yield of another crop in intercropping, the LER_T value will be equal to 1.0. If the value of LER_T is less than 1.0, sole crop is superior to intercropping, and if the value of LER_T is higher than 1.0, intercropping is more beneficial than sole crop².

Statistical analysis

For analysis of variance (ANOVA) the SAS Version 9.0.3 was used. For two growing seasons of 2020 and 2021 and all traits the combined ANOVA was done based on complete randomized block design with 15 treatments and three replicates. The data of LER_M , LER_F , and LER_T were not subjected to analysis of variance. The experimental data met the assumptions of normality and variance homogeneity and no transformation was needed. For comparison of the means the Duncan's multiple range test was used at $p \leq 0.05$.

Data availability

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

Declarations

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Author contributions statement

Z. A. C.: conducted the experiment, collected the data, analyzed the data, and wrote the manuscript, prepared figures and tables. **R. A.:** reviewed the final data, edited and reviewed the manuscript. **A. D. M. N.:** edited the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Figures

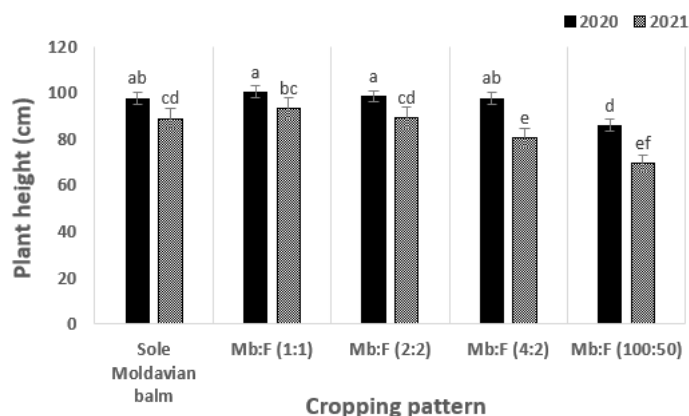


Figure 1

Plant height of Moldavian balm affected by interaction of year × cropping pattern. Different letters indicate significant differences at $p \leq 0.05$.

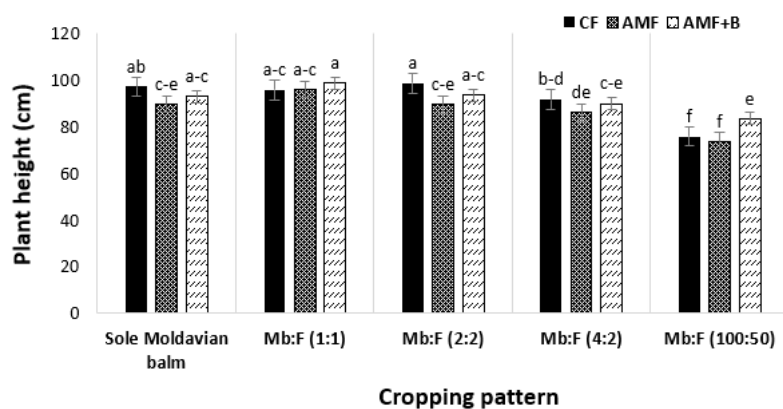


Figure 2
Plant height of Moldavian balm affected by interaction of cropping pattern $\times$ fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

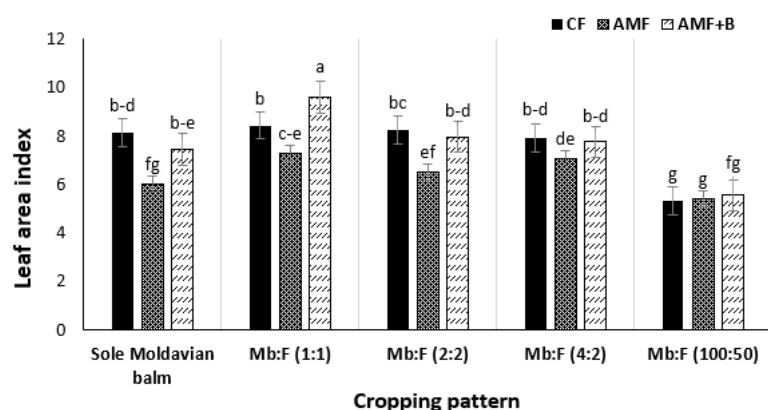


Figure 3
Leaf area index of Moldavian balm affected by interaction of cropping pattern $\times$ fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

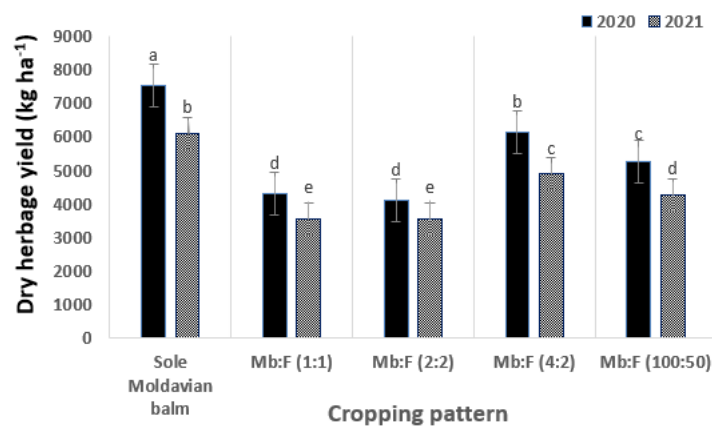


Figure 4
Dry herbage yield of Moldavian balm affected by interaction of year $\times$ cropping pattern. Different letters indicate significant differences at $p \leq 0.05$.

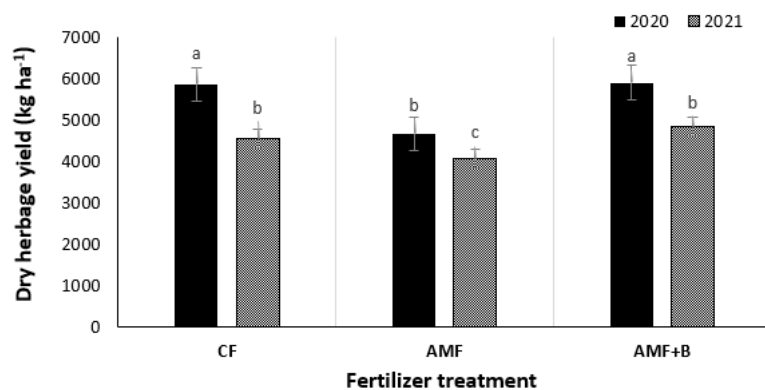


Figure 5

Dry herbage yield of Moldavian balm affected by interaction of year × fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

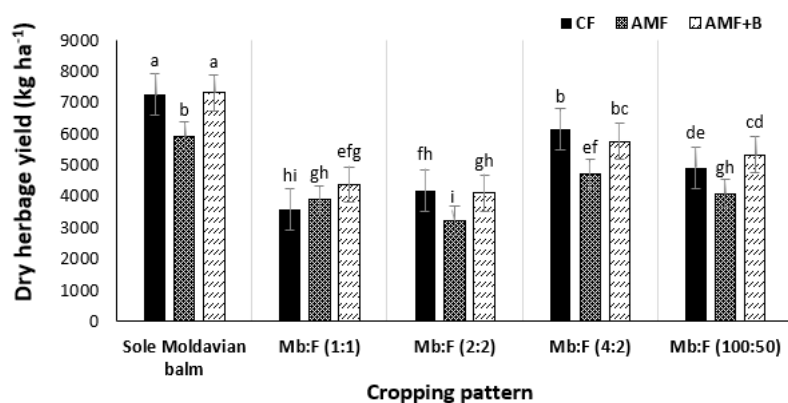


Figure 6

Dry herbage yield of Moldavian balm affected by interaction of cropping pattern × fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

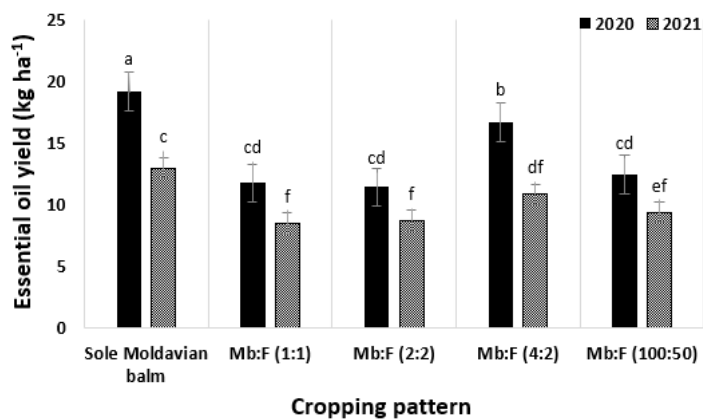


Figure 7

Essential oil yield of Moldavian balm affected by interaction of year × cropping pattern. Different letters indicate significant differences at $p \leq 0.05$.

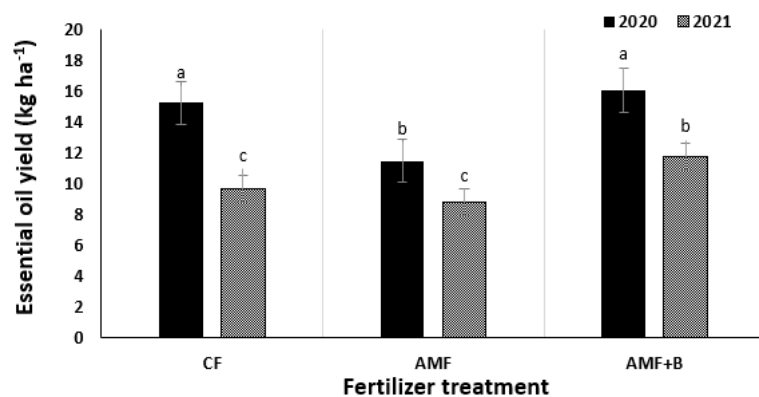


Figure 8

Essential oil yield of Moldavian balm affected by interaction of year × fertilizer treatment. Different letters indicate significant differences at $p \leq 0.05$.

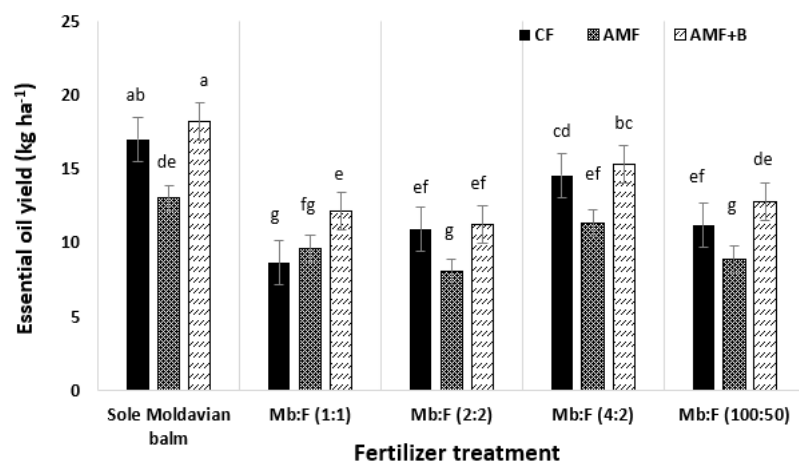


Figure 9

The interaction effect of cropping pattern × fertilizer treatment on essential oil yield of Moldavian balm. Different letters indicate significant differences at $p \leq 0.05$.