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INOCULATING MYCORRHIZA FUNGI AND GROWTH-PROMOTING BACTERIA AND MULCH OF PLANT RESIDUES IMPROVE YIELD AND ESSENTIAL OIL PRODUCTION OF ANISE (*Pimpinella anisum* L.)

Zahra Ebrahimi[1], Esfandiar Fateh[2]✉, Mohammad Mahmoodi Soorestani[2], Mansour Ghorbanpour[3]

[1] Agronomy and Plant Breeding Department, Shahid Chamran University of Ahvaz, Iran

[2] Production Engineering and Plant Genetics and Horticulture Department, Shahid Chamran University of Ahvaz, Iran

[3] Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-8-8349, Iran

ABSTRACT

This experiment aimed to investigate the effects of *Pseudomonas* growth-promoting bacteria, mycorrhizal fungi, and living mulch on morphological and physiological traits of a medicinal herb, *Pimpinella anisum*. The study was carried out using a factorial design in a randomized complete block design arrangement with three replications during the crop year of 2019–2020. The first factor includes the use of biological fertilizers (fungi and bacteria) at four levels: 1) control (without the use of biological fertilizers), 2) mycorrhizal fungi, 3) growth-promoting bacteria, 4) combined use of growth-promoting bacteria and fungi. The second factor includes mulch (plant residues) at four levels: 1) control, 2) wheat straw (3 t/ha), 3) berseem clover mulch (2 t/ha), 4) living berseem clover mulch, cultivated among the rows of anise. Cultivation of clover and anise was done simultaneously. Results showed that the combined use of biological fertilizers (*Glomus intraradices* and *Pseudomonas putida*) along with living clover mulch led to a significant increase in seed yield, harvest index, and the essential oil yield of anise. Moreover, under such conditions, the highest seed yield (930.8 kg/ha) and the highest essential oil yield (18.15 kg/ha) increased by 48% and 51% compared to the control, respectively. We found that the use of living clover mulch led to a significant increase in biological yield, seed yield, and the number of umbels and umbellets of anise. Accordingly, the highest biological yield of 5332 kg/ha was obtained, which was 55% higher than the control. The extraction of essential oil components showed that α-pinene and para-cymene showed the highest amount and significant components of essential oil. Moreover, the maximum amount of α-pinene (2.277%) observed upon fungi and living clover application along with inter-row clover cultivation (increased by 60% compared to the control), and the maximum value of para-cymene (0.2300%) was obtained upon bacteria and living clover inoculation along with inter-row clover cultivation (increased by 59% compared to the control). Finally, according to the perspectives of sustainable and organic agriculture in medicinal plant cultivation, the use of ecologically compatible inputs such as bacteria and fungi that stabilize food elements, as well as the use of living and non-living plant mulches, can be used as a tool to increase the quantitative and qualitative performance of the anise plant.

Key words: *Pseudomonas bacteria*, plant residues, berseem clover, medicinal herb, essential oil, living mulch, α-pinene, para-cymene

✉ e.fateh@scu.ac.ir

INTRODUCTION

Anise (*Pimpinella anisum*) is one of the most important medicinal herbs of the Apiaceae family. It is an annual and herbaceous plant. The origin of this plant is the western coast of the Mediterranean Sea, Egypt, and Asia Minor. So far, 150 species of the *Pimpinella* genus have been identified in Europe, Asia, and Africa. Species of this genus self-grow in some islands of the Aegean Sea [Omid Beygi 2013]. One of the ways to achieve the goals of sustainable agriculture is the use of microorganisms that play an essential role in providing the nutritional needs of plants [Ghorbanpour et al. 2014]. Biological fertilizers refer to products resulting from the activity of microorganisms that perform in connection with nitrogen fixation or providing phosphorus and other nutrients in the soil [Nourzadeh et al. 2014].

The relationship between plant roots and fungi, known as mycorrhizal symbiosis, is a crucial and beneficial association that has evolved. This mutually beneficial relationship allows both parties to thrive and grow together [Naseri et al. 2010]. Mycorrhizal fungi aid in the establishment of hormonal balance, osmotic balance, and swelling pressure while also increasing stomatal conductance and transpiration and improving nutrient accumulation and absorption in plants [Ghorbanpour et al. 2013a, b, Deepika and Kothamasi 2015, Saghafi et al. 2019]. Tarraf et al. [2017] found that the use of mycorrhizal fungi on sage (*Salvia officinalis* L.) resulted in increased active substance amounts and phosphorus absorption. Similarly, Floresa et al. [2010] reported that the joint application of mycorrhizal fungi and growth-promoting bacteria on common marigold (*Calendula officinalis*) improved nutrient absorption and dry yield. Shabahang et al. [2013] discovered that the use of mycorrhizal fungi on savory (*Satureja* sp.) resulted in an increase in plant height, essential oil yield, and percentage.

Among the organic conservation methods, plant residue mulching has a significant impact on many hydrological processes, soil biological ecosystems, and modification of soil, plant, atmosphere, and water cycle changes [Lou et al. 2011, Xu et al. 2022]. Straw is one of the cheap and valuable mulches in agriculture. Reports show that straw mulch can increase water storage in the soil, protect the soil from raindrops, reduce erosion and sedimentation, increase the roughness of the soil surface and reduce the amount and speed of surface flows, increase the amount of organic matter, and strengthen the soil structure [Wang et al. 2016]. Rezvani Moghadam et al. [2012] showed that the use of fenugreek as a living mulch in the cultivation of cumin medicinal plants led to an increase in the yield components of this plant.

Researchers have found that changing the root system structure can lead to increased plant growth by promoting nutrient absorption and carbohydrate allocation to the roots, reducing root activity, and synthesizing new proteins [Saghafi et al. 2013]. Regarding growth-promoting bacteria, it can be stated that these bacteria, through different mechanisms, including the production of plant growth-promoting hormones, siderophore production, increasing phosphorus absorption by the plant, nitrogen fixation, and the synthesis of enzymes that regulate the amount of ethylene in the plant, stimulate plant growth and increase biological yield and harvest index [Stajkovic et al. 2011 Ghorbanpour et al. 2011, Saghafi et al. 2018]. Another study on the cumin (*Cuminum cyminum* L.) plant revealed that the application of combined treatment of bio-fertilizers containing nitrogen-fixing and phosphate-dissolving bacteria had a positive and significant effect on vegetative traits, seed yield, and biological yield [Talaei et al. 2014]. Eblagh et al. [2013], in a study on ajowan (*Trachyspermum ammi* L.), showed that phosphate-solubilizing bacteria led to a significant increase in the percentage of essential oil due to the provision of photosynthetic materials. According to our hypothesis, the use of mycorrhizal fungi, as well as the use of living and non-living mulches, change the quantitative and qualitative yield of the anise medicinal plant. Therefore, the purpose of this research was to determine the effect of the employed treatments (inoculation with *Glomus intraradices* and *Pseudomonas putida*) along with living clover mulch on the yield and components of grain yield, active substances, and the components of the substance of anise (*Pimpinella anisum* L.).

MATERIAL AND METHOD

Experimental design and treatments

This study was carried out during the crop year of 2018–2019 in the educational farm no. 1 of the Production Engineering and Plant Genetic Department, Faculty of Agriculture, Shahid Chamran University of Ahvaz.

This experiment was conducted using a randomized complete block design as a factorial arrangement with three replications. The first factor includes the use of biological fertilizers (fungi and bacteria) in four levels: 1) control (no biological fertilizers), 2) mycorrhizal fungi, 3) growth-promoting bacteria, 4) combined use of growth-promoting bacteria and fungi. The second factor includes different mulches (plant residues) in four levels: 1) control, 2) wheat stubble mulch (3 t/ha), 3) berseem clover mulch (2 t/ha), 4) living berseem clover mulch, cultivated among the rows of anise. Cultivation of clover and anise was done simultaneously on October 20, 2019. The length of each plot was 3 m, and its width was 2 m. The method of planting anise seeds was considered linearly with a fixed row distance of 30 cm, where six rows of 3 m were planted inside each plot. The inoculation treatment was conducted using the reproduction process of hypha and mycelium in the culture bed of clover, and then the desired soil, along with the roots, was used for inoculation. The intended bacteria were also obtained from the Department of Soil Science, Urmia University. The active population of bacteria was 109 per gram of bio-fertilizer [Naseri et al. 2010]. The tested rhizobacteria strain (*Pseudomonas putida* strain 168) was previously isolated from the rhizospheric soil of field-grown safflower crop in Urmia University, Iran. The rhizobacteria were identified and tested for some plant growth-promoting traits such as siderophores, auxin (IAA-like substances) production, and phosphate solubilization capacity by Abbas-Zadeh et al. [2010], Naseri et al. 2010, Ghorbanpour et al. [2016]. The rhizobacteria strain was cultured in a 200 mL capacity flask containing 50 mL of nutrient broth supplemented with 5 g of L-tryptone, 2 g of NaCl, 2 g yeast extract, and 100 mL of distilled water at pH 7±0.2 with 200 µL of the strain, and allowed to incubate (30°C for four days) shaked at 150 rpm until the strain had reached a cell concentration of 1×10^9 CFU mL^{-1}, which was used as the treatment at the time of inoculation. For inoculation, 20 grams of anise seeds were soaked in 50 mL of the cell suspension for 1 h [Rahma and Kristina 2021]. The phosphate-buffered saline powder (PBS) could be used for dilution of the overnight culture until the optical density at 600 nm (OD600) was between 0.07 and 0.1. It is roughly equivalent to 1×10^8 CFU/mL. Mycorrhizal fungi, *Glomus intraradices*, were grown on nutrient agar or in liquid media with agitation (120 rpm min^{-1}) for 48 h at 25 ±2°C. *Glomus intraradices* inoculation was performed by planting the seed over a thin layer of the inoculum material at the time of sowing at the rate of approximately 1g/seed containing 60 viable spores (oven-dry bases) of the final mycorrhizal blend. The treated seeds were immediately cultivated after inoculation. Experimental design and different factors are represented in Figure 1.

Measured traits

At the time of the entire processing on May 9, 2020, after removing the border lines and also removing half a meter from the upper and lower sides of each plot from the middle lines, the harvest cutting was done. The traits that were measured included the seed yield components, including the number of umbels per plant and the number of umbeletts per umbel were determined in the selected plants. In order to determine the seed and biological yields, they were also harvested by removing the effect of the marginal remaining product. The plants harvested from the surface of each plot were placed in an oven at 70°C for 48 hours until completely dried and weighed for biological yield. Then, the anise seeds were separated from the stems and other appendages, and their dry weight was selected as the seed yield in each plot. Using the data of seed and biological yields, the harvest index of the samples was also calculated using the following relation [Kouchaki and Sarmadnia 1998]

$$HI = (G_Y/B_Y) \times 100$$

Essential oil yield

To extract essential oil, an essential oil extractor called Clevenger was used. The yield of essential oil was obtained from the percentage of essential oil in the seed yield. The components of essential oil, α-pinene, and para-cymene were measured using a GC mass device, which is specified in Table 1 [Adams 2001].

Variance analysis of the data obtained from the measured traits was done using SAS software, and a comparison of means was done using Duncan's multiple range test.

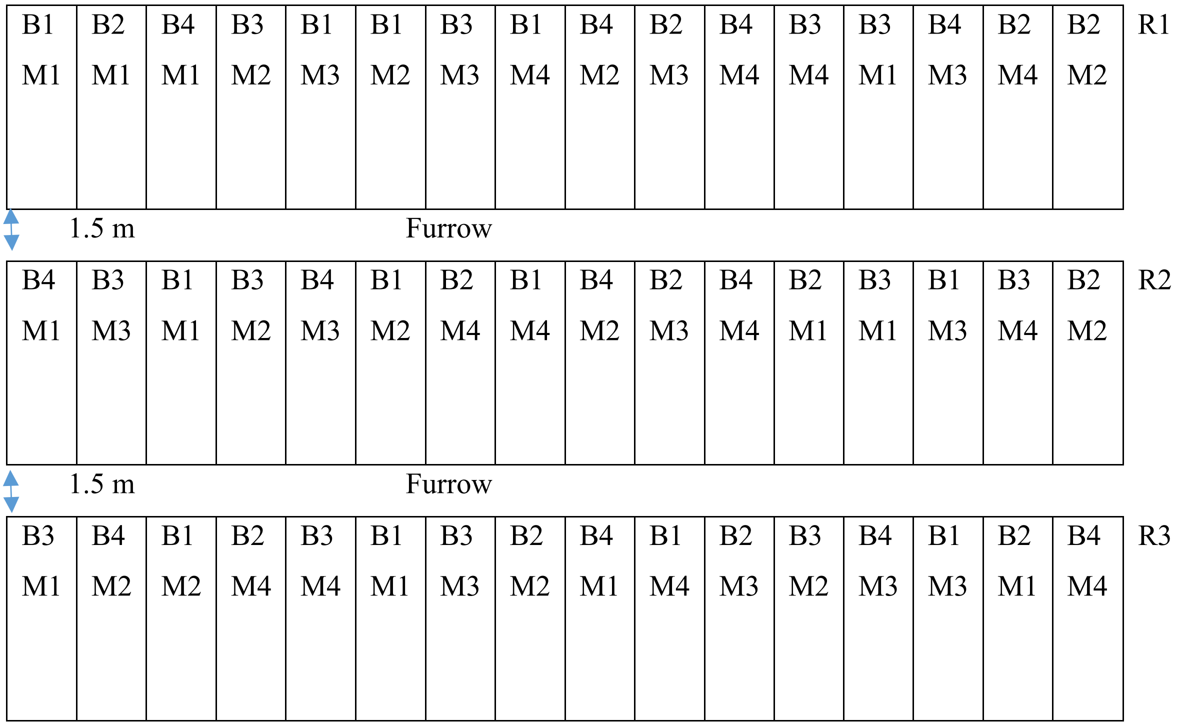

Fig. 1. Experimental design and schematic view of factors and treatments.
First factor: phosphate solubilizing bacteria (B) in 4 levels: B1 (control = without bacteria and fungi), B2 (*Mycorrhiza intradices*), B3 (*Pseudomonas putida*) and B4 (*Mycorrhiza intradices* + *Pseudomonas putida*)
Second factor: different mulch levels (M) in 4 levels: M1 (control = without mulch), M2 (wheat straw mulch = 3 t/ha), M3 (berseem lover straw mulch = 2 t/ha), and M4 (berseem clover living mulch cultivated in rows with anise). R1, 2, and 3 are replications

Table 1. Specifications of the GC/MS apparatus

Feature	Specification
GC device model	Agilent 6890
Mass device model	Agilent 5973
Column type	BPX5
Column length	30 m
Inner diameter of column	0.25 µm
Initial temperature of column	50°C
Final temperature of column	300°C
Carrier gas type	He
Detector	Mass

RESULTS AND DISCUSSION

Biological yield

The results of the analysis of variance (Tab. 2) showed that the biological fertilizer treatment and the interaction of biological fertilizer and plant mulch on the biological yield trait were significant at ($p < 0.05$) and $p < 0.01$respectively. Based on the results of the mean comparison, it was found that the highest biological yield (5332 kg/ha) was obtained in the condition of applying the mulch of berseem clover residues and the absence of fungus and bacteria, and the lowest biological yield with (2933 kg/ha) was related to the control treatment (no mulch and biological fertilizer) (Fig. 2). Based on the results, it can be stated that the use of plant mulch has a positive effect in increasing the biological yield through better soil moisture retention, releasing more nutrients, and improving the condition of soil organic matter, and it was found that

Table 2. Variance analysis of measured traits under the treatment of biological fertilizers and mulching

Source of variation (S.O.V)	df	Mean square (MS)							
		Biological yield	Seed yield	Harvest index	No. of umbels per plant	No. of umbellets per plant	Essential oil yield	α-Pinene	Para-Cymene
Replication	2	59565.554	14323.996	3.494	8.292	225.119	12.545	1.238	0.004
Biological fertilizer	3	1699721.857*	8359.422 ns	68.002**	4.934 ns	949.597 ns	3.577 ns	0.292*	0.003**
Mulch	3	499797.554 ns	6333.942**	108.476**	40.377**	3791.911**	28.577**	0.233*	0.005**
Biological fertilizer × mulch	9	21343.829**	3530.335**	73.973**	36.804**	1105.984*	16.532**	0.201*	0.002**
Error	30	459831.094	10150.657	11.052	6.792	520.165	4.622	0.085	0.0004
Coefficient of variation (CV%)		19.19	14.89	16.52	16.85	18.31	16.42	15.44	11.11

ns, * and ** indicate non-significant and significant differences at the probability level of 0.05% and 0.01%, respectively

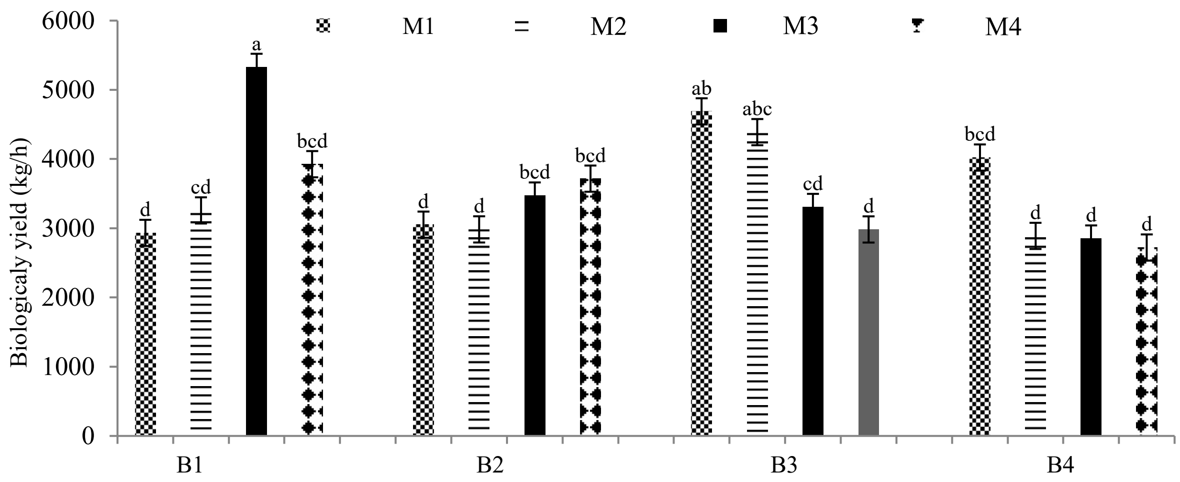

Fig. 2. Interaction of biological fertilizer and plant mulch on biological yield of anise plant. B1: control (without use), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria, *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

using plant mulch, the amount of biological yield has increased compared to the control. Also, it has been found that inoculation with growth-promoting bacteria increases the efficiency of absorbing water and nutrients and can increase the dry matter production of plants [Ansari et al. 2010, Ghorbanpour and Hatami 2013, Ghorbanpour et al. 2018]. Dastborhan et al. [2011] revealed that the application of growth -promoting bacteria in the German chamomile plant led to an increase in its biological yield. Aliabadi et al. [2008] stated that the coexistence of mycorrhizal fungi with coriander root led to an increase in biological yield, which was consistent with the results of this research.

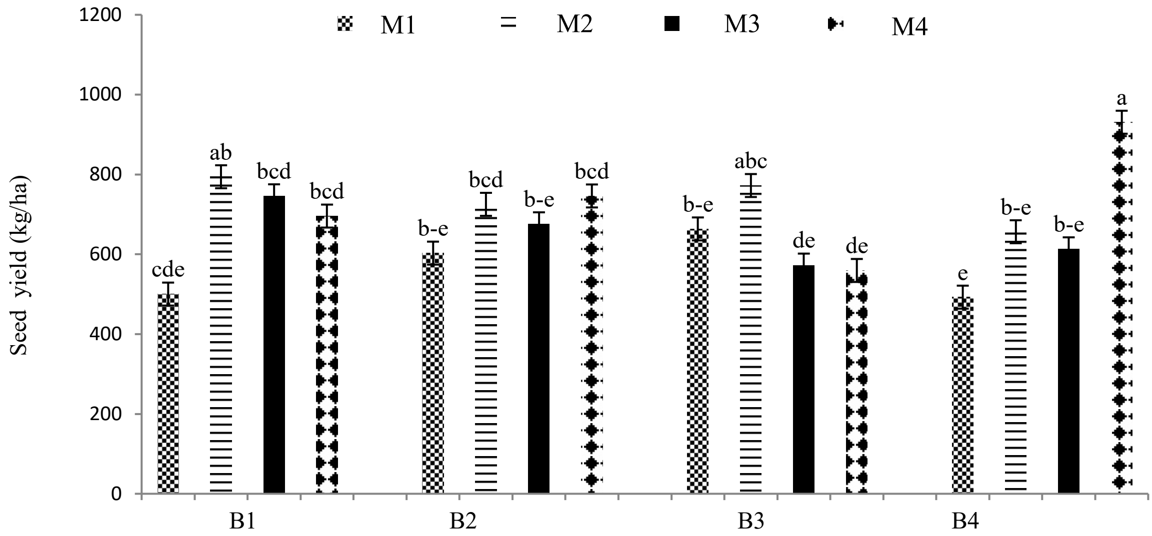

Fig. 3. Interaction of biological fertilizer and plant mulch on anise seed yield. B1: control (no biological fertilizer), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi, and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

Seed yield

Based on the analysis of the variance, the effect of plant mulch treatment and the interaction of biological fertilizer and plant mulch on the trait of seed yield was significant at ($p < 0.01$) probability (Tab. 2). Based on the comparison results, the highest amount of seed yield was obtained (930.8 kg/ha) in fungi and bacteria usage, along with the use of living clover and inter-row clover cultivation. Also, the lowest amount of seed yield (492.6 kg/ha) was obtained in fungi and bacteria usage and no application of herbal mulch (Fig. 3). It seems that the application of fungi and bacteria in the presence of living mulch can be more effective on seed yield. The reason may be the positive effect of the living mulch roots of the clover plant, which may have provided suitable conditions for the activity of the fungi and bacteria used due to the root exudations. The results of this research were consistent with the results of Habibzadeh et al. [2012] because these researchers showed that mycorrhizal fungi have a positive effect on nitrogen fixation, and phosphorus, leaf duration, maintaining and increasing leaf size, leading to an improvement in the rate of photosynthesis and an increase in photosynthetic production, which leads to increase in plant growth and allocation of nutrients to sinks (seeds), leading to increase in seed yield [Ghorbanpour and Hatami 2014]. Zamani et al. [2018] announced that the highest yield of Fennel seeds was obtained with 146.68 g/m^2 in the combined treatment of bacterial bio-fertilizer with the use of mycorrhizal fungi. These researchers expressed that it seems that the combined use of mycorrhizal fungi and growth-promoting bacteria has increased the efficiency of the photosynthetic materials transfer to the sinks due to the increase in the speed and duration of photosynthesis, which is effective in increasing seed yield.

Harvest index

The results of the analysis of variance (Tab. 2) showed that the treatment of biological fertilizer, plant mulch, and the interaction of biological fertilizer and plant mulch had a significant effect ($p < 0.01$) on the harvest index. Based on the mean comparison results, it was found that the highest value of the harvest index (34.39%) was obtained at the fungi and bacteria usage along with the application of living clover and inter -row clover cultivation. Also, the lowest value of the harvest index (12.56%) was obtained by using fungi and bacteria and not using plant mulch (Fig. 4). The results showed that the use of living mulch and fungi and bacteria together led to an increase in the harvest index because the use of living mulch increases cell growth

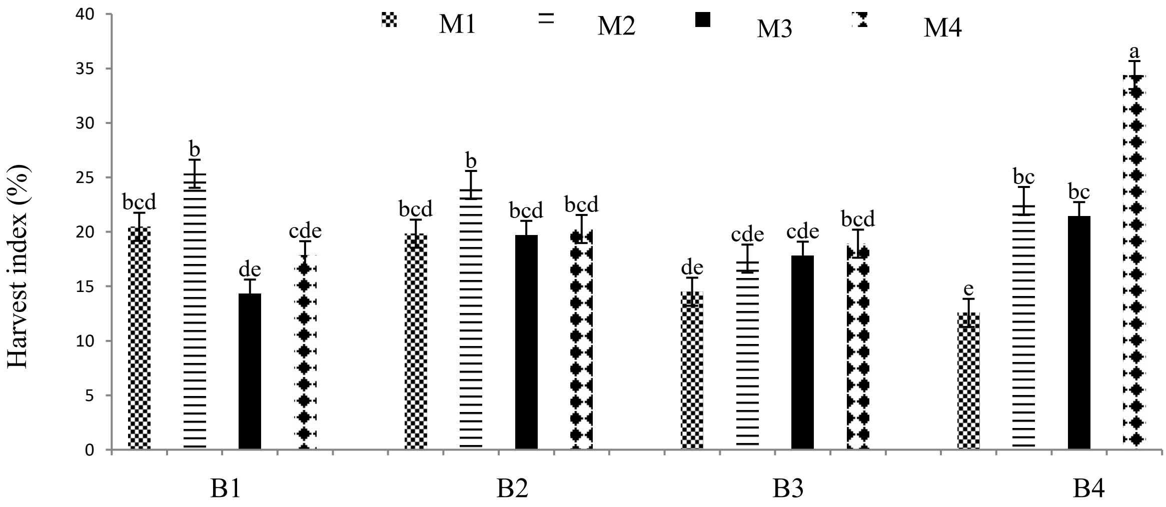

Fig. 4. Interaction of biological fertilizer and plant mulch on the harvest index of anise. B1: control (no biological fertilizer), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

and provides conditions for better absorption of fungi and bacteria. The combined application of fungi and bacteria leads to an increase in the activity of the soil microbial population and an increase in the synthesis of plant growth hormones. Rezvani Moghadam et al. [2012] showed that the use of fenugreek as a living mulch in the cultivation of the *Cuminum cyminum* medicinal plant led to an increase in the yield and yield components of this plant. Rahimzadeh and Pirzad [2017] expressed that mycorrhizal mycelium plays a crucial role in the soil and leads to the absorption of water from the very fine pores of the soil. Because the mycorrhizal fungi probably absorb more water by changing the root morphology and lengthening the root system of the host plant and increasing the level of absorption through the fungi hyphae, and leads to the improvement of plant relationships and increases photosynthesis and growth, and as a result, plant yield.

Number of umbels per plant

The results of the analysis of variance (Tab. 2) showed that the treatment of plant mulch and the interaction effects of biological fertilizer and herbal mulch on the trait of the number of umbels per plant were significant ($p < 0.01$). The mean comparison results showed that the highest number of umbels per plant (23.22) was obtained using clover mulch residues and no biological fertilizer, and the lowest number of umbels per plant (10.48) was obtained at control treatment (Fig. 5). Based on the results of the comparison, it was determined that the use of living clover mulch and the interaction effects of plant mulch and fungi and bacteria had a positive effect on the number of umbels per plant. Because the use of herbal mulch through optimal processing and improvement of nutrients and helping to increase the effects of fungi and bacteria led to the improvement of yield components, including the number of umbels per plant. Hashemzadeh et al. [2014] announced that the number of sub-branches increased due to the application of mycorrhizal fertilizers, nitroxin, and *Pseudomonas* growth-promoting bacteria in *Dill*. Also, Rezaei Chianeh et al. [2015] revealed that the use of mycorrhizal fungi along with *Azotobacter* in ajowan led to the improvement of plant growth and allocation of more photosynthetic substances. It led to an increase in the number of umbels in the plant. Senajek et al. [2017] expressed that the application of mycorrhizal fungi was effective in increasing soil phosphorus and increasing the number of flowers at *Chrysanthemum*.

The number of umbellets per plant

The results of the analysis of variance showed that the treatment of plant mulch ($p < 0.01$) and the inte-

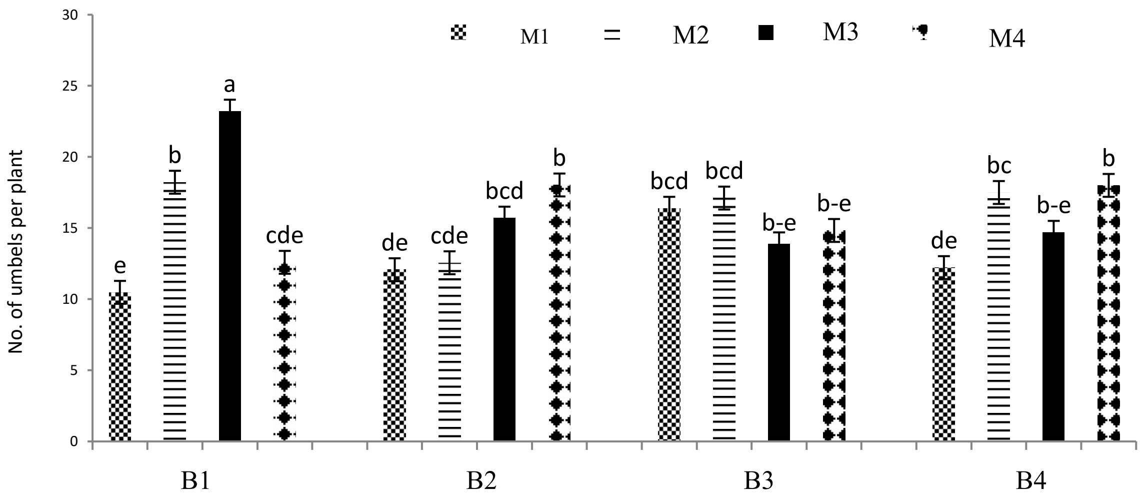

Fig. 5. Interaction of biological fertilizer and plant mulch on the number of umbels in anise. B1: control (no biological fertilizer), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

raction effects of biological fertilizers and plant mulch ($p < 0.05$) on the number of umbellets per plant were significant (Tab. 2). Based on the results of the mean comparison, it was found that the highest number of umbellets per plant (174.5) was obtained using living clover and without using biological fertilizer. Also, the lowest number of umbellets per plant (73.03) was obtained in the control treatment (no application of plant mulch and biological fertilizer) (Fig. 6). Based on the results, it was determined that the use of living mulch had a positive effect on the number of umbellets in the plant because the use of living mulch provided more nutrients to the *Anise* through the reduction of biomass and weed yield and increased the number of umbellets in the plant. Shabahang et al. [2013] showed that as a result of symbiosis with mycorrhizal fungi, the number of lateral branches increased in ajwain and fennel. Naseri et al. [2010] expressed that the use of growth -promoting bacteria in safflower led to an increase in the number of capitul in this plant. Shabahang et al. [2013] stated that as a result of symbiosis with mycorrhizal fungi, the number of lateral branches increased in ajwain and fennel. Kochchi et al. [2015] believed that as a result of plant inoculation with mycorrhizal fungi, the number of seeds in the umbel increased in ajwain and fennel.

Essential oil yield

According to the analysis of variance, the treatment of plant mulch and the interaction of biological fertilizers and plant mulch on essential oil yield were significant at $p < 0.01$ (Tab. 2). Mean comparison results showed that the highest amount of essential oil yield (18.15 kg/ha) was obtained at fungi and bacteria usage along with the application of living clover and inter-row clover cultivation treatment. Also, the lowest amount of essential oil yield (9.29 kg/ha) was obtained at fungi and bacteria usage and no use of plant mulch (Fig. 7). Khaosaad et al. [2006] reported that the application of mycorrhizal fungi had a significant increase in the amount of essential oil of marjoram. Moghadasan et al. [2016], in research on common marigolds, revealed that the use of mycorrhizal fungi led to an increase in dry yield, plant height, number of flowers, and essential oil yield. Also, Boyeri deh Sheikh et al. [2015], in an experiment on the catnip medicinal plant, reported that the use of some bio-fertilizers and phosphate-dissolving bacteria led to an increase in the fresh and dry weight of shoot parts of the plant, photosynthetic pigments, the rate of net photosynthesis and an increase in the amount of essential oil. Tarraf et al. [2017] showed that the application of mycorrhizal fungi on the sage medicinal plant had an effective role in increasing the amount of the plant's essential oil.

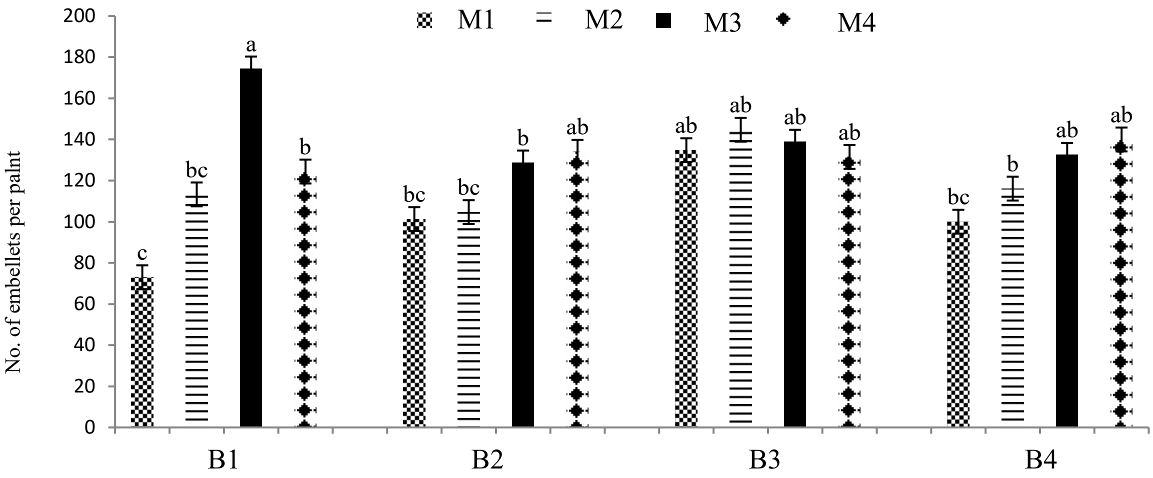

Fig. 6. Interaction of biological fertilizer and plant mulch on the number of umbellets in anise. B1: control (without use), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

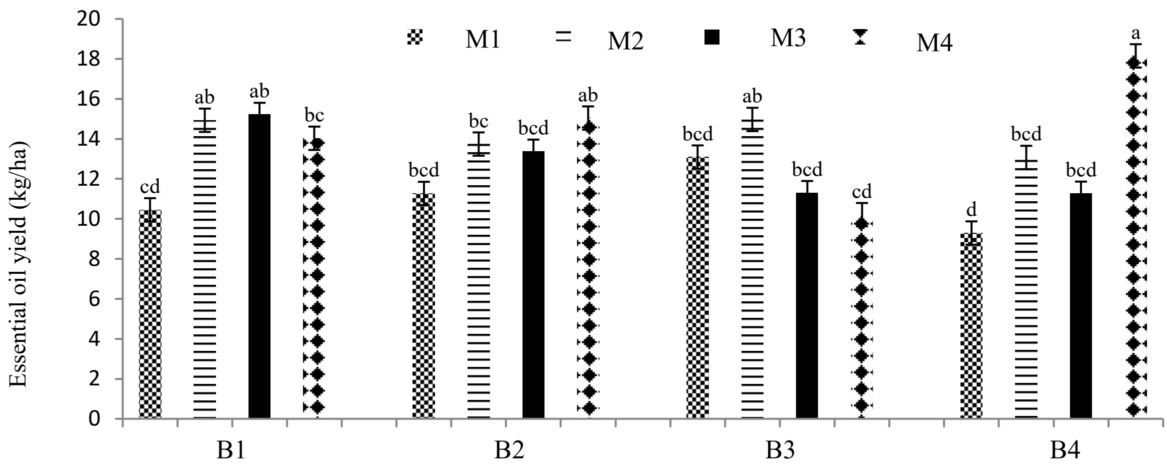

Fig. 7. Interaction of biological fertilizer and herbal mulch on the essential oil yield in anise. B1: control (without use), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

α-Pinene

The results of the analysis of variance showed that the treatment of plant mulch and the interaction effects of biological fertilizer and herbal mulch ($p < 0.05$) on the amount of α-pinene were significant (Tab. 2). Based on the mean comparison results, it was found that the highest amount of α-pinene was obtained (2.277) in the condition of fungi usage along with the application of living clover and inter-row clover cultivation. Also, the lowest amount of α-pinene was obtained (1.387) in the condition of fungi usage, and the lack of application of herbal mulch was obtained (Fig. 8).

Para-cymene

The results of the analysis of variance showed that the treatment of herbal mulch and the mutual effects of biological fertilizer and herbal mulch ($p < 0.01$) on the amount of para-cymene were significant (Tab. 2). Based

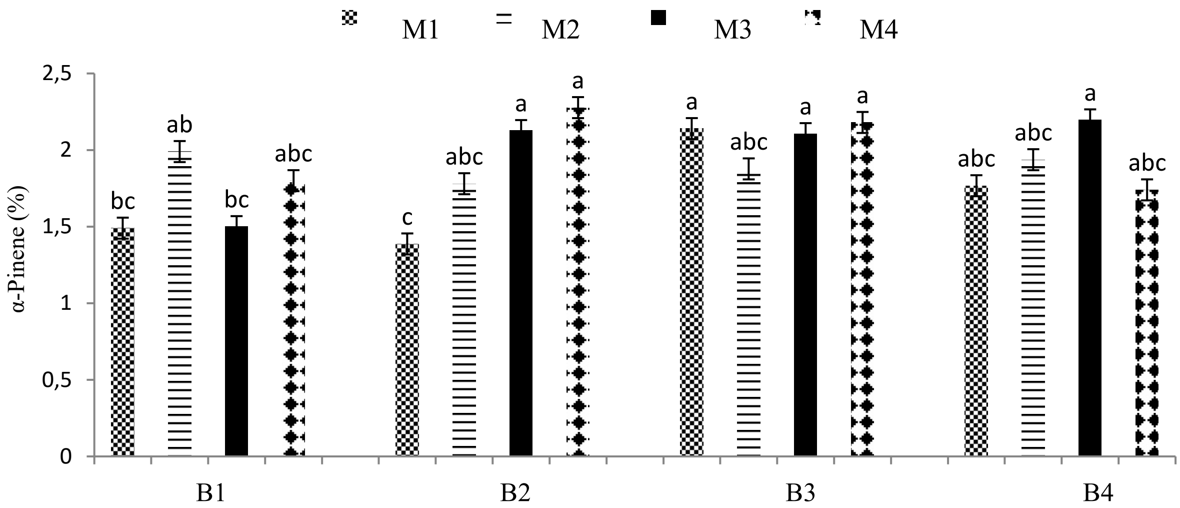

Fig. 8. IInteraction of biological fertilizer and herbal mulch on the α-pinene rate in anise. B1: control (without use), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

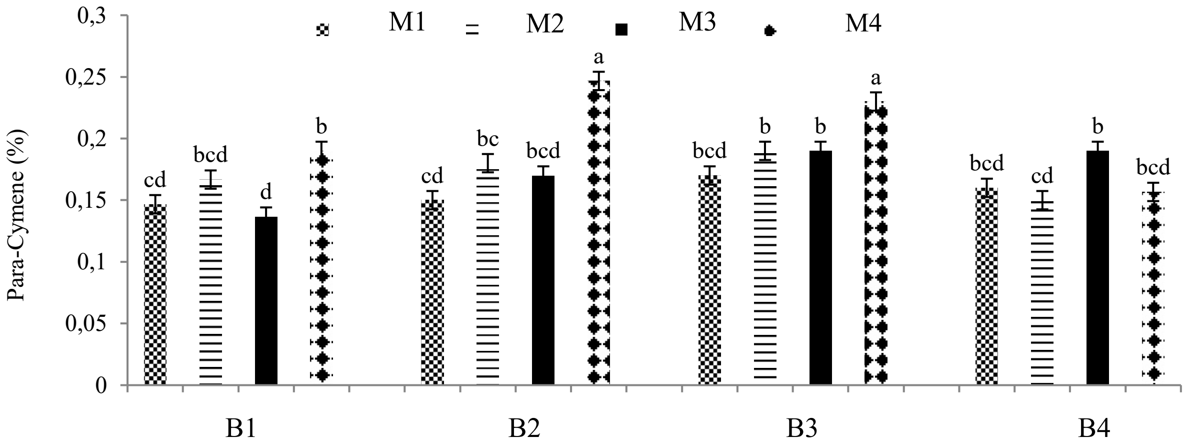

Fig. 9. Interaction of biological fertilizer and herbal mulch on the para-cymene rate in anise. B1: control (without use), B2: inoculation with *Glomus intraradices* fungus, B3: inoculation with growth-promoting bacteria *Pseudomonas putida*, B4: combination of bacteria and fungi and mulch (plant residues including M1: control, M2: wheat straw, M3: clover straw, and M4: living clover mulch)

on the results of the mean comparison, it was found that the highest amount of para-cymene was obtained (0.2300) in the conditions of using bacteria along with the use of living clover and inter-row clover cultivation. Also, the lowest amount of para-cymene was obtained (0.1367) in the condition of not using biological fertilizer and the use of clover as herbal mulch (Fig. 9).

Khalesvar et al. [2009] reported that due to the application of organic fertilizers, the highest amount of essential oils extracted from anise seeds included anethole and methyl chavicol. Ghorbanpour et al. [2013] showed that the use of *Pseudomonas* rhizospheric bacteria caused a significant increase in the dry weight of leaves, the dry weight of roots, and an increase in the amount of cis-thujone compound in the essential oil of sage plant. A sample of the GC/MS chromatogram for obtained essential oil under experimental treatments is presented in Figure 10.

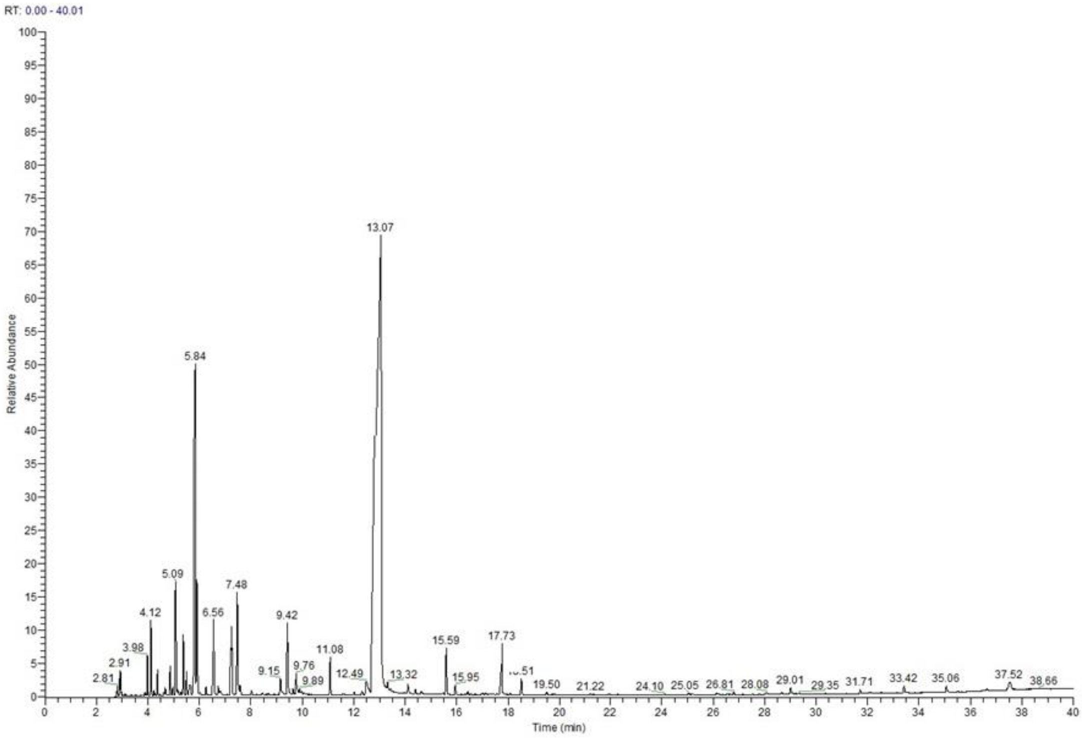

Fig. 10. The GC/MS chromatogram of obtained essential oil in Pimpinella anisum plants under experimental treatments

CONCLUSIONS

Based on the outcomes of the experiment, implementing sustainable agriculture practices by using biological fertilizers and herbal mulch has proven to have a positive impact on the quality and quantity of anise. It is recommended, in line with sustainable and biological agriculture principles, to use bio-compatible inputs like bacteria and fungi to stabilize nutrients and living or non-living herbal mulches to increase the yield of anise. The extraction process revealed that α-pinene and para-cymene essential oils were the most significant components in the essential oil.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

EF: conceptualization, experimental work, writing – original draft preparation, investigation, data curation, writing – original draft project, and administration. MMS: experimental work, writing, software, methodology, formal analysis, and administration. ZE: experimental work. MG: supervision, writing – review, and editing.

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