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Effect of inoculation of two Aztobacter and nitrogen fertilizer on of peppermint (*Mentha piperita*) essential oil

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Abstract. *Bidgoli RD. 2019. Effect of inoculation of two Aztobacter and nitrogen fertilizer on of peppermint (*Mentha piperita*) essential oil. Asian J Agric 3: 22-25.* This study was conducted to study changes of percentage and yield of essential oil of peppermint (*Mentha piperita*) as a split factorial in a randomized complete block design (RCBD) with three replications in 2016-2017. The experimental treatments were nitrogen at four levels 0, 50, 100 and 150 kg/ha as the main factor and two strains of PGPR **(***Azotobacter chroococcum* MZ11, MZ26) in two states of (use and non-use) was considered as subfactors. Results showed that use of these two *A. chroococcum* strains has led to an increase in the percentage and yield of peppermint essential oil more than all Nitrogen fertilizer treatments. Also, the highest peppermint essential oil percentage (0.73%) was obtained in the triple interaction of Nitrogen (100 kg/ha) *A. chroococcum* MZ11 and *A. chroococcum* MZ26. The highest essential oil yield (91.65 kg/ha) was observed under the influence of the triple interaction of Nitrogen (100 kg/ha), *A. chroococcum* MZ11 and *A. chroococcum* MZ26.

Keywords: *Aztobacter chroococcum,* essential oil, fertilizer, peppermint, plant yield

INTRODUCTION

This study was carried out to investigate the effect of biofertilizers on the essential oil of peppermint in 2018. Peppermint (*Mentha piperita* L.), which belongs to the family Lamiaceae, is a hybrid species obtained from the confluence of *Mentha spicata* and *Mentha aquatica* species (Foster 1996; Peirce 1999). This plant species is a perennial plant, with a height of 50 to 60 cm, a quadrangular stalk that is usually purple in color and smooth (Govedarica et al. 2003). The global peppermint essential oil production is about 8,000 tons/year. The main compounds of peppermint essential oil are menthol (29%), menthon (20-30%), and methyl acetate (1 to 3%). Extraction of peppermint essential oil from the aerial parts of the plant at the beginning of the flowering stage is usually by steam distillation method. About 30 to 70% of its essential oil is menthol and esters of menthol, and the rest are more than 40 other compounds (Afzal 2010). Other compounds found in the peppermint essential oil include flavonoids (12%), polymerized polyphenols (19%), carotene, tocopherol betaine and choline (Murrray 1995). Currently, peppermint is used for the treatment of irritable bowel syndrome (IBS), inflammatory bowel disease (Crohn and ulcerative colitis), gallbladder inflammation, biliary system defects and liver problems (Blumenthal 1998; Fleming 1998).

Peppermint is a long day plant (LDP), and its planting in long day conditions leads to increase in its production and yield. (Okan et al. 2004). Singh and Chatterjee (1989) stated that nitrogen increases the vegetative growth in the plant and leaf area index, number of sub-branches and flowering branches, for example in *Mentha sativa***,** with use of 150 kg/ha nitrogen obtained the highest of desired traits. Anvar et al. (2005) found that the application of 100 kg/ha nitrogen fertilizer increased the number of branches, number of leave pigments and the dry matter per unit and yield of essential oils. Masto et al. (2006) studied the effect of micronutrients and planting density on essential oil content and essential oil yield of the peppermint plant, and reported that the solution spraying of microelements produced the highest essential oil yield.

Valad Abadi et al. (2008) reported on the effects of different levels of drought stress and nitrogen on the *Calendula officinalis* L. and showed the effect of nitrogen on the percentage essential oil yield was significant at (1% level). Akbarinia et al. (2012) evaluated effect of fertilizer on the percentage and yield of *Trachyspermum ammi* essential oil. They stated with increasing nitrogen and phosphorus, grain yield increased up to 90 and 60 kg/ha, respectively. Chemical fertilizers had no effect on essential oil percentage and 60 kg/ha nitrogen, 40 kg/ha phosphorus and 25 tons of manure per hectare and 60 kg/ha nitrogen with 15 tons manure per hectare produced the highest grain and essential oil yield. Imam et al. (2014) reported that the use of nitrogen fertilizers increases the size, longevity, and freshness of leaves and shoots in the plant.

Also, Balyan and Sobti, (1990) reported that application rate of 80 kg/ha nitrogen resulted in the accumulation and highest increase of dry matter in basil. Indiscriminate use of chemical fertilizers, most especially, nitrogen, coupled with lack of organic fertilizers in recent years, has resulted in significant reductions in the amount of organic matter in Iranian agricultural soils (Malekooti 2018). Green fertilizers are plants that are modified to improve the physical, chemical, and biological properties of soils, and to supply essential nutrients for optimum plant growth in successive growing seasons (Cherr et al. 2016). In fact, increased plant growth by inoculation of Azotobacter, has been attributed to the hormones produced by these bacteria and root growth (Zaied et al. 2003). The use of green fertilizers in addition to nitrogen fertilizers causes the nutrients to be readily available for crop production during the growing season and achieves more performance compared to the sole application of chemical fertilizers (Aktar et al. 1993; Paramanic et al. 2014).

MATERIALS AND METHODS

Plant cultivation

This study was conducted in a split factorial randomized complete block design (RCBD) with 3 replications from fall 2016 to spring 2017. The nitrogen factor of urea source at four levels, 0, 50, 100, 150 kg/ha as the main factor and two types of Azotobacter (*Azotobacter chroococcum* MZ11, MZ26) in two states (use and non-use) as sub-factor were considered.

Rooted branches with lengths of 8 to 10 cm were cut from 2- to 3-year-old plants at 3 leaf stage and cultivated in 12 plots (4 treatments with 3 replications) of 25 m^2. The selected plants were same in terms of size and height and based on the main and subfactors. The nitrogen at four levels 0, 50, 100 and 150 kg/ha were the main factors and other treatments were divided into 4 groups $N_{0:}$ (MZ11, MZ26), $N_{50:}$ (MZ11, MZ26),), $N_{100:}$ (MZ11, MZ26)), $N_{150:}$ (MZ11, MZ26). The nitrogen treatments herein were applied as done previously to investigate the interaction of the treatments. Planting was done in early January and due to the high sensitivity of *Mentha piperita* to drought stress in the region, irrigation was carried out every 4 days. Also, weed control was done manually owing to the experimental design adopted and the medicinal properties of the peppermint plant, from the beginning of the vegetative stage to the end, weeding was carried out continuously. There was no incidence of pest invasion, hence chemical pest control was not carried out. This was achieved because of the scent emitted by the plant. Physical and chemical properties of soil are presented in Table 1.

Table 1. Physical and chemical properties of farm soil

Soil property	Value
Cu (ppm)	1.60
Mg (ppm)	24.80
Mn (ppm)	9.88
P (ppm)	12.50
K (ppm)	255.00
N (ppm)	0.08
$CaCO_3$ (ppm)	6.00
Texture	Loam
Sand (%)	38.00
Silt (%)	35.00
Clay (%)	27.00

After harvest operations, the yield of fresh weight was determined immediately and to determine the yield of dry weight, samples were dried at room temperature (25ºC) and in shade for 10 days and then weighed. In order to determine the percentage and yield of *Mentha piperita* essential oil obtained from the different treatments, the plant samples were dried and weighed at room temperature and in shade. The samples were subjected to laboratory analyses, wherein essential oil was obtained by the water distillation method. The Clevenger device was used to calculate the essential oil percentage dehydration by dry Sodium sulfate (Na_2SO_4). The essential oil yield was obtained from the multiplication of the essential oil percentage in the biological function divided to 100. Data were analyzed by MSTAT-C software, and mean comparison was carried with Duncan's method at 1% level of significance.

RESULTS AND DISCUSSION

Essential oil percentage

The results presented in Table 2 show that the individual effect of nitrogen on essential oil percentage of *Mentha piperita* was significant at 5% level and two *A. chroococcum* strains were significant at 1% level. According to Table 3 and the results of the independent effect of the treatments, the lowest and highest essential oil percentages were obtained from the control nitrogen (mean = 0.15%), and the *A. chroococcum* MZ26 treatment (mean = 0.44%), respectively. Omid Beigi (1995) and Anvar et al. (2005) reported that the application of 100 kg of nitrogen would increase the yield of the branches, the number of leaves, pigment of leaves and increase of dry matter yield per unit area and yield of essential oil. Also, Bist et al. (2000) found that with addition of nitrogen fertilizer to soil, percentage, and some components of *Anethum graveolens* essential oil increased.

These observations could be attributed to the soil's ability to retain more moisture because of improved soil structure upon the incorporation of the PGPR treatments. This ultimately resulted in the increased biological yield of the peppermint plant and its essential oil yield. On the other hand, the increase in essential oil from the application of different fertilizer treatments could be due to the readily availability of nutrients, such as nitrogen and phosphorus, for the formation of ATP and NADPH, which serve in the pathway to the formation of terpenoids and isoprenoids in the essential oils (Loomis and Corteau 1972).

The interactions of nitrogen fertilizer with incorporated *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage of essential oil were significant at 5% and 1% levels respectively, however, their triple effect was not significant (Table2). In the double interaction of Nitrogen and this two *A. chroococcum,* results showed the highest percentage of essential oil was obtained from 150 kg/ha nitrogen in combination with incorporated *A. chroococcum* MZ11 (0.62%), and the lowest essential oil percentage, obtained from the interaction of these two *A. chroococcum* (MZ11 and MZ26)as 0.16% (Table 4). In the essential oil yield, the highest and lowest values were obtained in these treatments too in a similar study, Valad Abadi et al. (2008) investigated the effects of different levels of nitrogen on *Calendula officinalis* and concluded that nitrogen increased the percentage and yield of its seed oil.

The interaction of *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage of essential oil was significant at 5% level and presented in Table 2. According to Table 3, *A. chroococcum* MZ26 increased the essential oil percentage more than *A. chroococcum* MZ26, but in terms of essential oil yield, MZ26 was more effective than MZ11. The triple interaction of nitrogen, and *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage of essential oil were significant ($p < 5\%$) (Table 2).

In the triple interaction of Nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 the highest percentage of essential oil of 0.48% was obtained from the treatment with 150 kg/ha nitrogen and the two *A. chroococcum* strains, and the lowest percentage with mean of 0.38% and the lowest from the 50 kg/ha nitrogen and the two *A. chroococcum* strains with a mean value of 0.38% (Table 5). Like the study by Akbarinia et al. (2012), fertilizer application resulted in an increase in essential oil content and essential oil yield in *Trachyspermum ammi*. They also stated that application of nitrogen and phosphorus up to 60 and 90 kg/ha, respectively resulted in increased grain yield size, longevity of leaves and branching, and the freshness of leaves in the plant.

Essential oil yield

The individual effect of nitrogen on essential oil yield of *Mentha piperita* was significant ($p < 5\%$) (Table 2). In this regard, the highest yield was obtained from the *A. chroococcum* strains (55.80 kg/ha), and the lowest was related from the control of nitrogen (25.23 kg/ha) (Table 3). According to the results, the increase in the use of nitrogen from zero to 150 kg/ha increased the essential oil percentage and essential oil yield (Table 4). These results are a clear indication of the role of nitrogen in increasing the vegetative growth in plants, hence, increase in total dry matter yield per unit area. A close analysis of the results revealed that the triple interaction of nitrogen, *A. chroococcum* MZ11, and *A. chroococcum* MZ26 had the most significant effect on the percentage and yield of essential oil, which implies that the combined effect of these treatments has an enormous effect compared to their individual and cross-linking effects.

The individual effects of *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on essential oil yield of *Mentha piperita* were significant at 1% level. (Table 2). The interactions of nitrogen fertilizer and *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the yield of essential oil were significant at 5% and 1%, respectively (Table 2). According to the results, it is obvious that the *A. chroococcum* MZ11 and *A. chroococcum* MZ26 complemented the role of the nitrogen fertilizer to effectively meet the plant requirement. Based on the results of Table 4, the combination of these two *A. chroococcum* strains with nitrogen fertilizer have a greater effect on the combination of these two *A. chroococcum* strains together. The combined effect of *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the yield of essential oil was significant at 5% (Table 2). In this regard the highest yield of essential oil obtained 91.65 kg/ha and the lowest yield, being 52.62 kg/ha (Table 5). The triple interaction of nitrogen and two *A. chroococcum strains* on the yield of essential oil were also significant at 1% (Table 2).

Table 2. Analysis of variance of Nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage and yield of essential oil of *Mentha piperita*

SOV	df	MS	
		Essential oil percentage	Essential oil yield
Replication	2	1.280^{ns}	6325648.333^{ns}
N	3	182.025^{*}	3325416.226^{*}
Error	6	20.335	358469.875
MZ11	1	2225.036^{**}	72136548.215^{**}
MZ11*MZ26	3	48.387^{*}	2569823.7012^{**}
MZ26	1	523.081^{**}	13269587.658^{**}
N*MZ11	3	11.685^{*}	2569875.325^{**}
N*MZ26	1	52.431^{**}	22564.548^{*}
N* MZ26*MZ11	3	83.152^{*}	203269.559^{*}
Error	24	25.325	1352648.562
CV (%)	-	13.23	8.2

Table 3. Comparison of the average effect of nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage and yield of essential oil of *Mentha piperita*

Treatment	Essential oil percentage	Essential oil yield (kg/ha)
Nitrogen (kg/ha)		
0	0.15d	20.23b
50	0.20c	27.53d
100	0.22b	30.45d
150	0.35a	45.60c
MZ11	0.43a	55.80b
MZ26	0.44a	49.50a

Table 4. Comparison of the average double interaction of nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the Percentage and yield of essential oil of *Mentha piperita*

Treatment	PGPR	Essential oil percentage	Essential oil yield (kg/ha)
Nitrogen (kg/ha)	MZ11		
0		0.25d	33.70c
50		0.30c	42.58c
100		0.39b	53.38b
150		0.62a	80.30a
Nitrogen (kg/ha)	MZ26		
0		0.22b	27.26d
50		0.28a	38.05ab
100		0.34a	44.33b
150		0.41a	55.90a
MZ11	MZ26	0.16c	21.38c

Table 5. Comparison of the average triple interaction of Nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage and yield of essential oil of *Mentha piperita*

Average		Treatment		
Essential oil yield (kg/ha)	**Essential oil percentage**	**MZ26**	**MZ11**	**Nitrogen (kg/ha)**
0.15i	20.23i	Non-use	Non-use	0
0.44gh	49.50gh	Use		
0.43ef	55.80ef	Non-use	Use	
0.16cd	21.38c	Use		
0.20i	27.53i	Non-use	Non-use	50
0.28h	38.05h	Use		
0.30fg	42.58fg	Non-use	Use	
52.62de	0.38de	Use		
0.22i	36.45i	Non-use	Non-use	100
0.34h	44.33gh	Use		
0.39cde	53.38cd	Non-use	Use	
65.09b	0.73b	Use		
0.35b	45.60b	Non-use	Non-use	150
0.41gh	55.90i	Use		
0.62a	80.30a	Non-use	Use	
91.65c	0.48c	Use		

Similarly, Banchio et al. (2009) reported that the application of biological fertilizers to basil, was very effective in increasing the biomass and yield of essential oils. Thus, the positive effects of biofertilizers such as incorporation of Aztobacter on improving the nutritional conditions in plants have been proven in this study.

According to the results obtained in this study, the application of different Aztobacter both singularly and in combination resulted in the increase in the yield of essential oil of peppermint. Thus, the incorporation of *A. chroococcum* MZ11 and *A. chroococcum* MZ26, especially by small-holder farmers will serve as a good alternative to application of chemical fertilizers. Generally, the use of crop techniques and environmental factors in the cultivation of crops to increase the amount of active ingredients is a very interesting and important subject matter, for example, the use of legumes as green fertilizers, nitrogen will be released gradually over a long period of time to enhance nitrogen absorption by plants during successive growing periods.

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