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Quantity and Quality of essential oil of basil (*Ocimum basilicum* L.) under biofertilizers application conditions

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ABSTRACT

The main objective of this study was to determine the effects of biofertilizers on quantity and quality of essential oil of basil essential oil content, geranial, caryophyllene, caryophyllene oxide and methyl chavicol in essential oil. The experiment was carried out as randomized complete blocks design with eight treatments and three replications at research field of Agriculture Company of Ran in Firouzkuh of iran in 2012 . The treatments were [1] *Azotobacter chroococcum* (A), [2] *Azospirillum lipoferum* (B), [3] *Bacillus circulans* (C), [4] A + B, [5] A + C, [6] B + C, [7] A + B + C and [8] control (without fertilizer application). The present results have shown that the highest essential oil content and the minimum caryophyllene oxide in essential oil were obtained after applying each three bioertilizers (A + B + C). The maximum geranial in essential oil and the minimum caryophyllene in essential oil were obtained by using two biofertilizers (A + C). Also, the highest methyl chavicol in essential oil was obtained after applying two biofertilizers (B + C).

Key Words Basil, Azotobacter, Azospirillum, Bacillus, Essential oil.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is one of the most important of vegetables, spice and medicinal plant. It is an annual and herbaceous plant, belonging to the Lamiaceae family. Its essential oils are synthesized and stored in glandular hairs and are used as flavorings in foods and beverages, as fragrances, as toiletry products such as mouth washes and dental creams, as fungicides, or insecticides in pharmaceutical and industrial products (Mondello et al. 2002; Khalid et al., 2006). The main constituents of basil essential oil has been reported as methyl chavicol, citral, linalool, geraniol and eugenol (Mondello et al. 2002; Sajjadi, 2006; Ziaei et al., 2012). Applying biofertilizers such as nitrogen fixing bacteria and phosphate solubilizing microorganisms has led to a decrease in the application of chemical fertilizers and has provided high quality agricultural products (Sharma, 2002). Free-living nitrogen fixing bacteria such as; *Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid,

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which could stimulate plant growth, absorption of nutrients, and photosynthesis (El Ghadban et al., 2006; Mahfouz and Sharaf Eldin, 2007). Phosphate solubilizing microorganisms such as; bacteria and fungi, are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Chen et al., 2006). Also, By using biofertilizers, quantity and quality of active substances of medicinal plants can be improved (Rashmi et al., 2008; Azzaz et al., 2009). Some studies have reported that nitrogen fixing bacteria such as *Azotobacter chroococcum* and *Azospirillum lipoferum* could cause increased quantity and quality of essential oil of some medicinal plants such as fennel (Abdou et al., 2004; Mahfouz and Sharaf Eldin, 2007; Azzaz et al., 2009; Moradi et al., 2011), lemon balm (Harshavardhan et al., 2007), turmeric (Velmurugan et al., 2008), hyssop (Koocheki et al., 2009), davana (Kumar et al., 2009) and dill (Darzi et al., 2012). Several other studies have reported that Phosphate solubilizing bacteria such as *Bacillus* sp and *Pseudomonas* sp could cause increased quantity and quality of essential oil of some medicinal plants such as lemon grass (Ratti et al.,2001), black cumin, borage (Shaalan, 2005a,b), basil (Rashmi et al., 2008), davana (Kumar et al., 2009), fennel (Abdou et al., 2004; Mahfouz and Sharaf Eldin, 2007; Darzi et al., 2009; Azzaz et al., 2009; Moradi et al., 2011) and Darzi et al, (2013) on anise. Therefore, the main objective of the present field experiment was to investigate the quantity and quality of essential oil of basil (*Ocimum basilicum*) under biofertilizers application conditions.

METHODS

Field Experiment

A field experiment, arranged in a randomized complete blocks design with three replications, was conducted in the Experimental field of the Agriculture Company of Ran, Firouzkuh, Iran during the growing season of 2012. The geographical location of the experimental station was 35º 45´ N and 52º 44´ E with the altitude of 1930 m. The eight treatments of experiment contain: [1] *Azotobacter chroococcum* (A), [2] *Azospirillum lipoferum* (B), [3] *Bacillus circulans* (C), [4] A + B, [5] A + C, [6] B + C, [7] A + B + C and [8] control (without fertilizer application). Inoculation was carried out by dipping the basil seeds in the cells suspension of 10^8 CFU/ml for 15 min. Several Soil samples (0–30 cm depth) were taken for the nutrient and trace element analysis prior to land preparation. Chemical and physical properties of the experimental soil is presented in Tables1. Each experimental plot was 3 m long and 2.4 m wide with the spacing of 10 cm between the plants and 40 cm between the rows. There was a space of one meter between the plots and 2 meters between replications. Basil seeds were directly sown by hand. There was no incidence of pest or disease on hyssop during the experiment. Weeding was done manually and the plots were irrigated weekly (as trickle irrigation system). All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. fifteen plants were randomly selected from each plot and the observations were recorded. In this study, quantity and quality of basil essential oil consisted of essential oil content and geranial, caryophyllene, caryophyllene oxide and methyl chavicol in essential oil were evaluated.

Extraction of Essential oil

In order to determine the essential oil content (%), a sample of 100 g of basil dried herb from the each plot were mixed with 500 ml distilled water and then were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Sajjadi, 2006; Darzi et al., 2012).

Identification of Essential oil Components

For identifying the essential oil components, essential oil fraction was collected and subjected to GC and GC/MS (gas chromatography and gas chromatography-mass spectrometry) analysis. For GC analysis

from a Younglin Acm600, equipped with HP-5 MS capillary column (30m X 0.25 µm) and for GC/MS analysis from an Agilent 6890 GC and Agilent 5973 MS, equipped with HP-5 MS capillary column (30m X 0.25 µm) was used.
Authentic reference substance of geranial, caryophyllene, caryophyllene oxide and methyl chavicol were used to establish the retention times (Sephidkon, 2002; Sajjadi, 2006).

Table 1. Some Traits of Physical and Chemical of soil in experiment site

Cu (mg/kg)	**Fe (mg/kg)**	**K (mg/kg)**	**P (mg/kg)**	**N (%)**	**O.C (%)**	**EC (ds/m)**	**pH**	**Texture**
1.2	8	720	48	0.127	1.86	1.55	7.6	Clay-Loamy

Statistical Analysis
All the data were subjected to statistical analysis (one-way ANOVA) using SAS software (SAS Institute, version 8, 2001). Differences between the treatments were performed by Duncan's Multiple Range Test (DMRT) at 5% confidence interval. Transformations were applied to the data to assure that the residuals had normal distribution (Zar, 1996).

RESULTS AND DISCUSSION

Essential oil content
The present results have indicated that essential oil content was significantly affected by the application of different treatments of biofertilizers (Table 2). The maximum essential oil content (0.226%) was obtained by applying each three biofertilizers (azotobacter + azospirillum + bacillus) and the minimum essential oil content (0.103%) was indicated by control treatment (Figure 1). Nitrogen fixing bacteria and phosphate solubilizing bacteria application through improvement of phosphorus and nitrogen uptake and eventually increase of biomass amount (Shaalan, 2005a,b; Mahfouz and Sharaf Eldin, 2007), has a positive effect on essential oil content. Our findings are in accordance with the observations of Ratti et al. (2001) on *Cymbopogon martini*, Shaalan (2005a,b) on *Nigella sativa* and *Borago officinalis*, Harshavardhan et al. (2007) on *Mellisa officinalis*, Mahfouz and Sharaf Eldin (2007), Azzaz et al. (2009) and Darzi et al. (2009) on *Foeniculum vulgare*, Mirshekari et al. (2010) on *Cyminum cuminum* and Darzi et al. (2013) on *Pimpinella anisum*.

Geranial in essential oil
The results presented in Table 2 have demonstrated that geranial in essential oil was influenced by the application of different treatments of biofertilizers, significantly. Among various treatments, two treatments of the application of azotobacter with bacillus (51.09%) and azotobacter plus azosprillum plus bacillus (49.86%) have indicated maximum increase in geranial in essential (Figure 2). Integrated application of nitrogen fixing bacteria and phosphate solubilizing bacteria, through the improvement of mineral elements absorption (Rashmi et al., 2008; Azzaz et al., 2009), caused optimal biomass production which leads to improvement of the essential oil quality. These findings are in accordance with the observations of Ratti et al. (2001) on *Cymbopogon martini*, Shaalan (2005b) on *Nigella sativa*, Harshavardhan et al. (2007) on *Mellisa officinalis*, Mahfouz and Sharaf Eldin (2007), Moradi et al. (2011)

on *Foeniculum vulgare*, Fallahi et al. (2010) on *Matricaria recutita*, Rashmi et al. (2008) on *Ocimum gratissimum* and Darzi et al. (2013) on *Pimpinella anisum*.

Caryophylene in essential oil

The results presented in Table 2 have revealed that studied various treatments had significant effects on the caryophyllene in essential oil. The maximum caryophyllene in essential oil (8.65%) was obtained by using nitrogen fixing bacteria (azotobacter) and the minimum caryophyllene percent (5.46%) was indicated by applying azotobacter plus bacillus (Figure 3) . The increase of the geranial in essential oil in treatment of integrated application of azotobacter with bacillus, has a negative effect on other constituents of essential oil and subsequently have decreased caryophyllene percent in this treatment. The present result is in agreement with previous studies on medicinal plants (Ratti et al., 2001; Abdou et al., 2004; Shaalan, 2005a,b; Harshavardhan et al., 2007; Mahfouz and Sharaf Eldin, 2007; Velmurugan et al., 2008; Darzi et al., 2009; Moradi et al., 2011; Rashmi et al., 2008; Darzi et al., 2013).

Caryophyllene oxide in essential oil

The results presented in Table 2 have showed that biofertilizers had significant effects on the caryophyllene oxide in essential oil. The minimum caryophyllene oxide in essential oil (0.827%) was obtained by integrated applying of biofertilizers (azotobacter + azospirillum + bacillus) and the highest caryophyllene oxide percent (2.72%) was indicated by applying azotobacter (Figure 4). The decrease of the caryophyllene oxide in essential oil in treatment of application of each three biofertilizers, was be caused of increase in some components of essential oil such as geranial percent in this treatment. The present result is in agreement with the report of Abdou et al. (2004), Darzi et al. (2009), Azzaz et al. (2009) and Moradi et al. (2011) on *Foeniculum vulgare*, Harshavardhan et al. (2007) on *Mellisa oficinalis*, Velmurugan et al. (2008) on *Curcuma longa* and Darzi et al. (2013) on *Pimpinella anisum*.

Methyl chavicol in essential oil

The present results have indicated that methyl chavicol in essential oil was significantly affected by the application of different treatments of biofertilizers (Table 2). The maximum methyl chavicol in essential oil (1.25%) was obtained by using integration of two bacteria of *Azospirillum lipoferum* and *Bacillus circulans* (Figure 5). Biostimulants application such as nitrogen biofertilizer and phosphatic biofertilizer through the improvement of biological activities of soil and nutrient elements absorption, caused more growth and biomass production which leads to improvement of the essential oil quality (Shaalan, 2005b; Mahfouz and Sharaf Eldin, 2007; Darzi et al., 2012). These findings are in accordance with the observations of Harshavardhan et al. (2007) on *Mellisa oficinalis*, Rashmi et al. (2008) on *Ocimum gratissimum*, Kumar et al. (2009) on *Artemisia pallens*, Moradi et al. (2011) on *Foeniculum vulgare*, and Darzi et al. (2013) on *Pimpinella anisum*.

Table 2. Analysis of variance of studied traits

S. O. V	df	MS				
		Essential oil content	Geranial in essential oil	Caryophyllene in essential oil	Caryophyllene oxide in essential oil	Methyl chavicol in essential oil
Replication	2	0.000279^{ns}	0.500000^{ns}	1.58820^{ns}	0.002112^{ns}	0.000472^{ns}
Treatments	7	0.004740**	616.781**	3.18955**	1.35060**	0.416370**
Error	14	0.001131	3.64285	0.700390	0.031083	0.003022

* and **: Significant at the 5 and 1% levels of probability, respectively.
ns: Non-significant.

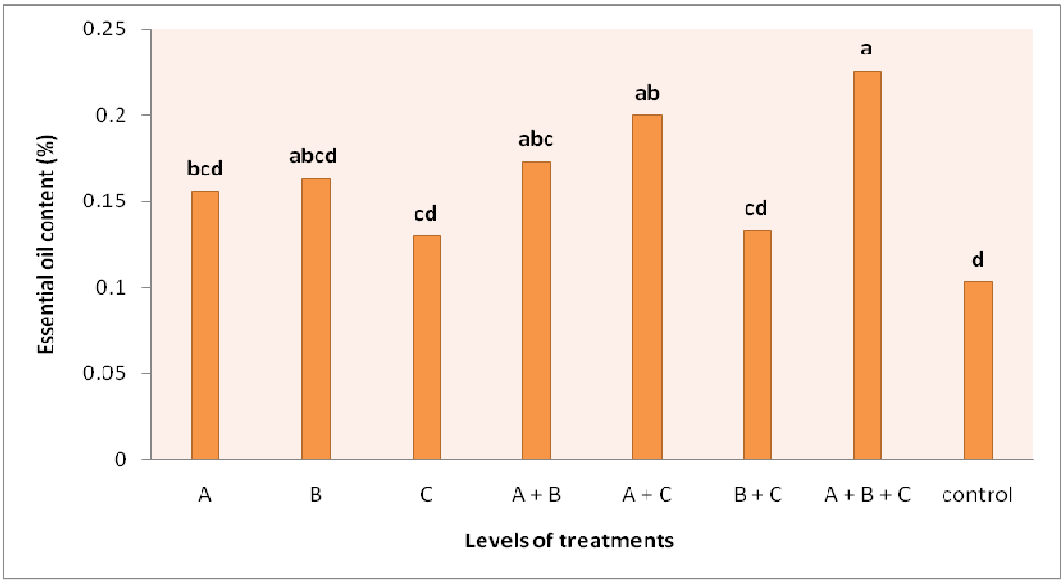

Figure 1. Mean comparison for essential oil content in different levels of biofertilizers treatments

A, B, C, A + B, A + C, B + C, A + B + C and control represent azotobacter, azospirillum, bacillus, azotobacter + azospirillum, azotobacter + bacillus, azospirillum + bacillus, azotobacter + azospirillum + bacillus and without fertilizer application, respectively.

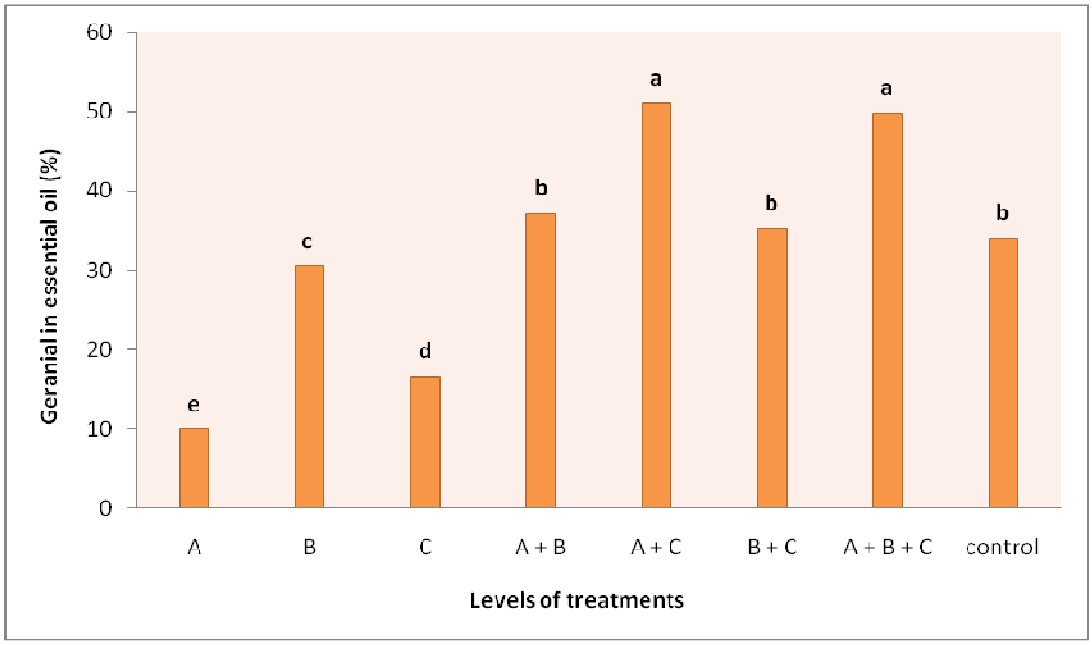

Figure 2. Mean comparison for geranial in essential oil in different levels of biofertilizers treatments

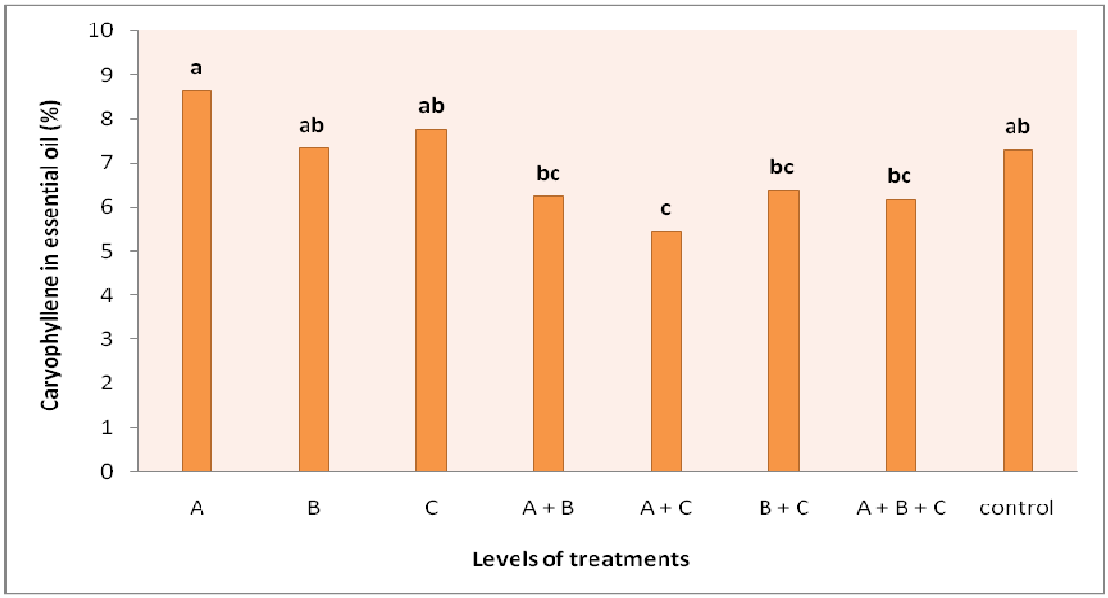

Figure 3. Mean comparison for caryophyllene in essential oil in different levels of biofertilizers treatments

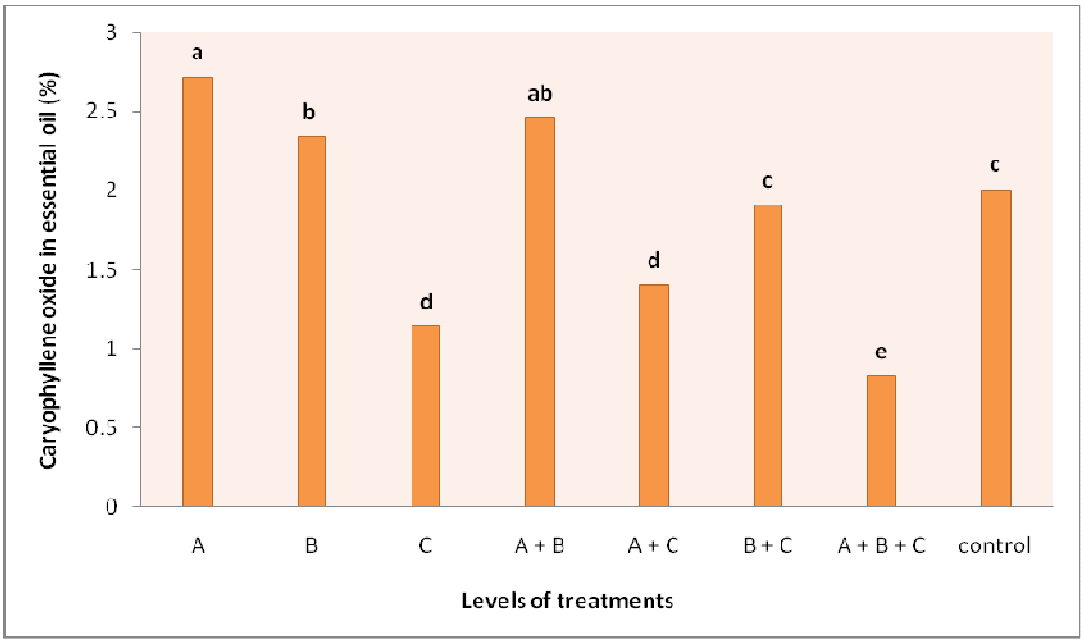

Figure 4. Mean comparison for caryophyllene oxide in essential oil in different levels of biofertilizers treatments

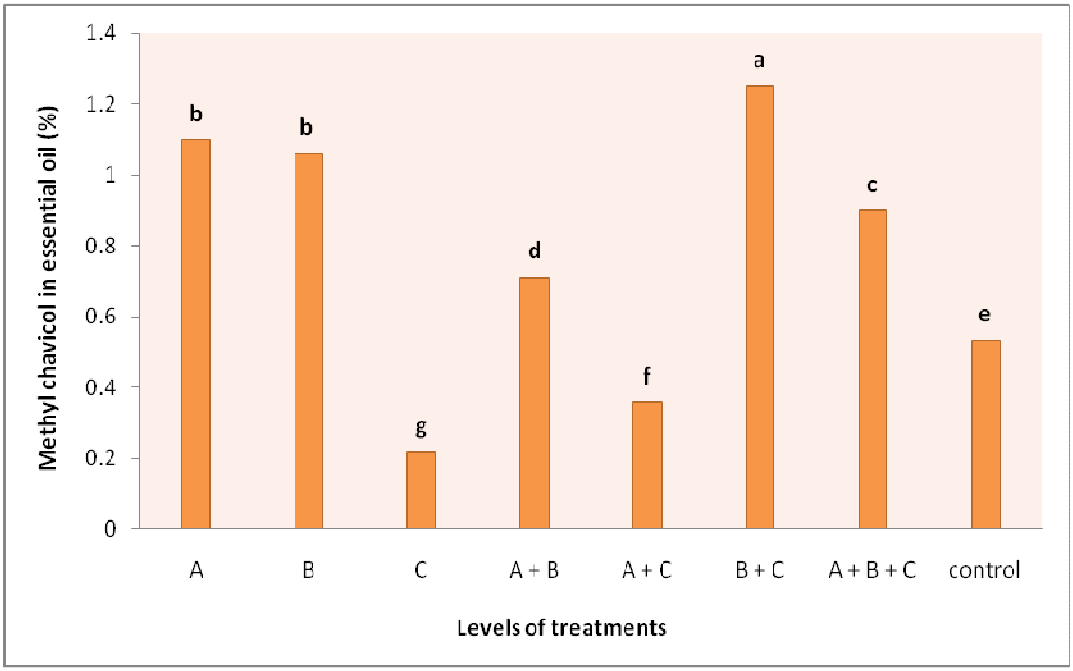

Figure 5. Mean comparison for methyl chavicol in essential oil in different levels of biofertilizers treatments

CONCLUSION

Conclusively, Integrated application of biofertilizers positively influenced on quantity and quality of basil essential oil, as the highest essential oil content and its quality were obtained by using each three biofertilizers (azotobacter + azospirillum + bacillus).

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