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Full Length Research Paper

Effects of the application of vermicompost and nitrogen fixing bacteria on quantity and quality of the essential oil in dill (*Anethum graveolens*)

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The main objective of this study was to determine the effects of vermicompost and nitrogen fixing bacteria on the quantity and quality of essential oil in dill essential oil content in seed, essential oil yield and carvone content, limonene content and dillapiole content in essential oil. The experiment was carried out at the Hamand Research Station of Damavand in 2011. Vermicompost (0, 4, 8 and 12 ton/ha) and nitrogen fixing bacteria, mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* (non-inoculated, inoculated seeds and inoculated seeds + spray on the plant base at stem elongation stage) were used as the effecting parameters. The present results have shown that the highest essential oil content in seed and carvone content in essential oil and the minimum dillapiole content in essential oil were obtained after applying 4 ton/ha vermicompost. The maximum essential oil yield was obtained after applying 8 ton/ha vermicompost. Nitrogen fixing bacteria also showed significant effects on essential oil content, essential oil yield and carvone content in essential oil. The maximum essential oil content and carvone content were obtained by using the nitrogen fixing bacteria once. The highest essential oil yield was obtained by using the nitrogen fixing bacteria twice.

Key words: Dill, vermicompost, Azotobacter, Azospirillum, essential oil.

INTRODUCTION

The intensive use of chemical fertilizers has side effects in polluting underground water, destroying microorganisms and reducing soil fertility. At present, using organic manures and biofertilizers, such as vermicompost and nitrogen fixing bacteria have led to a decrease in the application of chemical fertilizers and have provided high quality products free of harmful agrochemicals for human safety (Mahfouz and Sharaf Eldin, 2007; Malik et al., 2009). Vermicompost is the product of the degradation of organic matter through interactions between earthworms and microorganisms. Vermicompost contains most nutrients in plant available forms, such as nitrates, phosphates and exchangeable calcium and soluble potassium (Atiyeh et al., 2002). Vermicompost has large particulate surface area that provides many microsites for the microbial activity and strong retention of nutrients. It is rich in microbial population and diversity, particularly, fungi, bacteria and actinomycetes. It contains plant growth regulators and other growth-influencing materials produced by microorganisms (Edwards, 1998; Atiyeh et al., 2002; Arancon et al., 2005). 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, which could stimulate plant growth, absorption of nutrients and photosynthesis (El Ghadban et al., 2006; Mahfouz and Sharaf Eldin, 2007). Production of medicinal plants is mainly under the circumstances of sustainable agricultural system. In this system, management of environmental parameters is very critical. By using correct nutritional sources through organic manures and biofertilizers, quantity and quality of active substances, such as, essential oil in medicinal plants can be

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maximized. *Anethum graveolens*, commonly known as dill, is an herbaceous annual plant, which is native to mediterranean countries and Southeastern Europe. Dill seed and leaves are used as flavouring in sauces, vinegars, pastries and soups. The dill seeds have essential oil as an active substance, while carvone and limonene are the most important constituent of dill, which is used in pharmaceutical industry as a diuretic, stimulant and a carminative (Bailer et al., 2001; Singh et al., 2005; Callan et al., 2007).

Some studies have reported that vermicompost can increase the quantity and quality of essential oil in a few medicinal plants, such as, basil (Singh and Ramesh, 2002; Anwar et al., 2005; Geetha et al., 2009), coriander (Singh et al., 2009), fennel (Darzi et al., 2009; Moradi et al., 2011), chamomile (Haj Seyed Hadi et al., 2011) and cumin (Saeid Nejad and Rezvani Moghaddam, 2011).

Several other studies have reported that nitrogen fixing bacteria, such as *A. chroococcum* and *A. lipoferum* could cause increased quantity and quality of essential oil of some medicinal plants, such as celery (Migahed et al., 2004), 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), cumin (Saeid Nejad and Rezvani Moghaddam, 2010) and black cumin (Valadabadi and Farahani, 2011).

Therefore, the main objective of the present field experiment was to investigate the effects of vermicompost and nitrogen fixing bacteria on quantity and quality of the essential oil in dill (*A. graveolens*).

MATERIALS AND METHODS

Field experiment

A 4 × 3 factorial experiment, arranged in a randomized complete blocks designed with three replications, was conducted in the Experimental Station of the Research Institute of Forest and Rangeland, Damavand, Iran during the growing season of 2011. The geographical location of the experimental station was 35° 39´ N and 52° 5´ E with the altitude of 1800 m. The treatments consisted of different concentrations of vermicompost (0, 4, 8 and 12 ton/ha) and nitrogen fixing bacteria, with different mixture inoculation conditions of *A. chroococcum* and *A. lipoferum* (non-inoculated, seed inoculated and seed inoculated + spraying on the plant base at stem elongation stage). Inoculation was carried out by dipping the dill seeds in the cells suspension of 10^8 CFU/ml for 15 min. The vermicompost was prepared from cow dung by employing epigeic species of *Eisenia foetida*. The required quantities of vermicompost were applied and incorporated to the top 5 cm layer of soil in the experimental beds before the plantation of dill seeds. Several soil samples (0 to 30 cm depth) were taken for the nutrient and trace element analysis prior to land preparation. Chemical and physical properties of the experimental soil and vermicompost are presented in Tables 1 and 2. Nitrogen (20 kg/ha) was applied to the plots, based on the soil and vermicompost analysis, in the stem elongation stage.

Each experimental plot was 3 m long and 2 m wide with the spacing of 10 cm between the plants and 40 cm between the rows. There was a space of 1 m between the plots and 2 m between replications. Dill seeds were directly sown by hand into the field at a rate of 12 kg ha^{-1} to a depth of 2 cm. There was no incidence of pest or disease on dill during the experiment. Weeding was done manually and the plots were irrigated weekly. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation.

Twenty plants were randomly selected from each plot and the observations were recorded. In this study, the quantitative and qualitative traits of essential oil in dill essential oil content in seed, essential oil yield and carvone content, limonene content and dillapiole content in essential oil were evaluated.

Essential oil extraction and analysis

In order to determine the essential oil content (%), a sample of 100 g of dill seeds from the each treatment were crushed in electric grinder and were mixed with 500 ml distilled water and then were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The essential oil content was measured after dehydrating water by anhydrous sodium sulfate (Sephidkon, 2002; Kapoor et al., 2004). Essential oil yield also was calculated by using essential oil content. For identifying the essential oil components, essential oil fraction was collected and subjected to gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analysis. For GC analysis from a Younglin Acm600, equipped with HP-5 MS capillary column (30 m × 0.25 µm) and for GC/MS analysis from an Agilent 6890 GC and Agilent 5973 MS, equipped with HP-5 MS capillary column (30 m × 0.25 µm) was used.

Authentic reference substance of carvone, limonene and dillapiole were used to establish the retention times (Sephidkon, 2002).

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 ensure that the residuals had normal distribution (Zar, 1996).

RESULTS

Essential oil content in seed

The present results have indicated that essential oil content in seed was significantly affected by the application of vermicompost (Table 3). The most significant essential oil content (2.21%) was obtained by applying 4 ton vermicompost per hectare. Nitrogen fixing bacteria, had also, a significant effect on essential oil content (Table 3), as higher essential oil content (2.19%) was recorded by using nitrogen fixing bacteria once (inoculated seeds).

Essential oil yield

The results presented in Table 3 have demonstrated that essential oil yield was influenced by the application of

Table 1. Physical and chemical properties of soil on the experimental site.

Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	K (mg/kg)	P (mg/kg)	N (mg/kg)	O.C (%)	EC (ds/m)	pH	Texture
0.46	1.2	8.8	7.4	-	-	550	40	9.0	0.8	0.70	7.1	Loamy-clay

*Available form of nutrients was measured.

Table 2. Chemical characteristics of vermicompost.

K (%)	P (%)	N (%)	O.C (%)	O.M (%)	EC(ds/m)	pH
3.9	0.67	11.3	26.1	45	1.8	8.5

Table 3. Mean comparison of the quantitative and qualitative traits of essential oil in dill at various levels of vermicompost and nitrogen fixing bacteria**.**

Treatment	Essential oil content in seed (%)	Essential oil yield (kg/ha)	Carvon content in essential oil (%)	Limonene content in essential oil (%)	Dillapiole content in essential oil (%)
Vermicompost (ton/ha)					
v1	2.00^{b}	26.1^{c}	50.7^{c}	37.9^{a}	2.31^{a}
v2	2.21^{a}	43.0^{b}	55.6^{a}	36.0^{a}	1.81^{b}
v3	2.20^{a}	48.6^{a}	54.6^{a}	36.6^{a}	1.89^{b}
v4	2.14^{a}	46.1^{ab}	52.3^{b}	37.1^{a}	2.58^{a}
Nitrogen fixing bacteria					
n1	2.04^{b}	35.5^{c}	52.6^{b}	36.9^{a}	2.16^{a}
n2	2.19^{a}	41.4^{b}	53.8^{a}	36.3^{a}	2.15^{a}
n3	2.17^{a}	45.8^{a}	53.6^{a}	37.5^{a}	2.12^{a}

Means, in each column for each factor followed by at least on letter in common, are not significantly different at 5% probability level using Duncans' Multiple Range Test. v1, v2, v3 and v4 represent 0, 4, 8 and 12 ton/ha vermicompost, respectively. n1, n2 and n3 represent non-inoculated, inoculated seeds and inoculated seeds + spraying on the plant base at stem elongation stage, respectively.

vermicompost, significantly (Table 3). Among various treatments, the application of 8 ton vermicompost per hectare has indicated maximum increase in essential oil yield (48.6 kg/ha). Nitrogen fixing bacteria showed significant effect on essential oil yield (Table 3), as the highest essential oil yield (45.8 kg/ha) was obtained in the third treatment level of biofertilizer (a two-times application of *Azotobacter* + *Azospirillum*).

The present results show that the interaction of vermicompost and nitrogen fixing bacteria was significant (Figure 1). The highest essential oil yield (60.9 kg/ha) was obtained after the integrated application of 8 ton/ha vermicompost and twice using nitrogen fixing bacteria. The interaction of vermicompost and nitrogen fixing bacteria, on the essential oil yield, revealed that the application of 4 and 8 ton/ha vermicompost successively in the level of twice application of nitrogen fixing bacteria, resulted in a significant increase in essential oil yield.

Carvone content in essential oil

The results indicated that carvone content in essential oil was significantly affected by the application of vermicompost (Table 3). The highest carvone content was obtained with applying 4 ton/ha vermicompost (55.6%). Nitrogen fixing bacteria showed significant effect on carvone content in essential oil (Table 3), as the highest carvone content (53.8%) was obtained in the second treatment level of biofertilizer (once application of nitrogen fixing bacteria).

The present results show that the interaction of vermicompost and nitrogen fixing bacteria was significant (Figure 2). The highest carvone content (56.0%) was obtained after the integrated application of 4 ton/ha vermicompost and the use of nitrogen fixing bacteria once. The interaction of vermicompost and nitrogen fixing bacteria, on the carvone content, revealed that the

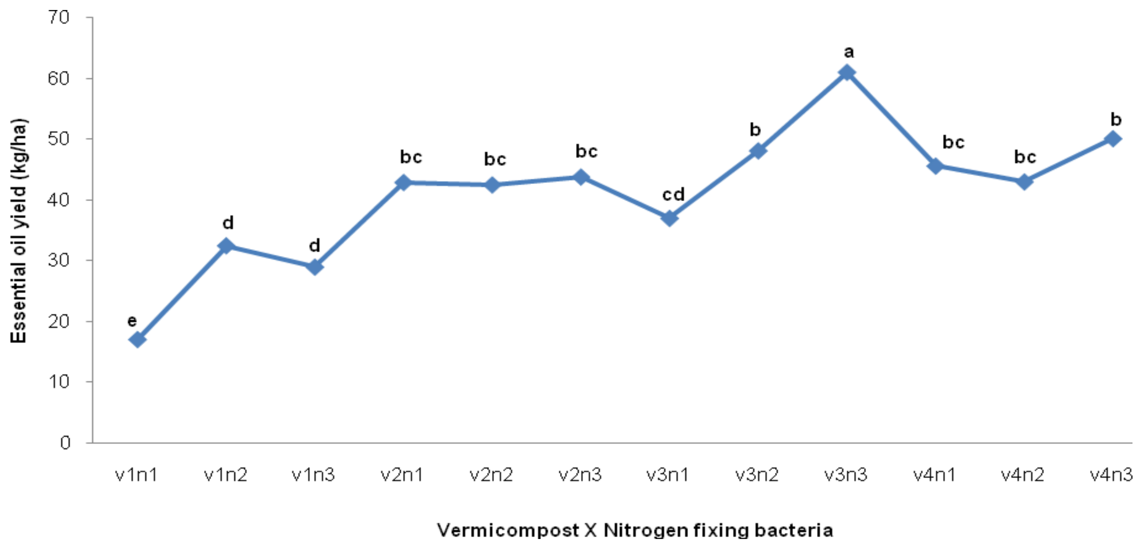

Figure 1. Mean comparison for essential oil yield after the intraction of different factors.

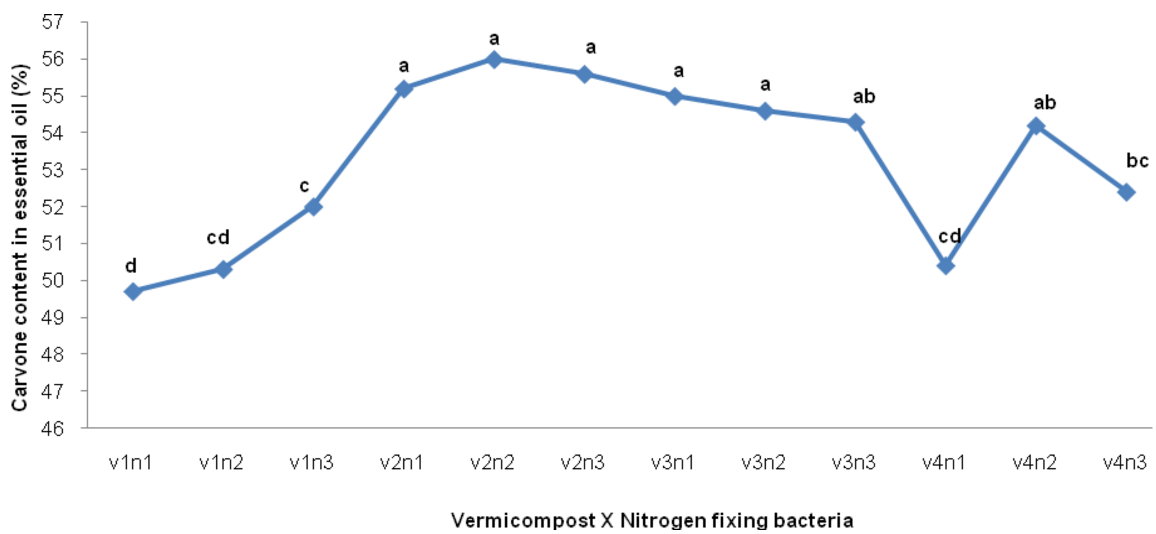

Figure 2. Mean comparison for carvone content in essential oil after the intraction of different factors.

application of 4 ton/ha vermicompost in the level of one time using of nitrogen fixing bacteria, which resulted in a significant increase in carvone content.

Limonene content in essential oil

The results indicated that limonene content in essential oil was not affected by vermicompost and nitrogen fixing bacteria (Table 3).

Dillapiole content in essential oil

The results presented in Table 3 have revealed that various levels of vermicompost had significant effects on the dillapiole content in essential oil. The minimum dillapiole content (1.81%) was obtained by using 4 ton/ha vermicompost. Nitrogen fixing bacteria did not show significant effect on dillapiole content (Table 3).

The present results show that the interaction of vermicompost and nitrogen fixing bacteria was significant (Figure 3). The maximum dillapiole content (3.54%) was obtained after the integrated application of 12 ton/ha vermicompost and twice application of nitrogen fixing bacteria. The interaction of vermicompost and nitrogen fixing bacteria, on the dillapiole content, revealed that the application of 4 and 8 ton/ha vermicompost, successively in the level of twice application of nitrogen fixing

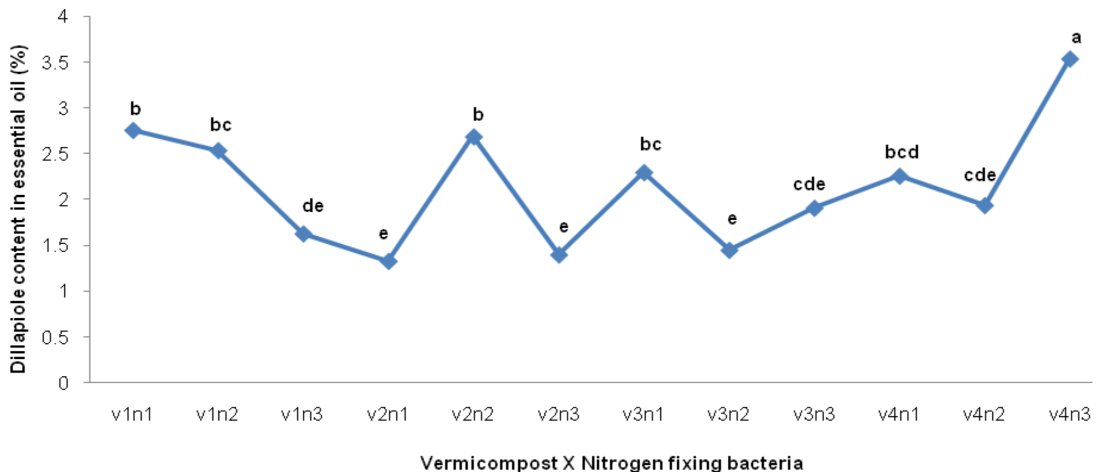

Figure 3. Mean comparison for dillapiole content in essential oil after the intraction of different factors.

bacteria, which resulted in a significant decrease in dillapiole content.

DISCUSSION

According to the present analysis, vermicompost application through increase of the mineral uptake, such as, nitrogen and phosphorus (Arancon et al., 2006; Zaller, 2007), has a positive effect on proper biomass production, and subsequently, the enhanced essential oil content in seed. Improved essential oil content of medicinal plants have previously been reported in the presence of optimal amounts of vermicompost (Anwar et al., 2005; Darzi et al., 2009; Geetha et al., 2009; Singh et al., 2009; Haj Seyed Hadi et al., 2011). Nitrogen fixing bacteria also have increased essential oil content by enhancing the nitrogen absorption (Migahed et al., 2004). The present result were derived from the improvement of nitrogen fixing bacteria' activities in soil at the second and the third treatments levels (inoculated seed and inoculated seed + spraying on plant base at stem elongation stage), which are in agreement with the previous studies carried out on the fennel and turmeric (Mahfouz and Sharaf Eldin, 2007; Velmurugan et al., 2008; Azzaz et al., 2009).

Increased essential oil yield in vermicompost treatments can be owing to the improvement of yield components, such as, essential oil content and seed yield. Our findings are in accordance with the observations of Singh and Ramesh (2002), Pandey (2005), Anwar et al. (2005), Saeid Nejad and Rezvani Moghaddam (2011) and Moradi et al. (2011). Nitrogen fixing bacteria, promoted essential oil yield through the enhancement of yield attributes. These result are in agreement with the investigation of Abdou et al. (2004) and Mahfouz and Sharaf Eldin (2007) on *Foeniculum vulgare*, Swaminathan et al. (2008) and Kumar et al. (2009) on *Artemisia pallens*, Koocheki et al. (2009) on *Hyssopus officinalis*, Saeid Nejad and Rezvani Moghaddam (2010) on *Cuminum cyminum* and Valadabadi and Farahani (2011) on *Nigella sativa*. These results likely show that the positive and synergistic effect of interaction between two factors is highly dependent on the effect of organic matter contained in vermicompost, on the activity of nitrogen fixing bacteria. Many reports have shown that the interaction between organic manures and biofertilizers can be beneficial for essential oil yield (Koocheki et al., 2009; Moradi et al., 2011; Valadabadi and Farahani, 2011).

Vermicompost has significantly influenced the carvone content in essential oil. On the other hand, vermicompost application through the improvement of biological activities of soil and mineral element absorption (Zaller, 2007), caused more biomass production and flowering promotion and subsequently seed ripening which leads to improvement of the essential oil quality (carvone content). These findings are in accordance with the observations of Anwar et al. (2005) on *Ocimum basilicum* and Darzi et al. (2009) and Moradi et al. (2011) on *F. vulgare*. The present results suggest that the influence of nitrogen fixing bacteria on the carvone content in essential oil was due to increased nitrogen uptake (Kalyanasundaram et al., 2008). The finding of this investigation is in agreement with the reports of Harshavardhan et al. (2007) on *Mellisa officinalis*, Mahfouz and Sharaf Eldin (2007) and Moradi et al. (2011) on *F. vulgare* and Velmurugan et al. (2008) on *Curcuma longa*. Many studies have reported that the interaction between organic manures and biofertilizers caused an increase in essential oil quality (Harshavardhan et al., 2007; Padmapriya and Chezhyian, 2009).

Applying proper amount of vermicompost, through increase of the carvone content in essential oil, has a negative effect on other components of essential oil, such as, dillapiole content and subsequently have decreased dillapiole content in essential oil. The present result is in agreement with the report of Anwar et al. (2005) on *O. basilicum* and Darzi et al. (2009) and Moradi et al. (2011) on *F. vulgare*.

Conclusion

Conclusively, the results of current experiment show that vermicompost and nitrogen fixing bacteria have stimulatory effects on the quantity and quality of the Essential oil in dill and thus have considerable potential for providing nutritional elements in essential oil production of dill, especially for the sustainable production systems.

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