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Original Article

Effects of Compost, Vermicompost and Nitrogen Fixing Bacteria on Content and Chemical Compositions of Essential Oil of Hyssop (*Hyssopus officinalis* L.)

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ABSTRACT

The main objective of this study was to determine the effects of compost, vermicompost and nitrogen fixing bacteria on content and chemical compositions of essential oil of *Hyssopus officinalis* L. from Firouzkuh region, Iran. The experiment was carried out as randomized complete block design with eight treatments and three replications at research field of Agriculture Company of Ran in Firouzkuh in 2012. The treatments were compost (20 ton ha^{-1}), vermicompost (12 ton ha^{-1}), nitrogen fixing bacteria (inoculated seeds with *Azotobacter chroococcum* and *Azospirillum lipoferum*), compost (10 ton ha^{-1}) + vermicompost (6 ton ha^{-1}), compost (10 ton ha^{-1}) + nitrogen fixing bacteria, vermicompost (12 ton ha^{-1}) + nitrogen fixing bacteria, compost (10 ton ha^{-1}) + vermicompost (6 ton ha^{-1}) + nitrogen fixing bacteria and control. The results showed that the highest content (0.200%) and yield (5.21 kg ha^{-1}) of essential oil were obtained after applying 12-ton ha^{-1} vermicompost plus nitrogen fixing bacteria. The maximum cis pinocamphone (63.56%) and beta phellandrene (9.71%) and the minimum trans pinocamphone (2.50%) in essential oil were obtained by using the 10 ton ha^{-1} compost, 6 ton ha^{-1} vermicompost plus nitrogen fixing bacteria. Generally, the highest essential oil quantity with integrated application of 12 ton ha^{-1} vermicompost and biofertilizer and the highest essential oil quality with integrated application of 10 ton ha^{-1} compost, 6 ton ha^{-1} vermicompost and biofertilizer were obtained.

1. Introduction

Hyssop (*Hyssopus officinalis* L.) is a perennial herb which grows wild and cultivated in temperate regions of Asia, Europe and America. The essential oil of hyssop is widely used as traditional drug in some parts of Asia and Europe to treat respiratory diseases. Hyssop is also used in food, pharmaceutical, flavor and cosmetic industries throughout the world. It is mainly used for antispasmodic, stomachic, antifungal, relax spasm and cough treatment. The main constituents of hyssop essential oil have been reported as pinocamphone, β pinene, limonene, pinocarvone and phellandrene (Cvijovic et al., 2010; Moro et al., 2011; Mohan et al., 2012; Khan et al., 2012; Zawislak, 2013). Chemical fertilizers have various negative environmental effects such as soil, water and air pollution, which decrease environment health. Therefore, it is necessary to use organic fertilizer and biofertilizers for quality increase of agricultural products (Migahed et al., 2004; Khalid et al., 2006). Organic fertilizers such as compost and vermicompost improve soil physical (structure and aggregation) and chemical properties (decrease soil pH and increase cation exchange capacity) which results in enhanced beneficial microbes' activity in soil and nutrients availability by plants (Hussein et al., 2006; Singh et al., 2009). Also, free living nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* as biofertilizer have the ability not only to fix nitrogen but also to release certain phytohormones of GA and IAA nature which could stimulate plant growth, absorption of nutrients and photosynthesis process (Migahed et al., 2004).

The effect of compost on the essential oil and its chemical constituents in medicinal and aromatic plants was studied by many researchers such as Khalil and El Sherbeny (2003) on Mentha species, Naguib (2003) and Ahmadian et al. (2010) on *Chamomilla recutita*, Khalid et al. (2006) on *Ocimum basilicum*, Hussein et al. (2006) on *Dracocephalum moldavica* and Moradi et al. (2011) on *Foeniculum vulgare*. These studies indicated that optimal compost additions markedly improved the productivity of mentioned plants and significantly increased essential oil and chemical constituents.

Some studies have reported that vermicompost can increase the concentration and composition of essential oil in a few medicinal plants such as basil (Singh & Ramesh, 2002; Anwar et al., 2005; Geetha et al., 2009), coriander (Singh et al., 2009), fennel (Moradi et al., 2011; Darzi et al., 2009), chamomile (Haj Seyed Hadi et al., 2011), cumin (Saeid Nejad & Rezvani Moghaddam, 2011), dill (Darzi et al., 2012) and anise (Darzi et al., 2013). Several other studies have reported that biofertilizers such as nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum lipoferum*) could cause increased quantity and quality of essential oil of some medicinal plants such as fennel (Moradi et al., 2011; Abdou et al., 2004; Mahfouz & Sharaf Eldin, 2007; Azzaz et al., 2009), lemon balm (Harshavardhan et al., 2007), turmeric (Velmurugan et al., 2008), hyssop (Koocheki et al., 2009), cumin (Saeid Nejad & Rezvani Moghaddam, 2010) and dill (Darzi et al., 2012).

The main object of this work was to record the best integrated treatment of organic manures (compost and vermicompost) and biofertilizer (nitrogen fixing bacteria) to obtain the highest quantity and quality of essential oil of Hyssop (*Hyssopus officinalis*) under the Firouzkuh environmental condition in Iran.

2. Material and Method

2.1 Field experiment

A field experiment, arranged in a randomized complete block 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 the experiment contained: compost (20 ton ha^{-1}), vermicompost (12 ton ha^{-1}), nitrogen fixing bacteria (inoculated seeds with *Azotobacter chroococcum* and *Azospirillum lipoferum*), compost (10 ton ha^{-1}) + vermicompost (6 ton ha^{-1}), compost (10 ton ha^{-1}) + nitrogen fixing bacteria, vermicompost (6 ton ha^{-1}) + nitrogen fixing bacteria, compost (10 ton ha^{-1}) + vermicompost (6 ton ha^{-1}) + nitrogen fixing bacteria and control (without fertilizer application). Inoculation was carried out by dipping the hyssop seeds in the cell's suspension of 10^8 CFU ml^{-1} for 15 min. The required quantities of compost and vermicompost were applied and incorporated to the top 5 cm layer of soil in the experimental beds before the plantation of hyssop seeds. 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 and compost and vermicompost are presented in Tables1 and 2.

Table 1. Physicochemical properties of farm soil

Texture	pH	Electrical Conductivity (dS/m)	Organic Carbon (%)	Total Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Fe (mg/kg)
Clay-loam	7.6	1.55	1.86	0.127	48	720	8

Table 2. Some chemical characteristics of the used compost and vermicompost.

	pH	EC (ds/m)	O.M (%)	N (%)	P(%)	K(%)
Compost	6.7	10.9	51	1.2	0.35	1.2
Vermicompost	8.5	1.8	45	2.13	0.67	3.9

2.2 Extraction of essential oil

For determining the essential oil content (%), about 100 g dried herb of hyssop (dried in shadow) sampled from each plot were selected and then were subjected to hydro-distillation (Clevenger type apparatus) for 3 hours (Darzi et al., 2012; Kapoor et al., 2004). Essential oil yield also was calculated with by using essential oil content and herb dry yield.

2.3 Identification of essential oil components

The essential oil was analyzed with gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) for identifying the essential oil components. For GC analysis. A Younglin Acm600, equipped with HP-5 MS capillary column (30m X 0.25 µm) and for GC/MS analysis an Agilent 6890 GC and Agilent 5973 MS, equipped with HP-5 MS capillary column (30m X 0.25 µm) was used. The components of the essential oil containing cis pinocamphone, trans pinocamphone and β phellandrene were identified by comparing their mass spectra with those held in a computer library or obtained using authentic compounds. The identities of the components were confirmed by comparing their retention indices, either with those of authentic compounds or with data published in the literature (Kapoor et al., 2004; Sephidkon, 2002).

2.4 Statistical analysis

Data analysis was conducted by SAS software (SAS, 2001) and mean comparison was done by the Duncan's multiple range tests at the 5% probability level. Data were transformed when necessary before analysis to satisfy the assumptions of normality (Zar, 1996).

3. Results

3.1 Essential oil compositions

The present results indicated that essential oil content was significantly affected by the application of different treatments of organic manures and biofertilizer (Table 3).

Table 3. Analysis of variance of the studied traits

S.O.V	df	Essential oil content	Essential oil yield	Cis pinocamphone in essential oil	Trans pinocamphone in essential oil	β phellandrene in essential oil
Replication	2	0.001^{ns}	10.1 **	2.39^{ns}	0.45^{ns}	1.29^{ns}
Treatments	7	0.002 **	4.56**	176.18 **	156.62 **	4.48**
Error	14	0.00076	1.1034	5.5949	0.50287	0.5768

The maximum essential oil content (0.200%) was obtained by applying 12 ton vermicompost per hectare plus nitrogen fixing bacteria and the minimum essential oil content (0.120%) was indicated by control treatment (Fig. 1).

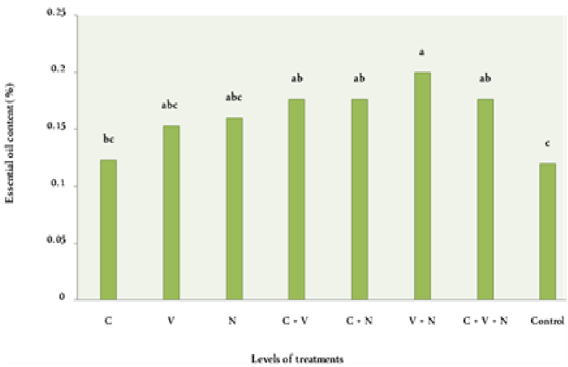

Fig. 1. Mean comparison for essential oil content in different levels of organic manures and biofertilizer treatments. C= 20 ton ha^{-1} compost, V= 12 ton ha^{-1} vermicompst, N= nitrogen fixing bacteria, C+V=50%C+50%V, C+N=20 ton ha^{-1} compost + nitrogen fixing bacteria, V+N= 12 ton ha^{-1} vermicompst + nitrogen fixing bacteria, C+V+N=50%C+50%V+ nitrogen fixing bacteria and Control= without fertilizer application.

3.2 Essential oil yield

The results presented in Ttable 3 demonstrated that essential oil yield was influenced significantly by the application of different treatments of organic manures and biofertilizer.

Among various treatments, the application of 12 ton vermicompost per hectare plus nitrogen fixing bacteria indicated maximum increase in essential oil yield (5.21 kg ha^{-1}) (Fig. 2).

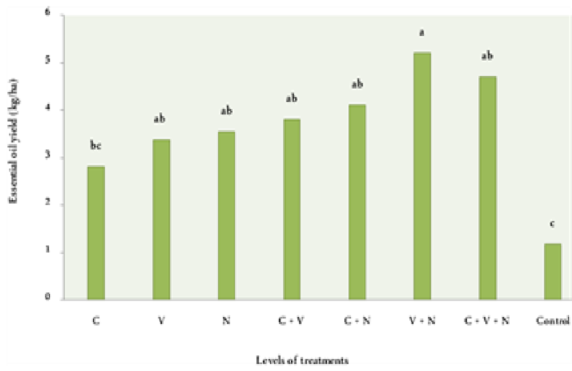

Fig. 2. Mean comparison for essential oil yield in different levels of organic manures and biofertilizer treatments. C= 20 ton/ha compost, V= 12 ton ha^{-1} vermicompst, N= nitrogen fixing bacteria, C+V=50%C+50%V, C+N=20 ton ha^{-1} compost + nitrogen fixing bacteria, V+N= 12 ton ha^{-1} vermicompst + nitrogen fixing bacteria, C+V+N=50%C+50%V+ nitrogen fixing bacteria and Control= without fertilizer application.

3.3 Cis pinocamphone in essential oil

The results presented in Table 3 revealed that the studied treatments had significant effects on the cis pinocamphone in essential oil. The maximum cis pinocamphone in essential oil (63.56%) was obtained by using 10-ton compost per hectare plus 6 ton vermicompost per hectare plus nitrogen fixing bacteria and the minimum cis pinocamphone percent (35.84%) was obtained by applying 20-ton compost per hectare (Fig. 3).

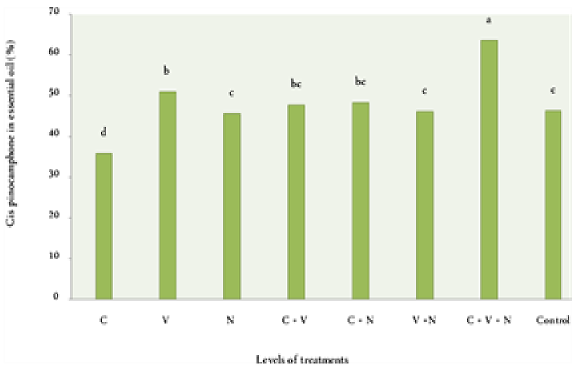

Fig. 3. Mean comparison for cis pinocamphone in essential oil in different levels of organic manures and biofertilizer treatments**.** C= 20 ton ha^{-1} compost, V= 12 ton ha^{-1} vermicompst, N= nitrogen fixing bacteria, C+V=50%C+50%V, C+N=20 ton ha^{-1} compost + nitrogen fixing bacteria, V+N= 12 ton ha^{-1} vermicompst + nitrogen fixing bacteria, C+V+N=50%C+50%V+ nitrogen fixing bacteria and Control= without fertilizer application.

3.4 Trans pinocamphone in essential oil

The results presented in Table 3 showed that various treatments of organic manures and biofertilizers had significant effects on the trans pinocamphone in essential oil. The minimum trans pinocamphone in essential oil (2.50%) was obtained by applying 10-ton compost per hectare plus 6 ton vermicompost per hectare plus nitrogen fixing bacteria (Fig. 4).

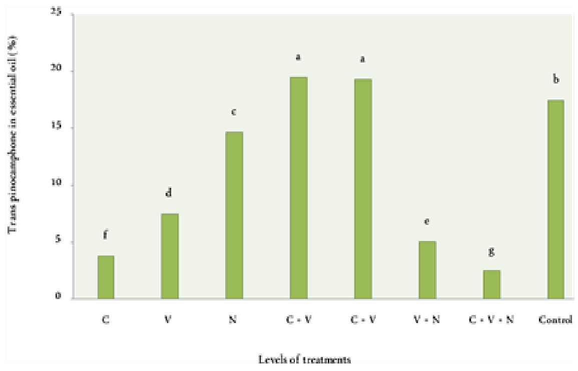

Fig. 4. Mean comparison for trans pinocamphone in essential oil in different levels of organic manures and biofertilizer treatments. C= 20 ton ha^{-1} compost, V= 12 ton ha^{-1} vermicompst, N= nitrogen fixing bacteria, C+V=50%C+50%V, C+N=20 ton ha^{-1} compost + nitrogen fixing bacteria, V+N= 12 ton ha^{-1} vermicompst + nitrogen fixing bacteria, C+V+N=50%C+50%V+ nitrogen fixing bacteria and Control= without fertilizer application.

3.5 β phellandrene in essential oil

The present results indicated that beta phellandrene in essential oil was significantly affected by the application of different treatments of organic manures and biofertilizer (Table 3). The maximum beta phellandrene in essential oil (9.71%) was obtained by using 10 ton compost per hectare plus 6 ton vermicompost per hectare plus nitrogen fixing bacteria (Fig. 5).

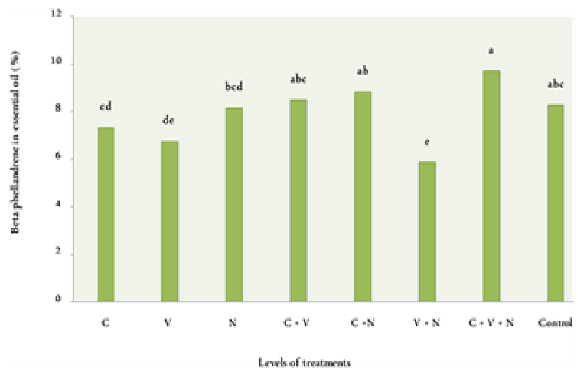

Fig. 5. Mean comparison for beta phellandrene in essential oil in different levels of organic manures and biofertilizer treatments. C= 20 ton ha^{-1} compost, V= 12 ton ha^{-1} vermicompst, N= nitrogen fixing bacteria, C+V=50%C+50%V, C+N=20 ton ha^{-1} compost + nitrogen fixing bacteria, V+N= 12 ton ha^{-1} vermicompst + nitrogen fixing bacteria, C+V+N=50%C+50%V+ nitrogen fixing bacteria and Control= without fertilizer application.

4. Discussion

Due to a high ability for absorption of mineral nutrients like N, P and K by plants and then increase of biomass amount (Arancon et al., 2006; Zaller, 2007), Vermicompost has a positive effect on secondary metabolites concentration such as essential oil and subsequently the enhanced essential oil content. Improved essential oil content of medicinal plants has previously been reported in the presence of optimal amounts of vermicompost (Singh et al., 2009; Anwar et al., 2005; Geetha et al., 2009; Darzi et al., 2009; Haj Seyed Hadi et al., 2011; Darzi et al., 2012).

Increased essential oil yield in treatment of 12 ton vermicompost per hectare plus nitrogen fixing bacteria can be owing to the improvement of yield attributes such as essential oil content and herb dry yield. Our findings are in accordance with the observations of Singh and Ramesh (2002) and Anwar et al. (2005) on *Ocimum basilicum*, Pandey (2005) and Kumar et al. (2009) on *Artemisia pallens*, Koocheki et al. (2009) on *Hyssopus officinalis*, Mahfouz and Sharaf Eldin (2007) and Moradi et al. (2011) on *Foeniculum vulgare*, Saeid Nejad and Rezvani Moghaddam (2011) on *Cuminum cyminum* and Darzi et al. (2012) on *Anethum graveolens*.

Integrated application of compost, vermicompost and nitrogen fixing bacteria, through the improvement of mineral elements absorption (Migahed et al., 2004; Zaller, 2007), caused optimal biomass production (herb dry yield) which leads to improvement of the essential oil composition (cis pinocamphone percent). These findings are in accordance with the observations of Naguib (2003) on *Chamomilla recutita*, Anwar et al. (2005) and Khalid et al. (2006) on *Ocimum basilicum*, Hussein et al. (2006) on *Dracocephalum moldavica*, Darzi et al. (2009) and Moradi et al. (2011) on *Foeniculum vulgare* and Darzi et al. (2012) on *Anethum graveolens*.

Using proper amounts of compost and vermicompost, plus nitrogen fixing bacteria through increase of the cis pinocamphone in essential oil, has a negative effect on other constituents of essential oil and subsequently have decreased trans pinocamphone in essential oil. The present result is in agreement with the report of Khalid et al. (2006) on *Ocimum basilicum*, Hussein et al. (2006) on *Dracocephalum moldavica*, Harshavardhan et al. (2007) on *Mellisa offisinalis*, Padmapriya and Chezhyian (2009) on *Curcuma longa*, Darzi et al. (2009) and Moradi et al. (2011) on *Foeniculum vulgare* and Darzi et al. (2012) on *Anethum graveolens*.

Organic fertilizers application through the improvement of biological activities of soil and nutrient elements absorption such as nitrogen and phosphorus, caused more growth and biomass production which leads to improvement of the essential oil quality (Hussein et al., 2006; Khalil & El-Sherbeny, 2003; Ahmadian et al., 2009).

These findings are in accordance with the observations of Khalid et al. (2006) on *Ocimum basilicum*, Hussein et al. (2006) on *Dracocephalum moldavica*, Moradi et al. (2011) on *Foeniculum vulgare*, and Darzi et al. (2012) on *Anethum graveolens*.

5. Conclusion

Integrated application of organic manures and biofertilizer positively influenced concentration and composition of hyssop essential oil. The highest essential oil content and essential oil yield were obtained by using 12 ton vermicompost per hectare plus nitrogen fixing bacteria (*Azotobacter* + *Azospirillum*). Also, maximum cis pinocamphone in essential oil (major compound) was observed by applying 10-ton compost per hectare plus 6 ton vermicompost per hectare plus nitrogen fixing bacteria.

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