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Response of Concentration and Composition of Essential oil of Coriander (*Coriandrum sativum* L.) to Cattle manure and Nitrogen fixing bacteria

Mohammad Taghi Darzi[1*], Mohammad Reza Haj Seyed Hadi[1]

1- Associate professor, Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Roudehen Branch, Roudehen , Iran.

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*Corresponding author:
Associate professor,
Department of Agronomy,
Faculty of Agriculture, Islamic Azad University, Roudehen Branch, Roudehen , Iran.
E-mail:
mt_darzi@yahoo.com

Abstract

In order to study the response of concentration and composition of essential oil of coriander (*Coriandrum sativum*) to cattle manure and nitrogen fixing bacteria, an experiment was conducted as factorial experiment in the base of randomized complete blocks design with twelve treatments and three replications at homand research station in Damavand of Iran in 2010. The factors were cattle manure in four levels (5, 10, 15 and 20 ton/ha) and nitrogen fixing bacteria in three levels (inoculation with azotobacter, inoculation with azospirillum and inoculation together). Results showed that the highest essential oil content and linalool and alpha pinene in essential oil were obtained with application of 10 ton/ha cattle manure. The maximum gamma terpinene and the minimum geranyl acetate in essential oil were obtained after applying 15 ton/ha cattle manure. Nitrogen fixing bacteria also showed significant effects on linalool, alpha pinene and geranyl acetate in essential oil (except essential oil content and gamma terpinene in essential oil) as the highest linalool in essential oil in treatment of inoculation with azotobacter, the maximum alpha pinene in essential oil in treatment of inoculation with azospirillum and the maximum geranyl acetate in inoculation together (azotobacter & azospirillum) were obtained.

Introduction

Coriander (*Coriandrum sativum*) is a herbaceous annual plant, which is native to mediterranean region. The coriander seeds have essential oil as an active substance, that linalool is the most important constituent of coriander and other essential oil components contain alpha pinene, gamma terpinene, limonene and etc, which are used in pharmaceutical industry as a antispasmodic and a carminative (Deiderichen., 1996; Carrubba et al., 2002). Cattle manures are the source of N and other nutrients for plants (such as phosphorus, potassium, calcium,, iron, zinc and copper) that can make valuable contributions to soil's organic matter, can improve physical fertility, and are a center for biological activities (Khalid & Shafei., 2005; Najm et al., 2012). 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 & Sharaf Eldin., 2007). By using correct nutritional sources through organic manures and biofertilizers, yield and active substance of medicinal plants can be maximized.

Several studies have reported that cattle manure can increase concentration and composition of the essential oil of some medicinal plants such as dill (Khlid & Shafei., 2005), sage (Kaplan et al., 2009; Kocabas et al., 2010), basil (Biasi et al., 2009), thyme (Ateia et al., 2009), fennel (Osman., 2009; Azzaz et al., 2009) and onion (Yassen & Khalid., 2009).

Some other studies have reported that nitrogen fixing bacteria such as Azotobacter chroococcum and Azospirillum lipoferum could cause increased essential oil content and it constituents in a few medicinal plants such as fennel (Mahfouz & Sharaf Eldin., 2007; Osman., 2009; Moradi et al., 2011), lemon balm (Harshavardhan et al., 2007), davana (Kumar et al., 2009), Thyme (Ateia et al., 2009), Basil (Biasi et al., 2009), turmeric (Padmapriya & Chezhyian., 2009) and Dill (Darzi et al., 2012).

Therefore, the main objective of the present field experiment was to investigate the response of concentration and composition of essential oil of

coriander (*Coriandrum sativum*) to cattle manure and nitrogen fixing bacteria.

Materials and Methods

• Field Experiment

A 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 2010. The geographical location of the experimental station was 35º 39´ N and 52º 5´ E with the altitude of 1800 m. The treatments consisted of different levels of cattle manure (5, 10, 15 and 20 ton/ha) and levels of nitrogen fixing bacteria (inoculation with azotobacter, inoculation with azospirillum and inoculation together). Inoculation was carried out by dipping the coriander seeds in the cells suspension of 108 CFU/ml for 15 min. The cattle manure was prepared from cow dung. The required quantities of cattle manure were applied and incorporated to the top 5 cm layer of soil in the experimental beds before the plantation of coriander 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 cattle manure are presented in Table1. Nitrogen (20 kg/ha) was applied to the plots, based on the soil and cattle manure 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 one meter between the plots and 2 meters between replications. Coriander seeds were directly sown by hand. There was no incidence of pest or disease on coriander 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.

• Extraction of Essential oil

In order to determine the essential oil content (%), a sample of 100 g of coriander seeds from the each plot 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 (Kapoor et al., 2004; 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 linalool, alpha pinene, gamma terpinene and geranyl acetate were used to establish the retention times (Sephidkon., 2002; Kapoor et al., 2004).

Table 1. Chemical and physical properties of the experimental soil and cattle manure

	Cu	Zn	Mn	Fe	Mg	Ca	K	P	N	O.C	EC	pH
	mg/kg								%		dS/m	
Soil	0.37	1.16	6.8	6.4	-	-	500	48	0.09	0.87	0.60	6.9
Cattle manure	71.3	199.1	957	2755	6000	16000	11000	3600	0.85	11.7	20.8	8.0

• 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

• Essential oil content

The results presented in Table 2 have demonstrated that essential oil content was influenced by the application of cattle manure, significantly. Among various treatments, the application of 10 ton cattle manure per hectare has indicated maximum increase in essential oil content (0.276%). Nitrogen fixing bacteria, also did not show significant effect on essential oil content.

Table 2. Mean comparison of the concentration and composition of essential oil of coriander at various levels of cattle manure

Treatments	Essential oil content (%DW)	Linalool (%)	Alpha pinene (%)	Gamma terpinene (%)	Geranyl acetate (%)
Cattle manure (ton/ha)					
c_1	0.196 d	70.75 c	8.85 c	4.88 ab	3.48 a
c_2	0.276 a	73.96 a	9.96 a	4.64 b	3.33 b
c_3	0.256 b	72.57 b	10.05 a	5.05 a	3.18 c
c_4	0.216 c	70.61 c	9.39 b	4.81 ab	3.26 bc

Means, in each column for each factor followed by at least on letter in common, are not significantly different at 5% probability level using Duncan's Multiple Range Test.

c_1, c_2, c_3 and c_4 represent 5, 10, 15 and 20 ton cattle manure per hectare, respectively.

The present results show that the interaction of cattle manure and nitrogen fixing bacteria was significant (Figure 1). The highest essential oil content (0.300%) was obtained after the integrated application of 15 ton/ha cattle manure and inoculation together of azotobacter and azospirillum. The interaction of cattle manure and nitrogen fixing bacteria, on the essential oil content, revealed that the application of 5, 10 and 15 ton/ha cattle manure successively in the level of inoculation together of azotobacter and azospirillum, which resulted in a significant increase in essential oil content.

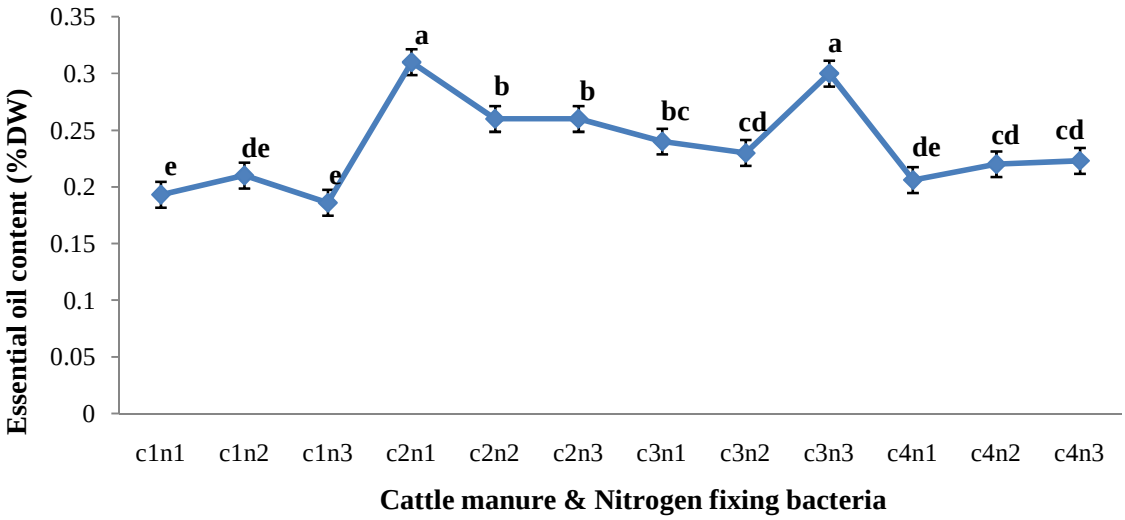

Figure 1. Mean comparison for essential oil content after the interaction of different factors
c1, c2, c3 and c4 represent 5, 10, 15 and 20 ton cattle manure per hectare, respectively.
n1, n2 and n3 represent inoculation with azotobacter, inoculation with azospirillum and inoculation with azotobacter & azospirillum, respectively.

- **Linalool in essential oil**

The results have indicated that linalool in essential oil was affected by the application of cattle manure (Table 2). The highest linalool in essential oil was obtained with applying 10 ton/ha cattle manure (73.96%). Nitrogen fixing bacteria showed significant effect on linalool in essential oil (Figure 2), as the highest linalool in essential oil (72.79%) was obtained in inoculation with azotobacter.

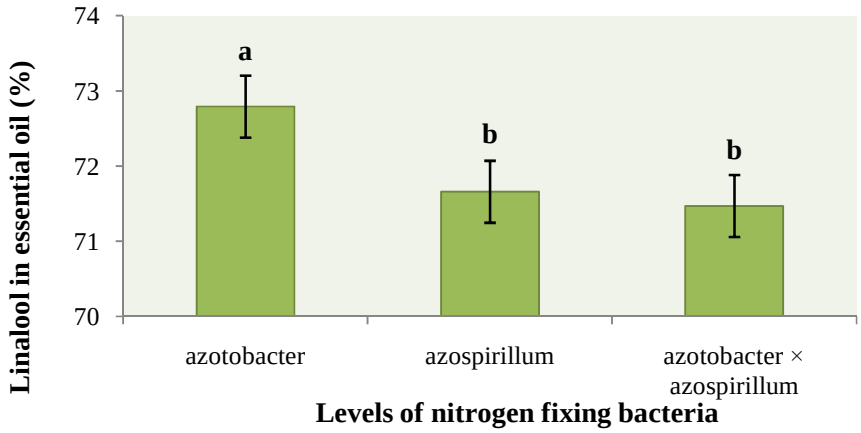

Figure 2. Mean comparison for linalool in essential oil in different levels of nitrogen fixing bacteria

- **Alpha pinene in essential oil**

The results presented in Table 2 have revealed that various levels of cattle manure had significant effects on the alpha pinene in essential oil. Among various treatments, the applications of 10 and 15 ton cattle manure per hectare have indicated maximum increase in alpha pinene in essential oil (9.96% and 10.05% respectively). Nitrogen fixing bacteria showed significant effect on alpha pinene in essential oil (Figure 3), as the highest alpha pinene (10.3%) was obtained in the treatment of inoculation with azospirillum.

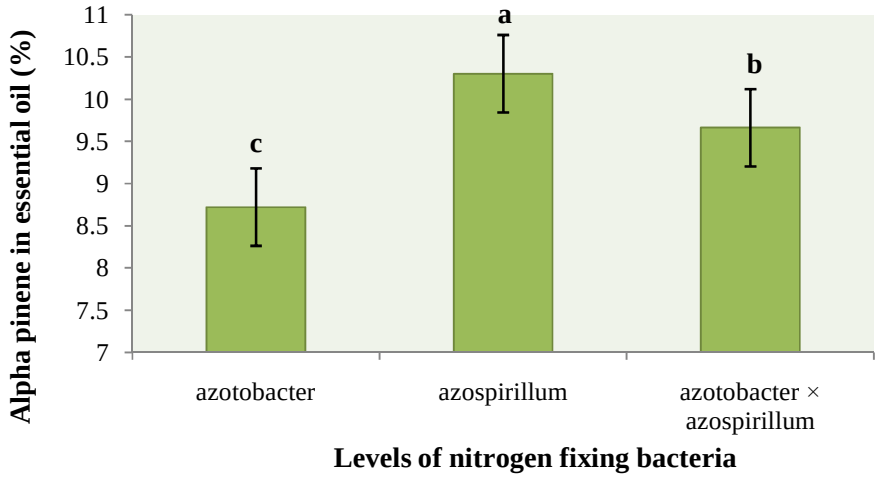

Figure 3. Mean comparison for alpha pinene in essential oil in different levels of nitrogen fixing bacteria

- **Gamma terpinene in essential oil**

Cattle manure showed significant effect on gamma terpinene in essential oil. The maximum gamma terpinene in essential oil were obtained with applying 15 ton/ha cattle manure (5.05%) (Table 2). Nitrogen fixing bacteria, also did not show significant effect on gamma terpinene in essential oil. The present results show that the interaction of cattle manure and nitrogen fixing bacteria was significant on gamma terpinene in essential oil (Figure 4). The highest gamma terpinene in essential oil (5.28%) was obtained after the integrated application of 15 ton/ha cattle manure and inoculation with azotobacter. The

interaction of cattle manure and nitrogen fixing bacteria on gamma terpinene in essential oil, revealed that the application of 10 and 15 ton/ha cattle manure successively in the level of inoculation with azospirillum, which resulted in a significant decrease in gamma terpinene in essential oil.

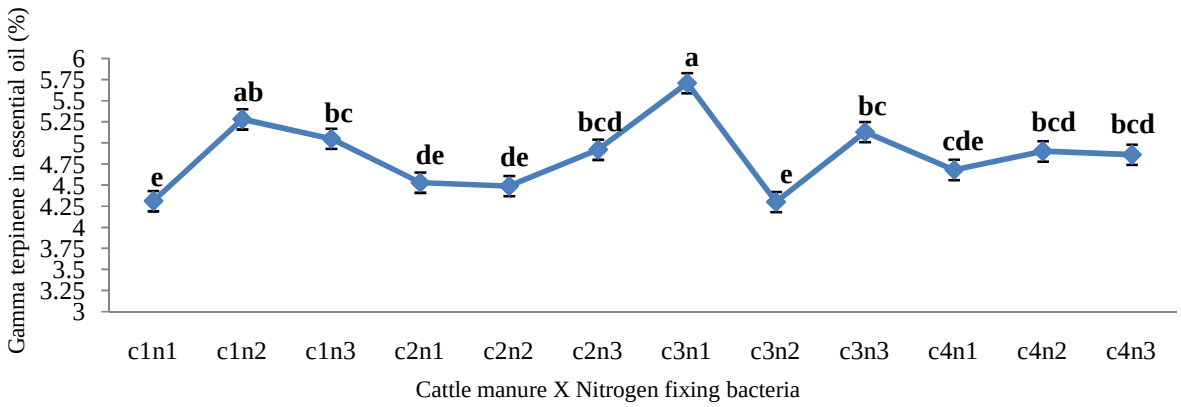

Figure 4. Mean comparison for gamma terpinene in essential oil after the interaction of different factors

- **Geranyl acetate in essential oil**

Cattle manure showed significant effect on geranyl acetate in essential oil. The minimum geranyl acetate in essential oil were obtained with applying 15 ton/ha cattle manure (3.18%) (Table 2). Nitrogen fixing bacteria showed significant effect on geranyl acetate in essential oil (Figure 5), as the highest geranyl acetate (3.40%) was obtained in the treatment of inoculation with N_1 & N_3. The present results show that the interaction of cattle manure and nitrogen fixing bacteria was significant on geranyl acetate in essential oil (Figure 6). The highest geranyl acetate in essential oil (3.63%) was obtained after the integrated application of 5 ton/ha cattle manure and inoculation with azotobacter. The interaction of cattle manure and nitrogen fixing bacteria on geranyl acetate in essential oil, revealed that the application of 10, 15 and 20 ton/ha cattle manure successively in the level of inoculation with azotobacter, which resulted in a significant decrease in geranyl acetate in essential oil.

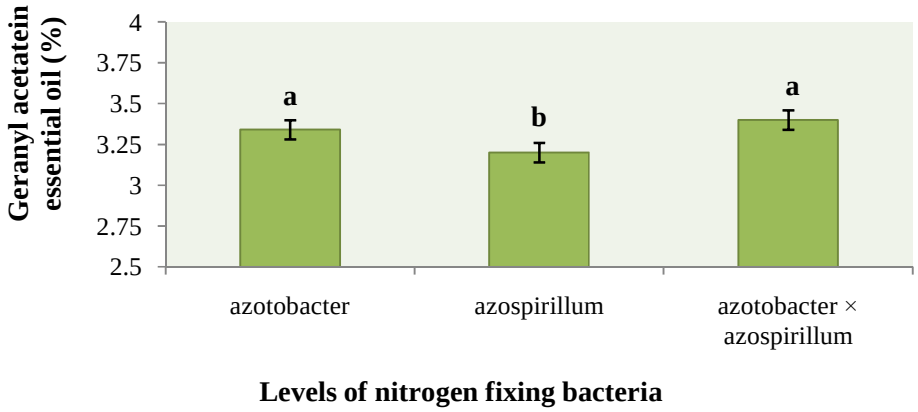

Figure 5. Mean comparison for geranyl acetate in essential oil in different levels of nitrogen fixing bacteria

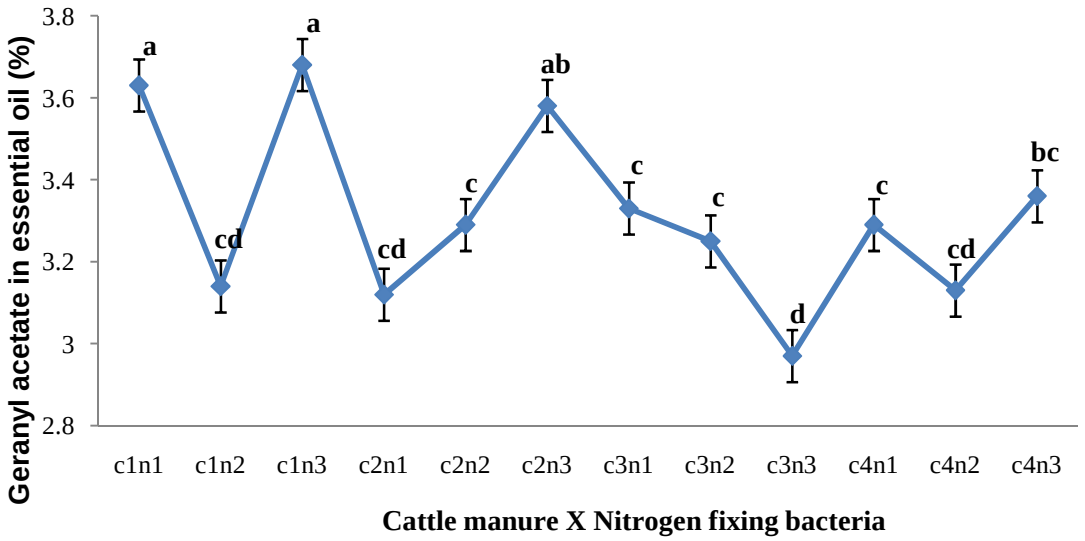

Figure 6. Mean comparison for gerany acetate in essential oil after the interaction of different factors

Discussion

According to the present analysis, cattle manure application through increase of the mineral uptake such as; nitrogen and phosphorus (Eghball et al., 2002; Badran & Safwat., 2004; Araya et al., 2006), 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 cattle manure (Khalid & Shafei, 2005; Kaplan et al., 2009; Azzaz et al., 2009; Yassen & Khalid., 2009; Kocabas et al., 2010). Results likely show that the positive and synergistic effect of interaction between two factors on essential oil content is highly dependent on the effect of organic matter, contained in cattle manure, on the activity of nitrogen fixing bacteria. Many reports have shown that the interaction between proper amounts of organic manures and bio fertilizers can be beneficial for essential oil content (Velmurugan et al., 2008; Singh et al., 2009; Azzaz et al., 2009; Moradi et al., 2011; Valadabadi & Farahani., 2011).

Cattle manure has significantly influenced the linalool and alpha pinene in essential oil. Using of optimal amount of Cattle manure through the improvement of biological activities of soil and mineral element absorption (Eghball et al., 2002; Mohapatra & Das., 2009), caused more biomass production and flowering promotion and subsequently seed ripening which leads to improvement of the linalool and alpha pinene in essential oil. These findings are in accordance with the observations of Khalid and Shafei (2005) on *Anethum graveolens*, Osman (2009) on *Foeniculum vulgare*, Padmapriya and Chezhyian (2009) on *Curuma longa*, Ateia et al. (2009) on *Thymus vulgaris* and Biasi et al. (2009) on *Ocimum basilicum*. The present results suggest that influence of inoculation with azotobacter on the linalool in essential oil was due to increased nitrogen uptake (Kalyanasundaram et al., 2008). This 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 *Foeniculum vulgare* , Velmurugan et al. (2008) on *Curcuma longa* and Darzi et al. (2012) on *Anethum graveolens*. Also, results suggest that influence of inoculation with azospirillum on the alpha pinene in essential oil was due to increased nitrogen uptake. This result is in agreement with the report of Padmapriya and Chezhyian (2009) on *Curcuma longa*.

Applying higher amount of cattle manure (15 ton/ha), through decrease of the some essential oil components such as linalool and geranyl acetate, has a positive effect on other constituents of essential oil such as; gamma terpinene and subsequently have increased gamma terpinene in essential oil. The present result is in agreement with the report of Khalid and Shafei (2005) on *Anethum graveolens* and Ateia et al. (2009) on *Thymus vulgaris*.

Results likely show that the negative and antagonistic effect of interaction between two factors on gamma terpinne and geranyl acetate in essential oil is highly dependent on the effect of amount of cattle manure, on the activity of azospirillum and azotobacter. Darzi et al. (2012) have shown that the integrated application of 8 and 12 ton/ha organic manure and nitrogen fixing bacteria on *Anethum graveolens* caused lower carvone percent in essential oil as compared to the experimental plants with 4 ton/ha organic manure application.

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