

## Effects of Biofertilizer and Plant Density on Essential Oil Content and Yield Traits of Ajowan (*Carum copticum*)

<sup>1</sup>Ardalan Ghilavizadeh, <sup>2</sup>Mohammad Taghi Darzi  
and <sup>2</sup>Mohammadreza Haj Seyed Hadi

<sup>1</sup>Roudehen Branch, Islamic Azad University, Roudehen, Iran

<sup>2</sup>Faculty of Agriculture, Department of Agronomy,  
Roudehen Branch, Islamic Azad University, Roudehen, Iran

**Abstract:** In order to study the effect of biofertilizer and plant density on essential oil content and yield traits of ajowan (*Carum copticum*), an experiment was conducted as factorial experiment in the base of randomized complete blocks design with twelve treatments and three replications at research field of Agriculture Company of Ran in Firouzkuh of Iran in 2011. The factors were biofertilizer (nitrogen fixing bacteria), mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* in four levels (non-inoculated, inoculated seeds, spray on the plant base at stem elongation stage and inoculated seeds + spray on the plant base at stem elongation stage) and plant density in three levels (12.5, 16.6 and 25 plants m<sup>-2</sup>). Results showed that biofertilizer had significant effects on studied traits. The highest, biological yield, seed yield, essential oil content and essential oil yield were obtained by using the biofertilizer twice (inoculated seeds + spray on the plant base at stem elongation stage). Plant density, also showed significant effects on mentioned traits except essential oil content. The maximum biological yield, seed yield and essential oil yield were obtained with 25 plants m<sup>-2</sup>.

**Key words:** Ajowan • *Azotobacter* and *Azospirillum* • Plant density • Essential oil

### INTRODUCTION

Ajowan (*Carum copticum*) belonging to the Apiaceae family is a grassy, annual plant with a white flower and small, brownish seeds which commonly grows in Iran, India, Egypt and Europe. The seeds have been used for their flavour and spice in food industry. Also, the ajowan seeds have essential oil as an active substance, which is used in pharmaceutical industry as a diuretic, antiemetic, analgesic, antiasthma, antispasmodic and a carminative [1-4]. Applying of biofertilizers such as nitrogen fixing bacteria has led to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful agrochemicals for human safety [5, 6]. 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 [6, 7].

By using correct nutritional sources through biofertilizers, growth and yield of medicinal plants can be maximized. Also, proper agronomic management include suitable plant density has a high influence on growth and yield of medicinal plants.

Several studies have reported that nitrogen fixing bacteria such as *Azotobacter chroococcum* and *Azospirillum lipoferum* could cause increased yield and essential oil in a few medicinal plants such as coriander [8], fennel [6, 9, 10], davana [11, 12], turmeric [13], hyssep [14], black cumin [15] and dill [16].

Some other studies have reported that suitable plant density can increase the essential oil and yield of some medicinal plants such as dill [17], coriander [18, 19], thyme [20], fennel [21], sesame [22], davana [23], peppermint [24] and anise [25, 26].

Therefore, the main objective of the present field experiment was to investigate the effects of biofertilizer and plant density on essential oil content and yield traits of ajowan (*Carum copticum*).

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
0.42	3.18	400	30	0.14	0.65	1.02	7.48	Clay-Loamy

## MATERIALS AND METHODS

**Field Experiment:** A factorial experiment, arranged in a randomized complete blocks designed with three replications, was conducted in the Experimental field of the Agriculture Company of Ran, Firouzkuh, Iran during the growing season of 2011. The geographical location of the experimental station was 35°45' N and 52°44' E with the altitude of 1930 m. The treatments consisted of biofertilizer, different inoculation conditions of mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* bacteria (non-inoculated, inoculated seeds, spray on the plant base at stem elongation stage and inoculated seeds + spray on the plant base at stem elongation stage) and different levels of plant density (12.5, 16.6 and 25 plants m<sup>-2</sup>). Inoculation was carried out by dipping the ajowan seeds in the cells suspension of 10<sup>8</sup> 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 Table 1. Nitrogen (50 kg/ha) and phosphorus (20 kg/ha) were applied to the plots, based on the soil analysis, before cultivation.

Each experimental plot was 3 m long and 2 m wide with the spacing of 40 cm between the rows. There was a space of one meter between the plots and 2 meters between replications. Ajowan 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.

Fifteen plants were randomly selected from each plot and the observations were recorded. In this study, quantitative and qualitative traits of ajowan consisted of biological yield, seed yield, essential oil content and essential oil yield were evaluated. For evaluating the biological yield, plants were put in the oven at 80°C for 48 h and dry weight was calculated using a digital balance (Sartorius B310S; ±0.01 g) [27, 28]. In order to determine seed yield, the plots were manually harvested following the air-drying of umbels at 20-24°C and then the seeds were removed from plants by hand [29].

**Essential oil Extraction:** In order to determine the essential oil content (%), a sample of 100 g of ajowan 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 of water by anhydrous sodium Sulfate [30, 31]. Essential oil yield also was calculated by using seed yield and essential oil content.

**Statistical Analysis:** All the data were subjected to statistical analysis (one-way ANOVA) using SAS software [32]. 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 [33].

## RESULTS AND DISCUSSION

**Biological Yield:** The results presented in Table 2 have demonstrated that biological yield was influenced by the application of biofertilizer, significantly. Among various treatments, treatment of inoculated seeds together spraying on the plant base (10806.9 kg/ha) have indicated maximum increase in biological yield. According to the present analysis, Positive effect of the biofertilizer twice on the biological yield was due to increased nitrogen uptake and growth improvement [6]. The result of present work are in agreement with the reports of Swaminathan *et al.* (2008) and Kumar *et al.* (2009) on *Artemisia pallens* [11, 12] and Kalyanasundaram *et al.* (2008) on *Acorus calamus* [34].

Plant density had also a significant effect on biological yield (Figure 1), as higher biological yield was recorded in treatment of 25 plants m<sup>-2</sup> (12384.4 kg/ha). The results clearly demonstrate the effectiveness of high plant density (25 plant m<sup>-2</sup>) in greater biological yield, that is in accordance with the observations Shareh and Rashed Mohassel. (2003) and Rasam *et al.* (2007) [25, 26].

**Seed yield:** The results presented in Table 2 have revealed that different levels of biofertilizer (nitrogen fixing bacteria) had significant effects on the seed yield. The maximum seed yield (1376 kg/ha) was obtained in

Table 2: Mean comparison of the quantitative and qualitative characteristics of ajowan at various levels of biofertilizer

Treatments	Biological yield (kg/ha)	Seed yield (kg/ha)	Essential oil content (%)	Essential oil yield (kg/ha)
Biofertilizer (nitrogen fixing bacteria)				
b1	9400 b	896.5 c	1.98 b	17.85 c
b2	9760.4 b	1084.4 b	2.33 ab	25.18 b
b3	9804.9 b	1204.5 b	2.54 a	30.96 b
b4	10806.9 a	1376.0 a	2.78 a	38.58 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.

b1, b2, b3 and b4 represent non-inoculated, inoculated seeds, spraying on the plant base at stem elongation stage and inoculated seeds + spraying on the plant base at stem elongation stage, respectively.

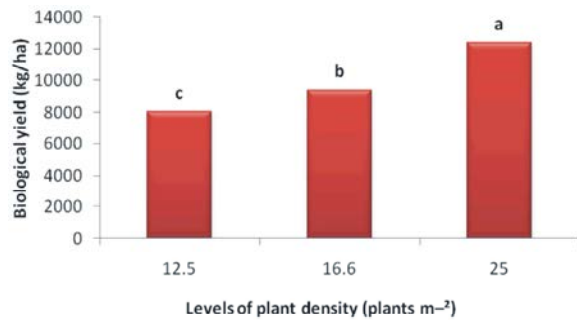


Fig. 1: Mean comparison for biological yield in different levels of plant density

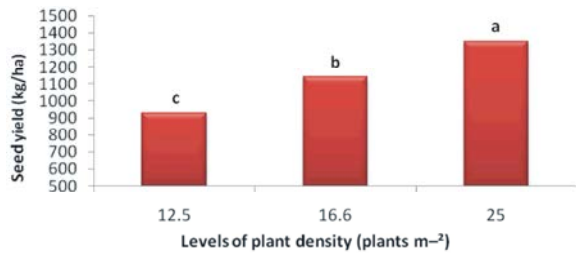


Fig. 2: Mean comparison for seed yield in different levels of plant density

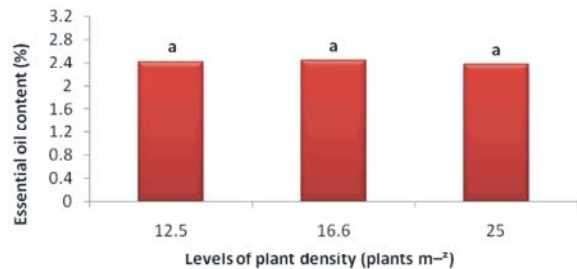


Fig. 3: Mean comparison for essential oil content in different levels of plant density

the fourth treatment level of biofertilizer (inoculated seeds + spraying). Increased seed yield in fourth treatment of biofertilizer can be owing to the improvement of growth and dry matter and eventually yield components. These results are in agreement with the investigation of Kumar *et al.* (2002) on *Coriandrum sativum* [8], Migahed *et al.* (2004) on *Apium graveolens* [27], Abdou

*et al.* (2004) and Mahfouz and Sharaf Eldin (2007) on *Foeniculum vulgare* [6, 9] and Valadabadi and Farahani (2011) on *Nigella sativa* [15].

Significant difference in seed yield was observed in various levels of plant density (Figure 2). The highest seed yield (1351.6 kg/ha) was obtained in 25 plants m<sup>-2</sup>. The comparison of plant density showed that maximum density (25 plant m<sup>-2</sup>) produced the highest grain yield. Akbarinia *et al.* (2005) reported that 30 plants m<sup>-2</sup> in spring grown obtained the most of grain yield in coriander (*Coriandrum sativum*) [19]. Present result is in agreement with the investigation of Gosh and Patra (1994) on *Sesamum indicum* [22], Darzi *et al.* (2001) on *Foeniculum vulgare* [21], Mcvicar *et al.* (2004) on *Coriandrum sativum* [18], Gowda *et al.* (2006) on *Trigonella foenum gracum* [35] and Rasam *et al.* (2007) on *Pimpinella anisum* [26].

**Essential oil content:** The results have indicated that essential oil content was affected by the application of biofertilizer (Table 2). Significant increase in essential oil content was observed in levels of biofertilizer application. The highest essential oil content was obtained in treatment of inoculated seeds together spraying on the plant base at stem elongation stage (2.78%). Nitrogen fixing bacteria have increased essential oil content by enhancing the nitrogen absorption [27]. The present result were derived from the improvement of nitrogen fixing bacteria' activities in soil at the third and fourth treatments levels (spraying on plant base 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 [6, 10, 13, 16].

Plant density was not showed significant effect on essential oil content (Figure 3).

**Essential Oil Yield:** The results presented in Table 2 have demonstrated that essential oil yield was influenced by the application of biofertilizer, significantly.

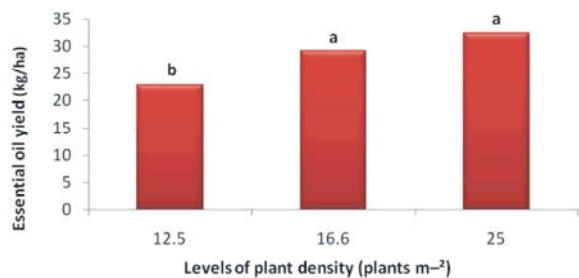


Fig. 4: Mean comparison for essential oil yield in different levels of plant density

Among various treatments, fourth level of biofertilizer (inoculated seeds + spraying) has indicated maximum increase in essential oil yield (38.58 kg/ha). Biofertilizer, promoted essential oil yield through the enhancement of yield attributes such as; essential oil content and seed yield. These result are in agreement with the investigation of Abdou *et al.* (2004) and Mahfouz and Sharaf Eldin (2007) on *Foeniculum vulgare* [6, 9], Swaminathan *et al.* (2008) and Kumar *et al.* (2009) on *Artemisia pallens* [11, 12], Koochehi *et al.* (2009) on *Hyssopus officinalis* [14], Saeid Nejad and Rezvani Moghaddam (2010) on *Cuminum cyminum* [36], Valadabadi and Farahani (2011) on *Nigella sativa* [15] and Darzi *et al.* (2012) on *Anethum graveolens* [16].

Plant density showed significant effect on essential oil yield (Figure 4), as the highest essential oil yield (32.41 kg/ha) was obtained in 25 plants m<sup>-2</sup>. Increased essential oil yield at the second and the third treatments levels of Plant density (16.6 and 25 plants m<sup>-2</sup>) can be owing to the improvement of some yield components such as; seed yield. This finding is in accordance with the previous observations [17, 20, 23, 24].

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