Effects of Nitroxin and plant density on grain yield and yield components of black cumin (*Nigella sativa*)

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ABSTRACT: In order to study the effect of nitroxin and plant density on grain yield and yield components of black cumin (*Nigella sativa*), 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 Firouzkouh of Iran in 2011. The factors were nitroxin (biofertilizer), 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⁻²). Results showed that the highest plant height, capsule number per plant, grain number per capsule, dry weight of plant and seed 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 studied traits except weight of 1000 grains. The maximum capsule number per plant and dry weight of plant were obtained in 12.5 plants m⁻², the highest grain number per capsule were obtained in 16.6 plants m⁻², and The maximum plant height and grain yield were obtained in 25 plants m⁻².

Key Words: Black cumin, Azotobacter, Azospirillum, Plant density, Grain yield.

INTRODUCTION

Black cumin (*Nigella sativa* L.) belonging to the Ranunculaceae family, is annual species that has originated from arid and semi-arid zones such as Iran and are used widely in traditional and industrial pharmacology. It is reported that intact black cumin seeds or their extracts contain anti-diabetic, antihistaminic, antihypertensive, anti-inflammatory, anti-microbial, antitumour, galactagogue and insect repellent effects (D’Antuno et al., 2002; Bannayan et al., 2008). Applying of biofertilizers such as Nitroxin (contain 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 (Youssef et al., 2004; Mahfouz and Sharaf Eldin, 2007). 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 Ghaban et al., 2006; Mahfouz and Sharaf Eldin, 2007). 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 huge 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 growth and yield in a few medicinal plants such as coriander (Kumar et al., 2002; Darzi et al., 2012), cerely (Migahed et al., 2004), black cumin (Shaalan, 2005; Valadabadi and Farahani, 2011), fennel (Tehlan et al., 2004; Abdou et al., 2004; Mahfouz and Sharaf Eldin, 2007) and hyssop (Koocheki et al., 2009).

Some other studies have reported that suitable plant density can increase the growth and yield of some medicinal plants such as fennel (Darzi et al., 2001), safflower (Dadashi, 2001), coriander (Bhati, 1995; Mvcicar et al., 2004; Akbarinia et al., 2005), fenugreek (Singh et al., 2005) and anise (Rasam et al., 2007).

Therefore, the main objective of the present field experiment was to investigate the effects of Nitroxin and plant density on grain yield and yield components of black cumin (*Nigella sativa*).
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 Nitroxin (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⁻²). Inoculation was carried out by dipping the black cumin seeds in the cells suspension of 10⁸ 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. Black cumin seeds were directly sown by hand. There was no incidence of pest or disease on black cumin 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.

Data were recorded for the plant height, capsule number per plant, grain number per capsule, weight of 1000 grains, dry weight of plant and grain yield. Fifteen plants were randomly selected from each plot and the observations were recorded. At the beginning of flowering, the plant height, from plant base to the tip of plant, was measured for each plot using a ruler (±0.1 cm) (Darzi et al., 2007; Azizi et al., 2008). Capsule number per plant and grain number per capsule was recorded at the end of growth season. In addition, the weight of 1000 grains was also determined. For evaluating the dry weight of plant, 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) (Kapoor et al., 2004). In order to determine grain yield, the plots were manually harvested and then the grains were removed from plants by hand.

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

<table>
<thead>
<tr>
<th>Cu (mg/kg)</th>
<th>Fe (mg/kg)</th>
<th>K (mg/kg)</th>
<th>P (mg/kg)</th>
<th>N (%)</th>
<th>O.C (%)</th>
<th>EC (ds/m)</th>
<th>pH</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42</td>
<td>3.18</td>
<td>400</td>
<td>30</td>
<td>0.14</td>
<td>0.65</td>
<td>1.02</td>
<td>7.48</td>
<td>Clay-Loamy</td>
</tr>
</tbody>
</table>

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

Plant height

The present results have indicated that plant height were significantly affected by the application of Nitroxin (Table 2). The most significant plant height (47.5 cm) was obtained in twice using of Nitroxin (inoculated seeds + spraying on the plant base at stem elongation stage). According to the present analysis, Nitroxin have increased plant height by enhancing the nitrogen content and the rate of photosynthesis (Fatma et al., 2006). The present result were derived from the improvement of nitrogen fixing bacteria’ activities in soil, which is in agreement with the previous studies carried out on the fennel, cerely, black cumin and hyssop (Tehlan et al., 2004; Migahed et al., 2004; Shaalan, 2005; Koocheki et al., 2009).

Plant density had also a significant effect on plant height (Figure1), as higher plant height (45 cm) was recorded in 25 plants m⁻². High plant density resulted in greater plant height, that is in accordance with the observations Dadashi (2001), Purcell et al. (2002) and Singh et al. (2005) on safflower, soybean and fenugreek respectively.
Capsule number per plant

The results presented in Table 2 have demonstrated that capsule number per plant was influenced by the application of Nitroxin, significantly. Among various treatments, treatment of inoculated seeds together spraying on the plant base at stem elongation stage has indicated maximum increase in capsule number per plant (63.5). Nitroxin has significantly influenced the capsule number per plant. On the other hand, nitrogen fixing bacteria application through the improvement of biological activities of soil and mineral element absorption, caused more biomass production and capsule number. These findings are in accordance with the observations Tehlan et al. (2004) on Foeniculum vulgare, Migahed et al. (2004) on Apium graveolens, Shaalan (2005) on Nigella sativa and Darzi et al. (2012) on Coriandrum sativum.

Plant density had also a significant effect on capsule number per plant (Figure 2), as higher umbel number per plant was recorded in two treatments of 12.5 plants m⁻² (56.7) and 16.6 plants m⁻² (56.5). Plant density has significantly influenced on the capsule number per plant, as With increase density and competition, decreased capsule number per plant. The present result is in agreement with the report of Bhati (1995) on Coriandrum sativum.

Grain number per capsule

The present results have indicated that grain number per capsule were significantly affected by the application of Nitroxin (Table 2). The most grain number per capsule (142.7) was obtained in twice using of Nitroxin (inoculated seeds + spraying on the plant base at stem elongation stage). Azotobacter and azospirillum application through the improvement of biological activities of soil and mineral element absorption, caused more biomass production and grain number per capsule.

Plant density had also a significant effect on grain number per capsule (Figure3), as higher grain number per capsule (138.3) was recorded in 16.6 plants m⁻². Plant density has significantly influenced on the grain number per capsule, as With increase density and competition, increased grain number per capsule.

Weight of 1000 grains

The results indicated that weight of 1000 grains was significantly affected by the application of Nitroxin (Table 2). The highest weight of 1000 grains was obtained with applying twice using of Nitroxin (1.50 g). Nitroxin has increased weight of 1000 seeds by the biomass production improvement (Roy and Singh, 2006). The present result is in agreement with the report of Darzi et al. (2007) on fennel.

The present results showed that Plant density had not significant effect on weight of 1000 grains (Figure 4).

Dry weight of plant

The results have indicated that dry weight of plant was affected by the application of Nitroxin (Table 2). Significant increase in dry weight of plant was observed in levels of biofertilizer application. The highest dry weight of plant was obtained in treatment of inoculated seeds together spraying on the plant base at stem elongation stage (55.24 g). Effect of Nitroxin on the dry weight of plant was due to increased nitrogen uptake and the growth rate improvement (Vande Broek, 1999). The result of present work are in agreement with the reports of Youssef et al. (2004) on Salvia officinalis, Kumar et al. (2009) on Artemisia pallens and Valadabadi and Farahani (2011) on Nigella sativa.

Plant density showed significant effect on dry weight of plant (Figure 5), as the highest dry weight of plant (53.25 g) was obtained in 12.5 plants m⁻². The results clearly demonstrate the effectiveness of plant density in the dry weight of plant, as Plants at low density (12.5 plant m⁻²) had more extensive branching and umbel development and greater dry weight. This finding is in accordance with the previous observations (Zahoor, 1991; Vergara et al., 1998; Rasam et al., 2007).

Grain yield

The results presented in Table 2 have revealed that different levels of Nitroxin had significant effects on the grain yield. The maximum grain yield (1337.7 kg/ha) was obtained in the fourth treatment level of Nitroxin (inoculated seeds + spraying). Increased seed yield in Nitroxin treatments can be owing to the improvement of yield components such as; capsule number per plant, grain number per capsule and dry weight of plant. These result are in agreement with the investigation of Kumar et al. (2002) and Darzi et al. (2012) on Coriandrum sativum, Migahed

Significant difference in grain yield was observed in various levels of plant density (Figure 6). The highest grain yield (1583.3 kg/ha) was obtained in 25 plants m\(^{-2}\). The comparison of plant density showed that maximum density (25 plant m\(^{-2}\)) produced the highest grain yield. Akbarinia et al. (2005) reported that 30 plants m\(^{-2}\) in spring grown obtained the most of grain yield in coriander. Present result is in agreement with the investigation of Darzi et al. (2001) on Foeniculum vulgare, Mcvicar et al. (2004) on Coriandrum sativum, Gowda et al. (2006) on Trigonella foenum gracum and Rasam et al. (2007) on Pimpinella anisum.

Table 2. Mean comparison of the some traits of black cumin at various levels of Nitroxin

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>capsule number per plant</th>
<th>Grain number per capsule</th>
<th>Weight of 1000 grains (g)</th>
<th>Dry weight of plant (g)</th>
<th>Grain yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b1</td>
<td>36.2 c</td>
<td>47.6 c</td>
<td>126 c</td>
<td>1.39 b</td>
<td>40.25 c</td>
<td>924.2 c</td>
</tr>
<tr>
<td>b2</td>
<td>42.7 b</td>
<td>53.9 b</td>
<td>131 bc</td>
<td>1.44 ab</td>
<td>46.85 b</td>
<td>1130 b</td>
</tr>
<tr>
<td>b3</td>
<td>41.9 b</td>
<td>55.7 b</td>
<td>135.6 ab</td>
<td>1.45 ab</td>
<td>47.08 b</td>
<td>1066.3 b</td>
</tr>
<tr>
<td>b4</td>
<td>47.5 a</td>
<td>63.5 a</td>
<td>142.7 a</td>
<td>1.50 a</td>
<td>55.24 a</td>
<td>1337.7 a</td>
</tr>
</tbody>
</table>

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.

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Figure 2. Mean comparison for capsule number per plant in different levels of plant density

Figure 3. Mean comparison for grain number per capsule in different levels of plant density
Figure 4. Mean comparison for weight of 1000 grains in different levels of plant density

Figure 5. Mean comparison for dry weight of plant in different levels of plant density
Figure 6. Mean comparison for Grain yield in different levels of plant density

REFERENCES


