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Original Article

Synergistic Effects of Plant Growth-Promoting Rhizobacteria (PGPR) and Planting Density on Agronomic Performance and Seed Yield of Ajowan (*Carum copticum* L.) Under Field Conditions

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ABSTRACT

To investigate the effects of plant growth-promoting rhizobacteria (PGPR) and plant density on yield components and seed yield of ajowan (*Carum copticum*), a factorial experiment based on a randomized complete block design (RCBD) with twelve treatments and three replications was conducted in 2018. The experimental factors included PGPR, specifically a consortium of *Azotobacter chroococcum* and *Azospirillum lipoferum*, applied at four levels: non-inoculated control, seed inoculation, foliar application at the stem elongation stage, and a combination of seed inoculation and foliar application at the same stage. Plant density was evaluated at three levels: 12.5, 16.6, and 25 plants per square meter. The results demonstrated that PGPR application significantly influenced all measured traits except plant height and thousand-seed weight. The highest values for umbel number per plant, plant dry weight, and seed yield were achieved with the combined treatment of seed inoculation and foliar application. Plant density also had a significant effect on the studied traits, with the exception of thousand-seed weight. The maximum umbel number per plant and plant dry weight were observed at the lowest density (12.5 plants m^{-2}), while the greatest plant height and seed yield were obtained at the highest density (25 plants m^{-2}).

1. Introduction

The application of biofertilizers, particularly nitrogen-fixing bacteria, has significantly reduced the reliance on chemical fertilizers and contributed to the production of high-quality crops free from harmful agrochemicals, thereby enhancing food safety for human consumption (Mahfouz and Sharaf Eldin, 2007; Moradi et al., 2010). Vermicomposts are organic materials resulting from the decomposition of organic matter through the synergistic activity of earthworms and microorganisms. Free-living nitrogen-fixing bacteria such as *Azotobacter chroococcum* and *Azospirillum lipoferum* not only fix atmospheric nitrogen but also synthesize phytohormones analogous to gibberellic acid and indole-3-acetic acid, which promote plant growth, nutrient uptake, and photosynthetic efficiency (El Ghadban et al., 2006; Mahfouz and Sharaf Eldin, 2007).

In recent years, interest in plant growth-promoting rhizobacteria (PGPR) has grown substantially due to their potential application as eco-friendly biofertilizers (Richardson et al., 2009; Compant et al., 2010). Moreover, the escalating costs of synthetic fertilizers and pesticides have positioned biofertilizers as a sustainable alternative. In Asia, intensive agricultural practices often involve excessive application of chemical fertilizers and herbicides, with their usage increasing markedly over the past five decades (Park et al., 2005; Adesemoye and Kloepper, 2009; Jin, 2012). Despite their short-term benefits in enhancing crop yields, the prolonged use of such inputs has proven detrimental to soil health and poses risks to human and animal well-being. As a result, current agricultural trends are shifting toward biotechnological approaches that support crop productivity while maintaining soil fertility (Fernando et al., 2005).

PGPR facilitate plant growth through multiple mechanisms, including biological nitrogen fixation, phytohormone production (e.g., auxins, gibberellins, cytokinins), suppression of phytopathogens, siderophore synthesis, and enhancement of nutrient availability in the rhizosphere (Islam et al., 2009; Dobbelaere et al., 2003; Beneduzi et al., 2008). Numerous studies have demonstrated the direct and indirect plant growth-promoting effects of these bacteria via such synergistic mechanisms.

The cultivation of medicinal plants is primarily conducted within sustainable agricultural systems, where precise environmental management is crucial. The use of appropriate nutrient sources, particularly biofertilizers, can significantly improve both the quantitative and qualitative traits of medicinal plant yields. *Anethum graveolens* (dill) is an annual herbaceous plant native to the Mediterranean region. Dill fruits and leaves are commonly used as flavoring agents in sauces, vinegars, pastries, and soups. The essential oil extracted from dill fruits, particularly its major constituent carvone, is widely utilized in the pharmaceutical industry as a diuretic, stimulant, and carminative (Bailer et al., 2001; Callan et al., 2007; Hassan et al., 2010).

Several investigations have reported that vermicompost enhances growth and yield in various medicinal plants, including basil (Singh and Ramesh, 2002; Anwar et al., 2005), garlic (Arguello et al., 2006), plantain (Sanchez et al., 2008), coriander (Singh et al., 2009), fennel (Darzi et al., 2007; Moradi et al., 2010), chamomile (Haj Seyed Hadi et al., 2011), cumin (Saeid Nejad and Rezvani Moghaddam, 2011), and anise (Darzi et al., 2012). Similarly, nitrogen-fixing bacteria such as *A. chroococcum* and *A. lipoferum* have been shown to enhance the growth and productivity of medicinal plants like coriander (Kumar et al., 2002), celery (Migahed et al., 2004), fennel (Abdou et al., 2004; Mahfouz and Sharaf Eldin, 2007), turmeric (Velmurugan et al., 2008), hyssop (Koocheki et al., 2009), and black cumin (Valadabadi and Farahani, 2011).

Therefore, the principal objective of the present field experiment was to evaluate the effects of organic manure and biofertilizers on the fruit yield and its components in dill (*Anethum graveolens*).

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In recent years, interest in plant growth-promoting rhizobacteria (PGPR) has grown substantially due to their potential application as eco-friendly biofertilizers (Richardson et al., 2009; Compant et al., 2010). Moreover, the escalating costs of synthetic fertilizers and pesticides have positioned biofertilizers as a sustainable alternative. In Asia, intensive agricultural practices often involve excessive application of chemical fertilizers and herbicides, with their usage increasing markedly over the past five decades (Park et al., 2005; Adesemoye and Kloepper, 2009; Jin, 2012). Despite their short-term benefits in enhancing crop yields, the prolonged use of such inputs has proven detrimental to soil health and poses risks to human and animal well-being. As a result, current agricultural trends are shifting toward biotechnological approaches that support crop productivity while maintaining soil fertility (Fernando et al., 2005).

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and biofertilizers on the fruit yield and its components in dill (*Anethum graveolens*).

2. Materials and Methods

2.1 Field Experiment

A factorial field experiment based on a randomized complete block design (RCBD) with three replications was conducted in Iran during the 2018 growing season. The treatments included: Plant growth-promoting rhizobacteria (PGPR) – a combination of *Azotobacter chroococcum* and *Azospirillum lipoferum* applied in four levels:

Control (non-inoculated), Seed inoculation, Soil drench at the stem elongation stage, Combined treatment (seed inoculation + soil drench at the stem elongation stage).

Plant density – applied at three levels: 12.5, 16.6, and 25 plants per square meter (plants m^{-2}).

For bacterial inoculation, ajowan seeds were immersed in a bacterial suspension with a concentration of 10^8 CFU/mL for 15 minutes. Prior to land preparation, composite soil samples were collected from a depth of 0–30 cm to determine the physicochemical characteristics. The results of the soil analysis are presented in Table 1.

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

Based on soil test recommendations, nitrogen (50 kg ha^{-1}) and phosphorus (20 kg ha^{-1}) were applied before sowing. Each experimental plot measured 3 m in length and 2 m in width, with a 40 cm row spacing. A 1-meter buffer was maintained between plots, and a 2-meter space was maintained between replications. Ajowan (*Carum copticum*) seeds were hand-sown directly into the soil. There were no recorded incidences of pest or disease during the experiment. Manual weeding was performed as necessary, and irrigation was applied weekly. Standard agronomic and plant protection practices were uniformly implemented across all plots throughout the growing season.

2.2 Soil and Agronomic Management

Prior to land preparation, composite soil samples (0–30 cm depth) were collected and analyzed for physicochemical properties, including texture, organic matter, pH, EC, and macronutrients. The results are presented in Table 1.

Based on the soil analysis, nitrogen (50 kg ha^{-1}) and phosphorus (20 kg ha^{-1}) fertilizers were applied uniformly before sowing. Each plot measured 3 m × 2 m with 40 cm row spacing. A buffer zone of 1 m was maintained between plots and 2 m between replications.

Ajowan seeds were manually sown. No significant incidence of pests or diseases was observed during the experiment. All weeding operations were carried out manually, and irrigation was performed weekly. All other agronomic and plant protection practices were maintained uniformly across the experimental plots.

2.3 Data Collection

Data were recorded for the following agronomic traits:

- Plant height (cm) – measured at the onset of flowering from the base to the apex of randomly selected 15 plants per plot using a calibrated ruler (±0.1 cm), as described by Darzi et al. (2007) and Azizi et al. (2008).
- Number of umbels per plant – recorded at physiological maturity.
- Thousand-seed weight (g) – determined by weighing 1000 air-dried seeds using a precision digital balance (Sartorius B310S; ±0.01 g).
- Plant dry weight (g) – samples were oven-dried at 80°C for 48 hours prior to weighing.
- Seed yield (kg ha^{-1}) – obtained by manual harvesting and threshing of each plot, followed by seed separation.

2.4 Statistical Analysis

All collected data were statistically analyzed using one-way analysis of variance (ANOVA) with SAS software (version 8.0; SAS Institute Inc., 2001). Duncan's Multiple Range Test (DMRT) was used to compare treatment means at a 5% level of significance ($p < 0.05$). Prior to analysis, data were tested for normality and homogeneity of variance. When required, appropriate transformations (e.g., log or square root) were applied to normalize data and ensure valid ANOVA assumptions, as recommended by Zar (1996).

3. Results

3.1 Plant Height

According to the results presented in Table 2, the application of plant growth-promoting rhizobacteria (PGPR) had no statistically significant effect on plant height. However, plant density showed a significant influence on this trait (Figure 1). The highest mean plant height (81.9 cm) was observed at a density of 25 plants m^{-2}, indicating that higher intra-specific competition may have promoted vertical growth.

Table 2. Mean comparison of the some characteristics of ajowan at various levels of plant growth promoter bacteria

Treatments	Plant height (cm)	Umbel number per plant	Weight of 1000 seeds (g)	Dry weight of plant (g)	Seed yield (kg/ha)
Plant growth promoter bacteria					
b1	68.0 a	54.9 b	1.04 a	50.13 b	896.5 c
b2	75.2 a	63.9 a	1.12 a	54.66 b	1084.4 b
b3	70.8 a	66.5 a	1.21 a	54.73 b	1204.5 b
b4	71.9 a	66.7 a	1.18 a	62.21 a	1376.0 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.

3.2 Number of Umbels per Plant

As shown in Table 2, the number of umbels per plant was significantly affected by the application of PGPR. Among the treatments, foliar spraying at the stem elongation stage (66.5 umbels per plant) and the combined treatment of seed inoculation plus foliar spraying (66.7 umbels per plant) resulted in the highest values, demonstrating the synergistic effect of dual inoculation.

Plant density also had a significant effect on this trait (Figure 2). The highest umbel number per plant was recorded under densities of 12.5 and 16.6 plants m^{-2}, suggesting that lower planting densities may facilitate better branching and reproductive development due to reduced competition for light and nutrients.

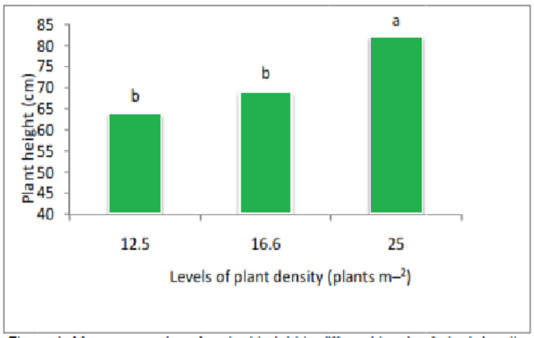

Figure 1. Mean comparison for plant height in different levels of plant density

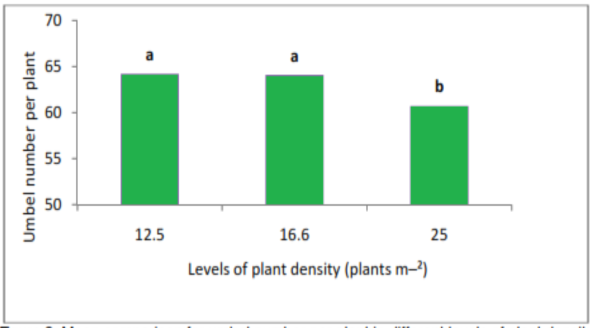

Figure 2. Mean comparison for umbel number per plant in different levels of plant density

3.3 Thousand Seed Weight

The results showed that the weight of 1000 seeds was not significantly influenced by either PGPR treatments or plant density (Table 2). This indicates that seed mass was genetically stable and less responsive to changes in microbial treatment and spacing under the experimental conditions.

3.4 Plant Dry Weight

Application of PGPR had a statistically significant effect on the dry weight of ajowan plants (Table 2). The highest plant biomass (62.2 g) was observed in the treatment involving both seed inoculation and foliar spraying at the stem elongation stage, highlighting the role of PGPR in enhancing plant vigor and biomass accumulation.

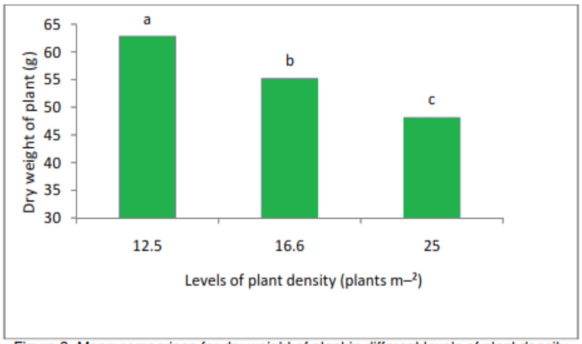

Figure 3. Mean comparison for dry weight of plant in different levels of plant density

3.5 Seed Yield

The data presented in Table 2 indicate that seed yield was significantly affected by both PGPR application and plant density. The highest seed yield was recorded in the treatment where seeds were inoculated and the plant base was sprayed during the stem elongation stage, confirming the additive effect of dual microbial applications on seed productivity.

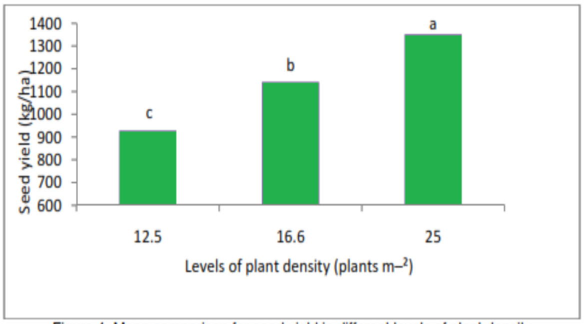

Figure 4. Mean comparison for seed yield in different levels of plant density

Plant density also influenced seed yield significantly. The maximum yield (1351.6 kg ha^{-1}) was achieved at the highest planting density (25 plants m^{-2}), likely due to increased total biomass and reproductive unit per unit area despite lower per-plant performance.

4. Discussion

According to the present analysis, vermicompost with high water-holding capacity and proper supply of macro- and micro-nutrients (Cavender et al, 2003; Arancon et al., 2006; Kumawat et al., 2006), has a positive effect on biomass production and subsequently enhanced plant height. Improved growth, development and height of medicinal plants and other crops have previously been reported in the presence of optimal amounts of vermicompost (Singh and Ramesh, 2002; Arguello et al., 2006; Darzi et al., 2012; Haj Seyed Hadi et al., 2011). According to the present analysis, biofertilizer increased plant height by enhancing the nitrogen content and the rate of photosynthesis (Migahed et al., 2004). The present result were derived from the improvement of nitrogen fixing bacteria' activities in soil at the third treatment level (inoculated seed + spraying on plant base at stem elangation stage), which are in agreement with the previous studies carried out on fennel, turmeric and hyssop (Mahfouz and Sharaf Eldin, 2007; Velmurugan et al., 2008; Koocheki et al., 2009).

Vermicompost significantly influenced flowering and umbel number per plant. On the other hand, vermicompost application through the improvement of biological activities of soil and mineral element absorption (Jat and Ahlawat, 2006; Zaller, 2007), caused more biomass production and umbel number. These findings are in accordance with the observations of Pandey (2005) on *Artemisia pallens*, Moradi et al. (2010) on *Foeniculum vulgare*, Saeid Nejad and Rezvani

Moghaddam (2011) on cumin and Darzi et al. (2012) on *Pimpinella anisum*.

According to the present analysis, vermicompost have increased weight of 1000 seeds by enhancing the rate of photosynthesis and the biomass production (Roy and Singh, 2006). The present result is in agreement with the report of Darzi et al. (2007) on *F. vulgare*.

The results clearly demonstrate the effectiveness of vermicompost in increasing the biomass yield. Vermicompost increases the growth rate because of the water and mineral uptake such as; nitrogen and phosphorus (Arancon et al., 2006; Zaller, 2007), which leads to the biomass yield improvement. This finding is in accordance with previous observations (Anwar et al., 2005; Moradi et al. 2010; Darzi et al. 2012; Saeid Nejad and Rezvani Moghaddam, 2011). Effect of nitrogen fixing bacteria on the biomass yield was due to increased nitrogen uptake (Mahfouz and Sharaf Eldin, 2007; Kalyanasundaram et al., 2008). The result of present work are in agreement with the reports of Swaminathan et al. (2008) and Kumar et al. (2009) on *A. pallens* and Valadabadi and Farahani (2011) on *Nigella sativa*.

Increased fruit yield in vermicompost treatments can be as a result of improvement of yield components such as; plant height, umbel number per plant and biomass yield. Our findings are in accordance with the observations of Saeid Nejad and Rezvani Moghaddam (2008), Sanchez et al. (2008), Singh et al. (2009), Moradi et al. (2010) and Darzi et al. (2012). Biofertilizer (nitrogen fixing bacteria), promoted fruit yield through the enhancement of yield attributes. These results are in agreement with the investigation of Kumar et al. (2002) on *Coriandrum sativum*, Migahed et al. (2004) on *Apium graveolens*, Abdou et al. (2004) and Mahfouz and Sharaf Eldin (2007) on *F. vulgare* and Valadabadi and Farahani (2011) on *N. sativa*. The results likely shows that the positive and synergistic effect of interaction between two factors on fruit yield is highly dependent on the effect of organic matter, contained in vermicompost, on the activity of nitrogen fixing bacteria.

5. Conclusion

It is clear from the present study that vermicompost and biofertilizer successfully manipulate the growth of dill, resulting in beneficial changes in yield and yield components. The highest fruit yield was obtained by using 8 ton vermicompost per hectare. Maximum fruit yield was observed twice by using nitrogen fixing bacteria (inoculated seeds + spraying on the plant base at stem elongation stage). Thus, combined application of vermicompost and nitrogen fixing bacteria can be helpful in the development, production and yield in dill.

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