Effect of bio-fertilizers on performance of Dill (*Anethum graveolens* L.)

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Abstract

Biological fertilizers are compounds comprising various free living microorganisms that are able to change the basic nutrients into available form through biological procedures. Being an important source of nutrients in sustainable agriculture, these fertilizers lead to better seed germination and development of root system. In order to investigate the effect of biological fertilizers on the medicinal plant, dill *(Anethum graveolens*), an experiment was conducted in completely randomized blocks with 3 replications using 4 treatments in Lueen plains in Saveh, central Iran. Treatments included nitroxin (Azospirillum and Azotobacter), *Pseudomonas florescence* containing phosphate solubilizing bacteria, mixture of nitroxin and *Pseudomonas florescence* fertilizers, and control (no fertilizer). Findings suggested that application of biological fertilizers significantly increased seeds performance, biological yield per plant, plant height, number of spikes per plant, number of seeds per spike, number of seeds per plant, and the weight of 1000 seeds. Among the treated plants, mixed treatment of nitroxin and *Pseudomonas florescence* caused maximum increase in most of the parameters under study. This was followed by nitroxin treatment which resulted in the second most pronounced difference with the control. The minimum and maximum seed performances were observed in mixed treatment of nitroxin and *Pseudomonas florescence* (16.31 g per plant) and control plants (9.8 g per plant), respectively. In general, results of the present study revealed that application of biological fertilizers plays a remarkable role in improving growth characteristics and yield compounds of *Anethum graveolens* and they can be viewed as a suitable replacement for chemical fertilizers.

Keywords: phosphate dissolving bacteria; *Anethum graveolens*; nitroxin performance

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Introduction

Biological fertilizers are complex of some microorganisms that mobilize main nutrients from unavailable form to available form and can improve root system and seed germination. Presently, these fertilizers are considered as a replacement for chemical fertilizers to improve soil fertility and crop production in sustainable agriculture which is based on ecologic principles. In this system, instead of external input such as chemical fertilizers and pesticides, vegetal residues, manure, organic and biologic fertilizers together with biologic pest controls are utilized to supplement the soil nutrients, control weeds and pests and, at the same time, increase biodiversity of the farm (Elsen, 2000).

Regarding the importance of medicinal plants and their role in human health, it is imperative to increase their biomass without application of harmful chemical fertilizers,

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Table 1
Physical and chemical properties of the soil in the experiment field

Tissue	Analysis of Particle Size			Available Phosphorous (ppm)	Available Potassium (ppm)	Percent of Total Nitrogen %N	Percent of Organic Matter (%OM)	Percent of Carbonate Calcium (%TNV)	PH	EC ds/m	Percent of Saturated Humidity (SP)
	Clay %	Silt %	Sand %								
S. C. L.	28	18	54	9.6	215	0.1	1.2	19.4	7.1	1.56	38

pesticides and herbicides. The most important advantages of growth promoting bacteria include production of growth inducing and regulating hormones, development of root system, improving water and nutrients uptake (Kravchenko et al., 1994), improving seed germination and generation of plantlets (Kloepper et al., 1998), interaction effect with rhizobiums, improving availability of phosphorous to the plants, biological fixation of nitrogen (Ishizuka, 2002), generation of ionophores especially siderophores and production of some antibiotic compounds such as bacteriocins to control infections (Tapia-Hernandez et al., 1990). Three most important growth inducing bacteria that are presently considered in crop production include Azospirillum and Azotobacter which fixate nitrogen, and Pseudomonas which solubilize phosphate.

Studies on symbiotic relationship between bacteria and plants have been mainly on cereals and grassy plants and only a few studies have been carried out on medicinal plants. Basil, artemisia *Nigella sativa* L, *Foeniculum vulgare* Mill and *Majorana hortensis* are the medicinal plants on which the effect of biological fertilizers have been studied. In a study on the medicinal plant *Scutellaria integrifolia*, inoculation of mycorrhiza increased root length and general plant growth in low phosphorous soils (Joshee et al., 2007). Inoculation of Azotobacter in *Rosmarinus officinalis* increased concentration of plant essence (Leithy et al., 2006). Application of bio-fertilizers Azospirillum and Azotobacter in the medicinal plant Salvia officinalis was reported to increase plant height and shoot dry and wet weights (Vande broek, 1999). In another study, inoculation of Caharanthus roseus plantlets with *Pseudomonas florescence* bacteria increased plant biomass and alkaloids levels in the plant under water stress (Abdul-Jaleel et al., 1991). Also a study by Ratti et al. (2001) showed that simultaneous application of mycorrhiza fungus with Azospirillum and bacillus increased biomass in the medicinal plant Cymbopogon maritinii. Moreover, in Thymus vulgaris, application of biological fertilizers made a significant increase in the plant growth (Youssef et al., 2004).

Dill (*Anethum graveolens* L.) is an aromatic annual grassy plant belonging to the Umbelliferae family that originally comes from Eastern Mediterranean. The entire vegetative organ contains essence. The most important essential oil compounds in this plant are d-carrone and phellandrene and the most important compounds from the fully grown seeds are d-carrone and limenene. *Anethum graveolens* L. was systematically planted in 14th century for medicinal purposes and as spice in most parts of the world. Europeans have used this plant's vegetative organ and seeds to treat headache and hemato-vascular diseases. *Anethum graveolens* L. is a laxative and carminative agent

Table 2
Analysis of variance for plant height, leaf length, stem diameter, and dry and wet weight plant.

		MEAN SQUARES				
Source of Variability	Degree of Freedom	Plant Height	Leaf Length	Stem Diameter	Wet Weight	Dry Weight
Repetition	2	57.341 ns	8.178 ns	1.203 *	367.261 ns	1.780 ns
Treatment	4	200.952 **	8.001 ns	17.196 **	1779.109 **	47.728 **
Error	8	11.892	8.952	0.172	86.534	1.197

ns: not significant; * and **significant at P≤0.05 and P≤0.01, respectively.

and is used to treat stomachache and digestion problems. An aromatic plant with sweet fragrance, dill is an appetizer with numerous applications in food industry. Considering the necessity of research on alternatives for chemical fertilizers and due to the insufficient studies about the effect of bio-fertilizers on growth and yield of medicinal plants in Iran, the present study aimed at investigation of the effects of bio-fertilizers on growth characteristics and yield of *Anethum graveolens*, and consequently, reducing the application of chemical fertilizers.

Materials and Methods

The experiment was conducted in completely randomized blocks with 3 replications using 4 treatments in Lueen plains in Saveh (49˚54' N and 35˚2' E), 1413 m above sea level in cultivation year 2011. The field was prepared by cultivating and twice perpendicular disc harrowing followed by smoothing with leveler and then making furrows in March. Before sowing, soil samples were collected from various parts of the field and physical and chemical analyses were carried out and the percent of main elements and components were determined (Table 1). Locally available seeds of Varamin cultivar were then sown in 4×2 plots of 6 rows in mid April.

In order to measure dry weight of the plants, samples were harvested and put into numbered paper bags to be sent to laboratory. Samples were oven dried at 50 °C for 72 h and then weighed with digital scale with ±0.01 g error of measurement.

The treatments in the experiment included 1. nitroxin bio-fertilizer (mixture of Azospirillum and Azotobacter), 2. *Pseudomonas florescence* containing phosphate solubilizing bacteria which are able to secrete a large amount of organic acids and phosphatase and therefore are able to release phosphate from minerals and organic compounds, and 3. mixture of nitroxin and *Pseudomonas florescence* fertilizers. Control plants received no fertilizer. Bio-fertilizers were applied after sowing and through irrigation. 5 L/hectare nitroxin was applied based on the recommendations of the manufacturer (Mehr Asia Biologic Technology Co.), through 2nd and 3rd irrigation. Following the recommendations of the manufacturer (Zist Fanavar Co.), one box of *Pseudomonas florescence* was added to 5 liter water and thoroughly mixed and filtered through a thin piece of fabric. The solution was then diluted with 100 liter water and applied through the first irrigation when the plants had 2-3 leaves. The solution was again applied after one month. For the plants treated with mixture of nitroxin and Pseudomonas florescence, the fertilizers were applied according to the relevant application time. Control plants did not receive any bio-fertilizers.

The sowing was done in 4×2 plots with 6 rows in each plot. The rows were set 30 cm apart from each other and thinning was done when plants were in 4-leaf stage so that in each row, the plants were 20 cm apart. During the experiment no herbicides, pesticides or fungicides were applied. In order to supply the plants with nutrients, recommended amount of manure, nitrogen and phosphate fertilizers were applied to the field during its preparation in March (urea: 100 kg per hectare; super phosphate triple: 50 kg per hectare; rotten manure: 10 tons per hectare). Weeding was done at two stages, 20 and 45 days after sowing. Flooding irrigation was done every 7 days.

In order to observe yield compounds before harvesting, the marginal rows in each plot and also plants grown up to 50 cm from the ends of each row were set aside and 10 plants were randomly selected from each plot. Number of seeds in each spike, number of spikes per plant, number of spike in each florescence, dry and wet weight of the plants, seed performance in each plant, and the weight of 1000 seeds were recorded. Finally statistical analysis was carried out using Spss software and the relevant graphs were prepared using Excel. Means were compared using Duncan test ($p \leq 0.05$).

Results

Plant height

The findings are displayed in Table 2. Comparison of the means for various treatments suggests that Pseudomonas florescence, nitroxin, and the mixture of nitroxin and *Pseudomonas florescence* fertilizers resulted in significant differences in the plant height and the highest and lowest plants were observed in the samples treated with the mixture of nitroxin and *Pseudomonas florescence* fertilizers, and control plants, respectively (Fig. I).

Stem diameter

No difference was observed between the stem diameters of the plants treated with nitroxin, and the mixture of nitroxin and *Pseudomonas florescence* fertilizers. On the other hand, the treatment with *Pseudomonas florescence* fertilizer resulted in a significantly longer stem diameter compared with the control. The longest and shortest stem diameters were recorded for the plants treated with the mixture of nitroxin and *Pseudomonas florescence* fertilizers, and control plants, respectively (Fig. II).

Wet plant weight

Comparison of the means of different treatments showed that Pseudomonas florescence, nitroxin, and the mixture of nitroxin and *Pseudomonas florescence* fertilizers resulted in significant differences in the wet weights of *Anethum graveolens* L. Moreover, nitroxin and the mixture of nitroxin and *Pseudomonas florescence* fertilizers were not significantly different regarding the wet plant weight. The highest and lowest wet plant weights were obtained with the mixture of nitroxin and *Pseudomonas florescence* treatment and control plants, respectively (Fig. III).

Dry plant weight

Significant differences were observed between dry plant weight of the plants treated with nitroxin, Pseudomonas florescence, and the mixture of nitroxin and *Pseudomonas florescence* fertilizers. On the other hand, treatment with *Pseudomonas florescence* fertilizer and control were not significantly different considering dry plant weight. The highest and lowest dry weights were observed in the mixture of nitroxin and *Pseudomonas florescence* fertilizers and control, respectively (Fig. IV).

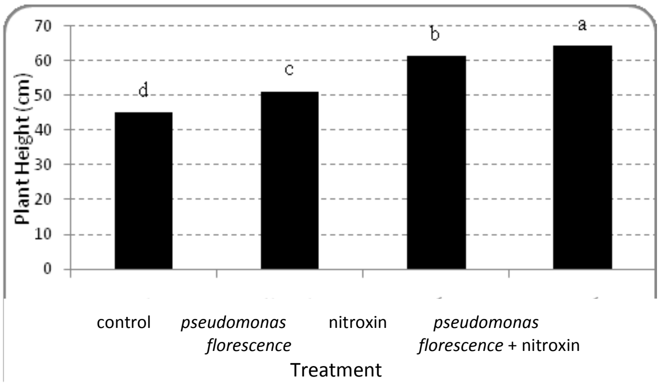

Fig. I. Effect of different treatments on the plant height

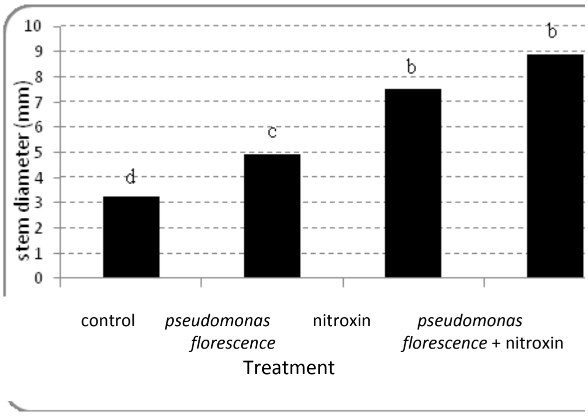

Fig. II. Effect of different treatments on stem diameter

Discussion

Fatma et al. (2006) reported a favorable result for the effect of Azospirillum and Azotobacter, and also phosphate solubilizing bacteria on the medicinal plant, *Majorana Hortensis*. Similarly, Krishna et al. (2008) reported an improvement in germination indexes such as percentage and speed of germination, viability, and also the length of roots and stems of Ocimum sanctum and Withania somniferum treated with Azospirillum and Azotobacter bio-fertilizers, phosphate solubilizing bacteria, nitrogen fixation bacteria, and a combination of these fertilizers.

Many research studies have mentioned the positive effect of microorganisms on improving the growth and performance of medicinal plants. In addition to nitrogen fixation, Azospirillum improves root growth through generation of stimulating compounds and this results in an increase in water and nutrients uptake and the general performance of the plant (Tilak et al., 2005). Subba Rao (1979) reported

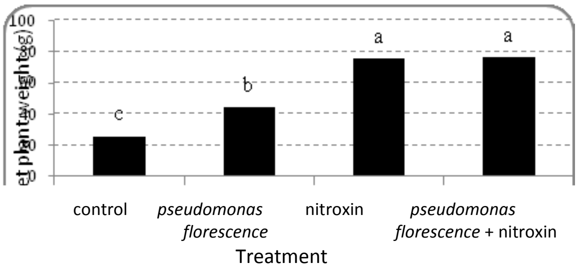

Fig. III. Effect of different treatments on wet plant weight

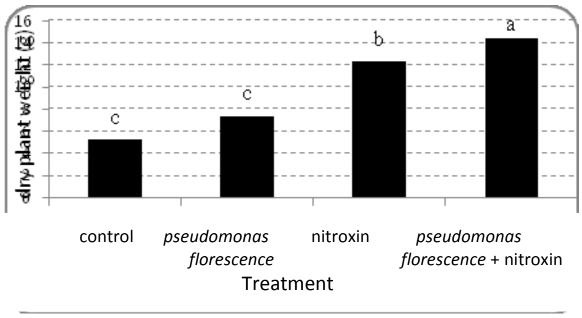

Fig. IV. Effect of different treatments on dry plant weight

that the most important growth stimulating bacteria are Azospirillum, Azotobacter, and Pseudomonas which in addition to biological fixation of nitrogen and solubilizing the soil phosphate, considerably affect plant growth regulators especially auxin, gibberellin and cytokinin and hence improve the plant performance. Azotobacter is able to produce antifungal compounds that fight plant diseases and improve viability and germination of the plantlets and, as a result, improve the overall plant growth (Chen, 2006).

Results of the present study are in agreement with those of Sanches Govin et al. (2005) who reported that application of biologic fertilizers in *Calendula officinallis L.* and *Matricaria recutita L.* improved the performance of the shoots in these medicinal plants. Also Youssef et al. (2004) reported an increase in plant height and dry and wet weight of the shoots in the first and second harvest.

The findings of the present study suggest that application of biological fertilizers had promising effects on dill and this is in agreement with infrequent research studies on the effect of these fertilizers on medicinal plants. Therefore, it is recommended that the mineral nitrogen and phosphate fertilizers be replaced with bio-fertilizers to reduce production costs and stop damages to the environment due to the use of chemical fertilizers especially nitrogen as nitrate.

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