

*Full Length Research Paper*

# Effects of the application of vermicompost and phosphate solubilizing bacterium on the morphological traits and seed yield of anise (*Pimpinella anisum* L.)

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Accepted 19 October, 2011

The main objective of this study was to determine the effects of biofertilizers on the morphological traits and seed yield in the anise plant height, umbel number per plant, weight of 1000 seeds, biological and seed yield. The experiment was carried out at the Hamand Research Station in Damavand in 2009. Vermicompost (0, 5 and 10 ton/ha) and phosphate solubilizing bacterium, *Bacillus circulans* (non-inoculated, inoculated seeds and inoculated seeds + spray on the plant base at stem elongation stage) were used as the effecting parameters. The results of present study demonstrated that the highest plant height, umbel number per plant, biological and seed yield were obtained after applying 10 ton/ha vermicompost. Phosphate solubilizing bacterium also showed significant effects on umbel number per plant, biological yield and seed yield. The maximum umbel number per plant, biological yield and seed yield were obtained using the phosphate solubilizing bacterium twice.

**Key words:** Anise, biofertilizers, vermicompost, seed yield.

## INTRODUCTION

Current trends in agriculture are centered on reducing the use of inorganic fertilizers by organic manure and the application of biofertilizers such as vermicompost and phosphatic biofertilizers (Gyaneshwar et al., 2002; Darzi et al., 2011). Vermicomposts are the products of the degradation of organic matter through interactions between earthworms and microorganisms. Vermicomposts are finely divided peat-like materials with high porosity, aeration, drainage, and water-holding capacity and usually contain most nutrients in the available forms such as nitrates, phosphates, exchangeable calcium and soluble potassium (Atiyeh et al., 2002; Arancon et al., 2004). Phosphate solubilizing microorganisms such as; bacteria and fungi, are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Chen et al., 2006). Production of medicinal plants is mainly under the circumstances of sustainable agricultural system.

In this system, management of environmental parameters is very critical. By using correct nutritional sources through biofertilizers, quantitative and qualitative yield of medicinal plants can be maximized. Anise (*Pimpinella anisum* L., family: Apiaceae) is a herbaceous annual plant, which is native to mediterranean region. It is primarily grown for its fruits, commercially called Anise seeds. The anise seeds have essential oil as an active substance, while is the most important constituent of anise, which is used in pharmaceutical, food, perfumery and flavouring industry (Tuncturk and Yildirim, 2006; Ozkan and Chalchat, 2006).

Several studies showed that vermicompost could increase the growth and yield of some medicinal plants and other crops such as turmeric (Vadiraj et al., 1998), basil (Anwar et al., 2005), garlic (Arguello et al., 2006), geranium (Chand et al., 2007), fennel (Darzi et al., 2007, 2008), chamomile (Azizi et al., 2008), plantain (Sanchez et al., 2008), strawberry (Arancon et al., 2004) and barley (Roy and Singh, 2006). On the other side, phosphate solubilizing bacteria such as *Bacillus* sp and *Pseudomonas* sp could lead to increase the growth and yield of a few medicinal plants such as lemon grass (Ratti

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**Table 1.** Physical and chemical properties of soil on the experimental site.

Cu	Zn	Mn	Fe	Mg	Ca	K	P	N	O.C	EC	pH	Texture
(mg/kg)									(%)	(ds/m)		
1.2	1.2	5.8	4.5	-	-	400	14	9.0	0.76	0.89	7.7	Loamy-Clay

\* Available form of nutrients was measured.

**Table 2.** Chemical characteristics of vermicompost.

Cu	Zn	Mn	Fe	Mg	Ca	K	P	N	O.C	EC	pH
(mg/kg)									(%)	(ds/m)	
0.0079	0.0015	0.067	0.98	1.47	3.89	0.65	0.4	1.2	10.6	2.55	7.1

et al., 2001), black cumin, borage (Shalan, 2005a, b), fennel (Darzi et al., 2007, 2008), basil (Rashmi et al., 2008) and other crops (Singh and Kapoor, 1998).

Keeping in view the nutritional properties, medicinal importance, and common use of anise and its chemical constituents, the present study was designed to investigate the effects of vermicompost and phosphate solubilizing bacterium on the morphological traits and seed yield in anise plant which was previously not well investigated. The results of the present study are presented in current communication.

## MATERIALS AND METHODS

### Field experiment

A 3x3 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 2009 to 2010. The geographical location of the experimental station was 35° 39' N and 52° 5' E with the altitude of 1800 m. The treatments consisted of different concentrations of vermicompost (0, 5, 10 ton/ha) and different inoculation conditions of phosphate solubilizing bacterium (non-inoculated, seed inoculated and seed inoculated + spraying on the plant base at stem elongation stage). *Bacillus circulans* was used as the test microorganism.

The bacterial cells were earlier isolated from soil and tested for phosphate solubilization under *in vitro* conditions using tricalcium phosphate as P source. Inoculum was prepared in Pikovskaya culture medium (Singh and Kapoor, 1998) at 30°C for 5 days using tricalcium phosphate as a P source. Inoculation was carried out by dipping the anise seeds in the cells suspension of 10<sup>8</sup> CFU/ml for 10 min. The vermicompost was prepared from cow dung by employing epigeic species of *Eisenia foetida*. The required quantities of vermicompost were applied and incorporated to the top 5 cm layer of soil in the experimental beds before the plantation of anise seeds. Several soil samples (0 to 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 vermicompost are presented in Tables 1 and 2. Nitrogen (20 kg/ha) was applied to the plots, based on the soil and vermicompost analysis, in the stem elongation stage.

Each experimental plot was 3 m long and 1.5 m wide with the spacing of 10 cm between the plants and 30 cm between the rows.

There was a space of one meter between the plots and two meters between replications. Anise seeds were directly sown by hand into the field at a rate of 15 kg ha<sup>-1</sup> to a depth of 3 cm. There was no incidence of pest or disease on anise 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, umbel number per plant, weight of 1000 seeds, biological yield and seed 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 ( $\pm 0.1$  cm) (Darzi et al., 2007; Azizi et al., 2008). Umbel number per plant was recorded at the end of growth season.

In addition, the weight of 1000 seeds was also determined. 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;  $\pm 0.01$  g) (Kapoor et al., 2004). In order to determine seed yield, the plots were manually harvested following the air-drying of umbels at 22 to 28°C and then the seeds were removed from plants by hand (Zheljzakov et al., 2008).

### 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

### Plant height

The present results have indicated that plant heights were significantly affected by the application of vermicompost (Table 3). The most significant plant height (50.1 cm) was obtained by applying 10 ton vermicompost per hectare. Phosphate solubilizing bacterium did not show significant effect on this trait. However, using bacterium twice (inoculated seeds + spraying on the plant base at stem elongation stage) caused more plant height

**Table 3.** Mean comparison of the quantitative characteristics of anise at various levels of vermicompost and phosphate solubilizing bacterium.

Treatments	Plant height (cm)	Umbel no./plant	Weight of 1000 seeds (g)	Biological yield (kg/ha)	Seed yield (kg/ha)
Vermicompost (ton/ha)					
V1	41.2 <sup>c</sup>	19.2 <sup>c</sup>	2.11 <sup>a</sup>	4507.3 <sup>c</sup>	1576.7 <sup>c</sup>
V2	46.3 <sup>b</sup>	27.7 <sup>b</sup>	2.16 <sup>a</sup>	6868.6 <sup>b</sup>	2441.7 <sup>b</sup>
V3	50.1 <sup>a</sup>	33.2 <sup>a</sup>	2.14 <sup>a</sup>	9797.2 <sup>a</sup>	2973.2 <sup>a</sup>
Phosphate solubilizing bacterium					
P1	44.3 <sup>b</sup>	23.7 <sup>c</sup>	2.12 <sup>a</sup>	6436.5 <sup>c</sup>	2112.8 <sup>c</sup>
P2	45.9 <sup>ab</sup>	26.8 <sup>b</sup>	2.08 <sup>a</sup>	6967.8 <sup>b</sup>	2307.2 <sup>b</sup>
P3	47.4 <sup>a</sup>	29.5 <sup>a</sup>	2.21 <sup>a</sup>	7768.8 <sup>a</sup>	2571.6 <sup>a</sup>

Means, in each column for each factor followed by at least one letter in common, are not significantly different at 5% probability level using Duncan's multiple range test. V1, V2 and V3 represent 0, 5 and 10 ton vermicompost per hectare, respectively. P1, P2 and P3 represent non-inoculated, inoculated seeds and inoculated seeds + spraying on the plant base at stem elongation stage, respectively.

(47.4 cm).

### Umbel number per plant

The results of present study, demonstrated that umbel number per plant was significantly influenced by the application of vermicompost (Table 3). Among various treatments, the application of 10 ton vermicompost per hectare has indicated maximum increase in umbel number per plant (33.2). Phosphate solubilizing bacterium also caused a significant effect on umbel number per plant (Table 3), and higher umbel number per plant (29.5) was recorded when phosphate solubilizing bacterium was used twice.

### Weight of 1000 seeds

The results indicated that 1000 seeds weight was not affected by vermicompost and phosphate solubilizing bacterium (Table 3).

### Biological yield

The results have indicated that biological yields were affected by the application of vermicompost (Table 3). Significant increase in biological yield was observed in two treatments of vermicompost application (5 and 10 ton/ha) as compared to the control experiment (non-vermicompost). The highest biological yields were obtained with applying 10 ton/ha vermicompost (9797.2 kg/ha).

Phosphate solubilizing bacterium showed significant effect on biological yield (Table 3), as the highest biological yield (7768.8 kg/ha) was obtained in the third treatment level of bacterium (a two-times application of

phosphatic biofertilizer). The present results showed that the interaction of vermicompost and phosphate solubilizing bacterium was also significant (Table 4). The highest biological yield (10589 kg/ha) was obtained after the integrated application of 10 ton/ha vermicompost and twice administration of phosphate solubilizing bacterium. The interaction of vermicompost and phosphatic biofertilizer, on the biological yield, revealed that the application of 5 and 10 ton/ha vermicompost successively increased the levels of phosphatic biofertilizer, which resulted in a significant increase in biological yield.

### Seed yield

The results presented in Table 3 have revealed that various levels of vermicompost had significant effects on the seed yield. The maximum seed yield (2973.2 kg/ha) was obtained by using 10 ton vermicompost per hectare. Significant increase in seed yield was observed in two treatments of phosphate solubilizing bacterium (once time and twice application of bacterium) as compared to the control (non-inoculated cultures; Table 3). The highest seed yield (2571.6 kg/ha), however, was found after a twice application of phosphate solubilizing bacterium.

## DISCUSSION

Vermicompost, with high water-holding capacity and proper supply of macro- and micro-nutrients (Edwards and Burrows, 1988; Atiyeh et al., 2002; Arancon et al., 2004), has a positive effect on biomass production and subsequently the 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 (Vadiraj et

**Table 4.** Mean comparison for biological yield after the interaction of different factors.

Treatment	Biological yield (kg/ha)
Vermicompost × Phosphate solubilizing bacterium (V × P)	
V1p1	4397.4 <sup>g</sup>
V1p2	4232 <sup>g</sup>
V1p3	4892.4 <sup>g</sup>
V2p1	5854.8 <sup>f</sup>
V2p2	6926 <sup>e</sup>
V2p3	7825.1 <sup>d</sup>
V3p1	9057.2 <sup>c</sup>
V3p2	9745.4 <sup>b</sup>
V3p3	10589 <sup>a</sup>

Means, in each column for each factor followed by at least one letter in common, are not significantly different at 5% probability level using Duncan's multiple range test.

al., 1998; Arguello et al., 2006; Darzi et al., 2007; Azizi et al., 2008). Vermicompost has significantly influenced the flowering and umbel number per plant.

On the other hand, vermicompost application through the improvement of biological activities of soil and mineral element absorption (Arancon et al., 2004), caused more biomass production and umbel number. These earlier findings are in accordance with the results of present experiment, and the observations on the *Fragaria ananasa* (Arancon et al., 2004). Similar, results were also obtained for several other plants such as *Artemisia pallens*, *Foeniculum vulgare* (Pandey 2005; Roy and Singh 2006; Darzi et al., 2007). Roy and Singh (2006) reported a large number of productive tillers of barley in response to vermicompost application. They have suggested that vermicompost affected the productive tillering through microbial stimulation and gradual mineralization of soil. According to the present analysis, phosphatic biofertilizers have promoted flowering and increased umbel number per plant by enhancing the phosphorus content and the rate of photosynthesis (Ratti et al., 2001). The present result were derived from the improvement of phosphate solubilizing microorganisms' activities in soil at the third treatment level (inoculated seed + spraying on plant base at stem elongation stage), which are in agreement with the previous studies carried out on the borage plant (Shalan, 2005a).

The results clearly demonstrated the effectiveness of vermicompost in increasing the biological yield. Vermicompost increases the growth rate because of water and mineral uptake such as; nitrogen and phosphorus, which lead to the biological yield improvement (Atiyeh et al., 2002; Arancon et al., 2004). This finding is in accordance with the previous observations (Anwar et al., 2005; Darzi et al., 2008). Effect of phosphate solubilizing bacteria on the biological yield was due to increased phosphorus uptake (Ratti et al., 2001; Shalan, 2005a, b). The result of the present work are in agreement with the reports of Omar (1998) on *Triticum aestivum*, Ratti et al. (2001) on *Cymbopogon*

*martini*, Rashmi et al. (2008) on *Ocimum gratissimum* and Darzi et al. (2011) on *Pimpinella anisum*. All the earlier reports and present results supported the fact of positive and synergistic effect on interaction between two factors which highly dependent on the effect of organic matter, containing vermicompost, on the activity of phosphate solubilizing bacteria. Many reports have shown that the interaction between biofertilizers can be beneficial for plant growth and yield (Hazarika et al., 2000; Ratti et al., 2001; Kumar et al., 2002; Darzi et al., 2008; Padmapriya and Chezhiyan, 2009).

Increased seed yield due to vermicompost treatments can be owing to the improvement of yield components such as; plant height, umbel number per plant and biomass/biological yield. Our findings are in accordance with the observations of earlier researchers (Mba 1996; Vadiraj et al., 1998; Darzi et al., 2007; Chand et al., 2007; Sanchez et al., 2008). Whilst, Roy and Singh (2006) demonstrated that increased supply of mineral elements, through vermicompost application, resulted in greater absorption and utilization of these elements, which resulted in better growth of barley having direct effect on the yield attributes as well as the grain yield. Arguello et al. (2006) have shown that the application of vermicompost on *Allium sativum* caused greater yield as compared to the experimental plants with no vermicompost application due to an earlier start of bulbification and lengthening of the bulb filling period. Phosphatic biofertilizer promoted seed yield through the enhancement of yield attributes. These results are in agreement with the earlier investigation on *Vigna radiata*, *Borago officinalis*, *Nigella sativa* and *P. anisum* (Singh and Kapoor, 1998; Shalan 2005a, b; Darzi et al., 2011).

## Conclusion

It is clear from the present study that biofertilizers successfully manipulate the growth of anise, resulting in beneficial changes in yield and yield components. The

highest biological and seed yield was obtained by using 10 ton vermicompost per hectare. Maximum biological and seed yield was observed twice by using phosphate solubilizing bacterium (inoculated seeds + spraying on the plant base at stem elongation stage). Thus, combined application of vermicompost and phosphate solubilizing bacterium can be helpful in developing of production and yield in anise.

## ACKNOWLEDGEMENTS

The authors wish to thank the Research division of Islamic Azad University, Roudehen Branch for providing the financial support to undertake this research project.

## REFERENCES

- Anwar M, Patra DD, Chand S, Alpesh K, Naqvi AA, Khanuja SPS (2005). Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. *Communications in Soil Science and Plant. Anal.*, 36(13-14): 1737-1746.
- Arancon N, Edwards CA, Bierman P, Welch C, Metzger JD (2004). Influences of vermicomposts on field strawberries: 1. Effects on growth and yields. *Bioresource Technol.*, 93: 145-153.
- Arguello JA, Ledesma A, Nunez SB, Rodriguez CH, Goldfarb MDD (2006). Vermicompost effects on bulbing dynamics, nonstructural carbohydrate content, yield, and quality of Rosado paraguay garlic bulbs. *Hort.Sci.*, 41(3): 589-592.
- Atiyeh RM, Arancon N, Edwards CA, Metzger JD (2002). The influence of earthworm- processed pig manure on the growth and productivity of marigolds. *Bioresource Technol.*, 81: 103-108.
- Azizi M, Rezwanee F, Khayat MH, Lackzian A, Neamati H (2008). The effect of different levels of vermicompost and irrigation on morphological properties and essential oil content of German chamomile (*Matricaria recutita*) C.V. Goral. *Iran. J. Med. Aroma. Plants*, 24(1): 82-93.
- Chand S, Pande P, Prasad A, Anwar M, Patra DD (2007). Influence of Integrated supply of Vermicompost and Zinc-enriched Compost with two graed levels of iron and zinc on the productivity of Geranium. *Communications in Soil Science and Plant. Anal.*, 38: 2581-2599.
- Chen YP, Rekha PD, Arun AB, Shen FT (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil. Ecol.*, 34: 33-41.
- Darzi MT, Ghalavand A, Rejali F, Sefidkon F (2007). Effects of Biofertilizers Application on yield and yield components in fennel (*Foeniculum vulgare* Mill.). *Iran. J. Med. Aroma. Plants*, 22(4): 276-292.
- Darzi MT, Ghalavand A, Rejali F (2008). Effect of mycorrhiza, vermicompost and phosphate biofertilizer application on flowering, biological yield and root colonization in fennel (*Foeniculum vulgare* Mill.). *Iran. J. Crop. Sci.*, 10(1): 88-109.
- Darzi MT, Haj Seyed Hadi MR, Rejali F (2011). Effect of vermicompost and phosphate biofertilizer application on yield and yield components in Anise (*Pimpinella anisum* L.). *Iranian J. Med. Aroma. Plants*, 4(50): 452-465.
- Edwards CA, Burrows I (1988). The potential of earthworm composts as plant growth media. In: Edwards, C.A., Neuhauser, E.F. (Eds.), *Earthworms in Environmental and Waste Management*. SPB Academic Publ. b.v., The Netherlands, pp. 211-220.
- Gyaneshwar P, Naresh Kumar G, Parekh LJ, Poole PS (2002). Role of soil microorganisms in improving P nutrition of plants. *Plant. Soil*, 245: 83-93.
- Hazarika DK, Taluk Dar NC, Phookan AK, Saikia UN, Das BC, Deka PC (2000). Influence of vesicular arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on nursery establishment and growth of tea seedlings in assam. Symposium No. 12, Assam Agricultural University, Jorhat- Assam, India.
- Kumar S, Choudhary GR, Chaudhari AC (2002). Effects of nitrogen and biofertilizers on the yield and quality of coriander (*Coriandrum sativum* L.). *Ann. Agr. Res.*, 23(4): 634-637.
- Mba CC (1996). Treated-cassava peel vermicomposts enhanced earthworm activities and cowpea growth in field plots. *Res. Conserv. Recycle*, 17: 219-226.
- Omar SA (1998). The role of rock-phosphate-solubilizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. *World J. Microb. Biot.*, 14: 211-218.
- Ozkan MM, Chalchat JC (2006). Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. *Ann. Microbiol.*, 56(4): 353-358.
- Padmapriya S, Chezhian N (2009). Effect of shade, organic, inorganic and biofertilizers on morphology, yield and quality of turmeric. *Indian J. Hort.*, 66(3): 333-339.
- Pandey R (2005). Mangement of *Meloidogyne incognita* in *Artemisia pallens* with bio-organics. *Phytoparasitica.*, 33(3): 304-308.
- Rashmi KR, Earanna N, Vasundhara M (2008). Influence of biofertilizers on growth, biomass and biochemical constituents of *Ocimum gratissimum*. *L. Biomed.*, 3(2): 123-130.
- Ratti N, Kumar S, Verma HN, Gautam SP (2001). Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martinii* var. motia by rhizobacteria, AMF and Azospirillum inoculation. *Microbiol. Res.*, 156: 145-149.
- Roy DK, Singh BP (2006). Effect of level and time of nitrogen application with and without vermicompost on yield, yield attributes and quality of malt barley (*Hordeum vulgare*). *Indian J. Agron.*, 51: 40-42.
- Sanchez GE, Carballo GC, Ramos GSR (2008). Influence of organic manures and biofertilizers on the quality of two Plantaginaceae: *Plantago major* L. and *Plantago lanceolata* L. *Revista Cubana de Plantas Medicinales.*, 13(1): 12-15.
- Shalan MN (2005a). Effect of compost and different sources of biofertilizers, on borage plants (*Borago officinalis*, L.). *Egyptian J. Agric. Res.*, 83(1): 271-284.
- Shalan MN (2005b). Influence of biofertilizers and chicken manure on growth, yield and seeds quality of *Nigella sativa*, L. plants. *Egyptian J. Agric. Res.*, 83(2): 811-828.
- Singh S, Kapoor KK (1998). Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions. *Mycorrhiza.*, 7: 249-253.
- Tunçturk M, Yildirim B (2006). Effect of seed rates on yield and yield components of anise (*Pimpinella anisum*). *Indian J. Agric. Sci.*, 76 (11): 679-681.
- Vadiraj BA, Siddagangaiah A, Poti N (1998). Effect of vermicompost on the growth and yield of turmeric. *South Indian Hort.*, 46: 176-179.
- Zar JH (1996). *Biostatistical Analysis*. Prentice-Hall, Upper Saddle River, New Jersey.