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Effect of *Azolla* Compost and Various Biofertilizers on Growth and Quality of Zinnia flower (*Zinnia Elegans* Thumbelina)

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Abstract

Zinnia is an annual plant with the long flowering period from late spring to mid-autumn. It is a popular plant for landscape. In the present study, the effect of two types of growth promoting bacteria and *Azolla* compost on some ornamental characteristics of *Zinnia* were evaluated in pot cultivation and greenhouse conditions. The factorial experiment was conducted in three replications based on a completely randomized design with two factors, including *Azolla* compost in 6 levels (0, 10, 20, 30, 40, 50 percent) and growth promoting bacteria in 3 levels (without bacteria, *Azospirillum* and *Azotobacter* bacteria). The results showed that growth promoting bacteria (*Azospirillum* and *Azotobacter*) and *Azolla* compost had a significant effect on the investigated factors. Treatment of 50% *Azolla* compost and *Azospirillum* compared to other treatments had higher leaf and root fresh weight, leaf nitrogen and plant height. Moreover, 10% *Azolla* compost and *Azospirillum* was the best treatment in terms of leaf and root dry matter content. According to conducted evaluation, *Azospirillum* with less percentage of compost had a significant effect on dry weight and *Azospirillum* with a higher percentage of compost had a significant effect on fresh weight. Besides, a higher percentage of compost in *Azospirillum* in treatment *Azospirillum* and 50% compost had a significant effect on leaf nitrogen and height of the stem.

Keywords: *Azolla* compost, *Azospirillum*, *Azotobacter*, Growth factors.

INTRODUCTION

Zinnia elegans is one of the ornamental flowers of Asteraceae family with colorful flower and it is native to Mexico (Karimi *et al.*, 2002; Dole and Wilkins, 2005). In order to increase the size of flowers, prevent the loss and yellowing of leaves, and increase the durability of inflorescence, treatments should be performed on them. They were flowering from June to autumn. Plant sizes varied range from dwarf varieties of less than 30 cm in height to 80 cm tall. Moreover, the size of the flowers varies from big to small. In general, *Zinnia* is a plant with high diversity (Ghasemi Ghahsare and Kafi, 2009).

One of the important problems that cause the ornamental flowers survive shortly with less quality is an incorrect use of the agricultural soil and inappropriate treatments. Replacing biological fertilizer instead of chemical fertilizer is the best method. This fertilizer not only has positive effects on a physical, chemical, and biological characteristic of the soil but also increases the production and saves the energy and costs (Ostadi Jafari *et al.*, 2012). The use of compost is known as a necessity in sustainable agriculture because it can supply many essential elements of the soil such as zinc, nitrogen, phosphorous and potassium, which is needed by plants (Ramesh *et al.*, 2009). Compost is a mixture of decayed organic materials decomposed by microorganisms in a warm, moist, and aerobic environment, releasing nutrients for plants (Das *et al.*, 2010). Moreover, compost may be used as a nutrients source and water storage in the soil; therefore, it might increase the efficiency of the water use, improve the soil conditioning, and solve the lack of humus or organic matter. Besides, the compost may provide some vitamins, hormones, and enzymes that cannot be added to the soil by chemical fertilizers (Sanati *et al.*, 2011).

To solve the lack of nitrogen, the use of bacteria with the ability of biological nitrogen fixation such as *Azospirillum* and *Azotobacter* should be an effective method (Sanati *et al.*, 2011). Today, due to the indiscriminate use of chemical fertilizers, organic matters of agricultural land was increased in Iran and soil composition has become a hard and undesirable texture (Rahimi *et al.*, 2012). Hajiboland *et al.* (2004) evaluated the effect of Omid wheat inoculation with *Azotobacter* separated from the rhizosphere of Poa pratensis and reported an increase in root growth and aerial parts and absorption of the nutrient. Bashan and Dubrovsky (1996) found that inoculation of both barley and sorghum with *Azospirillum* resulted in an increase in the total weight of aerial parts and root weight, plant height and number of the spikelet in barley. Tilak *et al.* (1998) by performing a greenhouse experiment showed an increase in barley dry matter yield due to the effect of seed inoculation with *Azospirillum* brasilense, *Azotobacter chroococcum*, 40 g of nitrogen per hectare. Tilak *et al.* (1982) demonstrated the effect of *Azotobacter* inoculation with *Azospirillum* on dry matter content of corn and sorghum aerial parts. The present study aims to evaluate the *Azolla* compost effect, based on the importance of using growth-promoting bacteria with chemical fertilizers to reduce the environmental risks and organic wastes. Therefore, A*zospirillum brasilense* and *Azotobacter chroococcum* in growing media containing various levels of *Azolla* compost on the *zinnia* flowers growth factors were studied.

MATERIALS AND METHODS

The present research was conducted in the greenhouse of Lahijan Ornamental Plants and Flower Research Station. The study aimed to evaluate the effect of the various ratio of *Azolla* compost with garden soil as growing media and growth promotion bacterias on ornamental characteristics and freshness of leaves and growth of *Zinnia* flowers. The factorial experiment was conducted in three replications and three plants in each experimental unit with162 pots. The experiment was completely randomized design with two factors including growing media in 6 levels (a0= 0%, a1=10%, a2=20%, a3=30%, a4=40%, and a5=50% *Azolla* compost) and factor of growth promoting bacteria in 3 levels (b0= without bacteria, b1= *Azospirillum* and b3= *Azotobacter*). F1 Japanese *Zinnia* seed was provided by Shokofeh Co. in Isfahan. To carry out this study, all seeds were planted in proper and same growing media in late March. Main media included uniformly mixed *Azolla* compost with garden soil, which was added to pots. After preparation of transplanting *Zinnia* flowers (after 40 days) and when they put on 2 leaves, the roots were washed and impregnated with liquid *Azospirillum* and *Azotobacter* and then were transferred to pots containing growing media. The liquid inoculation including *Azospirillum* and *Azotobacter* was provided by Soil Biology Research Division of Soil and Water Research Institute located in Meshkindasht in Karaj. All necessary cares including irrigation during the plant growing in the greenhouse were performed in terms of plant needs. Besides, all characteristics below were measured during growing period (5 months).

At the end of the growing cycle, the plants were picked up from the crown and fresh weight of leaves was recorded, then the number of leaves and lateral branches were counted. In the next step, the root was washed and then, fresh weight of the root was measured. To determine the dry weight of root and leaf, different parts of the plant were dried at 75° C for 48 hours (Mahboub Khomami & Mammadov Goshgar, 2014). The chlorophyll of leaves was measured by Mazumdar & Majmodar (2003) method. In order to measure nitrogen of soil, before cultivating the plant in the pot, the soil was sampled. Moreover, after cultivating the plant in the pots, the soil of each treatment was sampled and soil extracts were prepared in the laboratory. Growth media and leaf nitrogen was measured using the Kjeldahl method.

Statistical analysis

Data analyses were done using SAS software and means were compared based on LSD test at 5% of probability level. Moreover, charts and tables were drawn using Excel.

RESULTS AND DISCUSSION

Leaf dry matter

The effect of compost and bacteria at 1% on leaves dry matter content was significant (Table 1). As shown in Table 2, the maximum and the minimum leaves dry matter content were observed respectively in treatment a1b1 (13.73%) and treatment a4b2 (9.68%).

Fresh weight of leaves in each plant

The effect of compost and bacteria on fresh weight of leaves was significant a 1% probability (Table 1). As seen in Table 2, the maximum and the minimum leaves fresh weight were observed in treatment a5b1 (23.74 g) and control plants (10.73 g), respectively.

Root dry matter

The effect of compost and bacteria on root dry matter content was significant at the statistical level of 5% (Table 1). Among the effect of compost and growth promoting bacteria on root dry matter, the maximum and minimum root dry matter were observed in treatment a1b1 (16.51%) and control plants (10.45) respectively (Table 2).

Root fresh weight

Based on the results of ANOVA (Table 1), the interactive effects of compost and bacteria on root fresh weight were significant at the statistical level of 1%. As seen in Table 2, the maximum and the minimum root fresh weight were calculated in treatment a5b1 (10.17 g) and control plants (5.07 g), respectively.

Table 1. Analysis of variance of the effect of organic fertilizers and compost on leaves and roots dry matter percentage, fresh weight of leaves and roots, number of leaves on the plant, number of lateral branches per plant, plant height, chlorophyll a, b and total, shoot nitrogen and soil nitrogen after planting.

S.o.V	df	Mean Square (MS)											
		Leaf dry matter	Fresh weight of leaves in each plant	Root dry matter	Root fresh weight	Leaf number per plant	The number of branches per plant	Final plant height	Chlorophyll a	Chlorophyll b	Total chlorophyll	Shoot nitrogen	Soil nitrogen
Compost levels	5	2.605**	61.70**	15.23**	9.99*	1410**	32.56**	26.45**	25.79**	3.804**	48.01**	0.688**	15.17**
Types of bacteria	2	1.956*	1.88ns	13.75*	2.88ns	245.7ns	0.129ns	52.55**	5.59ns	2.815**	16.29*	0.092**	7.22**
Compost levels × Type of bacteria	10	3.463**	34.57**	5.72*	8.26**	331.1**	21.396**	39.67**	8.34**	1.55**	12.62**	0.005*	0.463**
Total error	25	0.531	4.36	2.70	2.19	88.51	1.11	4.303	2.28	0.33	3.25	0.002	0.0183
Cv (%)	-	6.11	13.17	12.86	20.41	12.83	22.95	4.28	10.88	9.16	8.92	4.67	0.758

*, ** and ns: Significant at $P<0.05$, $P<0.01$ and no significant respectively.

Table 2. The interaction of different substrates and organic fertilizers on the dry matter content of leaves and roots, leaves and root weight, total number of leaves per plant, number of lateral branches per plant, plant height, chlorophyll a, b and total, shoot nitrogen and soil nitrogen after planting.

	Leaf dry matter (%)	**Fresh weight of leaves in each plant (g)**	**Root dry matter (%)**	**Root fresh weight (g)**	**leaf number per plant**	**The number of branches per plant**	**Final plant height (cm)**	**Chlorophyll a (mg g-1 FW)**	**Chlorophyll b (mg g^{-1} FW)**	**Total chlorophyll (mg g^{-1} FW)**	**Shoot nitrogen (ppm)**	**Soil nitrogen (ppm)**
a0b0	11.98 cd	10.73 h	10.45 f	5.07 e	45.66 f	3.00 c	41.00 h	11.20 h	4.536 g	15.74 h	0.6566 f	14.93 k
a0b1	12.82 ac	14.93 eg	11.49 df	8.05 ad	69.33 ce	4.33 ac	51.89 ac	11.35 h	5.530 ef	16.88 gh	0.6900 f	16.65 i
a0b2	12.19 bd	11.87 gh	11.11 ef	7.03 ce	55.00 ef	3.33 bc	50.66 be	14.01 cg	6.470 cf	20.48 cf	0.6600 f	16.47 i
a1b0	12.19 bd	13.32 fgh	11.38 df	8.19 ad	62.00 de	4.33 ac	47.88 ef	11.30 h	6.933 ac	18.24 fh	0.6733 f	15.69 j
a1b1	13.73 a	12.07 gh	16.51 a	6.60 ce	57.33 ef	4.33 ac	42.44 gh	13.40 ch	4.983 fg	18.39 eh	0.7833 e	17.05 h
a1b2	11.56 de	15.61 df	11.72 cf	7.17 ce	68.66 ce	5.00 ab	48.55 ce	14.72 be	6.963 ac	21.69 bd	0.6966 f	17.34 g
a2b0	12.35 bd	14.05 eh	14.32 ac	5.19 e	61.00 df	4.66 ac	49.44 be	12.53 eh	5.883 df	18.41 eh	0.7866e	17.34 g
a2b1	12.37 d	16.78 ce	13.53 be	8.92 ac	70.00 ce	5.66 a	48.39 de	11.66 gh	5.593 ef	17.25 gh	0.9566 d	17.70 f
a2b2	11.59 de	14.25 eg	14.38 ac	6.45 de	74.00 bd	4.33 ac	48.88 cde	11.80 fh	5.516 ef	17.32 gh	0.9400 d	18.74 d
a3b0	11.67 ce	16.71 cf	12.96 cf	5.20 e	89.33 ab	5.33 a	44.77 fg	15.34 bd	7.493 a	22.83 ac	0.9400 d	17.93 f
a3b1	12.66 ad	11.94 gh	13.76 be	6.60 ce	65.66 ce	4.00 ac	44.66 fg	13.10 dh	6.023 df	19.12 dg	1.0933 c	18.18 e
a3b2	11.62 de	21.33 ab	16.07 ab	8.00 ad	100 a	5.66 a	51.50 bd	14.70 be	6.616 ad	21.32 ce	0.9700 d	19.00 c
a4b0	13.20 ab	20.35 ab	11.56 df	9.80 ab	87.66 ab	5.33 a	48.27 de	14.26 cf	6.700 ad	20.96 cf	1.0666 c	18.15 e
a4b1	10.75 ef	14.24 eg	14.01 ad	5.810 de	76.33 bd	4.66 ac	52.44 ab	16.95 ab	7.510 a	24.46 ab	1.3033 b	18.32 e
a4b2	9.68 f	18.53 bd	11.68 cf	6.68 ce	76.00 bd	4.00 ac	48.50 ce	15.88 bc	7.330 ab	23.21 ac	1.1300 c	19.36 b
a5b0	10.74 ef	19.13 bc	10.79 f	7.97 ad	94.33 a	5.00 ab	47.50 ef	18.63 a	6.830 ad	25.46 a	1.3433 b	19.37 b
a5b1	10.57 ef	23.74 a	12.65 cf	10.17 a	78.66 bc	5.00 ab	55.22 a	13.42 ch	5.900 df	19.32 dg	1.4866 a	19.18 bc
a5b2	12.53 ad	15.74 cf	11.67 cf	7.71 bd	88.00 ab	4.66 ac	49.77 be	15.46 bd	7.356 ab	22.81 ac	1.3633 b	20.09 a

In each column, means with the similar letters are not significant different ($P < 0.05$) using LSD test. a0= 0%, a1=10%, a2=20%, a3=30%, a4=40%, and a5=50% *Azolla* compost; b0= without bacteria, b1= *Azospirillum* and b3= *Azotobacter*.

Leaf number per plant

Based on the results of ANOVA (Table 1), the interactive effects of compost and bacteria on the number of leaves were significant at the statistical level of 1%. Among interactive effects, the maximum and minimum number of leaf were observed in treatment a3b2 (100) and control plants (45.66), respectively (Table 2).

The number of branches per plant

The interactive effect of compost and bacteria on the number of branches per plant was significant at 1% probability (Table 1). Among the effect of compost and growth promoting bacteria on the number of branches per plant, the maximum and minimum lateral branches were observed in treatment a3b2 and a1b2 (5.66) and control plants (3), respectively (Table 2).

Chlorophyll a

Based on the results of ANOVA (Table 1), the interactive effects of compost and bacteria on the chlorophyll a of *Zinnia* were significant at the statistical level of 1%. Among the effect of compost and growth promoting bacteria, the maximum and minimum of chlorophyll a were observed in treatment a5b0 (18.63 mg/g FW) and control plants (11.20 mg/g FW), respectively (Table 2).

Chlorophyll b

The interactive effect of compost and bacteria on chlorophyll b of *Zinnia* was significant at 1% probability (Table 1). Among the interactive effect of compost and growth promoting bacteria, the maximum and minimum amount of chlorophyll b were observed in treatment a4b1 and a3b0 (7.51 and 7.49 mg/g FW) and control plants (4.53 mg/g FW), respectively (Table 2).

Total chlorophyll

Based on the results of ANOVA (Table 1), the interactive effects of compost and bacteria on the total chlorophyll was significant at the statistical level of 1%. Among the effect of compost and growth promoting bacteria, the maximum and minimum amount of total chlorophyll were observed in treatment a5b0 (25.46 mg/g FW) and control plants (15.74 mg/g FW), respectively (Table 2).

Shoot nitrogen

Based on the results of ANOVA (Table 1), the interactive effect of compost and bacteria on the shoot nitrogen was significant at the statistical level of 5%. Among the interactive effects, the maximum and minimum amount of nitrogen in aerial part were observed in treatment a5b1 (1.49 ppm) and control plants (0.66 ppm), respectively (Table 2).

Soil nitrogen

Based on the results of ANOVA (Table 1), the interactive effect of compost and bacteria on the soil nitrogen was significant at the statistical level of 1%. Among the interactive effects, the maximum and minimum amount of soil nitrogen were observed in treatment a5b2 (20.09 ppm) and control plants (14.93 ppm), respectively (Table 2).

Final plant height

According to the results of ANOVA (Table 1), the interactive effect of compost and bacteria on the final plant height was significant at the statistical level of 1%. Among the interactive effects, the maximum and minimum height of plant were observed in treatment a5b1 (55.22 cm) and control plants (42.44 cm), respectively (Table 2).

DISCUSSION

The results of the present study were in agreement with those of researchers who studied on fresh and dry weight of leaves in plant. In the present study, an increase in fresh weight of leaf was associated with an increase in *Azolla* compost content and *Azosprillium* compared to control. According to the results, the existence of *Azosprillium* and a low percentage of *Azolla* compost resulted in an increase in the leaves dry matter content. Yahalom and Okon (2004) reported that inoculation with *Azosprillium* by increasing the size of the root system resulted in an increase in absorption of N, P, and K by the roots of barley and sorghum. An increase in absorption of P led to an increase in accumulation of dry matter in the plant. In a study, the effects of growth promoting rhizobacteria such as *Azotobacter* and *Azosprillium* on the increasing of dry matter and chickpea yield was evaluated. Therefore, it was reported that the use of a combination of these bacteria resulted in an increase in dry matter and protein yield compared to control plants (Rokhzadi *et al.*, 2008). Bashan and Dubrovsky (1996) found that inoculation of both barley and sorghum with *Azospirillum* resulted in an increase in the total weight of aerial parts and root weight, plant height and number of the spikelet in barley.

Chen *et al.* (1988) concluded that leaf color was significantly affected by growing medium. Mahboub Khomami and Padasht (2010) by using *Azolla* compost in growing media of *Ficus benjamina* conducted that *Azolla* compost could be provided the nutrient for the plant during the growth period. Generally, the conducted studies showed that *Azolla* compost could be considered as a source of organic nutrients for plants due to its nutrients content (Mahboub Khomami and Padasht Dehkaei, 2010). In the present study was observed that use of *Azospirilium* with different percentage of compost had a desirable effect on aerial parts of the plant due to an increase in nutrient absorption by this bacterium that resulted in an increase in growth of aerial parts. The results were in agreement with those of Yahalom and Okon (2004) which reported that inoculation of root with *Azospirilium* led to increased root biomass. The effect of these bacteria on increasing the length of root, which leads to an increase in penetration ability of the root, is one of the most important effects of these bacteria. Talik *et al.* (1982) reported the effect of *Azotobacter* and *Azosprillium* inoculation on dry matter content of aerial part in corn and sorghum. Moreover, in the other study was determined that use of *Azosprillium* led to an increase in nitrogen absorption (Askary *et al.*, 2009). According to various studies on nitrogen fixation by *Azospirillum*, it was determined that these bacteria played an important role in supply some parts of nitrogen for the plant. Pandey *et al.* (1998) reported a significant increase in nitrogen and phosphor in different parts of the plant and observed the positive effect of seed inoculation with *Azospirillum brasilense* on the yield of barley.

Increasing the leaf chlorophyll has a direct relation with the amount of nitrogen in growing media and amount of absorption by the plant. One of the effective factor on the accumulation of nitrogen in the plant and subsequently increasing the amount of chlorophyll is an increase in the capacity of nutrients storage in growing media. The leaves with a low level of nitrogen have less chlorophyll. Rohitashav *et al.* (1999) conducted a field experiment for two years. They inoculated the wheat and barley seeds with *Azotobacter chroococcum* and planted them with other non-inoculated seeds, as a control. Under different treatments of nitrogen use or no nitrogen use, they observed that forage dry matter yield, green leaves of each plant, and stem diameter was increased under the inoculation effect. Dashti *et al.* (1997) showed that soybean inoculation with plant growth promoting bacteria increased the plant growth.

Kapulnik *et al.* (1985) indicated that vegetative and generative growth of wheat and barley was affected by seed inoculation with *Azospirillum* and plant height, the wet and dry weight of forage production, the number of spikes in each plant and plant yield was increased. Hajiboland *et al.* (2004) evaluated the effect of 'Omid' wheat inoculation with *Azobacter* separated from the rhizosphere of Poa pratensis/smooth meadow-grass and reported an increase in root growth and aerial parts and absorption of the nutrient. In a research, *Azospirillum* with different levels of nitrogen fertilizer were used for wheat that was irrigated by salty water (seawater). These bacteria caused an increase in plant height (plant and root dry weight) and finally, enhanced the yield. Moreover, these bacteria resulted in a decrease in the concentration of Na and an increase in N-P-K (Alamri and Mostafa, 2009).

CONCLUSION

In the last century, chemical fertilizer had a major role in producing agricultural products, but one of the most important changes that occurred in agricultural production policies as well as agricultural research, not only was an increase in production but also was an increase in production based on principles and objectives of sustainable agriculture and ecologic (Reyhani Tabar *et al.*, 2002). Use of growth promoting bacteria on the different plant was investigated and excellent results were achieved, but the study on ornamental plants such as *Zinnia* was very limited.

According to the studies on *Azosprillium* and compost, it was observed that combination of *Azosprillium* and a low percentage of compost had a significant effect on root and leaf dry matter content in a1b1 (10% Azolla compost and Azosprillium) treatment. Besides the combination of *Azosprillium* with a high percentage of compost, had a significant effect on root and leaf fresh in a5b1 (50% *Azolla* compost and *Azosprillium*) treatment.

Azotobacter and *Azosprillium* with different content of compost had a significant effect on chlorophyll b and total. The maximum nitrogen in leave and final height was observed in a5b1 treatment (*Azosprillium* and 50% compost). Moreover, the maximum number of leaves was determined in a3b2 treatment (30% Azolla compost and *Azotobacter*). The effect of different content of compost and growth promoting bacteria on the number of lateral branches was significant.

The results showed that generally growth promoting bacteria (*Azotobacter* and *Azosprillium*) and *Azolla* compost had significant effect on considered factors, but treatment a5ba (50% *Azolla* compost and *Azosprillium*) and a1b1 (10% *Azolla* compost and *Azosprillium*) was the best treatment and had the maximum significant effect on various factors compared to control group.

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