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Research article

INVESTIGATION OF BIOFERTILIZERS INFLUENCE ON VEGETATIVE GROWTH, FLOWER QUALITY, BULB YIELD AND NUTRIENT UPTAKE IN GLADIOLUS (*GLADIOLUS GRANDIFLORUS L.*)

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ABSTRACT: The study was aimed to assess the effect of different bio-fertilizer on growth and flower quality characteristics of Gladiolus (*Gladiolus grandiflorus L.*) An experiment with complete randomized block design (RCBD) having five biofertilizer treatments containing N-fixing bacteria (Azotobacter, Azospirillum), Rhizobium and P solubilizing bacteria with control and three replications was completed under green house conditions. The present results have shown that all the vegetative and reproductive growth accomplished successfully by application of biofertilizers. However, the treatment containing Azospirillum (T₄) gained highest values in terms of plant height, florets spike⁻¹, Spike length, Florets fresh weight and earlier sprouting than rest of the treatments. The role of biofertilizers in cormels production and nutrient uptake, T₄ had also superiority with more cormels plant⁻¹ and played leading role in nutrient (NPK) absorption than the control one. So, in this experiment Biofertilizer has been identified as an alternative to chemical fertilizer in order to increase soil fertility and crop production in sustainable farming.

Keywords: Biofertilizer, plant growth, flower quality, nutrient uptake, Gladiolus

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus L.*) is an important ornamental and commercial flower known as queen of the bulbous plants, belongs to Iridaceae family. It is the second most popular cut flower in Pakistan after Rose species that is grown for both as potted and aesthetic cut flower in the country [1]. Gladiolus is one among the top ten elite cut flowers due to their different shapes, dazzling colors, varying sizes and excellent vase life. It is considerable demand in both domestic and export markets and over 450 acres annually are under the cultivation of Gladiolus in Punjab province [2]. Today, Agrochemicals are being used excessively in crop production due to high trend in industrialization and population explosion in the world. Their continuous application has introduced major challenges for farmers in the form of soil infertility, nutrient imbalance, accumulation of toxic chemicals in the soil and food products which have an adverse effect on the soil productivity, ecosystem destruction, environmental degradation and also affecting the yield and quality of the product [3]. In that condition, sustainable agricultural practices have become a very difficult job for commercial growers now a days. To cope with all these problems a cheaper, better and safer way is necessary in order to improve the soil fertility status, maximize the agricultural productivity with minimum Eco hazards. All these criteria can be achieved through application of bio-fertilizers which is known as "microbial inoculants", these are the products containing the living cells (Mainly bacteria & fungi) that naturally activate the microorganisms found in the soil, restoring the soil fertility and improve physico-chemical and biological properties of soil [4] and [5]. These essential substances are the bio-stimulants, which act as a growth booster by inflicting positive effects on plant nutrition and crop protection against stress and diseased conditions [6]. Certain strains of bio-fertilizers which are being commercially used in horticultural crops are; *Azotobacter*, *Azospirillum*, *phosphate solubilizing bacteria* and VAM fungi. As reported in numerous studies, *Azospirillum* and *Azotobacter* are well known symbiotic N-fixing bacteria which help the plants indirectly through better nitrogen (N) fixation or improving the nutrient availability in the soil.

They have the ability to fix 20-200 kg N ha⁻¹ and increase crop yield by 10-50% ([7]; [8]; [9] and [10]). While, Phosphate Solubilizing Bacteria (PSB) are used to increase the availability of phosphorus in soil. Application of 120:65:62.5 kg NPK per ha⁻¹ + phosphobacteria + Azospirillum showed better results in vegetative and reproductive growth in gladiolus [11]. The increase in growth characteristics like plant height, early flowering, nutrient uptake were observed in French marigold and Rosesby *Azospirillum* inoculation [12] and [13]. Treated the bulbs with bio-fertilizers (*Azotobacter chroococcum* & *Azospirillum lipoferum*) increased plant height, leaf area, tepal diameter, growth and quality of tulip flower as well as bulb yield [14]. Bio-fertilizers seems to be a feasible option for sustained agriculture on a commercial and profitable scale. In addition, they are eco-friendly, easily available and cost effective [15]. Present project was formulated to investigate the potential role of bio-fertilizers application for enhancing growth, yield and improving quality of *Gladiolus grandiflorus* in a sustainable agricultural production system in order to reduce the amount of excessive chemical material released to the environment.

MATERIAL AND METHODS

The experiment was conducted in Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (latitude 31°30' N, longitude 73°10' E and altitude 213 m), Pakistan, during 2011-2012 to elucidate the effect of bio-fertilizers on growth, yield and quality of *Gladiolus grandiflorus* L. viz. "White friendship". Before starting the experiment, soil samples from various blocks of experimental field were collected randomly to assess physio-chemical properties (pH, EC, texture, organic matter contents and N, P, K and Zn contents) of the soil status that are given below in table 1. Soil was thoroughly tilled, leveled and blocks were laid out according to Randomized Complete Block Design. A basal dose of NPK was applied @ 200:180:200 kg ha⁻¹ before laying out the blocks. There were five treatments; control, azotobacter, azospirillum, rhizobium and phosphorus solubilizing bacteria (PSB), were taken in the study which were purchased from the National Institute for biotechnology & Genetic Engineering (NIBGE) Faisalabad, as a trade name "Biopower". Healthy and uniform size (10-12cm) corms were dipped in a thick slurry of respective carrier based Azotobacter and Azospirillum liquid fertilizer for half an hour and dried in shade for 30 min. Treated Corms were planted during 2nd week of October, 2011 according to layout of the experiment with 10cm plant to plant distance and on 60cm spaced ridges. Ten corms were planted in each treatment and each treatment was replicated thrice with a total of 150 corms used in this experiment. All other cultural practices like weeding, plant protection measures, earthing up etc, were same for all treatments during entire period of study. Plants were allowed to grow and data regarding growth, flowering and corm indices were collected using standard procedures.

Table 1: The results of soil analysis.

Soil properties	Soil depth (6-12 inch) mg kg ⁻¹	Adequate ranges mg kg ⁻¹
Organic matter %	0.50	> 1.29%
Ph	8.08	7
EC (dS m ⁻¹)	2.05	3
Zn Content (mg kg ⁻¹)	1.2	>1
Nitrogen (%)	10.12	>20
Available Phosphorus (mg kg ⁻¹)	8.2	>15
Exchangable Potasium (mg kg ⁻¹)	195	>150

Statistical Analysis

All data was analyzed statistically by using analysis of variance technique and treatment means were compared according to least significant difference (LSD) at 5% level [16].

RESULTS AND DISCUSSION

Influence of biofertilizers on vegetative and reproductive parameters

Concerning the evaluation of vegetative growth, statistically significant results are presented in table 1. Those treatments which were directly under the biofertilizer influence produced exclusive vegetative growth than the control treatment. In case of days to sprouting, T₄ (Azospirillum) had significant superiority which took least days of sprouting (17.58 days) followed by T₃, T₂ and T₁. The maximum days for sprouting were observed in T₀ control (24.34 days).

This might be due to inoculation with bacterial mixtures provided a more balance nutrition for plants as well as optimum absorption of bio fertilizers by corms accelerated the physiological process and improved the germination phenomenon. These results are in accordance with the findings of [17]. Application of 120:65:62.5 kg NPK ha⁻¹ + phosphor bacteria + Azospirillum showed better results with respect to days to sprouting in Gladiolus [11]. Treatment comprising Azospirillum as biofertilizers has a significant effect on the plant height. The increase in plant height was due to the presence of a readily available form of nitrogen. Azospirillum improves plant macro and micro nutrients absorption. Seeds inoculated with Azospirillum improved growth factors such as plant height in Jasmine and tuberose plants confirmed by [18] and [19]. These results are in line with the findings of [20] who reported that biofertilizers increases plant height by enhancing the nitrogen content and the rate of photosynthesis. Similar dominant effect of Azospirillum biofertilizer was also examined in early flowering which could be possible in 68.15 days with more number of flowers per plant (13.12) than rest of treatment. Early flowering and increase in number of florets per spike is dependant on food material prepared as result of photosynthesis in leaves [21]. In roses, earlier flowering due to inoculation with *Azospirillum* was observed [13]. This was due to induced cytokinin synthesis and rapid assimilation of photosynthates resulting in early transformation of the auxillary bulb from vegetative to reproductive phase.

It was inferred from the above results that the biofertilizers application with Azospirillum significantly increases the spike's length (74.04 cm) and fresh weight (38.54 g) as compared to control. The increase in spike length might be due to the availability of nutrition and elevated levels of macronutrients which have positive effect on floral characteristics. The similar results were obtained by [22] who mentioned that the treatment receiving Azospirillum sp. + Phosphate solubilizing bacterium + Vermi compost + NPK (25% of recommended dose) improved the flower quality and increase the flower yield of *Petunia hybrid* flower. Fresh weight increased due to biological fixation of nitrogen and phosphorus in root portion of plants resulting in absorption of more nutrients and its utilization. Moreover, *Azospirillum* has a role in nitrogen fixation and is also involved in the production of IAA, GA and cytokinin like substances which enhance the growth of plants. These findings are in accordance with the results of [23] stated that application of bio-fertilizers like Azospirillum, Phosphate solubilizing bacterium enhanced the flower fresh weight in carnation.

Table 2: Effect of different Biofertilizers on the growth, and quality characteristics of *Gladiolus grandiflorus* cv. white friendship.

Treatments	Days to sprouting (days)	Plant height (cm)	Days to spike emergence (days)	Florets per spike (number)	Spike length (cm)	Fresh weight of spike (g)
T ₀	24.34	64.34	84.23	7.13	58.33	24.00
T ₁	21.86	72.19	79.34	9.05	64.56	32.25
T ₂	19.45	74.04	71.87	9.87	67.89	34.66
T ₃	18.17	71.81	70.45	10.45	70.19	36.33
T ₄	17.58	79.56	68.15	13.12	74.04	38.54
S.Em	0.38	0.23	0.44	0.32	0.04	0.48
C.V at 5 %	3.30	0.56	1.01	5.66	0.13	2.52
T ₀ : Control, T ₁ : Rhizobium, T ₂ : Phosphorous solubilizing bacteria (PSB), T ₃ : Azotobacter, T ₄ : Azospirillum.						

Influence of biofertilizers on post-harvest attributes in Gladiolus.

In this study, Biofertilizers have played a stimulating role towards physiological response in nutrient accumulation as well post-harvest growth while significant differences are shown in table 3. Application of *Azospirillum* (T₄) excellent performed in maximum cormels production (31.95 corm plant⁻¹) with their higher weight (9.65 g), longest vase life (11.6 days) and better macro nutrient uptake percentage (4.76% N, 0.43% P and 3.63% K) with respect to all other treatments. A marked increase in both number of cormels plant⁻¹ and cormel's weight may be attributed to better availability of phosphorous, which is required in particularly for corm growth. Better cormels production might be due to corms inoculated with biofertilizers have stored more carbohydrates through effective photosynthesis. The increase in corms weight might be due to storage of carbohydrates and nitrogen compounds in the corms. The carbohydrates and soluble nitrogen compounds translocates from leaves to corms.

Corms act as sink source for storage of food, reported by [24]. Vase life is an important criterion to assess the postharvest quality of cut flowers. Our study revealed the preservative role of biofertilizers in *Gladiolus* flower longevity, when corms were treated with this supplement. Increment in vase life might be due to reduction in ethylene synthesis which has a harmful effect for flower life. Biofertilizers regulate nutrient uptake process and prolonged vase phenomenon. Our findings are harmony with [14] who reported that *Azotobacter*+ *Azospirillum* inhibit the action of ethylene and extend the vase life of tulip for 10-12 days.

Positive impact of biofertilizers for nutrient uptake (NPK) by leaves exhibited remarkable difference than control (T_0) are presented in table No, 3. These treatment application significantly increased nitrogen content, this could be attributed to the rapid absorption of these elements by the plant surface and their translocation in the plant [25]. Similar results were obtained by [26] on chrysanthemum and [27] on cauliflower. The role of phosphate solubilizing bacteria increases in the availability of phosphorus in soil through the secretion of phosphatase enzyme which leads to transfer organic phosphorus to their available forms [28] and [29]. Consequently, it enhances phosphorus absorption and accumulation in plant tissues. The increment in "K" percentage might be due to the effect of different strain groups and nutrients mobilizing microorganism which help in availability of metals and their forms in the composted material and increased levels of extracted minerals [30].

Table 3: Effect of different Biofertilizers on the vase life, corm/cormels production and nutrient uptake characteristics of *Gladiolus grandiflorus* cv. white friendship.

Treatments	Vase life (Days)	Corms		Cormels		Nutrient contents uptake by leaves in percentage		
		corms/plant	Wt.(g)	cormels/plant	Wt.(g)	N %	P%	K%
T₀	9.34	1.09	27.45	17.32	4.56	1.25	0.09	2.88
T₁	10.53	1.34	31.67	21.56	5.78	2.05	0.26	3.56
T₂	10.78	1.48	34.37	25.87	7.13	2.61	0.31	3.58
T₃	10.96	1.28	35.21	27.67	7.44	3.95	0.40	3.56
T₄	11.67	1.78	38.78	31.95	9.65	4.76	0.43	3.63
S.Em	0.27	0.054	0.19	0.048	0.02	0.11	0.03	0.53
C.V at 5%	4.40	6.58	0.99	0.34	0.72	6.60	7.12	8.95
T₀: control, T₁: Rhizobium, T₂: Phosphorous solubilizing bacteria (PSB), T₃: Azotobacter, T₄: Azospirillum.								

CONCLUSION

In general it appears that, as expected, *Gladiolus* as a cut flower significantly responded to bio fertilization which positively affected plants growth characters and flower yield. So, this is a little effort which was done to improve the soil structure and texture, reduces soil pollution, reduced extensive fertilizer application which is beneficial for the present problems of high cost of fertilizers and environmental pollution. Our findings may give applicable advice to farmers for crop management and concern on fertilizer strategy in alternative way of action.

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REFERENCES

- [1] Riaz, T., S. N. Khan and A. Javid, 2007. Scenario of gladiolus production in Punjab, Pakistan. Pak. J. Bot. 39(7): 2389-2393.
- [2] Manzoor, R., A.S. Shaukat and H.B. Mazahar, 2001. Economics of floriculture in Pakistan: A case study of Lahore market. Pak. Econ. Soc. Rev. 2: 87-102.
- [3] Eman, A.A., A.E. Monem, M.M.S. Saleh and E.A.M. Mostafa, 2008. Minimizing the quantity of mineral nitrogen fertilization grapevine by using humic acid organic and biofertilizers. Res. J. Agric. Sci, 4: 46-50.

- [4] Vessey, J. K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*, 255: 571-586.
- [5] Stevenson, T. L., 1959. Dehydrogenase activity in some soils under pasture. *J. Soil Biol. Biochem*, 3: 97-110.
- [6] Asghar, H. N., Z. A. Zahir, M. Arshad, A. Khalig, 2002. Plant growth regulating substances in the rhizosphere: microbial production and functions. *Adv Agron*, 62:146-151.
- [7] Bashan, Y., 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. *Bio.technol. Adv*, 16:729-770.
- [8] Persello, F., L. Nussaume and C. Robaglia, 2003. Tales from the underground: molecular plant-rhizobacteria interactions, *Plant Cell Environ*, 26 (2):189-199.
- [9] Kennedy, I.R., A.T.M.A., Choudhury and M. L. Kecskes, 2004. Non symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited. *S. Bio. Biochem*, 36 (8): 1229-1244.
- [10] Bashan, Y. Holguin., 1997. Azospirillum-plant relationships: environmental and physiological advances. *Can. J. Microbial*, 43:103-121.
- [11] Srivastava, R. and M., Govil, 2005. Influence of biofertilizers on growth and flowering in gladiolus cv. American Beauty, *ISHS Acta Horticulture* 742: International Conference and Exhibition on Soil less Culture (ICESC).
- [12] Balasubramanian, J., 1989. Studies on the combined effect of Azospirillum VA mycorrhizal and inorganic fertilizers on growth performance of French marigold (*Tegetesputula L.*). *S.I.H*, 37(4): 311.
- [13] Preethi, T.L., C.M. Pappiah and S. Anbu, 1999. Studies on the effect of Azospirillum sp. nitrogen and ascorbic acid on the growth and flowering of Edward rose (*Rosa bourboniana Desp.*). *J. South Indian Hort.*, 47(1-6): 106-110.
- [14] Khan, F. U., M. A. A. Siddique, F. A. Khan and I. T. Nazki, 2009. Effect of biofertilizers on growth, flower quality and bulb yield in tulip (*Tulipagesneriana*). *Indian Journal of Agricultural Sciences*. 79 (4): 248-251.
- [15] Gewaily, E., M. Fatma, I. El-Zamik, Tomader, T. EL -Hadidy, Abd El- Fattah H. I. and Seham H. Salem, 2006. Efficiency of biofertilizers, organic and inorganic amendments application on growth and essential oil of marjoram (*Majoranahortensis L.*) plants grown in sandy and calcareous soils. *Zagazig J. Agric. Res.* 33: 205-230.
- [16] Steel, R.G.D., J.H. Torrie and D.A. Dicky, 1997. Principles and Procedures of Statistics. A Biometric Approach, 3rd ed. McGraw Hill Book Co., New York.
- [17] Belimov, A.A., A.P. Kojemiakov and C.V. Chubarliyeva, 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria, *Plant and Soil*. 173: 29-37.
- [18] Manonmani, R., 1992, Effect of soil inoculation of Azospirillum and phosphor bacteria and graded level of N and P biofertilizers on growth and yield of *Jasminiumsambac Ait. Cv. Gundumali*. M.Sc. (Hort.) Thesis, Tamil Nadu Agric. Univ. Coimbatore.
- [19] Wange, S.S. Patil, P.L. and Patil, J.J., 1995, Effect of biofertilizers alone and with nitrogen levels on tuberose cv. Single petated. *J. Soil and Crops*, 5(2): 97-99.
- [20] Migahed, H.A., A.E. Ahmed and G.B.F. Abde, 2004. Effect of different bacterial strains as biofertilizer agents on growth, production and oil of *Apiumgraveolens* under calcareous soil. *Arab Univ. J. Agric. Sci*, 12(2): 511-525.
- [21] Brijendra, S. B. Singh, 1986. Foliar application of fertilizer mixtures for *Chrysanthemum*. *South Indian Hort.* 34: 367-369.
- [22] Moghadam, M. Z and M. Shoor, 2013. Effects of Vermi-compost and Two Bacterial Bio-fertilizers on some Quality Parameters of *Petunia*. *Not Sci Biol*, 5(2):226-231
- [23] Rajesh, B., D. Sandeep, S.R. Dhiman and J. Ritu, 2006. Effect of Biofertilizers and Biostimulants on Growth and Flowering in Standard Carnation (*Dianthus Caryophyllus Linn.*) *Journal of Ornamental Horticulture*, 9 (4): 282-284.
- [24] Nazki, I.T and S. Arora, 2000. Effect of GA3 and nitrogen on gladiolus. *J. Ornament. Hortic*, 124-127.
- [25] Mengel, K. and E. Kirkby, 1987. Principles of Plant Nutrition. International Potash Institute, (4th ed.). Worblaufen-Bern, Switzerland. 687 pp.
- [26] Khattab, M. and M.R. Hassan, 1980. Effect of different ratios and level of fertilizer on the vegetative growth and flower production of chrysanthemum. *Alex. J. Agric. Res.*, 28:225-231.
- [27] Kalyani, D.P., C. Ravishankar, R. N. Pillai and D. Manohar Prasad, 1992. Studies on the effect of nitrogen and Azospirillum on dry matter and nutrient uptake of cauliflower. *Veg. Sci*, 19(2): 147-151.

- [28] Abou El-yazeid A, Abou-Aly Eand M, Moussa, 2007. Enhancing growth productivity dissolving microorganism (Bio-phosphor) combined with boron foliar. *Agric Biol Sci*, 3 (4): 274-86.
- [29] Eid, R.A., S.A. Sedera and M. Attia, 2006. Influence of Nitrogen fixing bacteria incorporation with Organic/inorganic Nitrogen fertilizers on growth, flower yield and chemical composition of *Celosia Argentea*. *World journal of Agricultural Sciences*, 2(4): 450-458.
- [30] Yada, K.K., A.Tanwar and A. Aggarwal, 2013. Impact of Arbuscular Mycorrhizal Fungi and *Pseudomonas fluorescens* with Various Levels of Superphosphate on Growth Enhancement and Flowering Response of *Gerbera*. *Journal of Ornamental Plants*, (3): 161-170.