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# MICROBIAL INOCULATION INCREASES THE NUTRIENT UPTAKE EFFICIENCY FOR QUALITY PRODUCTION OF *Gladiolus grandiflorus*

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Gladiolus is a commercially important bulbous cut flower grown for its attractive multi-colored spikes. The present study was conducted to investigate the effect of microbial culture on growth and production of gladiolus cultivar White Prosperity, under field conditions. The experimental design was Randomized Complete Block Design (RCBD) having five treatments ($T_0$ = control, $T_1$ = *Rhizobium*, $T_2$ = Phosphorus Solubilizing Bacteria (PSB), $T_3$ = *Azotobacter* and $T_4$ = *Azospirillum*) and three replications. The microbial cultures were applied on the corm at planting time using the dip method followed by shade drying before planting. The results revealed that the gladiolus responded well to microbial culture and significant improvement was observed in different vegetative and flowering traits, corms productivity and leaf chemical composition of gladiolus. Among different treatments, considerable increase in both vegetative and reproductive attributes was observed when corms were treated with *Azospirillum*. Similarly, significant increase in total chlorophyll content, protein contents, total soluble sugars and accumulation of nutrients (N, P and K) was seen in leaves by *Azosprillum* application followed by *Azotobacter*.
**Keywords:** Nutrient availability, gladiolus, *Azosprillum, Rhizobium*, phosphorus solubilizing bacteria (PSB), *Azotobacter*

## INTRODUCTION

Floriculture has great export potential to develop faster as a sub-sector of agriculture due to its high demand in floral trade worldwide (Anderson *et al*., 2010). Gladiolus (*Gladiolus grandiflorus* L.) is an all-time favorite for the cut-flower industry as it occupies a leading position among cut flowers due to its elegant appearance and spikes of different hues and having long vase life (Bose *et al*., 2003; Anderson *et al*., 2012; Sajjad *et al*., 2014). In order to attain good quality spike, corms and cormels production in gladiolus, the continuous and indiscriminate application of chemical fertilizers has created a disproportion of nutrients which adversely affected the soil structure and health (Younis *et al*., 2013). Therefore, the integrated usage of the nutrients to get quality product without any environmental hazard is of prime concern. The use of organic soil amendments and microbial culture along with the judicious use of chemical fertilizers can improve biological and physico-chemical properties of the soil, moreover improving the nutrient uptake efficiency. During the current decade, microbial culture proved to be an important component of integrated nutrient application in agriculture and therefore seems a viable potential for efficient use of microorganisms for maximizing crop production. Application of microbes is an integral part of environment friendly sustainable organic agriculture (Bloemberg *et al*., 2000).

Organic cultivation practices in agriculture intends to increase biological cycles, biodiversity and soil biological activities in order to attain optimal organic system that are ecologically, socially and economically sustainable (Younis *et al*., 2010; Tariq *et al*., 2012). Soil microbes play a significant part in different ecological processes like: decomposition of organic matter (OM), homeostasis, nutrient cycling and supporting crop growth and health as bio-fertilization (Han *et al*., 2007). Certain strains of microbes are reported as plant growth-promoting rhizobacteria (PGPR), which can be applied as inoculant biofertilizer (Kennedy *et al*., 2004). These bacteria contain species of *Azospirillum* and *Azotobacter* and these were considered to stimulate growth, health and pest resistance in plants (Kennedy *et al*., 2004). Previous findings have proved the positive effect of microbial cultures in the production of gladiolus as the application of different microbial cultures like *Azotobacter* and phosphate solubilizing bacteria had improved the different vegetative and floral characteristics significantly compared to control in gladiolus (Srivastava and Govil, 2005). Therefore, present study was designed to assess the impact of different microbial cultures application for enhancing yield and improving quality of *Gladiolus grandiflorus* in a sustainable agricultural production system.

## MATERIAL AND METHODS

Present study was conducted at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, during 2011-12. Soil was thoroughly tilled, leveled and blocks were laid out according to Randomized Complete Block Design (RCBD). There were five treatments viz. control ($T_0$), *Rhizobium* ($T_1$), phosphorus solubilizing bacteria ($T_2$), *Azotobacter* ($T_3$) and *Azospirillum* ($T_4$). The bio-fertilizers were applied once at the time of planting using corm dip method for 30 minutes followed by shade drying before planting. Inoculum of *Azotobacter*, *Azospirillum* and *Rhizobium* were obtained from Soil Bacteriology Section, Ayub Agriculture Research Institute, Faisalabad while inoculum Phosphorus Solubilizing Bacteria (PSB) was obtained from Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

Corms of gladiolus cv. 'White Prosperity' were planted during 2$^{nd}$ week of October, 2011 at 10 cm plant to plant distance and on 60 cm spaced ridges. Six corms were planted in each replication and each treatment was replicated thrice. 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 on different productive and qualitative traits (plant height (cm), days to sprouting (days), dry weight of leaves (g), fresh weight of leaves (g), number of floret spike$^{-1}$, length of spike (cm), diameter of corms (cm) and days to spike emergence) were collected using standard procedures. Staking was also done by fixing bamboo sticks to support the spikes. The spikes were harvested with the help of a sharp secateur during morning hours (7:30 AM) when the lowermost 1-2 florets started showing colors. Immediately after harvesting, the spikes were kept in a bucket containing water and brought to the laboratory. The stem of each spike was given a slant cut at the base to increase the absorption area.

Plant samples from all treatments were also analyzed for uptake pattern of N, P and K contents using method of Chapman and Parker (1961) while total soluble proteins were calculated using following relationship;

$$\text{Protein contents} = \text{N\%} \times 6.25$$

All data were analyzed statistically by using ANOVA (analysis of variance) technique and treatment means were compared according to LSD (Least Significant Difference) test at 5% level of significance (Steel *et al*., 1997).

## RESULTS

The results regarding the effect of different microbial cultures on days to sprouting of gladiolus corms are given in Figure 1 as treatment means. Means indicated significant superiority of $T_4$ (*Azospirillum*), 6.25 days and $T_3$ (*Azotobacter*), 6.58 days on all the treatments. Both $T_4$ and $T_3$ behaved statistically alike and took minimum days to sprout. However, maximum (8.17) days were recorded in $T_0$ (control) and it was statistically at par with $T_1$ (*Rhizobium*) with 7.92 days. It was clear from these results that corms treated with *Azospirillum* followed by those treated with *Azotobacter* sprouted early as compared to control. Effect of microbial inoculation as bio-fertilizers on plant height of gladiolus is given in Figure 2 as treatment means. Among the treatments, highest plant height (126.67cm) was recorded in $T_4$ (*Azospirillum*) followed by $T_2$ (PSB) 116.08 cm and $T_3$ (*Azotobacter*) 114.6 cm respectively. Both $T_2$ and $T_3$ showed statistically similar response for this vegetative trait. The lowest plant height 88.5 cm was noticed in $T_0$ (control).

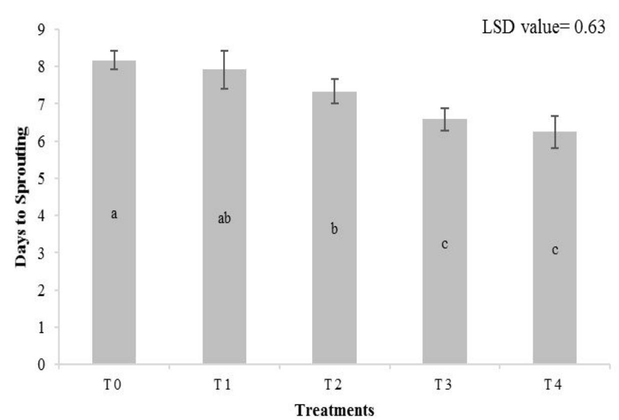


**Figure 1. Comparison of means for effect of microbial cultures on days to sprouting of *Gladiolus grandiflorus*** [Control ($T_0$), *Rhizobium* ($T_1$), phosphorus solubilizing bacteria ($T_2$), *Azotobacter* ($T_3$) and *Azospirillum* ($T_4$)]

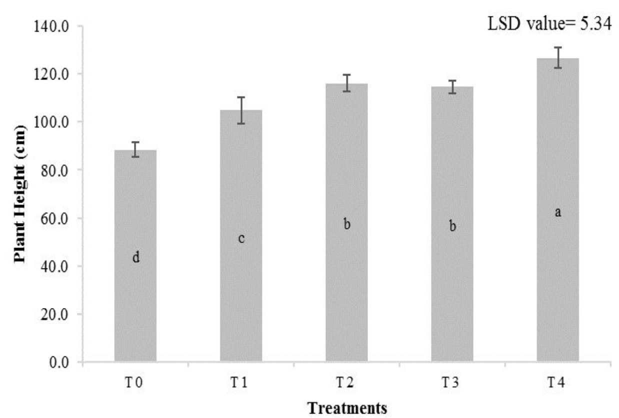


**Figure 2. Comparison of means for effect of microbial cultures on plant height of *Gladiolus grandiflorus*** [Control ($T_0$), *Rhizobium* ($T_1$), phosphorus solubilizing bacteria ($T_2$), *Azotobacter* ($T_3$) and *Azospirillum* ($T_4$)]

**Table 1. Comparison of means for effect of microbial inoculation on plant growth and flowering of *Gladiolus grandiflorus***

| Treatment | FWL (g) | DWL (g) | DFE | NFS | SL (cm) | DC (cm) |
|---|---|---|---|---|---|---|
| Control | 11.16 e | 4.01 e | 76.67 e | 8.44 d | 34.83 d | 1.74 b |
| *Rhizobium* | 12.04 d | 5.12 d | 75.75 d | 11.69 c | 45.33 c | 2.08 a |
| Phosphorus solubilizing bacteria | 15.25 c | 6.52 c | 71.83 c | 12.32 c | 51.33 b | 2.04 a |
| *Azotobacter* | 18.45 b | 7.43 b | 70.92 b | 13.63 b | 52.83 b | 2.31 a |
| *Azospirillum* | 20.63 a | 9.08 a | 69.00 a | 14.82 a | 56.58 a | 2.12 a |
| LSD | 0.83 | 0.45 | 1.41 | 1.06 | 4.73 | 0.29 |

Fresh Weight of leaves= FWL, Dry Weight of Leaves =DWL, Days to spike emergence =DSE, Number of floret spike= NFS, Length of spike= SL, Diameter of corms DC (cm)

**Table 2. Comparison of means for effect of microbial inoculation on physiological analysis of *Gladiolus grandiflorus***

| Treatment | N (%) | P (%) | K (%) | TCC (mg/g) | TSP (%) | TSS (%) |
|---|---|---|---|---|---|---|
| Control | 0.773 e | 0.095 e | 1.24 e | 49.471 c | 0.40 e | 1.27 e |
| *Rhizobium* | 1.994 d | 0.259 d | 2.02 d | 60.055b | 1.34d | 1.49 d |
| Phosphorus solubilizing bacteria | 2.472 c | 0.281 c | 2.52 c | 64.643b | 1.83 c | 1.61 c |
| *Azotobacter* | 3.558 b | 0.355 b | 3.02 b | 73.202 a | 2.46 b | 1.72 b |
| *Azospirillum* | 4.296 a | 0.453 a | 3.74 a | 77.288 a | 3.45 a | 1.79 a |
| LSD | 0.1008 | 0.0067 | 0.0174 | 6.3244 | 0.010 | 0.0548 |

Nitrogen content =N, Phosphorus content = P, Potassium content =K, Total Chlorophyll Contents =TCC, Total soluble Protein =TSP, Total soluble Sugars =TSS

Table 1 showed that all treatments differ significantly with respect to leaf biomass. Both fresh and dry weight of leaves per plant was significantly increased by all the inoculated treatments as compared to the control. However, the maximum fresh and dry weight of leaves per plant (20.63 & 9.08g) was obtained under $T_4$ (*Azospirillum*) followed by $T_3$ containing *Azotobacter* (18.45 & 7.43g). Plants grown in control treatment depicted lowest leaf fresh (11.16g) and dry weight (4.01g). The inoculation treatments also significantly improved the different flowering characters (Table 1). The days of spike emergence was found to be earlier (69.00 days) in $T_4$ *(Azotobacter)* followed by $T_3$ (70.92 days), $T_2$ (71.83 days) and $T_1$ (75.75 days) whereas, the maximum delay was found in control treatment (76.67 days). The data pertaining to the number of florets per spike revealed that all treatments significantly increased the number of florets per spike over control. The maximum number of florets per spike (14.82) were obtained in $T_4$ when *Azospirillum* was used as inoculum followed by $T_3$ (13.63), $T_2$ (12.32) and $T_1$ (11.69). The response of both $T_2$ and $T_1$ was similar statistically for number of florets per spike while least number of florets per spike (8.44) were recorded in $T_0$ (control). The spike length was also enhanced significantly (Table 1) and it was the maximum with the application of *Azospirillum* as $T_4$ (56.58cm). Both $T_3$ and $T_2$ behaved statistically alike with a spike length of 52.83 and 51.33cm respectively, while minimum spike length 34.83cm was obtained in control. The diameter of corms was significantly increased by all the inoculation treatments as compared to the control (1.73cm). The response to all inoculation treatments was same for corm diameter. However, the maximum diameter of corms (2.31cm) was seen in $T_3$ (*Azotobacter*).

Data regarding the distribution pattern of nitrogen, phosphorus and potash contents in leaves showed significant differences among all treatments, the corms treated with *Azospirillum* depicted maximum (4.30% N, 0.45% P & 3.73% K) concentrations of these elements. However, the minimum percentage of these elements were recorded in leaves from control plants (Table 2). The total chlorophyll contents were also significantly increased by all the inoculation treatments as compared to the control (49.47 mg/g). The maximum chlorophyll contents in leaves (77.29 mg/g) were obtained under $T_4$ (*Azospirillum*) followed by $T_3$ (73.20 mg/g) and both these treatments statistically non-significant. Similarly, both $T_2$ and $T_1$ showed similar response with respect to total chlorophyll contents. The results regarding the effect of microbial inoculation on total soluble proteins and total soluble sugars had a significant effect as compared to control. The maximum protein (3.45%) and total soluble sugars (1.79%) were observed in $T_4$ (*Azospirillum*) whereas, the plants with control treatments had minimum ratio (0.40 & 1.27%).

The results showed that the treatments comprising *Azospirillum* as inoculant had a significant effect on the plant height. The increase in plant height was due to the presence of readily available form of nitrogen as *Azospirillum* improved nutrient's availability. The correlation value (0.95) showed that the nitrogen in leaves and plant height are positively correlated, as the nitrogen contents in leaves increased the plant height also increased

**Table 3. Correlation between different parameters for effect of microbial cultures on *Gladiolus grandiflorus***

| | PH | FWL (g) | DWL (g) | DSE | NFS | SL (cm) | DC (cm) | N (%) | P (%) | K (%) | TCC (mg/g) | TSP (%) | TSS (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DTS | -0.89** | -0.997* | -0.98* | 0.97** | -0.92** | -0.90** | -0.75* | -0.98** | -0.93** | -0.97* | -0.97* | -0.97* | -0.95* |
| PH | | 0.90* | 0.95* | -0.94* | 0.97* | 0.99* | 0.74* | 0.94* | 0.96** | 0.96** | 0.95** | 0.96* | 0.97* |
| FWL (g) | | | 0.98** | -0.98** | 0.91* | 0.90* | $0.71^{NS}$ | 0.97* | 0.93* | 0.97* | 0.96* | 0.97* | 0.94* |
| DWL (g) | | | | -0.98** | 0.95* | 0.94* | $0.71^{NS}$ | 0.98** | 0.97* | 0.995** | 0.98** | 0.99** | 0.97* |
| DSE | | | | | -0.91* | -0.94* | $-0.69^{NS}$ | -0.95* | -0.92* | -0.97* | -0.95* | -0.96* | -0.95* |
| NFS | | | | | | 0.98** | $0.86^{NS}$ | 0.98** | 0.99** | 0.98** | 0.99** | 0.97* | 0.99** |
| SL (cm) | | | | | | | $0.83^{NS}$ | 0.95* | 0.97* | 0.96* | 0.97* | 0.95* | 0.99** |
| DC (cm) | | | | | | | | $0.81^{NS}$ | $0.81^{NS}$ | $0.76^{NS}$ | $0.85^{NS}$ | $0.74^{NS}$ | $0.86^{NS}$ |
| N (%) | | | | | | | | | 0.99** | 0.99** | 0.996** | 0.99** | 0.99** |
| P (%) | | | | | | | | | | 0.99** | 0.98** | 0.99** | 0.98** |
| K (%) | | | | | | | | | | | 0.99** | 0.998** | 0.98** |
| TCC (mg/g) | | | | | | | | | | | | 0.98** | 0.99** |
| TSP (%) | | | | | | | | | | | | | 0.97* |

* = Significant (P<0.05); ** = Highly significant (P<0.01); NS= Non Significant

Plant Height = PH, Days to Sprouting= DTS, Nitrogen content =N, Phosphorus content = P, Potassium content =K, Total Chlorophyll Contents =TCC, Total soluble Protein =TSP, Total soluble Sugars =TSS, Fresh Weight of leaves= FWL, Dry Weight of Leaves =DWL, Days to spike emergence =DSE, Number of floret spike= NFS, Length of spike= SL, Diameter of corms DC

(Table 3). The correlation value (0.98) between number of florets and protein contents in leaves showed the positive relation (Table 3). A significant positive correlation was observed between the spike length and nitrogen (0.98), phosphorus (0.98) and potassium (0.99) contents.

A positive correlation (0.99) between nitrogen and phosphorus contents in leaves was observed (Table 3). The results showed that the treatments comprising the microbial inoculations have a significant effect on the protein contents. The correlation value (0.99) between the nitrogen contents and proteins contents in leaves shows that both are positively correlated, as the nitrogen levels increased the amount of protein also increased (Table 3). The correlation value (0.87) between nitrogen contents and chlorophyll content showed that both are positively correlated.

## DISCUSSION

Treatments with bacterial inoculation provided balance nutrients for gladiolus plants and efficient uptake of nitrogen and phosphorus through roots is due to interaction between nitrogen fixing and phosphate solubilizing bacteria (Belimov *et al*., 1995). Therefore inoculation with the bio-fertilizers *Azotobacter*, phosphate solubilizing bacteria, VA-mycorrhiza and farmyard manure application enhanced vegetative and floral qualities of gladiolus compared with control treatment. Application of 120:65:62.5kg/ha NPK + phosphobacteria + *Azospirillum* showed better results with respect to days to sprouting, fresh weight and dry weight per plant. This was due to better availability of nutrients for the production of the bulbs (Srivastava *et al*., 2005). Dahiya *et al*. (2001) mentioned that increments in nitrogen level increased growth of tuberose and the treatments comprising *Azospirillum* as inoculant have a significant effect on the plant height. This increase in plant height was due to the presence of readily available form of nitrogen. *Azospirillum* improved plant macro and micro nutrient absorption. Similarly, it was observed that seeds inoculated with *Azospirillum* improved growth factors such as plant height (Brajeshwar *et al*., 2007; Migahed *et al*., 2004).

Increase in fresh weight of leaves due to the inoculation might be attributed to biological fixation of nitrogen and solubilization of phosphorus in root portion of plants resulting in absorption of more nutrients and its utilization. Moreover, *Azospirillum* had a role in nitrogen fixation and also involved in the production of indole-3-acetic acid (IAA), gibberellic acid (GA) and cytokinin like substances which enhanced the growth of plants. Phosphorous solubilizing bacteria helped in solubilization and mobilization of phosphorous in soil. The correlation value (0.96) showed a strong positive correlation between chlorophyll contents in leaves and fresh weight of leaves (Table 3). These findings are in accordance with the results of Selosse *et al*. (2004) who reported that the inoculation with fixative bacteria increased the absorption potential of plant and dry material in *Epipactis* plants. Kumar and Haripriya (2010) concluded that the increase in fresh weight of leaves might be attributed to the nutrient accumulation in the leaves. Some bacteria in the inoculated treatments not only fix the nitrogen, but also solubilized the phosphorus in the soil, activated the plant growth hormones, natural enzymes, antibiotics and different compounds such as volatile gases, that enhanced the vegetative growth (Astaraei and Koocheki, 1997). Nitrogen an essential component of protein, nucleic acid and many important substances like chlorophyll, which are required for vegetative growth and might be responsible for increase in dry matter accumulation in leaves (Dahiya *et al*., 2001; Viradia and Singh, 2004; Younis *et al*., 2014). The correlation value (0.99) showed a strong positive correlation between dry weight of leaves and protein contents in leaves; as the protein contents increased, dry weight of leaves also increased (Table 3). Inoculation with *Azospirillum* significantly increased the spike length as compared to control. The increase in spike length might be due to the availability of nutrition to the plant. Using *Azospirillum* and *Azotobacter* inoculants both individually or in combination, improved the flower quality and also increased the flower production. The increase in spike length might be due to elevated levels of macro-nutrients which have a positive effect on floral characteristics. Similarly, early flowering and increase in number of florets per spike is dependent on food material prepared as a result of photosynthesis in leaves (Brijendra and Singh 1986). In roses, the early flowering due to inoculation with *Azospirillum* was observed (Preethi *et al*., 1999). This was due to induced cytokinin synthesis and rapid assimilation of photosynthates resulting in early transformation of the axillary bulb from vegetative to reproductive phase. The increase in number of florets per spike is dependent on food material prepared as a result of photosynthesis in leaves. Carbohydrates are the major nutrient taking part in the development of flowers and may cause an increase in number of flowers (Brijendra and Singh, 1986). The results showed that the treatments comprising inoculation with different microbes have significant impact on the corms diameter; this was reported due to more accumulation of photosynthates in corms (Samia and Mahmoud, 2009; Yadav *et al*., 2002). The treatments of microbial inoculations significantly increased nitrogen content as compared with the control treatment. This could be attributed to the rapid absorption of these elements by the plant surface and their translocation in the plant (Mengel and Kirkby, 1987). The role of phosphate solubilizing bacteria excels in phosphorus availability in soil by secreting phosphatase enzyme which promoted to change organic unavailable phosphorus to its available forms (El-Ghandour *et al*., 2009). Therefore, it increases phosphorus absorption and more phosphorus accumulates in plant tissues. The significant effect of microbial inoculants was observed which may be due to the effect of different strain groups and nutrients mobilizing microorganisms which help in nutrient availability and increased levels of extracted minerals (El-Karmany *et al*., 2000). These results are in line with the findings of Youssef and Talaat (2003) who reported that bio-fertilizers may increase the total nitrogen percentage in rosemary plants which in turns increased the protein contents. Bekheta and Mahgoub (2005) concluded that the increase in nitrogen level led to change in amino acids quantity and specific proteins in carnation plants. Plants treated with microbial inoculations showed more sugar contents (stored carbohydrates) through effective photosynthesis. The correlation value (0.99) between sugar contents and protein contents showed a positive correlation, as the protein contents increased the level of total soluble sugars also increased. The beneficial effect of nitrogen on photosynthetic pigments as observed in this study might be due to its role in increasing the rates of photochemical reduction (Kumar *et al*., 1988). Chlorophyll contents are one of the most important criteria to determine the health of the plant, because chlorophyll contents are directly related to physiological activities to manufacture food (Richardson and Simpson, 2011).

***Conclusion*:** The obtained results from the study revealed that corms treated with *Azospirillum* produced a considerable increase in both vegetative and reproductive attributes as well as total chlorophyll content, protein content, total soluble sugars and accumulation of nutrients (N, P and K). Similarly, *Azotobacter,* phosphorus solubilizing bacteria and *Rhizobium* also improved the quantity and quality characters compared to control. Therefore, it can be concluded that microbial inoculation facilitated efficient nutrient's uptake which ultimately produce plants of superior quality.

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