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Research Article

# INOCULATION EFFECT OF *Azospirillum* ISOLATES ON YIELD PARAMETERS AND NUTRIENT CONTENT OF TUBEROSE (*Polianthes tuberosa* L.) cv. Mexican Single

**AVINASH M.*[1] AND SWAMY M.[2]**
[1]Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru, 560065, Karnataka, India
[2]Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Raichur, 584104, Karnataka, India
*Corresponding Author: Email - avi.mukhtedar@gmail.com

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**Abstract-** *Azospirillum* species are plant growth-promoting bacteria whose beneficial effects have been assumed to be moderately due to production of phytohormones (including IAA and gibberellins). Forty isolates were isolated from rhizoplane of tuberose and five efficient *Azospirillum* isolates were screened based on *in vitro* IAA production and $N_2$ fixation and used to study their beneficial effect on yield and nutrient content of Tuberose, which revealed that *Azospirillum* isolate ATR-39 was found significantly superior over other treatments.

**Keywords**- *Azospirillum, Tuberose, Gibberellins, IAA, Nitrogen fixation*

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## Introduction

Tuberose (*Polianthes tuberosa* L.) is essentially a florist's flower and leading commercial crop because of its multipurpose uses as cut flower, loose flower as well as its potential in perfume industry. Tuberose grows successfully in the warm plains of India. The commercial cultivation of tuberose in India is confined in West Bengal (Ranaghat, Kolaghat and Panskura), Karnataka, Andhra Pradesh, Tamil Nadu and Maharashtra. Tuberose occupies a prime position among the commercially grown flowers in India. So, in the view of the above; there is utmost need to use of fertilizers in balance proportions along with integration of bio fertilizers and their efficient managements. Since, negligible information is available regarding the effect of bio fertilizers on tuberose, experimental studies were carried out to find out the effect of *Azospirillum* isolates on flowering yield, biomass and nutrient content of Tuberose cv. Mexican single. Yield of flowers per unit area is the function of genotype and environment and hence proper genotype if used under proper environment will give higher yield. The flower yield of the crop can also be increased by manipulation of cultural practices. In this direction, one of the common cultural practices viz., nutrient application especially nitrogen, phosphorous and its management is of great importance for increasing the higher yield. Tuberose crop is the heavy feeder and responds well to the application of manures and fertilizers [1]. It is also observed that excessive application of nitrogenous fertilizers is not good for the crop as it not only decreases the yield apart from harming the plant growth and soil health [2]. The use of only inorganic fertilizers is also not sufficient to increase the flower yield. In order to improve deficient status of N in soil, the use of bio-fertilizer like *Azospirillum* is advocated, which fixes atmospheric nitrogen in the soil and thus maintaining the nutrient reserve of the soil and results in higher yield of flower. The use of *Azospirillum* also reduces inorganic fertilizers to an extent of 25 percent. The studies conducted elsewhere on use of bio-fertilizers in tuberose is limited and sometimes pertains to the particular zone.

## Materials and Methods

The pot culture experiment was carried out to study the effect of inoculation of *Azospirillum* isolates on yield, biomass and nutrient content of Tuberose (Mexican single) at the College of Agriculture, Raichur during 2015-16. The details of the materials used and the techniques adopted during the course of investigation are described below. The experimental pots were filled with red soil. Composite soil sample (0-15cm depth) was collected from fields before initiation of the experiment. The soil was air-dried, powdered and allowed to pass through 2mm sieve and was analyzed for physical and chemical properties. Soil is mixed with sand and FYM with the proportion of 3:2:1. The details of initial properties of soil used in pots along with the methods employed for their estimation are furnished in Table. Early sprouting variety Mexican single of Tuberose was collected from Horticulture department, UAS Raichur. Bulbs of Tuberose were completely dipped into different treatment conditions (5 efficient isolates of *Azospirillum*, one reference strain and control) for 1-2 minutes for uniform distribution of bioinoculant. The inoculated bulbs were planted in pots at the rate of 4 bulbs per pot. Each treatment was replicated thrice. The observations on plant growth parameters were recorded at periodical of intervals (30 DAP, 60 DAP and 90 DAP). Immediately after planting, the plots were irrigated. The irrigations were scheduled at regular interval. Irrigation was stopped when the crop attained physiological maturity. To check the growth of weeds and to keep the pots free from weeds during the cropping period hand weeding was undertaken at an interval of 15 days in the experimental pots to keep the pots free from weeds. The observations on plant growth parameters were recorded at 30, 60 and 90 DAP. The plant samples were analyzed for N uptake, P uptake, shoot dry weight content. Number of flowers produced in each spike of the selected plants was counted and average was worked out. The average length of the floret was computed from fully opened randomly selected ten flowers. The length of the flower was measured from pedicel to the throat of flower tube with the help of a scale and expressed in cm. The yield of flowers at each harvest from the net pot was recorded from each treatment. The total yield of flowers per pot was determined by addition of weight of flowers at each harvest. Flowers in the net pot were harvested separately and weighed treatment wise. Based on total net pot yield, yield of flowers per hectare was calculated.

Table-1 *Influence of Azospirillum isolates on number of flowers and length of flower in Tuberose*

| | Treatments | Number of flowers | Length of flower (cm) |
|---|---|---|---|
| $T_1$ | Control | 12.67 | 3.87 |
| $T_2$ | ATR-06 | 31.33 | 6.00 |
| $T_3$ | ATR-19 | 29.33 | 5.92 |
| $T_4$ | ATR-32 | 30.00 | 5.97 |
| $T_5$ | ATR-36 | 26.00 | 5.86 |
| $T_6$ | ATR-39 | 34.33 | 6.17 |
| $T_7$ | Reference *Azospirillum* strain (ACD-15) | 20.00 | 5.77 |
| | S. Em ± | 0.667 | 0.106 |
| | C.D. @ 1.0 % | 2.042 | 0.325 |

(Values are means of three replications)

Table-2 *Influence of Azospirillum isolates on yield of flowers in Tuberose*

| | Treatments | Flower yield per pot (g) | Flower yield per hectare (t/ha) |
|---|---|---|---|
| $T_1$ | Control | 30.13 | 1.26 |
| $T_2$ | ATR-06 | 86.09 | 3.59 |
| $T_3$ | ATR-19 | 43.65 | 1.82 |
| $T_4$ | ATR-32 | 73.90 | 3.08 |
| $T_5$ | ATR-36 | 37.11 | 1.55 |
| $T_6$ | ATR-39 | 99.74 | 4.16 |
| $T_7$ | Reference *Azospirillum* strain (ACD-15) | 85.87 | 3.58 |
| | S. Em ± | 1.455 | 0.064 |
| | C.D. @ 1.0 % | 4.457 | 0.195 |

(Values are means of three replications)

**Chemical analysis of plant sample for Nutrient uptake**

The tuberose plant collected at harvesting stages of individual treatment were dried in an oven at 65ºC till constant weight was observed and further ground to fine powder in Willey mill with stainless steel blades. The powdered samples were used for nutrient analysis.

**Shoot nitrogen content in plant**

Nitrogen content of shoot was estimated by modified micro kjeldhal method [3] at the time of harvest. 0.5g of dried shoot and root samples were digested with 5ml of concentrated $H_2SO_4$ and 200mg digestion calalyst ($K_2SO_4$:$CuSO_4$:Selenium) (100:10:1 ratio) until the contents become clear. After cooling, the volume was made up to 25ml with distilled water. Then 5ml of aliquot was transferred to micro kjeldhal distillation unit. An aliquot of 10 ml of 40 percent sodium hydroxide was added and steam distilled. Ammonia evolved was collected over 2 percent boric acid (20ml) containing 2 drops of double indicator (83.3mg bromocresol green and 16.6mg methyl red indicator dissolved in 10 ml of 95% ethanol) and back titrated against 0.05 N $H_2SO_4$. Nitrogen uptake was expressed as percentage at different growth stages. The total N uptake was calculated for each treatment separately using the following formula.

$$\text{Nitrogen (\%)} = \frac{\text{[Titer value x N. } H_2SO_4 \text{ x Dilution factors]}}{\text{[Weight of plant sample (gm) x 100]}}$$

**Digestion of plant sample**

A known quantity of powdered tuberose plant sample was pre-digested with concentrated nitric acid for overnight. Further, digestion was done with 5ml of di-acid mixture ($HNO_3$:$HClO_4$, 10:4) until white residue was developed. Then, the residue was dissolved in 6N HCl and volume was made up to 50ml. Blank was prepared in the same way without plant material.

**Shoot P content in plants**

The oven dried shoot samples were ground to fine powder separately in a willey mill and used for estimation of phosphorous content (mg/plant). Five hundred mg of root/shoot sample was taken in a 250ml capacity conical flask and was added with 2.5ml of concentrated $HNO_3$. The flasks were swirled to moisten the entire sample and then placed on a hot sand both for 30 minutes and then on an electric hot plate at 180ºC to 200ºC. The suspension was boiled until taken nearly to dryness.

**Wet oxidation**

Five ml of tri-acid mixture (conc. $HNO_3$, Conc. $H_2SO_4$ and 60% $HClO_4$ in the ratio of 10:1:4) was added to pre-digested sample and further digestion was carried out at 180ºC to 200ºC on a digestion mantle until the content in the flask became clear white. The contents of the flasks were cooled, 10-15ml of 6N HCl added and stirred well. The acid digest was transferred to 50ml volumetric flask and the volume was made up to 50ml with the distilled water. From this wet oxidized digested sample, P was estimated by Vanadomolybdate- phosphoric yellow colour method. Ten ml of wet oxidized digested sample was taken in a 50ml volumetric flask and 10ml Vanadomolybdate reagent was added. The volume was made up to 50ml with the distilled water and allowed to react for 30 minutes. The intensity of yellow colour developed was read 490nm using a spectronic-2D spectrophotometer. The P content was obtained by the standard curve prepared using $KH_2PO_4$.

**Results and Discussion**

In the present study efficient isolates of *Azospirillum* had a marked effect on the flower yield of the crop. Significantly higher flower yield was recorded in ATR-39 (4.91 t $ha^{-1}$) over control (0.30 t $ha^{-1}$). The increase in yield in the treatment ATR-39 might be due to the efficient use of nitrogen with less loss through leaching but fixation in the soil and thus available for cell division, protein synthesis and photosynthesis. *Azospirillum* which produces the growth promoting substances *viz*., IAA or GA like substances, Vitamin $B_{12}$, thiamine, riboflavin ($B_2$) *etc*, which might have helped in increasing the number of leaves in these treatments (ATR-39) might have increased the photosynthetic efficiency of the surface leading to the increased production of flowers and flowers yield. The similar results were obtained by Bankar (1987) [4]; Jitendra *et al*. (2012) [5]; Baskaran *et al*. (2014) [6] in tuberose crop and Ahmad *et al*. (2014) [7] in gladiolus.

The higher yield may be attributed to the higher number of flowers per spike [Table-1]. The significantly higher number of flowers per spike was recorded in the treatment ATR-39 than control. The significant higher flower yield (t $ha^{-1}$) in ATR-39 might be due to increase in number of flowers per spike due to beneficial effects of *Azospirillum* bulb inoculation. The similar results were obtained by Bankar (1987) and Koley and Pal (2011) [8] in tuberose.

The higher flower yield (t $ha^{-1}$) [Table-2] is an expression of other yield contributing characters like length of spike which accommodates more number of flowers, length of the flower which denotes the size of flower and weight of flowers which is directly proportional to the yield of flowers per unit area and weight of flowers.

All the characters were greatly influenced by the efficient isolates of *Azospirillum* inoculation. The significantly higher length of spike, length of flower and weight of individual flower recorded in ATR-39 might be due to the constant availability of nitrogen throughout the crop period required for cell division and protein synthesis which ultimately enhances growth of the plants. The *Azospirillum* bulb treatment helped to restore the nitrogen reserve in the soil which might have helped to get higher flower yield. Length of spike depends on the vegetative growth that increases with increase in height of the plant, which could be attributed to the beneficial effects of *Azospirillum*. Thus, the higher flower yield (t $ha^{-1}$) in these treatments might be due to better size of flower (spike and flower) and weight of individual flower. The lesser length of spike or flower might be due to lesser vegetative growth and delayed sprouting of bulbs, which might have contributed lesser yield in control. The similar results were in the flower yield (t $ha^{-1}$) was increased due to increase in size of flower and weight of individual flower as influenced by split application of fertilizer was reported by Bankar (1987) in tuberose, while the beneficial effect of *Azospirillum* was reported by Jitendra *et al*. (2012) in tuberose.

Table-3 *Influence of Azospirillum isolates on nutrient content in Tuberose*

| Treatments | | Nitrogen (%) | Phosphorous (%) |
|---|---|---|---|
| $T_1$ | Control | 1.86 | 0.11 |
| $T_2$ | ATR-06 | 2.33 | 0.21 |
| $T_3$ | ATR-19 | 2.12 | 0.17 |
| $T_4$ | ATR-32 | 2.24 | 0.20 |
| $T_5$ | ATR-36 | 2.00 | 0.15 |
| $T_6$ | ATR-39 | 2.41 | 0.23 |
| $T_7$ | Reference *Azospirillum* strain (ACD-15) | 1.99 | 0.14 |
| | S. Em ± | 0.044 | 0.005 |
| C.D. @ 1.0 % | | 0.134 | 0.015 |

(Values are means of three replications)

The nitrogen and phosphorous content [Table-3] at harvest of flowers increased significantly in the treatment ATR-39. The significantly lesser N and P content was observed in control. Increased N content of *Azospirillum* inoculated plants in the field at different stages during plant development has been observed in sorghum. Mostafa and Abo-Baker (2010) [9] studied the effect of bio-and chemical fertilizers separately and in different combinations on the growth of sunflower. Saha *et al*. (1985) [10] studied that influence of the seed inoculation of mustard (cv. Baruna) with *Azospirillum lipoferum* on $N_2$-fixation in rhizosphere, association of the bacteria with the roots and grain yield and N uptake. A significant increase in grain yield and nutrient uptake was also observed due to inoculation. The mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include, the ability to produce phytohormons, asymbiotic $N_2$ fixation, synthesis of antibiotics against phytopathogenic microorganisms, production of siderophores, enzymes and fungicidal compounds and also solubilization of mineral phosphates and other nutrients [11].

## Conclusion

The experimental results from the study revealed that bulbs of Tuberose treated with *Azospirillum* isolates produced a considerable increase in both vegetative and reproductive attributes as well as accumulation of nutrients (N and P). Among five isolates ATR-39 produced more flower yield and helped in more accumulation of nutrients, this may because isolate ATR-39 may adapt well to environment and produced significantly higher plant growth promoting substances which ultimately increased flower yield and accumulated more nutrient than other isolates and reference strain. Therefore, it can be concluded that inoculation of Tuberose bulbs with isolate ATR-39 facilitated efficient nutrient's uptake and production of plant growth promoting substances which ultimately produce significantly higher flower yield.

**Application of research:** Results from experiment can used and suggested for the application of biofertilizers for growth and yield of crops

**Research Category:** Agricultural Microbiology

**Abbreviations:** DAP- Days after planting, IAA- Indole acetic acid

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**Study area / Sample Collection:** College of Agriculture, Raichur

**Cultivar / Variety / Breed name:** Tuberose (*Polianthes tuberosa* L.) cv. Mexican Single

**Conflict of Interest:** None declared

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Ethical Committee Approval Number: Nil

## References

[1] Rameshwar A. (1976) *Ind. Hort*., 21, 22-23.
[2] Sadhu M.K. and Bose T.K. (1973) *Ind. Hort.,* 18, 17-20.
[3] Jackson M. L. (1973) *Soil Chemical Analysis, Prentice Hall of India Private Limited, New Delhi*, 38-82.
[4] Bankar G. J. (1987) *Ph. D. Thesis, Univ. Agric. Sci., Bangalore, India*.
[5] Jitendra K., Ajay K., and Singh K. P. (2012) *Ind. J. Agric. Res*., 46 (2), 192-195.
[6] Baskaran V., Misra R.L., Singh S.K. and Abirami K. (2014) *Ind. J. Hort*., 71(2), 237-241.
[7] Ahmad A., Tahir M., Rashid H., Ajmal B., Sajjad Raza, Najam-ud-Din and Adeel A. (2014) *Int. J. Plant, Animal Envt. Sci*., 4(1), 2231-4490.
[8] Koley S. and Pal A. K. (2011) *J. Crop Weed*, 7(2), 241-243.
[9] Mostafa G. and Abo-Baker A. A. (2010) *Asian J. Crop Sci*., 2, 137-146.
[10] Saha K.C. Sannigrahi S. and Mandal L.N. (1985) *Plant Soil*, 87, 273-280.
[11] Gholami A., Shahsavani S. and Nezarat S. (2009) *World Academy of Science, Engineering and Technology*, 49, 1-54.