Bangladesh J. Bot. **51**(3): 565-572, 2022 (September) DOI: https://doi.org/10.3329/bjb.v51i3.62003

RHIZOSPHERIC FUNGAL AND BACTERIAL BIO-AGENTS IN FLOWERING AND BULB OF TUBEROSE (*POLIANTHES TUBEROSA* L.)

RANJAN K SRIVASTAVA*, NAVNEET PAREEK[1], SATISH CHAND, BD BHUJ, KRITIKA PANT AND SHEEBA BELWAL

Department of Horticulture, G.B. Pant University of Agriculture & Technology, Pantnagar, India

Keywords: Tuberose, Agro-aquasorb, *Aspergillus* sp., *Penicillium* sp., *Bacillus subtilis, Bacillus megaterium,* Flowering, Bulb yield

Abstract

The present investigation was carried out to study the influence of rhizospheric fungal and bacterial bio-agents on the flowering and bulb parameters of tuberose with eight treatments [Strain-A i.e. *Aspergillus* sp., Strain-P i.e. *Penicillium* sp., Strain-A + Agro-aquasorb (AS), Strain-P + Agro-aquasorb, *Bacillus subtilis* (BS) + *Bacillus megeterium* (BM), BS + AS, BM + AS along with control (AS)]. Among these treatments, treatment T_4 i.e., Strain A+AS (*Aspergillus sp.*10^6 propagules/ml+ Agro-aquasorb 40 g/bulb) exhibited superiority for most of the parameters *viz*., floret diameter (4.83 cm), spike length (82.12 cm), number of spikes per m^2 area (39.99), maximum number of florets per spike (51.87) and weight of spike (94.41g) whereas treatment T_2 [Strain-A (*Aspergillus* sp.)] resulted in higher yield (316.06).

Introduction

Tuberose (*Polianthes tuberosa* L.) is an important commercial flower crop and is popular due to its sweet fragrance and long keeping quality of spike. Flowers have funnel shaped perianth tubular, waxy white and about 25 mm long. The flower buds are either single or double.Besides, the spikes are useful as cut flowers for vase decoration and bouquets, the individual florets are used for making *veni*, garland, button holes and essential oil extraction. The average yield of concrete from tuberose flowers is 0.15 per cent and the yield of absolute from concrete ranged from 30 - 45 per cent of concrete (Kahol *et al.* 2002). Thus, it is regarded as one of the important cut flower crops from aesthetic as well as commercial points of view. The various pharmacological studies indicates that the plant has anti-oxidant, anti-microbial, anti-viral, anti-inflammatory, antiulcer, immunomodulatory and neuropharmacological properties (Khatun and Hossain 2020). This crop also represents sensuality and is used in aromatherapy because of its ability to open the heart and calm the nerves, restoring joy, peace and harmony.

Bio-agents (*Azotobacter*, *Azospirillum*, phosphorus solubilizing bacteria and AM fungi) have proved their suitability in different flower crops such as rose, tuberose, carnation, marigold, aster, jasmine *etc.* These bioagents not only help in improving the nutrient uptake by the plants, releasing growth hormones and antibiotics but also improving the quality of product. The integrated plant nutrient supply system (IPNS) which involves the combined use of different nutrient sources such as chemical fertilizers, organic manures and biofertilizers helps in judicious use of chemical fertilizers (Chaudhary 2009). Nowadays, the importance and management of beneficial microorganisms have increased and led to the establishment of commercial trends around the world (Srivastava and Govil 2007). Meena *et al*. (2022) examined the efficacy of

*Author for correspondence: <ranjansrivastava25@gmail.com>. [1]Department of Soil Science, G.B. Pant University of Agriculture & Technology, Pantnagar, India.

different bio-agents in marigold and reported that application of *Pseudomonas fluorescens* 250 g per plot area was found better in flowering parameters *viz.* minimum number of days to first flower bud emergence (35.53 days), minimum days taken to 50 per cent flowering (65.33 days), longest duration of flowering (32.75 days), maximum number of flower plucked (6.80), and *Trichoderma viride* 250g per plot area was found better in disease parameters. It is reported that the 10-20 per cent of crop yield can be increased with biofertilizer use as a supplement (Brown 1972). Biofertilizers have been identified as alternative to chemical fertilizers in order to increase soil fertility and crop production in sustainable farming (Ali *et al.* 2014). Thus the present investigation was carried out to assess the influence of fungal and bacterial bio-agents on flower and bulb characteristics of tuberose cv. Suvasini and to study the status of rhizospheric microorganisms as influenced by these bio-agents.

Materials and Methods

The present investigation was conducted at Model Floriculture Centre of the university which is situated at the foothills of the Himalayas at 29^0 North Latitude and 79.3^0 East longitude at an altitude of 243.84 meter above the mean sea level. The maximum temperature during the growing period ranged from 26.9 to 39.4°C and minimum temperature 12.2 to 27.9°C. The experimental field had sandy loam soil having pH 6.68, organic carbon (0.60%), available N, P and K as 231.91, 18.34 and 135.97 $Kgha^{-1}$, respectively. Initially, the population of fungus, bacteria and PSB was 144.73 X 10^3, 31.43 X 10^8 and 86.3 X 10^5 cfu/g of soil, respectively.

The experiment was raised conducted under open field conditions by planting the tuberose bulb (1.5-2.0 cm in diameter) of var. Suvasini in 1.5 x 1.5 m size plot at 30 x 30 cm spacing at a depth of 5 cm in the month of April. The experiment was laid out in a randomized block design with four replications. At the time of planting, the bulbs were subjected to different treatments as given below:

S.N.	Code	Treatment	Microbial load/dose
1.	T1	Control, AS (Agro-aquasorb)	40 g/bulb
2.	T_2	Strain-A (*Aspergillus* sp.)	10^6 propagules/ml
3.	T_3	Strain-P (*Penicillium* sp.)	10^6 propagules/ml
4.	T_4	Strain-A + AS	10^6 propagules/ml+40 g/bulb
5.	T_5	Strain-P + AS	10^6 propagules/ml+40 g/bulb
6.	T_6	Strain-BS (*Bacillus subtilis*)	10^8 cfu/ml
7.	T_7	Strain-BM (*Bacillus megaterium*)	10^8 cfu/ml
8..	T_8	Strain-BS + AS	10^8 cfu/ml+ 40 g/bulb
9.	T_9	Strain-BM + AS	10^8 cfu/ml+ 40 g/bulb

Two fungal strains (*Aspergillus* sp. and *Penicillium* sp.) were used as Zn solubilizing bio-agents and two bacterial strains (*Bacillus megaterium* and *Bacillus subtilis*) were used as Phoshpate solubilizing bio-agents. The chemical fertilizers, nitrogen in the form of urea, phosphorus in the form of single super phosphate and potassium in the form of muriate of potash were applied according to RDF (150:150:150Kg/ha).

The fungal and bacterial bio-agents were procured from the Soil Microbiology Laboratory of the Department of Soil Science of the university. The load of bacterial cells was 10^8cfu (colony forming unit) per ml and of fungal cells was 10^6 propagules per milliliter. Ten milliliter broth of

the said concentration for bacteria and fungus cells were dissolved in 10 ml sterile water and 20 ml of the solution was used to dip 25 bulbs of tuberose variety for each treatment.

Initially composite soil sample was collected from 10-12 places before laid out of experiment and then from respective treatment at the termination of experiment at 0-15 cm depth by following 'V' notch method. Soils collected from all the places from each treatment were mixed thoroughly using quadrant method till it remains 500 g. A part (100 g) of soil sample was separately kept in the deep freezer for biological analysis. Soil samples were air dried and sieved through 2 mm for further chemical analysis *viz.*, pH, organic carbon (%), available N, P and K using standard methods of Jackson (1973), Walkley and Black (1934), Subbiah and Asija (1956), Olsen (1954) and Jackson (1973), respectively. Total bacterial and fungal population in soil were estimated in initial as well as final soil samples by using serial dilution plate count method. Prepared samples were serially diluted up to 10^{-8} dilution. Three different nutrient medium Potato Dextrose Agar, Soil Extract Agar and Pikovskaya's were used to enumerate fungus, bacteria and PSB, respectively. The plates were incubated at a temperature of 28 ± 2°C for 48 to 72 hrs. In order to prepare the serial dilution of soil sample, 10 g of soil was weighed and placed into 90 ml sterile water blank 150 ml capacity conical flask fitted with cotton plug and shaken to disperse the soil and this established 1 : 10 or 10^{-1} dilution. Then 1 ml from 10^{-1} dilution was transferred into 9 ml cotton plugged and sterile water blank in the test tube under aseptic condition to prepare 10^{-2} dilution. Likewise, the serial dilutions were prepared up to 10^{-8} dilution. The number of bacterial and fungal colonies were counted manually in the Petri plates and expressed as cfu per gram and propagules per ml, respectively of each soil sample.

Data recorded during the course of experiment were statistically analyzed (Panse and Sukhatame 1978). Valid conclusions were drawn by performing analysis of variance. To evaluate the significance of the difference between means of two treatments critical difference (at 5 per cent level of significance) was calculated, using following formula:

$$CD = \sqrt{\frac{2 \times E.M.S}{r}} \times t$$

Where,

CD = critical Difference

t = table value of t at 5 per cent level of significance at error degree of freedom

r = number of replications

EMS = error mean square

Results and Discussion

From the results it is evident that the treatments had hastened the spike emergence and the treatment T_5 (Strain-P + AS) resulted in significantly earlier spike emergence (47.80 days) as compared to other treatments while the treatment T_1 (Control, Agro-aquasorb) took longest time (Table 1). The findings of the present investigations are in agreement with the findings reported by Chaudhary (2009). The earliest flowering was observed in T_6 (Strain-BS) (16.93 days) which was statistically at par with T_1 (Control, AS Agro-aquasorb) (17.40 days), T_9 (Strain-BM + AS) (17.47 days), T_5 (Strain-P + AS) (18.27 days), T_8 (Strain-BS + AS) (18.40 days), and T_3 (Strain-A *Aspergillus* sp.) (18.63 days) whereas treatment T_2 (strain A *Aspergillus* sp.) took maximum days (19.97days) in flowering commencement. The treatment T_1 (Control, AS Agro-aquasorb) had earliest lowest floret opening (23.93 days) whereas in treatment T_4 (Strain-A + AS) lowest floret

took maximum time (26.73 days) to opening as compared to other treatments. The findings are in accordance with those reported by Kukde *et al*. (2004) who reported early opening of first pair of florets, better flower quality parameter in case of tuberose bulb treated with *Azotobacter* and PSB (at 2.5 g per kg bulb). The results further revealed that the tuberose bulb treated with *Azotobacter* and PSB at 2.5 g/kg bulb gave early opening of first pair of florets. The treatments had significant effect on days taken to lowest floret withers in treatment T_2 lowest floret withering was earliest (6.67 days) which was significantly lower than rest of the treatments, whereas in treatment T_8 lowest floret took maximum time (8.67 days) to withering. A perusal of data reveals that treatments had significant effect on days to 50% flowering (Table 1). The 50% flowering was earliest (8.4 days) in T_1 (Control, AS Agro-aquasorb) which was significantly quicker than rest of the treatments whereas longest time to 50% flowering was taken by T_9 (12.20 days) which was statistically higher than rest of the treatments. However, non-significant differences in duration for 50% flowering were observed among treatments T_2 (12.12 days) and T_9 (12.20 days); among T_3 (9.87 days), T_5 (9.73 days) and T_8 (9.80 days) and also among T_6 (10.60 days) and T_7 (10.40 days) (Table 1). The findings of this investigation are in conformity with the findings reported by Dalve *et al.* (2009) who reported that days to 50% flowering was positively influenced by the application of both the bio-agents in combination with nitrogen. The maximum duration of flowering was observed in T_3 (26.53 days) which was statistically at par with and followed by T_4 (24.47 days), T_9 (24.40 days), T_2 (22.73 days), T_5 (22.27 days), and it was minimum in T_6 (19.07 days). The results of this investigation are in agreement with the findings of Singh *et al.* (2008) who studied the effect of biofertilizer and graded dose of nitrogen on growth and flower yield of calendula (*Calendula officinalis*).

Table 1. Effects of bacterial and fungal bio-agents on floral characters of tuberose cv. Suvasini.

Treatments	Spike emergence (Days)	Flowering commencement (Days) *	Lowest floret open (Days)*	50% flowering (Days)**	Lowest floret wither (Days)**	Flowering Duration (Days)**
T_1 Control, AS (agro-aquasorb)	71.47	17.40	23.93	8.40	8.6	19.47
T_2 Strain-A (*Aspergillus* sp.)	51.87	19.97	24.80	12.13	6.67	22.73
T_3 Strain-P (*Penicillium* sp.)	62.20	18.63	25.07	9.87	7.47	26.53
T_4 Strain-A +AS	63.00	19.47	26.73	10.6	8.30	24.47
T_5 Strain-P +AS	47.80	18.27	24.73	9.73	7.47	22.27
T_6 BS- *Bacillus subtilis*	52.46	16.93	24.53	10.60	7.67	19.07
T_7 BM-*Bacillus megaterium*	69.93	19.50	25.20	10.40	7.40	21.86
T_8 BS + AS	70.92	18.40	26.00	9.80	8.67	20.87
T_9 BM + AS	48.13	17.47	25.27	12.20	8.13	24.40
S.Em.±	6.45	0.59	0.92	0.13	0.14	1.47
CD at 5%	2.45	1.77	NS	0.40	0.43	4.45

*from day to spike emergence, **from day to lowest floret opening.

A perusal of data presented in Table 2 indicates that the treatments had non-significant effect on number of florets per spike. However, the maximum number of florets per spike was recorded in T_2 (51.87) and it was minimum in T_8 (40.00). The above findings are in accordance with the results reported by Prabhat Kumar *et al.* (2003) who studied the response of vesicular arbuscular mycorrhizas (VAM) and phosphobacterin on China aster (*Callistephus chinensis*). The diameter of

lowest floret varied significantly with different treatments. The maximum lowest floret diameter was in T_3 (4.99cm) which was statistically at par with T_2 (4.94 cm), T_4 (4.83), T_7 (4.84 cm), T_8 (4.85 cm) and T_9 (4.96 cm) while the minimum lowest floret diameter was observed in T_1 (4.21 cm) (Table 2). Chaudhary *et al.* (2013) reported that the application of 50 g N/plant + *Azotobacter* and *Azospirillum* each @ 1 ml/plant (T_9) produced maximum diameter of flower which was significantly higher than rest of the treatments in gladiolus.

Table 2. Effects of bacterial and fungal bio-agents on floral characters of tuberose cv. Suvasini.

Treatments	Florets/ spike (number)	Lowest floret diameter (cm)	Opened florets/spike (number)	Spike Length (cm)	Spikes / m^2 (number)
T_1 Control, AS (agro-aquasorb)	43.06	4.23	36.07	79.21	28.14
T_2 Strain-A (*Aspergillus* sp.)	51.87	4.94	43.53	80.08	37.77
T_3 Strain-P (*Penicillium* sp.)	42.93	4.99	36.93	74.36	36.29
T_4 Strain-A+ AS	46.40	4.83	43.60	82.12	39.99
T_5 Strain-P + AS	48.13	4.51	43.27	75.47	34.81
T_6 BS- *Bacillus subtilis*	45.33	4.97	38.20	78.48	32.58
T_7 BM-*Bacillus megaterium*	43.40	4.84	36.47	83.37	34.81
T_8 BS + AS	40.00	4.85	33.80	81.23	34.81
T_9 BM + AS	41.87	4.96	36.80	74.44	37.77
S.Em.±	2.95	0.07	2.71	0.29	0.95
CD at 5%	NS	0.23	NS	0.86	2.86

The treatments had non-significant effect on number of opened florets per spike (Table 2). However, the maximum number of opened florets per spike was recorded in T_4 (43.60) followed by T_2 (43.53), T_5 (43.27), T_7 (38.20), T_3 (36.93), T_9 (Strain-BM + AS) (36.80), T_7 (36.47), T_1 (36.07) and was minimum in T_8 (33.80). The results are more or less similar with the findings of Chaudhary (2009) who reported that the maximum spike length (89 cm), number of florets per spike (49.2) were recorded in treatment *Azotobacter* (20 g/litre) + PSB (20 g/litre) which was statistically at par with treatment PSB (20 g/litre) + VAM (10 g/bulb) in tuberose. The spike length was enhanced significantly due to the bacterial and fungal bio-agents treatments. The maximum spike length was recorded in T_7 (83.37 cm) and minimum in plants treated with T_3 (74.36 cm). However, T_3 (74.36 cm) and T_9 (74.44 cm) were found to be statistically at par. It might be because phosphorus plays an indispensable role in chlorophyll synthesis, cell division and synthesis of growth hormones and might had produced the higher spike length. Lal *et al.* (2010) reported similar results in tuberose *cv.* Single when plants were treated with vermicompost and PSB @ 1 kg/m^2 and 2 g/bulb, respectively and produced highest spike length (77.70 and 77.86 cm, respectively) and maximum number of spikes per plant (1.49 and 1.49, respectively). The data recorded for average spike yield had significant effect of bacterial and fungal bio-agents treatments as depicted in Table 2. The maximum number of spike per m^2 was recorded in T_4 (Strain-A + AS) (39.99) and minimum was recorded in T_1 (28.14). However, spike yield under treatments T_2 (37.77), T_3 (36.29), T_5 (34.81), T_7 (Strain-BM (*Bacillus megaterium*) (34.81), T_8 (Strain-BS + AS (34.81) and T_9 (37.77) were to be found at par. Similar results were reported by Srivastava and Govil (2007) who advocated that biofertilizers significantly improved floral characters and for quality spike production, PSB was found more effective in gladiolus cv. American Beauty. Wasim *et al.* (2014) also reported that the floral characters were found to be

better in treatments having 75% RDF + *Azotobacter* + PSB and 100% RDF + *Azotobacte*r + PSB in tuberose (*Polianthes tuberosa* L.) cv. Mexican Single. (Similar finding was also observed by Anop Kumari *et al.* (2015) who reported that the tuberose bulbs treated with *Azotobacter* and PSB at 2.5 g/kg bulb gave better flower quality parameter and maximum yield of flowers per ha.

The different treatments and their combinations had non-significant effect on diameter of bulb which, however, ranged from 21.73 mm in treatment T_4 to 29.75 mm in treatment T_6 (BS- *Bacillus subtilis*) (Table 3). The number of bulbs per plant was significantly influenced by the treatments. The maximum numbers of bulbs were obtained in T_2 (28.44) and minimum in T_5 (23.51). A significant effect of different bio-agent treatments was recorded on the number of bulblets per plant. The maximum number of bulblets per plant was obtained in T_7 (13.33) and minimum was obtained in T_6 (BS- *Bacillus subtilis*) (6.66). Furthermore, bulblets under T_1 (Control) (7.33), T_2 (8.00), T_4 (7.33), T_5 (9.67), T_6 (8.00), T_8 (9.66) and T_9 (6.67) were found to be statistically at par (Table 3). The number of bulbs per square meter was maximum (316.06) in treatment T_2 Strain-A (*Aspergillus* sp.) which was significantly higher than rest of the treatments, followed by T_9 which recorded 282.32 bulbs per square meter whereas it was minimum (261.24) in T_5. These results are in agreement with the results of Lal *et al.* (2010) who reported that treatment with 120:65:62.5 kg NPK/ha + Az + PSB resulted in the highest number of bulbs in tuberose cv Single.

Table 3. Effects of bacterial and fungal bio-agents on bulb characters and yield in tuberose cv. Suvasini.

Treatments	Bulb diameter (mm)	Bulbs/plant (number)	Bulblets/ plant (number)	Bulbs/ m^2 (number)
T_1 Control, AS (agro-aquasorb)	21.88	24.52	7.33	272.43
T_2 Strain-A (*Aspergillus* sp.)	25.10	28.44	8.00	316.06
T_3 Strain-P (*Penicillium* sp.)	23.42	24.18	6.66	268.74
T_4 Strain-A + AS	21.73	24.58	7.33	273.13
T_5 Strain-P + AS	25.50	23.51	9.67	261.24
T_6 BS- *Bacillus subtilis*	29.45	24.36	8.00	270.73
T_7 BM-*Bacillus megaterium*	28.03	24.34	13.33	270.51
T_8 BS + AS	24.87	23.75	9.66	263.88
T_9 BM + AS	23.74	25.41	6.67	282.32
S.Em.±	2.18	0.84	1.23	9.36
CD at 5%	NS	2.54	3.69	28.17

The data presented in Table 4 showed that the fungal and bacterial bio-agents had significant effect on increasing the colony forming units of fungus per gram of soil. The maximum colony forming units of fungus per gram of soil was observed maximum in the T_4 (446.22 cfu) which were found to be at par with T_2 (419.00 cfu) and T_9 (399.11cfu) whereas it was minimum in T_7 (58.83 cfu). However, fungus colonies under treatment T_3 (214.11 cfu), T_5 (160.11 cfu), T_6 (155.89 cfu) and T_8 (231.44 cfu) were found to be statistically at par. In case of bacteria maximum colony forming unit per gram of soil was obtained in T_7 (31.46 cfu) which was statistically at par with T_6 (30.95 cfu) and T_1 (27.83cfu) whereas it was minimum in T_8 (8.82 cfu). The treatments had significant effect also on the colony forming unit of Phosphate solubilizing bacteria (PSB) per g of soil (Table 4). The maximum cfu was recorded in T_6 (515.78 cfu) which

was statistically at par with T_9 (375.56), T_8 (398.33) and T_7 (414.33) whereas it was minimum (204.67 cfu) in T_1 (control) which was statistically at par with T_2 (233.55 cfu), T_3 (270.8) and T_4 (284.55 cfu). The above results are in conformity with the finding of Barman *et al.* (2003) who reported increased P availability in response to PSB application. This might be due to the fact that phosphate solubilizing bacteria provide phosphate by solubilizing the insoluble phosphate by secreting organic acids (Dave and Patel 2003). The synergistic effect between VAM and PSB has been studied by Sandhya *et al.* (2013) who reported that VAM and PSB treated lavender plants recorded increased plants dry biomass and P-uptake. Similar synergistic interactions were reported between *Glomus. fasciculatum* and *Aspergillus niger* with each organism stimulating the growth of the each other resulting in subsequent improvement in plants growth and P uptake (Gopalkrishana 1980). The findings of above results are in accordance with the results reported by Kravchenko *et al.* (2013) who studied the effects of Bradyrhizobium and *Bacillus* sp. inoculation on soybean plants for soil biological and physico-chemical parameters and reported that the rhizospheric bacterial populations were significantly affected by the inoculation.

Table 4. Effects of bacterial and fungal bio-agents on micro-organisms (PSB, *Aspergillums and Penicillium*).

Treatments	Fungus(10^3) cfu/ g soil	Bacteria (10^8) cfu/ g soil	PSB(10^5) cfu/g soil
T_1 Control, AS (agro-aquasorb)	71.17	27.83	204.67
T_2Strain-A (*Aspergillus* sp.)	419.00	14.40	303.22
T_3 Strain-P (*Penicillium* sp.)	214.11	12.20	233.55
T_4 Strain-A + AS	446.22	16.53	270.78
T_5 Strain-P + AS	160.11	9.58	284.55
T_6 BS- *Bacillus subtilis*	155.89	30.95	515.78
T_7 BM-*Bacillus megaterium*	58.83	31.46	414.33
T_8 BS + AS	231.44	8.82	398.33
T_9 BM + AS	399.11	17.26	375.56
S.Em.±	33.32	2.43	57.62
CD at 5%	97.74	7.13	173.476

It may be concluded that the inoculation of *Aspergillus* sp. (@10^6 propagules/ml at the time of planting could be beneficial for better vegetative growth and higher yields of flower spikes and bulbs.

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(Manuscript received on 26 June, 2020; revised on 27 August, 2022)