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Effect of microbial consortium on increasing nitrogen, phosphorous and potasium content of *Gloriosa superba* L.

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Abstract
The present study intended to develop a microbial consortium which has good scope and wide adoptability for *Gloriosa superba* L. The plant growth promoting rhizobacterial (PGPR) isolates *Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens*, were used. The inoculation increased the nitrogen uptake, phosphorous and potassium the increase was the most significant in T_7 (consortium), which recorded nitrogen uptake of (204.50 mg plant^{-1}), phosphorus uptake of (155.40 mg plant^{-1}) and potassium uptake of (150.30mg plant^{-1}) on atharvest.

Keywords: *gloriosa superba*, consortium, medicinal plant, NPK content

1. Introduction
Gloriosa superba is a perennial tuberous climbing herb is widely distributed in tropical and sub-tropical parts of India including foothills of Himalayas [1]. It is a one of the important medicinal plant which has attained economic significance in recent times. The large scale cultivation of medicinal plants is gaining importance nowadays due to its pharmaceutical value and therefore several agronomic practices are tried to enhance the biomass production and also to increase the biochemical constituents (active principles).

Gloriosa superba is a well-known non-wood forest plant that has long been in regular demand among the practitioners of traditional medicine in tropical African and Asian countries since antiquity. In India, it is a much used plant in Ayurvedic and Unani systems of medicines [2, 3]. It is used either as a single drug or in combination with other drugs. Herbal medicine recommends *G. superba* for the treatment of urinary and reproductive systems, respiratory, skin diseases, cardiovascular troubles, and many other disorders. The seeds of *G. superba* are highly priced in the world market as sources of colchicine, chemical that has been used in the past as a remedy against gout, a disease caused by deposits of uric acid in the joints [4].

Gloriosa superba cure against leprosy, colics, chronic ulcers, haemorrhoids, skin parasites, head lice and tumours [5, 6, 7, 8, 9, 10].

The effect of plant growth promoting rhizobacteria on plant growth when used in the form of biofertilizer which is mainly performed by indirect means, fixation of atmospheric nitrogen, solubilisation of minerals such as phosphorous and synthesis of phytohormones [11].

Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible. Plant growth promoting bacteria may be important for plant nutrition by increasing N and P by the plants and playing a significant role as PGPR in the biofertilization of crops.

The mechanisms of plant growth stimulation by associative bacteria are mobilization of nutrients, stimulation of root growth by production of phytohormones and antagonism against soil borne plant pathogens [12]. Inoculation of canola seeds with *Pseudomonas putida* GR 12-2, which produces low levels of IAA, resulted in two or three fold increases in the length of seedling roots [13].

Successful examples of inoculation of maize, canola, wheat and other crops with PGPR species *Bacillus, Pseudomonas* have been achieved both in laboratory and field trials [14]. The microbial inoculums *Bacillus megaterium* and *Bacillus mucilaginous* not only increased the plant growth, but also improved nutritional assimilation of plant (total N, P and K). Plant growth promoting bacteria have an ability to convert nutritionally important elements from unavailable to available form through biological process [15].

Plant growth promoting rhizobacterial strains *viz*., *Azotobacter chroococcum* GAt -1, *Bacillus megatherium* GBm -18 and *Pseudomonas fluorescens* GPf -21 were selected from 24 isolates each of *Azotobacter, Bacillus* and *Pesudomonas* obtained from 24 different locations of five districts of Tamil Nadu where *Gloriosa superba* L. in commercially grown.

2. Materials and methods
2.1 Field Experiment
The field experiment was conducted at the backyard of the Department of Microbiology, Faculty of Agriculture,

Annamalai University, Annamalai Nagar. The plant growth promoting rhizobacterial (PGPR) isolates *Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens* were used.

2.2 Estimation of total nitrogen content of the plant

The nitrogen content of the plant sample was estimated by Microkjeldahl method. The plant samples were dried first in shade and then dried in an oven at 60º C till constant weight was obtained, the dried samples were powdered, sieved and the nitrogen content was estimated as follows.

One hundred mg of the sample was transferred to 50 ml pyrex micro kjeldahl flask. A quarter teaspoonful of digestion mixture (10 g of reagent grade potassium sulphate, 1g of cupric sulphate, 0.1 g of selenium metal powder) and 4 ml of salicyl sulphuric acid mixture (0.1 g of salicylic acid), 1 g of sodium thiosulphate and 30 ml of concentrated were introduced. The contents were slowly heated till frothing ceased and then heated strongly; completion of digestion was indicated by the solution turning bluish green. After cooling about 15 ml of distilled water was added to the flask and cooled. The contents were transferred into the distilled and excess of 0.1 N sulphuric acid (10 ml) containing few drops of methyl red. Distillation was continued for 15 minutes. The contents were back titrated with 0.1 N potassium hydroxide, till the appearance of golden yellow colour.

Nitrogen in the sample was calculated using the factor 1 ml of 0.1 N sulphuric acid = 0.0014 g of nitrogen

2.3 Estimation of total phosphorus content of the plant

The phosphorus content of the plant was estimated using vanadomolybdate method [16]. The oven dried plant material of about 0.5 g was taken and transferred to 50 ml of Pyrex microkjeldahl digestion flask and digested in 10 ml tri acid mixture consisting of nitric acid, per chloric acid and Sulphuric acid (10:4:1). When the digestion was complete the contents were cooled and 40 ml of distilled water was added. It was then thoroughly shaken and filtered through what man No. 44 filter paper. The filtrate was collected in a 100 ml volumetric flask.

The digestion flasks containing the residue were washed several, times with small quantities of warm dilute nitric acid (1:19) and the volume was raised to the mark with distilled water.

An aliquot of 10 ml was taken in a volumetric flask and to that a few drops of 2, 4, dinitrophenol indicator was added followed by 4 N Sodium carbonate (Na_2CO_3) to obtain a yellow colour which was later discharged by the addition of 6 N nitric acid to bring about pH of 3.0. One ml of 1.2 N hydrochloric acid and 2 ml of nitric acid were added and diluted with water to raise the volume to 4.0 ml. Immediately 2.5 ml each of 0.25 per cent ammonium metavanadate and a 4 per cent ammonium molybdate were added and shaken. Then the volume was allowed to stand for 30 min. Readings were taken in klette summerson photometric colorimeter using a blue filter. Known concentration of Phosphorus solution were simultaneously developed and read in the colorimeter.

2.4 Estimation of potassium content of the plant

Two gram of grounded, oven dried oxidized samples were dissolved in 10 molar hydrochloric acid and allowed for further digestion on steam bath for 20 minutes. Then evaporated the solution to dryness and heated the residue in an oven for 30 minutes at 105˚C. Further, the residues were allowed to cool returned to the water bath and added 10 ml of 10 molar hydrochloric acid. Then, transferred 2 ml of the digested samples were made upto 100 ml in volumetric flask with distilled water. Then aspirate the Blank and each standard into the BWB Flame Photometer, in turn, and enter each value when prompted. The amount of Potassium in the plant material sample can be calculated taking into account the dilution factor.

3. Results

3.1 Nitrogen uptake

The nitrogen uptake of *Gloriosa superba* in general, increased in all the treatments at all sampling periods. The inoculation increased the nitrogen uptake and the increase was the most significant in T_7 (consortium), which recorded nitrogen uptake of (204.50 mg plant^{-1}) on atharvest, followed by dual inoculation was (134.20 mg plant^{-1}) with T_5, (127.30 mg plant^{-1}) with T_6 and (113.60 mg plant^{-1}) with T_4,. Single inoculation was (104.32 mg plant^{-1}) with T_3 followed by T_1 and T_2. The uninoculated control recorded (80.50 mg plant^{-1}) in T_8 *Gloriosa superba* (Table - 1).

3.2 Phosphorus uptake

The phosphorus uptake of *Gloriosa superba* in general, increased in all the treatments at all sampling periods. The inoculation increased the phosphorus uptake and the increase was the most significant in T_7 (consortium), which recorded phosphorus uptake of (155.40 mg plant^{-1}) on atharvest, followed by dual inoculation was (130.20 mg plant^{-1}) with T_5, (122.70 mg plant^{-1}) with T_6 and (115.30 mg plant^{-1}) with T_4. Single inoculation was (99.46 mg plant^{-1}) with T_3, followed by T_1 and T_2. The uninoculated control recorded (87.95 mg plant^{-1}) in T_8 *Gloriosa superba* (Table-2).

3.3 Potassium uptake

The potassium uptake of *Gloriosa superba* in general, increased in all the treatments at all sampling periods. The inoculation increased the potassium uptake and the increase was the most significant in T_7 (consortium), which recorded potassium uptake of (150.30mg plant^{-1}) on atharvest, followed by dual inoculation was (134.67mg plant^{-1}) with T_5, (127.42 mg plant^{-1}) with T_6 and (115.72 mg plant^{-1}) with T_4, Single inoculation was (107.42mg plant^{-1}) with T_3, followed by T_1 and T_2. The uninoculated control recorded (72.45 mg plant^{-1}) in T_8 *Gloriosa superba* (Table-3).

4. Discussion

The rhizosphere microorganisms have increased because they play significant role in the maintenance of soil. The use of N fixing bacteria that assimilate gaseous nitrogen from the atmosphere includes symbionts of *Rhizobium* and free living rhizobacteria in *Azotobacter*. The PGPR strains such as *Azotobacter*, phosphate solubilizing *Bacillus* and *Pseudomonas* when tuber treated increased the N, P and K uptake of N, P and K in different crop plants such as rice, wheat, sorghum, maize, sugarcane, banana, forage crops and

medicinal plants.

The single, dual inoculant effect observed in the present study was in conformity with the earlier reports published on several other crops [17, 18].

Table 1: Effect of plant growth promoting rhizobacterial (PGPR) inoculants on Nitrogen uptake *Gloriosa superba*

SI. NO	Treatments	Nitrogen uptake mg plant^{-1}			
		90 DAS	120 DAS	150 DAS	Atharvest
1.	T_1–*A. chroococcum* (GAt -1)	40.67	60.46	71.20	96.53
2.	T_2 – *B.* megaterium (GBm - 18)	38.42	57.30	67.60	89.20
3.	T_3 – *P. fluorescens*(GPf - 21)	43.73	68.50	77.40	104.32
4.	T_4 - *A. chroococcum* (GAt -1) +*B. megaterium* (GBm -18)	45.60	73.60	82.37	113.60
5.	T_5–*A.chroococcum* (Gat -1) + *P. fluorescens*(GPf - 21)	53.40	88.00	95.60	134.20
6.	T_6–*B.megaterium* (GBm - 18) +*P. fluorescens*(GPf - 21)	49.36	80.40	90.57	127.30
7.	T_7 – Consortium *A. chroococcum* (GAt -1) + *B. megaterium* (GBm - 18) + *P. fluorescens* (GPf - 21)	56.37	90.50	110.20	204.50
8.	T_8 - Control (Un inoculated)	34.12	51.00	62.70	80.50
SED		1.094	1.194	2.487	2.835
CD (P = 0.05)		2.200	2.400	5.000	5.699

Table 2: Effect of plant growth promoting rhizobacterial (PGPR) inoculants on phosphorus uptake *Gloriosa superba*

SI. NO	Treatments	Phosphorus uptake mg plant^{-1}			
		90 DAS	120 DAS	150 DAS	Atharvest
1.	T_1–*A. chroococcum* (GAt -1)	37.47	61.57	81.46	95.30
2.	T_2 – *B.* megaterium (GBm - 18)	32.50	56.40	78.30	91.73
3.	T_3 – *P. fluorescens*(GPf - 21)	41.60	67.50	88.67	99.46
4.	T_4 - *A. chroococcum* (GAt -1) +*B. megaterium* (GBm -18)	47.20	72.70	94.28	115.30
5.	T_5–*A.chroococcum* (Gat -1) + *P. fluorescens*(GPf - 21)	57.60	87.52	109.50	130.20
6.	T_6–*B.megaterium* (GBm - 18) +*P. fluorescens*(GPf - 21)	52.73	80.20	101.65	122.70
7.	T_7 – Consortium *A. chroococcum* (GAt -1) + *B. megaterium* (GBm - 18) + *P. fluorescens* (GPf - 21)	63.40	100.20	120.60	155.40
8.	T_8 - Control (Un inoculated)	28.35	50.50	71.00	87.95
SED		4.500	3.283	3.482	3.632
CD (P = 0.05)		6.000	6.600	6.999	7.301

Table 3: Effect of plant growth promoting rhizobacterial (PGPR) inoculants on potassium uptake of *Gloriosa superba*

SI. NO	Treatments	Potassium uptake mg plant^{-1}			
		90 DAS	120 DAS	150 DAS	Atharvest
1.	T_1–*A. chroococcum* (GAt -1)	37.45	58.32	87.65	99.68
2.	T_2 – *B.* megaterium (GBm - 18)	31.65	56.42	80.45	90.54
3.	T_3 – *P. fluorescens*(GPf - 21)	42.63	60.42	93.74	107.42
4.	T_4 - *A.chroococcum* (GAt -1) +*B.megaterium* (GBm -18)	48.54	65.73	100.50	115.72
5.	T_5–*A.chroococcum* (Gat -1) + *P. fluorescens*(GPf - 21)	58.47	74.46	117.20	134.67
6.	T_6–*B.megaterium* (GBm - 18) +*P. fluorescens*(GPf - 21)	53.26	69.34	109.83	127.42
7.	T_7 – Consortium *A. chroococcum* (GAt -1) + *B. megaterium* (GBm - 18) + *P. fluorescens* (GPf - 21)	64.50	83.57	126.15	150.30
8.	T_8 - Control (Un inoculated)	28.95	53.50	63.72	72.45
SED		2.482	2.502	3.537	3.597
CD (P = 0.05)		4.990	5.030	7.110	7.230

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