

Effect of Efficient Isolates of PGPR on Growth and Yield of Chilli (*capsicum annuum l*.) Under Pot Culture Conditions

Priti S.Sabnis^{*1}, Mahadevaswamy¹ and Nagraj S.B.¹

Abstract: A survey work was conducted to screen and isolate efficient PGPR strains from TBP area Karnataka. Among all the 80 strains of PGPR six efficient strains were selected on the basis of their IAA production, nitrogen fixation, siderophore production, P-solubilization and biocontrol potential for our study. It was noticed that chilli seedlings treated with these efficient strains of PGPR showed increase in the plant height, number of branches per plant, shoot dry weight, root dry weight, shoot N and P content and number fruits plant⁻¹. Among Pseudomonas treatment the treatment inoculated with PS-5 strain significantly increases the plant height (42.96 cm), number of branches plant⁻¹ (6.67), shoot dryweight (12.08 gm plant⁻¹), root dry weight (0.98 gm plant⁻¹), shoot N content with 2.23% of nitrogen and P content with 0.21% of phosphorous plant⁻¹ and number fruits plant⁻¹ (52 fruits plant⁻¹) over all other treatment and control. Out of Azospirillum treatment, the plant inoculated with AZP-26 significantly perform better with respect to plant height (39.34 cm plant⁻¹), number of branches plant⁻¹ (6.00), shoot dry weight (11.83 gm plant⁻¹), root dry weight (0.80 gm plant⁻¹), shoot N content (2.70% N plant⁻¹), shoot P content (0.18% of P plant⁻¹) and number fruits plant⁻¹ (48 fruits plant⁻¹) compared to control and other treatments.

Keywords: PGPR, Azospirillum, Pseudomonas.

INTRODUCTION

Chilli grown as one of the important spice crop all over the world. India is the largest producer of chilli in the world. Plant growth promoting rhizobacteria (PGPR) constitute approximately 2-5% of the total rhizomicrobial population (Antoun and Kloepper, 2001 and Kloepper et al., 1980). Root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria (PGPR) (Kloepper et al. 1980). PGPR are known to induce resistance against various plant pathogens in different crops ranging from cereals, pulses, ornamentals, vegetables, plantation crops (Alagawadi and Gaur, 1992; Bashan and Holquin, 1997; Riggs et al., 2001 and Muthuraju and Jaysheela, 2005). The important traits of PGPRs include fixation of atmospheric nitrogen, solubilization of insoluble inorganic phosphates, production of plant hormones, siderophores,

bacteriocins *etc.* These organisms also provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms (Mishra *et al.*, 2005). Hence, an attempt was made to study the effect of different PGPR strains on chilli.

MATRIAL METHODES

A survey work was conducted by Priti *et al.*,2013 to screen and isolate efficient PGPR strains from TBP area Karnataka. Among all the 80 strains of PGPR six efficient strains were isolated (AZP-15, AZP-35, AZP-26 and PS-13, PS-23, PS-5) on the basis of their IAA production, nitrogen fixation, siderophore production, P-solubilization and biocontrol potential. Hence it's necessary to study the efficacy of these six efficient strains under pot conditions.

Effect of Selected Isolates of PGPR on the Growth and Yield of Chilli Plants.

Three efficient strain each of *Pseudomonas* and *Azospirillum* were further tested for their efficiency

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Table 1 Treatment details					
Sl. No.	Particulars	Details			
1. 2. 3.	Design Replications Treatments	Completely randomized design Three T_1 - Control T_2 - Efficient <i>Pseudomonas</i> isolate-1(PS-13) T_3 - Efficient <i>Pseudomonas</i> isolate-2(PS-23) T_4 - Efficient <i>Pseudomonas</i> isolate-3(PS-5) T_5 - Reference strain of <i>Pseudomonas</i> (<i>P. fluorescence</i>) T_6 - Efficient <i>Azospirillum</i> isolate-1 (AZP-35) T_7 - Efficient <i>Azospirillum</i> isolate-2(AZP-26) T_8 - Efficient <i>Azospirillum</i> isolate -3(AZP-15) T_0 - Reference of <i>Azospirillum</i> (<i>A.brasilense</i>)			
4 5. 6.	Crop Variety Soil	Chilli Pusa jwala Black			

Table 1

to promote growth and yield of chilli under pot culture conditions.

Soil

A black soil collected from the Agricultural Research Station, University of Agricultural Sciences, Raichur. The unsterilized soil was filled in one foot earthen pots at the rate of 5kg pot⁻¹. The soil had pH of 7.6; EC 0.13dSml¹and available P 12.06 kgha⁻¹.

Seedlings

Chilli (Capsicum annum L.) seeds of variety pusa jwala was sown in pots filled with medium black soil and FYM mixture (500 g pot⁻¹). The pots were kept in green house and watered regularly to maintain moisture at 60 percent. The seedlings were transplanted after 35 days after sowing.

Treatments

The details of treatments are given in Table 1.

Preparation and Application of Pseudomonas Inocula

Talc powder and King's B broth was used as a substrate for mass multiplication of efficient strains of Pseudomonas. PS-5, PS-13, PS-37 the substrate were first tyndalised then air dried and passed through 350 mesh sieves to obtain fine powder. Mass cultures of efficient strains of Pseudomonas were obtained by adding steriledistilled water to 24 hr old growth on King's B and 15 ml of such bacterial suspensions were aseptically added to separate 1000 ml King's B broth and incubated at incubated at 28 ± 2°C for 24 hr.

500ml of such bacterial suspensions (10⁸ cfu ml⁻¹) from king's B broth cultures were added to the 1 kg of substrate. Then inoculated substrates were mixed properly and used for root dipping, the root systems of 35 days old chilli seedlings were washed in tap water then dipped for 30 minutes in the suspension.

Preparation and Application of Azospirillum Inocula

Nitrogen free malate broth of 150 ml was prepared and inoculated in 500 ml conical flasks separately and incubated at 28 ± 2°C under shaking at 100 rpm for three days to give an optical density of 0.5. Lignite as carrier was sterilized at 121°C and 15 psi pressure for two hours and inoculated with broth cultures of Azospirillum as well as reference strains (Azospirillum brasilense).

The inoculants were prepared by mixing bacterial inoculam (10⁸ CFU ml⁻¹) and lignite in the ratio 1:2.5 followed by curing for 24 hrs. For seedling root dipping, biofertilizers slurry was prepared by dissolving 100 g of biofertilizer in one litre of water and 35 days old chilli seedlings were dipped for 5-10 minutes and transplanted into pots containing soil without any rinse. They were thinned to maintain four seedlings per pot. Three replications were maintained for each treatment, optimum soil moisture was maintained at 60% by providing required quantity of water. Necessary plant protection measures were taken as per the recommended packages of practices.

Transplantation and Maintenance

The inoculated seedlings were transplanted in pots at the rate of five seedlings pot⁻¹. Each treatment was replicated thrice. The pots were kept in green house and were watered regularly to maintain moisture at 60% water holding capacity to avoid leaching out of loses and to maintain optimum moisture throughout the period. The observations on plant growth parameters and nutrient content were recorded at different days of intervals viz, 30, 60, 90 days after transplantation and at harvest.

Observations

The observations on plant growth parameters were recorded at 30, 60, 90 DAT and at harvest. The plant samples were analyzed for shoot P concentration, shoot and root dry matter content.

Plant Growth Parameters

Plant height number of branches yield parameters

were counted at 30, 60, 90 DAT and at harvest and Total number of fruits plant⁻¹ were recorded at the time of harvest.

Dry matter content

The root and shoot portions of the uprooted plants were separated and oven dried separately at 60°C to constant weight. The shoot and root dry weights were recorded in different days of intervals and expressed in grams per plant.

Chemical Analysis

Shoot P concentration

The oven dried shoot samples were ground to fine powder separately in a Willey mill and used for estimation of phosphorous content.

Estimation of phosphorous

Five hundred mg of root/shoot sample was taken in a 250 ml capacity conical flask and was added with 2.5 ml 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 180°C to 200°C. The suspension was boiled until taken nearly to dryness.Further digestion of root/shoot sample is done by Wet oxidation method

From this wet oxidized digested sample, P was estimated by Vanadomolybdate-phosphoric yellow colour method (Jackson, 1973). Ten mili litre of wet oxidized digested sample was taken in a 50 ml volumetric flask and 10 ml Vanadomolybdate reagent was added. The volume was made up to 50 ml with the distilled water and allowed to react for 30 minutes. The intensity of yellow colour developed was read at 490 nm using a spectronic-2D spectro-

Table 2 Plant height of chilli at different stages of crop as influenced by efficient PGPR isolates isolated from chilli rhizosphere

Treatment	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	At harvest
T ₁ -Control	23.25	35.18	42.83	64.23
T ₂ -Efficient <i>Pseudomonas</i>	41.07	52.04	70.60	84.30
isolate-1 (PS-13)				
T ₃ -Efficient Pseudomonas	39.37	50.29	69.69	79.62
isolate-2 (PS-23)				
T ₄ -Efficient Pseudomonas	42.96	57.25	73.52	85.08
isolate-3 (PS-5)				
T ₅ -Reference strain	39.17	50.33	69.03	79.07
(Pseudomonas fluorescence)				
T ₆ -Efficient Azospirillum	39.01	50.19	64.70	78.81
isolate-1 (AZP-35)				
T ₇ - Efficient Azospirillum	39.34	50.53	66.40	80.09
isolate-2 (AZP-26)				
T ₈ - Efficient Azospirillum	38.81	49.77	63.66	78.28
isolate-3 (AZP-15)				
T ₉ - Reference strain	38.21	49.69	61.12	78.24
(Azospirillum brasilense)				
S. Em ±	1.06	1.41	1.86	2.23
C.D. at 1%	3.20	4.24	5.60	6.71

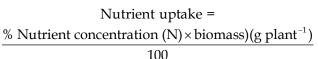
Note: Values are mean of three replications.

photometer. The P content was obtained by the standard curve prepared using KH₂PO₄.

Shoot N content

Shoot and root nitrogen content (mg plant⁻¹)

Nitrogen content of shoot and root was estimated by modified Micro kjeldhal method (Jackson, 1967) at 30, 60 DAS and at harvest. The total N uptake was calculated for each treatment separately using the following formula.



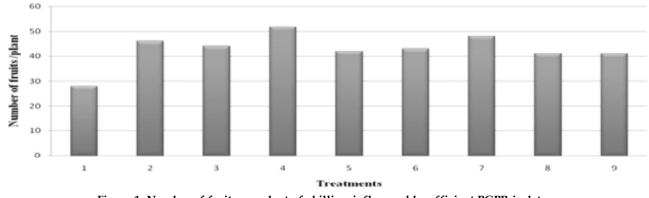


Figure 1: Number of fruits per plant of chilli as influenced by efficient PGPR isolates

Table 3 Number of branches of chilli at different stages of crop as influenced by efficient PGPR isolates					
Treatment	eatment Number of branches per plant				
	30 DAT	60 DAT	90 DAT	At harvest	
T ₁ -Control	2.56	4.47	5.67	7.00	
T ₂ -Efficient Pseudomonas					
isolate-1 (PS-13)	5.34	6.67	13.47	15.34	
T ₃ -Efficient Pseudomonas	5.13	6.08	12.00	14.33	
isolate-2 (PS-23)					
T ₄ -Efficient Pseudomonas	6.67	7.34	14.67	16.67	
isolate-3 (PS-5)					
T ₅ -Reference strain	5.00	6.01	12.00	14.68	
(Pseudomonas fluorescence)					
T ₆ -Efficient Azospirillum	5.11	6.01	10.67	12.66	
isolate-1 (AZP-35)					
T-Efficient Azospirillum	6.00	6.33	11.33	13.67	
isolate-2 (AZP-26)					
T ₈ -Efficient Azospirillum	4.78	5.60	9.34	12.33	
isolate-3 (AZP-15)					
T ₉ -Reference strain	4.33	5.33	9.67	12.00	
(Azospirillum brasilense)					

Note: Values are mean of three replications.

0.13

0.41

RESULT AND DISCUSSION

S. Em ±

C.D. at 1%

Effect of PGPR Strains on Plant Growth, Nutrient Uptake and Yield of Chilli

0.17

0.51

0.31

0.94

0.37

1.12

Plant growth parameters, dry matter and N and P content

Application of PGPR to the chilli plant significant increase in the plant height and number of branches, dry matter content, N and P content uptake at different stages of crop growth.

The highest plant height (42.96 cm) (Table 2) and number of branches (6.67) (Table 3), root 12.08 gm plant⁻¹ (table 4) and shoot (0.98gm plant⁻¹) (Table 5) dry matter content, maximum nutrient uptake of 2.23 per cent of nitrogen (Table 6) and 0.21 per cent of phosphorous plant⁻¹ recorded in PS-5 strain, (Table 7) and followed by T₂-Efficient *Pseudomonas* isolate-1 (PS-13), T₃-Efficient *Pseudomonas* isolate-2 (PS-23) and T₅-Reference strain (*P. fluorescence*) respectively. The lowest number of branches and plant height root and shoot dry matter and N and P uptake was observed in control at 30 DAT. The same trends were observed at 60 DAT, 90 DAT and at harvest.

Similarly, the inoculation of *Azospirillium* strains to the chilli significantly recorded maximum plant height, number of leaves root dry matter, shoot dry

Table 4		
Shoot phosphorus concentration at different stages of chilli		
crop as influenced by the inoculation of efficient PGPR		
isolates		

Treatment	Shoot phosphorous content (%)			
	30 DAT	60 DAT	90 DAT	At harvest
T ₁ -Control	0.10	0.12	0.11	0.10
T ₂ -Efficient Pseudomonas	0.19	0.22	0.21	0.19
isolate-1 (PS-13)				
T ₃ -Efficient Pseudomonas	0.15	0.19	0.17	0.16
isolate-2 (PS-23)				
T ₄ -Efficient Pseudomonas	0.21	0.24	0.22	0.21
isolate-3 (PS-5)				
T ₅ -Reference strain	0.15	0.19	0.18	0.15
(Pseudomonas fluorescence)				
T ₆ -Efficient Azospirillum	0.14	0.18	0.17	0.14
isolate-1 (AZP-35)				
T ₇ -Efficient Azospirillum	0.18	0.20	0.19	0.18
isolate-2 (AZP-26)				
T ₈ -Efficient Azospirillum	0.13	0.17	0.16	0.15
isolate-3 (AZP-15)				
T ₉ -Reference strain	0.13	0.17	0.15	0.14
(Azospirillum brasilense)				
S. Em ±	0.01	0.01	0.01	0.01
C.D. at 1%	0.03	0.03	0.03	0.03

Note: Values are mean of three replications.

 Table 5

 Shoot nitrogen concentration at different stages of chilli crop as influenced by efficient PGPR isolates

Treatment	ent Shoot nitrogen content (%)			
	30 DAT	60 DAT	90 DAT	At harvest
T ₁ -Control	1.08	2.17	2.29	2.17
T ₂ -Efficient <i>Pseudomonas</i> isolate-1 (PS-13)	2.16	3.02	3.19	3.14
T ₃ -Efficient <i>Pseudomonas</i> isolate-2 (PS-23)	2.05	2.97	3.1	3.06
T ₄ -Efficient <i>Pseudomonas</i> isolate-3 (PS-5)	2.23	3.19	3.27	3.20
T ₅ -Reference strain (<i>Pseudomonas fluorescence</i>)	2.00	2.68	3.19	2.91
T_6 -Efficient <i>Azospirillum</i> isolate-1 (AZP-35)		3.43	3.58	3.52
T_7 -Efficient <i>Azospirillum</i> isolate-2 (AZP-26)	2.70	3.68	3.8	3.75
T_8 -Efficient <i>Azospirillum</i> isolate-3 (AZP-15)	2.42	3.33	3.41	3.37
T ₉ -Reference strain	2.40	3.2	3.25	3.18
(Azospirillum brasilense) S.Em± C.D. at 1%	0.06 0.18	0.08 0.24	0.09 0.27	0.09 0.27

Note: Values are mean of three replications.

matter and N and P content as shown in Table (2, 3, 4, 5, 6 and 7). The seedlings treated with efficient strain of *Azospirillum* AZP-26 were recorded higher

Treatment Root dry weight (g) 30 DAT 60 DAT 90 DAT At harvest
T ₁ -Control 0.47 0.67 0.78 1.20
T ₂ -Efficient <i>Pseudomonas</i> 0.89 1.33 1.96 2.80
isolate-1 (PS-13)
T ₃ -Efficient <i>Pseudomonas</i> 0.80 0.98 1.92 2.42
isolate-2 (PS-23)
T ₄ -Efficient <i>Pseudomonas</i> 0.98 1.16 2.34 2.95
isolate-3 (PS-5)
T ₅ -Reference strain 0.77 0.85 1.83 2.22
(Pseudomonas fluorescence)
T ₆ -Efficient <i>Azospirillum</i> 0.74 0.87 1.63 1.90
isolate-1 (AZP-35)
T ₇ -Efficient <i>Azospirillum</i> 0.80 0.90 1.00 2.19
isolate-2 (AZP-26)
T ₈ -Efficient <i>Azospirillum</i> 0.70 0.86 1.92 1.80
isolate-3 (AZP-15)
T ₉ -Reference strain 0.69 0.70 1.00 1.72
(Azospirillum brasilense)
S.Em ± 0.01 0.02 0.04 0.05
C.D. at 1% 0.04 0.07 0.13 0.18

Note: Values are mean of three replications.

plant height (39.34 cm), number of branches (6.00) and root dry matter (0.80 gm plant⁻¹), shoot dry matter (11.83 gm) and 2.70% N and 0.18% of P plant⁻¹ of chilli followed by T₆-Efficient of strain of *Azospirillum* isolate-1 (AZP-35)-1, T₈-Efficient *Azospirillum* isolate-3 (AZP-15) and reference *Azospirillum* strain respectively and which was significantly superior over all other treatments at 30 DAT. Whereas, lower plant height and number of branches observed in uninoculated. The same trends were observed at 60 DAT, 90 DAT and at harvest.

The application of PGPR significantly help to increase the plant height and number of branches might be due to the production plant growth promoting substances which help to enhance the shoot and root growth (Kumar *et al.*, 2002). The PGPR Species of *Pseudomonas* and *Azospirillum* produce phytohormones or growth regulators that cause crops to have greater amounts of fine roots which have the effect of increasing the absorptive surface of plant roots for uptake of water and nutrients. These PGPR are referred to as Biostimulants and they produce the phytohormones include indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production (Black, 1968).

Glick (1995) reported that seedling application with these organisms has emerged as a powerful Table 7 Shoot dry weight of chilli at different stages of crop as influenced by efficient PGPR isolates

Treatment	Shoot dry weight (g)			
	30 DAT	60 DAT	90 DAT	At harvest
T ₁ -Control	7.04	10.11	14.72	16.90
T ₂ -Efficient <i>Pseudomonas</i>	11.93	21.81	27.17	31.33
isolate-1 (PS-13)				
T ₃ -Efficient Pseudomonas	11.81	21.61	26.75	30.55
isolate-2 (PS-23)				
T ₄ -Efficient Pseudomonas	12.08	22.00	27.30	32.95
isolate-3 (PS-5)				
T ₅ - Reference strain	11.26	21.20	26.67	30.00
(Pseudomonas fluorescence)				
T ₆ - Efficient Azospirillum	10.80	18.64	24.63	29.00
isolate-1 (AZP-35)				
T ₇ - Efficient Azospirillum	11.83	21.20	25.88	29.46
isolate-2 (AZP-26)				
T ₈ - Efficient Azospirillum	10.77	18.22	23.73	27.76
isolate-3 (AZP-15)				
T ₉ - Reference strain	10.50	18.00	25.70	27.50
(Azospirillum brasilense)				
S.Em±	0.31	0.54	0.70	0.80
C.D. at 1%	0.94	1.63	2.10	2.42

Note: Values are mean of three replications.

technology to enhance plant growth and yield besides providing protection against diseases.

However, another mechanism with which it is possible to explain similar effects was the synthesis and exudation of plant growth promoting substances like IAA and GA (Tien *et al.*, 1979). IAA and GA are known to enhance the shoot length root length and also the plant growth (Brown, 1974 and Bar and Okon, 1993).

The above results were similar with findings of Datta *et al.* (2011) reported that remarkable increase in growth characteristics of chilli such as plant height ,number of branches per plant, total number of fruits, fruit-weight, and yield was recorded in plants with inoculation of PGPR under pot culture and field conditions. The results clearly demonstrate the rhizo competence and plant growth enhancing efficacy of these PGPR strains. These PGPR also increase the root surface area for uptake of nutrient and water which significantly increase the plant growth.The increase in N and P uptake due to single inoculation of N2-fixers and phosphate solubilizers are reported by Parashar *et al.* (1999) and Defreitas *et al.* (1997).

Yield parameters

The present study revealed that, seedling inoculation of *Azospirillum* and *Pseudomonas* significantly increase

Table 8				
Number of fruits plant ⁻¹ of chilli as influenced by efficient				
PGPR isolates				

PGPR isolates	
Treatment	No. of fruit per plant
T ₁ -Control	28
T ₂ -Efficient <i>Pseudomonas</i> isolate-1 (PS-13)	46
T ₃ -Efficient <i>Pseudomonas</i> isolate-2 (PS-23)	44
T ₄ -Efficient <i>Pseudomonas</i> isolate-3 (PS-5)	52
T ₅ -Reference strain (<i>Pseudomonas fluorescence</i>)	42
T ₆ -Efficient <i>Azospirillum</i> isolate-1 (AZP-35)	43
T_7 -Efficient <i>Azospirillum</i> isolate-2 (AZP-26)	48
T ₈ -Efficient <i>Azospirillum</i> isolate-3 (AZP-15)	41
T ₉ -Reference strain (<i>Azospirillum brasilense</i>)	41
S. Em ±	1.21
C.D. at 1%	3.65

Note: Values are mean of three replications.

the yield parameters of chilli. The results are presented in Table 8.

The higher number of fruits was observed in case of treatment T_4 with the inoculation of efficient strain of *Pseudomonas* PS-5 with 52 number of chillis plant⁻¹ which significantly superior over treatment T_2 -Efficient *Pseudomonas* isolate-1 (PS-13) (46 fruits plant⁻¹), T_3 -Efficient *Pseudomonas* isolate-2 (PS-23) (44 fruits plant⁻¹) and T_5 -Reference strain (*P. fluorescence*) (42 fruits plant⁻¹) whereas, least number of fruits was recorded in uninoculated control with 28 number of fruits plant⁻¹.

Similarly among the *Azospirillum* inoculations the treatment T_7 recoded highest number of fruits plant⁻¹ *i.e.*, 48 fruits plant⁻¹ over the treatment T_6 -Efficient *Azospirillum* isolate-1 (AZP-35) (43fruits plant⁻¹), T_8 -Efficient *Azospirillum* isolate-3 (AZP-15) (41 fruts plant⁻¹) and T_9 -Reference strain (*A. brasilense*) (41 fruits plant⁻¹). The lower fruits per plant was noticed in control (T_1) compared to all other treatments.

These results are similar with findings of Datta (2011) studied the effect of PGPR on chilli crop in pot culture experiment and reported that inoculation of these PGPR strain to the chilli isolated from chilli rhizospere significantly increase in the average plant height plant canopy width, total number of plant fruit weight , fruit length. And he also reported these results might be due to ability of PGPR strains strain to solubilize the phosphorous and they also produce the IAA which helps to increase the plant growth.

Glick (1995) reported that *Pseudomonas* make up a dominant population in soil and rhizosphere and exert growth promoting influence on a variety of plant species on account of their strong competitive behaviour, colonization potential and sustainability. Seed bacterizaiton with these organisms has emerged as a powerful technology to enhance plant growth and yield besides providing protection against diseases.

Earnapalli (2005) who reported that all the PGPR strains tested promoted growth and challenge inoculation with the viral pathogen significantly decreased growth, growth components and fruit yield of tomato at all periods of observation.

CONCLUSIONS

The present investigation was carried out tostudythe effect of efficient isolates of PGPR on growth and yield of chilli (*capsicum annuum l*) under pot culture conditions. Rhizobacteria can play an important role in providing nutrients and in stimulating the biochemical activities that may increase the beneficial attributes of the plant. Therefore, according to our investigation PS-5 strain of *Pseudomonas* isolated from Raichur and *Azospirillum* strain AZP-26 isolated from Yerdhal location of TBP region of Karnataka significantly perform better in pot culture conditions, indicated their potential to be used as combined inoculations under field conditions as well as for the preparation of growth and yield of chilli.

ACKNOWLEDGEMENT

This study was supported by Department Agricultural Microbiology,College of Agriculture, U.A.S.,Raichur,Karnataka.

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