International Journal of Microbiological Research 5 (3): 144-151, 2014
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2014.5.3.81232

Interaction Effect of Combined Inoculation of Pgpr on Growth and Yield Parameters of Chilli Var K1 (*Capsicum annuum* L.)

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Abstract: In this study, soil samples were collected from ten different locations. The PGPR organisms *viz*., *Azospirillum, Pseudomonas, Azotobacter* and *Bacillus* were isolated from ten different soil samples purified and maintained for further studies. The selected PGPR isolates *viz*., Azs-2, Ps-2 and Azo-4 were inoculated either individually and along with *Bacillus* BS-2 and the interaction effect on chilli var. K-l was studied under pot culture experiment. All the inoculation of PGPR organisms increased the growth and yield parameters of chilli when compared to uninoculated control. The combined inoculation of PGPR recorded the maximum vigour index of 1460 and germination percentage of 99.0%. It also recorded the highest plant height of 78.65 cm and plant dry weight of 7.38 g plant^{-1}. The yield parameters such as number of fruits plant^{-1}, fruit weight and fruit yield were also at its maximum level due to interaction effect of PGPR. A maximum of 28.00 number of fruits plant^{-1}, 41.25 g fruit^{-1} and fruit yield of 1080.00 g plant^{-1} was recorded. It was confirmed that the interaction effect was more when the microbial inoculants applied as a consortium than individual inoculation.

Key words: Chilli · *Azospirillum* · *Pseudomonas* · *Azotobacter* and *Bacillus*

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly [1, 2]. The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration [3]. It is also suggested that PGPR can also prevent the deleterious effects of stresses from the environment [4]. Globally, India contributes one fourth to world production of chilli with an area of 8.53 lakh ha and production of 8.74 lakh tonnes with a productivity of 1016 kg ha^{-1} [5]. In India, chilli is extensively grown in the states of Andhra Pradesh, Orissa, Maharashtra, West Bengal, Karnataka, Rajasthan and Tamil Nadu.

Chilli, the fruit of *Capsicum annuum* L, is one of the most important commercial crops in India. With an annual production of 1.1 million tones, India is the largest producer of chilli in the world [6]. Owing to its high cash value and consumption rate the annual trade of chilli is approximately 17% of total spice trade in the world [7] and is about 33% in India. However, the yield of chilli in India is substantially low when the large area (930,000 hectares) of production is considered [8]. A large amount of herbicides, pesticides and fertilizers is applied every year to achieve maximum productivity of chilli and to meet the growing demand, the use of chemical fertilizers in India has increased 170 times in last 50 years [9]. Chilli is now gaining more importance in the global market because of its value added products like chilli powder, oleoresin, capsanthin and chilli oil etc. Chilli powder is the most important ground spice item exported from India.

MATERIALS AND METHODS

A well grown chilli plant with roots intact was uprooted from the field and excess soil was removed. The soil adhered to root surfaced and in between root was collected and used as rhizosphere soil. The rhizosphere soil was collected in ten different places around the

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Cuddalore district and is used to isolate the PGPR bacteria such as *Azospirillum, Pseudomonas, Bacillus* and *Azotobacter*. Based on the sample collection, the isolates were designated as Azs-1 to Azs-10, Ps-1 to Ps-10, Bs-1 to Bs-10 and Azo-1 to Azo-10 respectively. Efficient PGPR isolates were selected and used in field experiment.

Preparation of Pots and Treatment Details: Cement pots of size 2'×1'×2' were filled with pot mixture. The pots were watered and treated seeds were sown. The fertilizer schedule of 60: 40: 20 kg of NPK acre^{-1} was followed based on the treatments. The pots were arranged according to the treatments. Broth culture of *Pseudomonas* sp., *Azospirillum, Bacillus* and *Azotobacter* were prepared. The seeds of Chilly variety K-1 were treated with the cultures as per the treatment combination using carboxy methyl cellulose as binder. The treated seeds were shade dried and used for sowing. The experiment was conducted following the completely randomized block design with three replications and twelve treatments. The treatments were: T_1- Control; T_2 - *Azospirillum*; T_3 -*Azotobacter*; T_4 - *Pseudomonas*; T_5 - *Bacillus*; T_6 - *Azospirillum* + *Azotobacter*; T_7 - *Azospirillum* + *Bacillus*; T_8 - *Azotobacter* + *Bacillus*; T_9 - *Pseudomonas* + *Bacillus*; T_{10} -*Azospirillum* + *Azotobacter*; T_{11} - *Pseudomonas* + *Azotobacter* and T_{12} - *Azospirillum* + *Azotobacter* + *Pseudomonas* + *Bacillus*.

Biometric Observations: Five plants were chosen for each treatment for recording the biometric observation. Plant samples were taken at periodic intervals *viz*., 25th, 50th, 75th and 100th days after sowing. The biometric observations such as plant height, plant dry weight and yield of chilly were recorded.

Germination Percentage: The germination count of chilli was recorded on 15 DAS the germination percent arrived at using the formula

Germination% = Total number of seeds germinated/ Total number of seeds sown x 10

Vigour Index: The vigour index was worked out as on 15 DAS by multiplying the germination percentage with plant height and expressed as whole numbers.

Determination of Plant Height: The height of the plant from the surface of the soil to top most leaf was measured at periodical intervals *viz*., 25th, 50th, 75th, days after sowing.

Determination of Plant Dry Weight: The weight of the sample was determined in an oven at 60°C until a constant weight was obtained and expressed in (g) on oven dry basis.

Fruit Yield: The fruit yield of five plants were taken from each treatment and expressed as g per plant.

Number of Fruits: The number of fruits of five plants in each treatment was recorded and expressed as number of fruits plant^{-1}.

Fruit Weight: The average fruit weight of five plants selected as randomly recorded and expressed as g fruit^{-1}.

Estimation of Nitrogen Content of the Plant: The nitrogen content of the plant was estimated by Microkjeldahl method. The plant samples were initially dried and then dried in an oven at 60°C till constant weight was obtained. The dried samples were powdered, sieved and the nitrogen content was estimated.

Estimation of Phosphorus Content of the Plant: The phosphorus content of the plant was estimated using vanadomolybdate method [10].

Statistical Analysis: The experimental data were analyzed by following the method of Panse and Sukhatme [11].

RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR) constitute approximately 2-5% of the total rhizomicrobial population. Evidence of the beneficial effects of PGPR has been accumulating for the past 150 years. PGPR have been demonstrated to increase growth and productivity of many commercial crops including rice, wheat, cucumber, maize, cotton, black pepper and banana. A few studies have isolated and characterized the PGPR and phosphate solubilizing bacteria from chilli rhizosphere, the effect of PGPR on chilli growth and productivity under field conditions has hitherto not been investigated. Co-inoculation of PGPR has been demonstrated as a sustainable approach in plant health management. Prudent application of binary or multiple mixtures of PGPR inoculants can expand the spectrum of biocontrol activity. Therefore, individual and combined effects of PGPR on the yield and productivity of host plant should also be assessed [12, 13].

Table 1: Effect of individual and combined inoculation of PGPR on germination percentage and vigour index of Chilli var K-1

Treatments	Germination percentage	Vigour index
T_1	68.20	756
T_2	70.08	896
T_3	72.18	946
T_4	90.36	1328
T_5	81.31	1089
T_6	79.43	879
T_7	80.00	1024
T_8	94.53	1219
T_9	92.76	1339
T_{10}	82.38	1179
T_{11}	97.69	1359
T_{12}	99.00	1460.00
SED	1.2908	59.148
CD(p=0.05)	2.5816	118.2976

Table 2: Effect of individual and combined inoculation of PGPR on plant dry weight of chilli var K-1

	Plant dry weight (g plant^{-1})			
	Sampling period in days			
Treatments	25th day	50th day	75th day	100th day
T_1	0.56	1.76	2.30	4.01
T_2	1.45	2.10	4.08	5.34
T_3	1.37	2.25	3.10	5.04
T_4	1.48	2.35	3.61	5.20
T_5	0.98	2.05	3.24	3.26
T_6	1.57	2.08	3.98	5.86
T_7	0.99	2.00	3.01	3.48
T_8	1.60	2.41	4.65	5.44
T_9	1.90	2.56	4.11	6.64
T_{10}	1.46	1.03	3.06	6.02
T_{11}	2.03	2.62	5.02	7.03
T_{12}	2.58	3.67	4.43	7.38
SED	0.045	0.03	0.035	0.04
CD(p=0.05)	0.09	0.06	0.07	0.08

A pot culture experiment was conducted to study the interaction effect of inoculation of efficient isolates of PGPR *viz*., *Azospirillum azs-2, Pseudomonas Ps-2, Azotobacter* a*zo-4* and *Bacillus Bs-2* on growth and yield parameters of chilli variety K-1. The individual and combined inoculation effect of PGPR on germination percentage and vigour index of chilli were studied and the results are given in Table - 1. It was observed that all the treatment recorded significant difference in germination percentage and vigour index of chilli var. K-1 over control due to the inoculation of microbial inoculants. The treatment T_{12}, Azs + Azo + Ps + Bs recorded the maximum germination percentage of 99.0 and vigour index of 1460. Among the individual inoculation, *Pseudomonas* Ps-2 (T_6) was found to be best in increasing the germination percentage and vigour index followed by *Azospirillum* Azs-2 (T_4) and *Azotobacter* Azo-4 (T_5). Among the dual inoculation, the treatment T_9 (Ps 2 + Bs) recorded the maximum germination percentage of 97.69 and vigour index of 1359 followed by Azs + Bs and Azo + Bs. It was also observed that the interaction effect was more pronounced in the combined inoculation of all PGPR than single and dual inoculation.

It was observed that the plant height of chilli was significantly increased by all the treatments over control. The maximum plant height of 78.36 cm was recorded by the treatment *Azs + Azo + Ps + Bs* on 100 DAS. Among the individual inoculation, the *Pseudomonas* Ps-2 was found to be the best and recorded 16.00 cm of plant height on 25th DAS followed by Azs-2 (15.20) and Azo-4 (13.86). Among the dual inoculation, the treatment T_9 (*Pseudomonas + Bacillus*) recorded the maximum plant height of 18.37 cm on 25 DAS followed by *Azs + Bs* (17.18) and *Azo + Bs* (16.69). Though increase in plant height on 25 DAS was not statistically significant, but as the advancement of sampling periods, the differences between the treatments were statistically significant. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR (Figure – 1).

The individual and combined effect of PGPR on plant dry weight of chilli was studied and the results are presented in Table - 2. It was observed that the plant dry weight of chilli was significantly increased by all the treatments tested over control. The maximum plant dry weight of 7.38 g plant $^{-1}$ was recorded by the treatment *Azs+ Azo + Ps + Bs* on 100 DAS. Among the individual treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded 1.60 of plant dry weight on 25th DAS followed by *Azs-2* (1.48) and Azo-4 (0.98). Among the dual inoculation, the treatment T_9 (*Ps+Bs*) recorded the maximum plant dry weight of 2.03 25 DAS followed by *Azs+ Bs* (1.90) and Azo + Bs (1.46). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

The individual and combined effect of PGPR on number of fruits of chilli was studied and the results are presented in Figure - 2. It was observed that the number of fruits in chilli was significantly increased by all the treatments tested over control. The maximum number of fruits 28.0 plant^{-1} was recorded by the treatment *Azs + Azo+ Ps + Bs* on 130 DAS. Among the individual

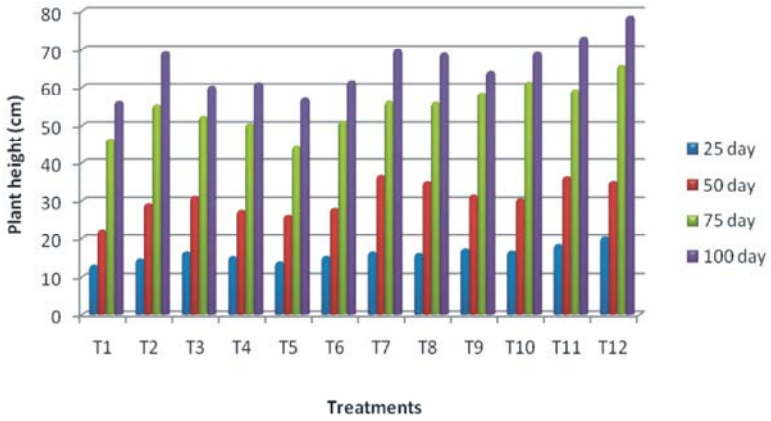

Fig. 1: Effect of individual and combined inoculation of PGPR on plant height of chilli var K-1

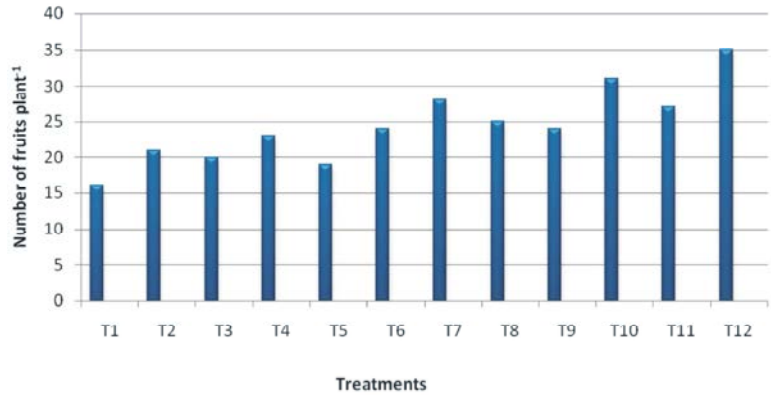

Fig. 2: Effect on PGPR isolates on increase in number of fruits per plant

Table 3: Effect of individual and combined inoculation of PGPR on fruit yield in chilli

Treatments	Fruit yield (g plant^{-1}) Pot culture study
T_1	78.00
T_2	89.05
T_3	83.07
T_4	100.00
T_5	80.00
T_6	106.00
T_7	128.00
T_8	115.00
T_9	106.00
T_{10}	136.00
T_{11}	121.05
T_{12}	160.02
SED	4.225
CD(p=0.05)	8.45

treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded 21.0 number of fruits on 130 DAS followed by *Azs -2* (20.0) and *Azo-4* (18.0). Among the dual inoculation the treatment T_9 (*Ps* + *Bs*) recorded the maximum number of fruits 26.0 number of fruits on 130 DAS followed by Azs +*Bs* and *Azo*+ *Bs*. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

It was observed that the plant fruit yield of chilli was significantly increased by all the treatments tested over control. The maximum fruit yield of 1080.00 (g plant^{-1}) was recorded by the treatment *Azs* + *Azo* + *Ps* + *Bs*. Among the individual treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded the 798.00 g plant^{-1} of fruit yield followed by *Azs-2* (648.00) and *Azo-4* (580.00). Among the dual inoculation, the treatment T_9 (*Ps* + *Bs*) recorded the maximum fruit yield of 920.00 g plant^{-1} followed by *Azs* + *Bs* (718.00) and Azo + *Bs* (619.00). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR Table 3.

It was observed that the fruit weight of chilli was significantly increased by all the treatments tested over control. The maximum fruit weight of 41.25 (g plant^{-1}) was

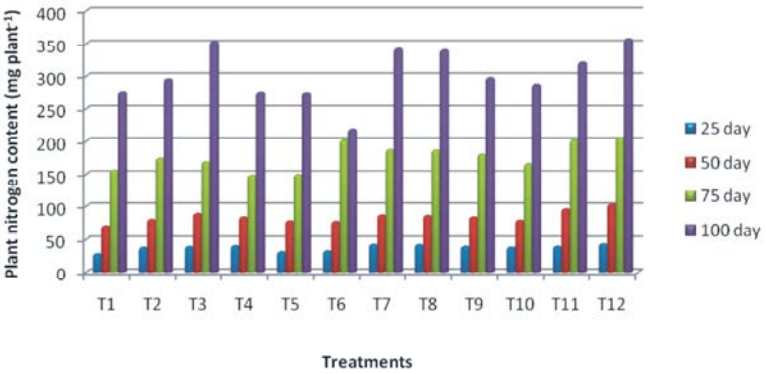

Fig. 3: Effect PGPR isolates on the increase in plant nitrogen content

Table 4: Effect of individual and combined inoculation of PGPR on fruit weight in chilli

Treatments	Fruit weight (g fruit^{-1})
T_1	5.09
T_2	6.02
T_3	7.00
T_4	6.00
T_5	6.25
T_6	6.51
T_7	6.03
T_8	8.12
T_9	8.06
T_{10}	7.63
T_{11}	9.00
T_{12}	9.21
SED	0.435
CD(p=0.05)	0.87

Table 5: Effect of individual and combined inoculation of PGPR on plant phosphorous content in chilli variety K-1

	Plant phosphorous content (mg plant^{-1})			
	Sampling period in days			
Treatments	25th	50th	75th	100th
T_1	2.29	7.96	11.37	22.48
T_2	3.16	7.26	15.82	28.30
T_3	3.38	8.08	17.25	21.39
T_4	3.78	5.60	13.80	17.34
T_5	3.09	6.60	12.89	15.94
T_6	4.26	5.85	13.32	27.98
T_7	3.21	6.87	12.96	15.77
T_8	4.35	5.80	13.09	26.90
T_9	4.72	7.62	16.28	28.89
T_{10}	4.10	6.50	12.97	26.73
T_{11}	4.98	9.68	16.72	29.97
T_{12}	5.02	8.76	18.25	30.99
SED	0.028	0.025	0.024	0.0255
CD(p=0.05)	0.056	0.050	0.048	0.051

recorded by the treatment *Azs* + *Azo* + *Ps* + *Bs*. Among the individual treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded 37.62 g plant^{-1} of fruit weight followed by *Azs* (35.20) and *Azo* (34.50). Among the dual inoculation of the treatment T_9 (*Ps* + *Bs*) recorded the maximum fruit weight of 39.40 (g plant^{-1}) followed by *Azs* +*Bs* (36.20) and *Azo* + *Bs* (35.89). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR Table 4.

It was observed that the Plant nitrogen content of chilli was significantly increased by all the treatments tested over control. The maximum Plant nitrogen content of 358.05 (mg plant^{-1}) was recorded by the treatment *Azs* + *Azo* + *Ps* + *Bs* on 100 DAS. Among the individual treatments, the inoculation of *Azospirillum azs-2* was found to be the best and recorded 40.50 mg plant^{-1} of plant nitrogen content followed by *Ps-2* (32.58) and *Azo* -4 (31.38) on 25 DAS. Among the dual inoculation of the treatment *Azospirillum* + *Bacillus* recorded the maximum plant nitrogen content of 42.56 (mg plant^{-1}) followed by *Ps-2* +*Bs -2* (39.59) and *Azo* + *Bs* (38.26). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

The individual and combined effect of PGPR on plant phosphorus content of chilli was studied and the results are presented in Table - 5. It was observed that the Plant nitrogen content of chilli was significantly increased by all the treatments tested over control. The maximum Plant phosphorus content of 30.99 (mg plant^{-1}) was recorded by the treatment *Azs-2* + *Azo-4* + *Ps-2* + *Bs-2* on 100 DAS. Among the individual treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded 4.26 mg plant^{-1} of plant phosphorus content followed by *Azs-2* (3.78) and *Azo* -4 (3.09) on 25 DAS. Among the dual inoculation of the treatment T_9 - *Pseudomonas* + *Bacillus* recorded the maximum plant Phosphorus content of 4.98 (mg plant^{-1}) followed by *Azs-2* (4.72) and *Azo* (4.10) on 25

DAS. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

Rini and Sulochana [13] evaluated the isolates of *Trichoderma* (*Trichoderma harzianum* TR20 and *Trichoderma pseudokoningii* TR17) and fluorescent pseudomonads (*Pseudomonas fluorescens* P28 and P51) (Alone and in combination) under greenhouse and field conditions for efficacy in suppressing rhizoctonia root rot incidence and promoting plant growth in chilli. The combination, *Trichoderma harzianum* (TR20) + *Pseudomonas fluorescens* (P28), was most effective in reducing disease incidence (66.7% more efficient than the control), but was at par with copper oxychloride (0.3%). Highest yield per plant was recorded in the treatment combination TR20 + P28, followed by *Trichoderma pseudokoningii* (TR17) + *Pseudomonas fluorescens* (P51). *Trichoderma pseudokoningii* (TR17) and *Trichoderma harzianum* (TR20) when applied alone also significantly increased the yield per plant and was superior to both the pseudomonads applied individually.

Samrah Tariq *et al*. [14] seven strains of *Pseudomonas aeruginosa* were isolated from inner roots of healthy chilli plants growing under field condition. *In vitro* test cell free culture filtrate of some strains showed nematicidal activity against *Meloidogyne javanica* root knot nematode by killing the 2nd stage juveniles and by retarding the egg hatching. In dual culture plate assay, one strain of *Pseudomonas aeruginosa* inhibited the radial growth of all the four test root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *Fusarium oxysporum* by producing the zone of inhibition. While other strains caused growth inhibition of at least 2 or 3 test fungi. Some bacterial strains also caused lysis of fungal hyphae. In screen house, application of some of these bacterial strains caused significant suppressive effect on root rotting fungi and root knot nematode infecting chilli roots. Some *Pseudomonas aeruginosa* strains also showed positive impact on plant growth by increasing the plant height and fresh shoot weight and were found to produce indole-acetic acid at varying degree.

Berg [15] evaluated the combined strategy of chilli fruit rot and powdery mildew control consisting of reduced fungicide dose and biological control with antagonistic *Pseudomonas fluorescens* (Pf1). The sensitivity of *Pseudomonas fluorescens* to fungicide azoxystrobin at different concentrations was tested by turbidometric method. The grown bacterium (Pf1) was tolerant to all concentrations of azoxystrobin. In two field trials, Pf1 tested in combination with reduced concentration of azoxystrobin was highly efficient in management of both diseases of chilli. Biological control of *Colletotrichum capsici* and *Leveillula taurica* with *Pseudomonas fluorescens* (Pf1) was effective but less so than fungicide alone at the standard dose. However, combination of the biological control agent with a 50% reduction of fungicide dose was as effective as the standard fungicide alone. Application of *Pseudomonas fluorescens* along with azoxystrobin significantly increased the survival of Pf1 in the phylloplane of chilli.

Edgar Omar Rueda – Puente *et al*. [16] carried out two germination to study the effect of salinity, temperature regime and inoculation with PGPB and AMF growth factors measured on germination (Percentage and rate), plant height, root length and produced biomass (Fresh and dry matter). The results indicated that from four studied ecotypes, Mazocahui was the most outstanding of all, showing the highest germination under saline and non saline conditions. However, the PGPB and AMF influenced the others variables evaluated. The first step to obtain an ideal ecotype of *Capsicum annum* var. *aviculare*, which grows in the northwest of México and promoting this type of microorganisms as an efficient and reliable biological product. Studies of the association of PGPB and AMF with the *Capsicum annum* var. *aviculare*. Mazocahui ecotype are recommended to determine the extent to which these observations can be reproduced under field conditions.

Muthukumar *et al*. [17] tested the efficacy of nine bacterial endophytes were isolated from stem and root portions of chillies and tested against *Pythium aphanidermatum* under glasshouse condition. Out of these nine bacterial endophytes, EBC 5, EBC 7 and EBC 6 recorded the minimum mycelial growth with maximum inhibition zone of pathogen over control. In the present study, chilli seeds treated with these endophytes in combination (EBC 5and EBC 6) recorded the lowest incidence of pre and post-emergence damping-off at seven and 14 days after sowing when compared to individual treatment. This was followed by seed treatment with EBC 5 and EBC 7 in combination. The combination (EBC 5 and EBC 6) treatment also increased the germination percentage, shoot length and root length of chilli plants significantly.

Moumita Datta *et al*. [18] isolated 15 bacteria from chilli rhizosphere and their morphological, biochemical, plant growth promoting and biocontrol characteristics were elucidated. Plant growth and yield attributes increased significantly when the 15 rhizospheric isolates

were applied to a local chilli cultivar 'Suryamukhi' in pots. On the basis of their performance in the pot experiment, three rhizobacteria (C2, C25 and C32) were selected for further study in field. The 16S rDNA sequencing has identified C2 and C25 strains as *Bacillus* spp. and C32 strain as *Streptomyces* sp. Remarkable increase in growth characteristics such as total number of fruits, fruit-weight and yield was recorded in plants with combined inoculation under field conditions. The results clearly demonstrate the rhizocompetence and plant growth enhancing efficacy of these strains.

CONCLUSION

From the present study, it was concluded that the plant growth promoting rhizobacteria *Azospirillum, Pseudomonas, Azotobacter* and *Bacillus* have the capacity to produce plant growth promoting substances and induce the growth of chilli plant. Among the twelve treatments the treatment 12 gave greatest results to induce the growth of plant and fruits.

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