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RESEARCH ARTICLE

Effect of Plant Growth Promoting Rhizobacteria for the Growth and Yield of *Coleus Forskohlii*

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A B S T R A C T

The effect of microbial consortium consisting plant growth promoting rhizobacteria (PGPR) like *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* were tested separately and in combination on *Coleus forskohlii* for pot culture experiment. The combinations of above mentioned PGPR strains significantly increased plant height, number of tubers, tuber length and tuber yield in *Coleus forskohlii* when compared to the uninoculated control. Plant growth promoting rhizobacteria (PGPR) exhibit direct and indirect mechanisms as plant growth promoters and biological control agents. Direct mechanism by PGPR, include the provision of bio-available phosphorus for plant uptake, nitrogen fixation for plant. The results of this study suggest that the PGPR applied in combination have yield of *Coleus forskohlii.*

INTRODUCTION

Coleus forskohlii is an important medicinal plant. This crop rose into prominence by virtue of its alkaloid, forskolin, a diterpense present in the swollen primary roots (tubers) and it is a great potential in future due to the expected increase in demand form forskolin widely used in the treatment of heart diseases, glaucoma, asthma and certain types of cancers (Shah *et al.,* 1980). Forskolin content has been found to vary from 0.07 to 0.59% of dry tubers and just 1 g of forskolin costs $85, showing the importance of mthis crop (Gowda, 2000).

An intensive practice that warrants high yield and quality requires the extensive use of chemical fertilizers, which are costly and may create environmental problems. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices (Esitken *et al.,* 2005). In this context, the use of biofertilizers containing Plant Growth Prompting Rhizobacteria (PGPR) strains instead of synthetic chemicals may serve as an effective alternative and environmental friendly practice to improve plant growth through the supply of plant nutrients and soil productivity (O'Connell, 1992). Moreover, it has been found that exploiting these PGPR strains for the growth promotion could reduce that need for chemical fertilizers as well as the cost of cultivation.

Among different group of biofertilizers; nitrogen fixing and phosphorous solubilizing bacteria may be considered to be important since they improve plant nutrition. Plant Growth Prompting Rhizobacteria (PGPR) in the biofertilization of crops (Karlidag *et al.,* 2007) has been a well known fact that these PGPR strains may promote growth either by fixation of atmospheric nitrogen or by solubilization, if minerals such as phosphorous (Karthikeyan *et al.,* 2007; 2008) and they can also promote growth production of plant growth regulators (Klopper and Schroth, 1978; Jaleel *et al.,* 2007).

This PGPR activity was reported in species belonging to *Azospirillum, Azotobacter, Pseudomonas* and Phosphate solubilization bacteria (Rodriguez and Fraga, 1999; Sturz and Sudhakar *et al.,* 2000; Karlidag *et al.,* 2007). The occurrence of *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* in the rhizosphere of medicinal plants such as *Coleus forskohlii* has been documented earlier (Attia and Saad, 2001; Priya, 2010). Furthermore, the strains are also known to stimulate growth and yield in *Catharanthus roseus* and other medicinal plants (Karthikeyan *et al.,* 2008) and *Withania somnifera* (Thosar *et al.,* 2005; Rajasekar and Elango, 2011).

However, reports regarding the bioinoculation effect of these PGPR strains in medicinal plants and particularly in *Coleus forskohlii* have been scarce. Hence the present study was undertaken to investigate the growth promoting effects on *Coleus forskohlii.*

MATERIALS AND METHODS

Bacterial strains, Culture conditions, Media and Treatments

All bacterial strains used in the present study were isolated from the rhizosphere soil of *Coleus forskohlii*, *Azospirillum lipoferum* grown in N free semisolid medium (NFb) *Azotobacter chrococcum*, grown in Waksman Base Medium for routine use and maintained in Waksman broth with 15 %

glycerol. Pseudomonas fluorescens King's B Agar Medium and *Bacillus megaterium* were grown on picovskya medium for routine use and for long term storage they were maintained in nutrient broth with 15 % glycerol at -80ºC. They isolates were designated as *Azospirillum lipoferum* CFAzs-3, *Azotobacter chrococcum* CFAzt-8, *Pseudomonas fluorescens* CFPf-18 and *Bacillus megaterium* CFPb-16. For each experiment a single colony was transferred to 500 ml flasks containing NFb, Waksman base no.77 broth, King's B broth and picovskya broth grown aerobically in flasks on a rotating shaker (150 rpm) for 48 hrs. The bacterial suspensions were than diluted in sterile water to a final concentration of 10^9 CFU/ml, and the resulting suspensions were treated with *Coleus forskohlii* plants and control plants were dipped in sterile water.

Field experiments

The seedling of *Coleus forskohlii* was raised in the pot culture yard, Department of Microbiology, Faculty of Agriculture, Annamalai University in the year January 2010 to June 2010. For planting, 10 to 12 cm long terminal cuttings having three to four pairs of leaves were dipped in the PGPR inoculums and planted in the field. There were fifteen treatments as given below, each with three replications.

T_1	-	*Azospirillum lipoferum* - CFAzs-3
T_2	-	*Azotobacter chroococcum* -CFAzt-8
T_3	-	*Pseudomonas fluorescens* - CFPf-18
T_4	-	*Bacillus megaterium* - CFPb-16
T_5	-	*Azospirillum lipoferum*- CFAzs-3+ *Bacillus megaterium* - CFPb-16
T_6	-	*Azospirillum lipoferum* - CFAzs - 3+*Pseudomonas fluorescens* - CFPf-18
T_7	-	*Azospirillum lipoferum* - CFAzs-3+ *Azotobacter chroococcum* - CFAzt-8
T_8	-	*Bacillus megaterium*- CFPb-16+ Pseudomonas *fluorescens* - CFPf-18
T_9	-	*Pseudomonas fluorescens*- CFPf-18 + *Azotobacter chroococcum* - CFAzt-8
T_{10}	-	*Azospirillum lipoferum*- CFAzs-3+ *Bacillus megaterium* - CFPb-16+ *Pseudomonas fluorescens*- CFPf-18
T_{11}	-	*Azospirillum lipoferum*- CFAzs-3+ *Bacillus megaterium* - CFPb-16+ *Azotobacter chroococcum* CFAzt-8
T_{12}	-	*Bacillus megaterium* - CFPb-16 + *Azotobacter chroococcum*- CFAzt-8+*Pseudomonas fluorescens* - CFPf-18 T_{13} - *Azospirillum lipoferum* - CFAzs-3+*Pseudomonas fluorescen s* - CFPf - 18+ *Azotobacter chroococcum*- CFAzt 8
T_{14}	-	*Azospirillum lipoferum* - CFAzs-3+ *Bacillus megaterium* - CFPb-16+ *Pseudomonas fluorescens* - CFPf-18+ *Azotobacter chroococcum*- CFAzt-8
T_{15}	-	Uninoculated (control)

Fifteen treatment plots (three plants per pot) were prepared and irrigated immediately for a better accommodation. Three replications were maintained for each treatment subsequent irrigation was done two times in a week to keep the optimum moisture level of the soil.

Growth promoting effects of bacterial treatments were evaluated by determining the plant height, Number of tubers, tuber length and wet weight and dry weight of tubers yield on 45, 90, 135, and 180 days after planting (DAP).

Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) as per procedures described by Gomez and Gomez (1984). Values represent mean ±SD for three samples in each group. P values are <0.05 were considered as significant.

RESULT AND DISCUSSION

Effect of PGPR inoculants on plant height of Coleus forskohlii

There was significant variation ($P \leq 0.05$) in plant height of *Coleus forskohlii* seedlings treated with *Azospirillum, Azotobacter*, *Pseudomonas* and *Bacillus* obtained the maximum plant height at all sampling periods.

The plant height of *Coleus forskohlii* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_{14}) recorded in at 180 DAP the maximum plant height of 68.3 cm plant^{-1} followed by treatments T_{13}, T_{10} and T_{11} respectively. Among the dual inoculants treatments, T_6 recorded the plant height of 59.8 cm plant^{-1} followed by T_7, T_9 and T_8. In the single inoculation T_1 recorded the plant height 59.1 cm plant^{-1} followed by T_3 respectively. The uninoculated treatment T_{15} recorded the minimum plant height (Table-1).

Effect of PGPR inoculants on number of tubers per plant of Coleus forskohlii

There was significant variation ($P \leq 0.05$) in number of tubers per plant of *Coleus forskohlii* seedlings treated with *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* obtained the maximum number of tubers per plant at all sampling periods.

The plant height of *Coleus forskohlii* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_{14}) recorded in at 180 DAP the maximum number of tubers per plant of 18.4 cm plant^{-1} followed by treatments T_{13}, T_{10} and T_{11} respectively. Among the dual inoculants treatments, T_6 recorded the number of tubers per plant of 15.6 cm plant^{-1} followed by T_7, T_9 and T_8. In the single inoculation T_1 recorded the number of tubers per plant 15.0 cm plant^{-1} followed by T_3 respectively. The uninoculated treatment T15 recorded the minimum number of tubers per plant (Table-2).

Effect of PGPR inoculants on tuber length per plant of Coleus forskohlii

There was significant variation ($P \leq 0.05$) in tuber length per plant of *Coleus forskohlii* seedlings treated with *Azospirillum, Azotobacter*, *Pseudomonas* and *Bacillus* obtained the maximum number of tubers per plant at all sampling periods.

The tuber length of *Coleus forskohlii* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_{14}) recorded in at 180 DAP the maximum tuber length per plant of 18.2 cm plant^{-1} followed by treatments T_{13}, T_{10} and T_{11} respectively. Among the dual inoculants treatments, T_6 recorded the tuber length per plant of 13.7 cm plant^{-1} followed by T_7, T_9 and T_8. In the single inoculation T_1 recorded the tuber length per plant 13.3 cm plant^{-1} followed by

Table 1 Effect of plant growth promoting rhizobacteria (PGPR) inoculation on plant height of *Coleus forskohlii*

S.No	Treatments	Plant height (cm) / plant^{-1} 45 DAP	90 DAP	135 DAP	180 DAP
1	T_1- *Azospirillum* (CFAzs-3)	19.2	36.1	47.2	59.1
2	T_2- *Azotobacter* (CFAzt-8)	15.6	29.4	41.5	52.8
3	T_3- *Pseudomonas* (CFPf-18)	17.9	32.5	44.7	56.1
4	T_4- *Bacillus* (CFPb-16)	14.5	27.3	39.8	48.4
5	T_5- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16)	18.8	35.6	46.4	56.3
6	T_6- *Azospirillum* (CFAzs-3)+ *Pseudomonas* (CFPf-18)	21.6	39.1	50.4	59.8
7	T_7- *Azospirillum* (CFAzs-3)+ *Azotobacter* (CFAzt-8)	19.4	35.9	47.8	56.0
8	T_8- *Bacillus* (CFPb-16)+ *Pseudomonas* (CFPf-18)	18.9	36.3	45.8	54.6
9	T_9- *Pseudomonas* (CFPf-18)+ *Azotobacter* (CFAzt-8)	20.1	38.2	49.2	58.9
10	T_{10}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Pseudomonas* (CFPf-18)	25.7	45.7	54.8	64.2
11	T_{11}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Azotobacter* (CFAzt-8)	23.2	42.6	53.9	62.4
12	T_{12}- *Bacillus* (CFPb-16) + *Azotobacter* (CFAzt-8) + *Pseudomonas* (CFPf-18)	22.4	40.9	51.5	58.9
13	T_{13}- *Azospirillum* (CFAzs-3)+ *Pseudomonas* (CFPf-18) + *Azotobacter* (CFAzt-8)	26.6	46.5	55.3	66.0
14	T_{14}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Pseudomonas* (CFPf-18) + *Azotobacter* (CFAzt-8)	28.4	48.5	56.9	68.3
15	T_{15}- Uninoculated (control)	12.5	25.0	36.5	45.8

Table 2 Effect of plant growth promoting rhizobacteria (PGPR) inoculation on number of tubers per plant^{-1} of *Coleus forskohlii*

S.No	Treatments	Number of tubers / plant^{-1} 45 DAP	90 DAP	135 DAP	180 DAP
1	T_1- *Azospirillum* (CFAzs-3)	5.7	11.6	13.5	15.0
2	T_2- *Azotobacter* (CFAzt-8)	5.0	10.9	12.5	14.0
3	T_3- *Pseudomonas* (CFPf-18)	5.2	11.3	13.2	14.5
4	T_4- *Bacillus* (CFPb-16)	4.5	10.5	12.0	13.9
5	T_5- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16)	5.5	11.8	12.4	14.7
6	T_6- *Azospirillum* (CFAzs-3)+ *Pseudomonas* (CFPf-18)	6.4	12.2	13.8	15.6
7	T_7- *Azospirillum* (CFAzs-3)+ *Azotobacter* (CFAzt-8)	6.0	11.5	13.6	15.3
8	T_8- *Bacillus* (CFPb-16)+ *Pseudomonas* (CFPf-18)	5.3	10.8	12.7	14.8
9	T_9- *Pseudomonas* (CFPf-18)+ *Azotobacter* (CFAzt-8)	6.0	11.5	13.1	15.0
10	T_{10}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Pseudomonas* (CFPf-18)	6.2	12.8	13.3	16.5
11	T_{11}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Azotobacter* (CFAzt-8)	6.4	12.1	13.5	16.2
12	T_{12}- *Bacillus* (CFPb-16) + *Azotobacter* (CFAzt-8) + *Pseudomonas* (CFPf-18)	6.1	12.5	13.0	15.9
13	T_{13}- *Azospirillum* (CFAzs-3)+ *Pseudomonas* (CFPf-18) + *Azotobacter* (CFAzt-8)	6.5	12.2	13.6	17.1
14	T_{14}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Pseudomonas* (CFPf-18) + *Azotobacter* (CFAzt-8)	7.5	13.5	15.0	18.4
15	T_{15}- Uninoculated (control)	4.5	8.2	9.3	12.5

Table 3 Effect of plant growth promoting rhizobacteria (PGPR) inoculation on tubers length per plant^{-1} of *Coleus forskohlii*

S.No	Treatments	Tubers length (cm) / plant^{-1} 45 DAP	90 DAP	135 DAP	180 DAP
1	T_1- *Azospirillum* (CFAzs-3)	2.8	6.3	10.2	13.3
2	T_2- *Azotobacter* (CFAzt-8)	2.0	5.4	8.2	11.7
3	T_3- *Pseudomonas* (CFPf-18)	2.3	5.6	8.8	12.2
4	T_4- *Bacillus* (CFPb-16)	1.8	4.5	7.4	10.8
5	T_5- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16)	2.9	6.5	9.6	12.6
6	T_6- *Azospirillum* (CFAzs-3)+ *Pseudomonas* (CFPf-18)	3.0	6.8	10.0	13.7
7	T_7- *Azospirillum* (CFAzs-3)+ *Azotobacter* (CFAzt-8)	2.9	6.4	9.7	13.0
8	T_8- *Bacillus* (CFPb-16)+ *Pseudomonas* (CFPf-18)	2.6	6.0	9.2	12.5
9	T_9- *Pseudomonas* (CFPf-18)+ *Azotobacter* (CFAzt-8)	2.8	6.3	9.5	12.9
10	T_{10}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Pseudomonas* (CFPf-18)	4.0	8.2	11.6	15.7
11	T_{11}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Azotobacter* (CFAzt-8)	3.8	7.6	10.9	14.8
12	T_{12}- *Bacillus* (CFPb-16) + *Azotobacter* (CFAzt-8) + *Pseudomonas* (CFPf-18)	3.4	7.4	11.5	13.8
13	T_{13}- *Azospirillum* (CFAzs-3)+ *Pseudomonas* (CFPf-18) + *Azotobacter* (CFAzt-8)	4.4	8.6	12.2	17.4
14	T_{14}- *Azospirillum* (CFAzs-3)+ *Bacillus* (CFPb-16) + *Pseudomonas* (CFPf-18) + *Azotobacter* (CFAzt-8)	4.7	9.5	14.8	18.2
15	T_{15}- Uninoculated (control)	1.5	4.0	6.3	10.4

T_3 respectively. The uninoculated treatment T_{15} recorded the minimum tuber length per plant (Table-3).

Effect of PGPR inoculants on tuber tuber yield of Coleus forskohlii

There was significant variation ($P \leq 0.05$) in tuber wet weight and dry weight per plant of *Coleus forskohlii* seedlings treated with *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* obtained the maximum number of tubers per plant at all sampling periods.

The tuber wet weight and dry weight of *Coleus forskohlii* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_{14}) recorded in at 180 DAP the maximum tuber wet weight and dry weight per plant of 135.20 and 67.10 g plant^{-1} followed by treatments T_{13}, T_{10} and T_{11} respectively. Among the dual inoculants treatments, T_6 recorded the tuber wet weight and dry weight per plant of 96.66 and 47.10 g plant^{-1} followed by T_7, T_9 and T_8. In the single inoculation T_1 recorded the tuber wet weight and dry weight per plant of 94.56 and 42.88 g plant^{-1} followed by T_3 respectively. The uninoculated treatment T_{15} recorded the minimum tuber length per plant (Fig.1 a & b).

Fig 1a Effect of plant growth promoting rhizobacteria (PGPR) inoculation on tuber wet weight (g/ plant^{-1}) of *Coleus forskohlii*

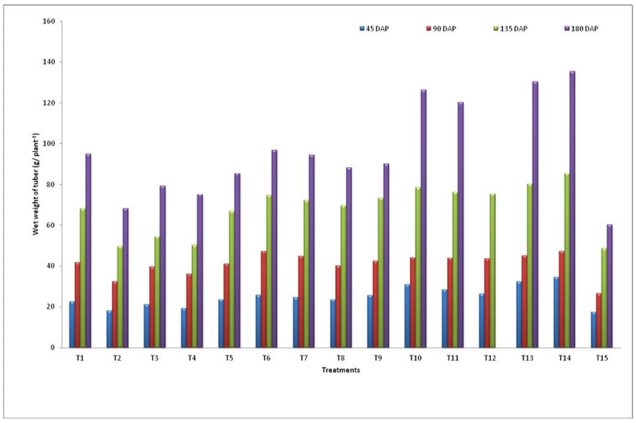
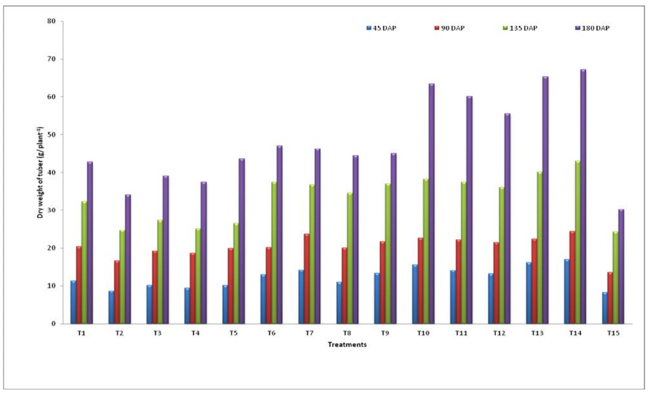


Fig 1b Effect of plant growth promoting rhizobacteria (PGPR) inoculation on tuber dry weight (g/ plant^{-1}) of *Coleus forskohlii*



Root inoculation with PGPR promoted significant increase in growth and yield content but the growth response varied between different rhizobacterial strains. However in general the growth response was found to be enhanced when the PGPR strains were applied in combination. This growth response was more effective in terms of an increased plant growth and yield compared to control. Earlier reports had shown that combined inoculation of sorghum with *Azospirillum lipoferum, Azotobacter chrococcum, Pseudomonas fluorescens* and *Bacillus megaterium* significantly increased grain yield. The stimulatory effects of this PGPR strains on the yield and growth of these crops were attributed to the N_2 fixation ability, plant growth regulator production and phosphate solubilizing capacity (Cakmakci *et al.,* 2007; Kevinvessey, 2003; Karlidag *et al.,* 2007; Priya, 2010; Rajasekar and Elango, 2011).

CONCLUSIONS

The combination of microbial consortium strains were found to have a great potential for use as bioinoculants to increase production in medicinal plants and other crops.

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