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Research Article

Bio-Management of Root-Rot Disease Caused by *Macrophomina phaseolina* in *Coleus forskohlii*

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ABSTRACT

Root rot disease in medicinal Coleus is a serious problem in all medicinal Coleus growing areas. Hence attempts were made to manage the disease through PGPR bioformulations. PGPR are a group of free-living saprophytic bacterial microorganisms that live in the plant rhizosphere and aggressively colonize the root system. Plant growth-promoting rhizobacterial (PGPR) strains (designated as Cf 60, Cf 7, Te 1, Av 30) were tested for their efficacy against the Coleus root rot pathogen *Macrophomina phaseolina* under *in vitro*, glasshouse and field conditions. Application of PGPR strains recorded maximum plant height, number of branches, fresh and dry weight of the plants with less disease severity in individual as well as combination treatments in Coleus. The perusal of data and ANOVA analysis indicates that root rot appeared in those treatments where PGPR inoculated singly than in combination. Maximum root-rot index (78.0%) and more disease severity was observed in only pathogen treated plants (control) without PGPR, followed by Cf 7+Cf 60 (29.5%), Te 1+Cf 60 (26.6%), Av 30+Cf 60 (23.0%), Cf 7+Te 1+Cf 60 (20.8%), Cf 7+Av 30+Cf 60 (18.0%), Te 1+Av 30+Cf 60 (16.46%) and Cf 7+Te 1+Av 30+Cf 60 (13.33%) *i.e.*, in all treatments with PGPR applications, the expression of symptoms was less and disease severity was less. The expression of symptoms and protection of the plants from infection of Coleus depended on PGPR strains, even in individual or combinations of PGPR strains. Cf 7+Te 1+Av 30+Cf 60 recorded maximum plant height, number of branches, more fresh and dry weight, total biomass with least disease severity. The study revealed the probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance against disease by PGPR bioformulations. The bio control agents not only controlled dry root rot, but also promoted plant growth and this give them an advantage over the use of chemical fungicides against root rot in disease management.

Key words: Biomanagement, PGPR, *M.phaseolina*,*Coleus forskohlii*

INTRODUCTION

Coleus (*Coleus forskohlii* Briq.) is cultivated mainly for their medicinal values in India. Among the several production constraints losses due to diseases are much concerned. Among the various diseases, root rot disease caused by *Macrophomina phaseolina* is the most devastating disease, which causes reduction in the tuber yield, forskolin content and finally complete death of the plant[1]. The control of Coleus root rot disease has been almost exclusively based on the application of chemical pesticides. Several effective pesticides have been recommended for use against this pathogen, but they are not considered to be long-term, solutions, due to concerns of expense, exposure risks fungicide residues and other health and environmental hazards. There is a vital need for alternative methods of control for Coleus root rot. So far, effective and ecologically sound management practices have to be developed for managing this disease.

PGPR are a group of free-living saprophytic bacterial microorganisms that live in the plant rhizosphere and aggressively colonize the root system. They are studied as plant growth-promoters for increasing agricultural production and as biocontrol agents against plant diseases [2,3]. Suppression of growth of soil-borne phytopathogens by producing allelochemicals by PGPR include a) siderophores, b) antimicrobial compounds, c) mycolytic enzymes eg., chitinase, β-1, 3-glucanase, lipases, proteases etc. and parasitism [4,5]. The production of allelochemicals by PGPR facilitates aggressive colonization and defensive inhabitation of rhizosphere niches [6,7]. On the other hand, microorganisms as biological control agents often exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for the suppression of plant pathogens. For this reason, we evaluated multiple PGPR strains for their ability to promote Coleus plant growth and their effectiveness against root rot disease under glasshouse and field conditions. Induced systemic resistance occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens. Once natural plant-resistance mechanisms are activated, increased defensive capacity is maintained for prolonged periods against multiple pathogens[8].

Similar studies have been done by some of the scientists.

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The maximum biomass yield and alkaloid content recorded in the combined inoculation of PGPRs in *Catheranthus roseus* and can be used as a good tool in the enhancement of biomass yield and alkaloid contents [9] as well as for biocontrol. A field study undertaken by Boby and Bagyaraj [10] for controlling the wilt disease caused by *Fusarium chlamidosporum* using three biocontrol agents viz., *Glomus mosseae, Pseudomonas fluorescens, Trichoderma viride* in Coleus. It resulted in maximum growth, yield and root forskolin concentration in Coleus and showed disease severity index of 33.28% compared to uninoculated control plants (85.5%). Bioefficacy of rhizobacteria on root knot/wilt complex in Coleus and Ashwagandha[11]; Bio Control Potential of *Pseudomonas fluorescens* against Coleus root rot disease was done by Pulla Reddy Akkim et al.[12] and Vanitha and Ramjegathesh [13].

Keeping these points in view, the present investigation on soil borne pathogens of Coleus was undertaken to evolve the efficacious plant growth promoting rhizobacteria (PGPR) bioformulations against fungi under glasshouse along with field conditions. In previous paper PGPR isolates was studied by testing the plant growth promoting activities i.e., production of indole acetic acid (IAA), lytic enzymes, hydrocyanic acid (HCN), volatile metabolites etc.[14]. Therefore, the objectives of the current study have been developed based on biological control strategy for this disease that is economically viable, environmentally safe durable and is an alternative to agrochemicals.

MATERIALS AND METHODS

Sample collection, isolation and selection of PGPR strains

Samples of rhizosphere soils were collected from different medicinal plants grown at the botanical garden, Osmania University, Central Institute of Medicinal and Aromatic Plants (CIMAP) centre, ANGRAU, SILTA Agro farms Pvt. Ltd. Hyderabad, India. Intact root system was dug out and the rhizospheric soil samples were carefully taken in plastic bags and stored at 4°C. A total of 25 soil samples were collected from the different medicinal plants located in various regions for the isolation of rhizosphere bacterial isolates.

Rhizobacteria (PGPR) were isolated from the rhizospheric soil samples by serial dilution plate technique[15]. Samples were serially diluted with sterile distilled water (10^{-1} to 10^{-7}) and each dilution was used for pouring on nutrient and King's B agar plates. After incubation for 48 h at 30°C, colonies were picked from these plates and maintained as pure cultures in nutrient agar slants with periodic transfer to fresh media. The bacterial strains were screened and selected for their plant growth promoting activities as described in previous paper[14] and antifungal activity by using dual culture plate technique.

Antifungal activity

Macrophomina phaseolina was isolated from diseased plants by using PDA (potato dextrose agar) medium. The pathogen was identified using standard mycological literature. The bacterial isolates were screened for the ability to inhibit *M. phaseolina* by employing dual culture method [16] on PDA plates.

The bioagent (bacteria) and the pathogen were inoculated side by side on a petri plate containing solidified PDA medium. The width of the inhibition zones between the pathogen and bacteria was categorized as strong, moderate and weak. Three replications were maintained for each isolate with one control by maintaining only pathogen. They were incubated at 28^0 C. Observations were recorded when there was a full growth of pathogen in the control plate (4-7 days). The diameter of the colony of the pathogen was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by Rabindran and and Vidyasekaran[17].

$$I = \frac{C - T}{C} \times 100$$

Where;

I = Per cent inhibition

C = Radial growth of the pathogen in control

T = Radial growth of pathogen in treatment

Glasshouse and field experiments

Experiments were conducted to study the efficacy of selected PGPR strains *i.e.*, the effect of *Pantoea agglomerans* (Cf 7), *Pseudomonas putida* (Te 1), *Psedomonas* sp. (Av 30) on plant growth promotion and biocontrol efficacy *of Bacillus subtilis* (Cf 60).

Preparation of inoculums

The *Pantoea agglomerans* (Cf 7), *Pseudomonas putida* (Te 1), *Psedomonas* sp. (Av 30) and *Bacillus subtilis* (Cf 60) bacterial cultures were grown in respected media (broth) for 48 h. on a shaker and centrifuged at 5500rpm for 7min, the supernatant discarded and the pellets containing bacterial cells was suspended in 250 ml of 0.01M $MgSo_4$ solution. The colony-forming units of bacteria were 24×10^5 /ml inoculum [10].

Bacterization of Coleus cuttings and Growth conditions

Uniform sized, pencil-thick Coleus cuttings (12 cm long) were selected and planted in poly bags containing 2 kg of 1:1 sterilized soil and sand. Bacterial inoculum10ml/bag was added to the planting hole as per the treatment, before planting the cuttings. The cuttings with sterile water served as control. The poly bags were kept on benches in moisturized conditions in glasshouse for 40 days for rooting and suitably watered. After 40 days, plants were removed and maintained in field conditions in order to harden the plants. Observations on plant height, number of branches, root length, number of tubers and total biomass of the Coleus plants were recorded at 60, 90, 150 and 180 days after planting.

Inoculation of fungal pathogen

The test pathogen *Macrophomina phaseolina* was mass cultured on sterilized seeds by inoculating 5mm mycelial discs in flasks containing 150g of sorghum seeds. Flasks were incubated for 15-20 days at 30°C. The mycelia inoculum was mixed in the field soil. When Pathogen was multiplied well in field, soil becomes a wilt sick soil. Ten plants were maintained for each treatment. The treatments were individual and in combinations: PGPRs (Cf 7, Te 1,

Table.1. Efficacy of PGPR strains on yield parameters and disease incidence in Coleus under wilt sick soil field conditions

PGPR strain treatments	Shoot length (cm) DAP				No. of branches	No. of tubers	Tuber length (cm)	Dia. of tuber (cm)	Shoot weight (gm)		Root weight (gm)		Total biomass (gm)	
	60	90	150	180					Fresh	Dry	Fresh	Dry	Fresh	Dry
Cf7+Cf 60	11.0	12.3	16.0	20.6	3.6	3.3	3.3	1.5	24.33	3.33	5.83	1.16	30.16	4.49
Te1+ Cf 60	11.6	13.0	16.1	20.0	4.6	4.0	4.3	1.6	27.33	3.33	5.53	1.46	32.86	4.79
Av30+ Cf 60	12.0	13.6	16.7	23.3	5.0	4.3	6.1	1.6	28.33	3.93	6.30	1.60	34.63	5.53
Cf7+ Te1+Cf 60	13.6	15.3	17.1	27.6	5.6	4.6	6.0	1.4	32.33	4.50	7.56	1.76	39.89	6.26
Cf7 +Av30+Cf 60	16.3	16.2	17.6	28.0	7.0	5.0	6.3	1.9	36.00	4.66	8.50	2.43	44.50	7.09
Te1 + Av 30+Cf 60	18.3	16.5	18.5	30.3	6.3	5.6	6.6	2.2	34.66	5.36	9.36	2.73	44.02	8.09
Cf7+Te1+ Av 30+Cf60	20.0	17.2	19.3	32.0	7.3	6.0	7.5	2.4	38.66	6.10	10.83	3.20	49.49	9.30
Control (Cf 60)	10.6	13.0	15.3	21.0	3.6	2.6	3.1	1.2	20.33	3.06	5.30	1.10	25.63	4.16

DAP- Days after planting; No. Number; Diam- Diameters

Av 30) with biocontrol agent (*Bacillus subtiliis* (Cf 60)) in wilt sick soil to test the plant growth promotion as well as biocontrol efficacy of PGPR.

Transplanting

An experiment was conducted for Coleus at Botanical garden, Dept. of Botany, Osmania University, Hyderabad. A completely randomized block design was maintained. 100 plants for PGPR's with biocontrol agent (*Bacillus*) in wilt sick soil conditions. Hardened plants were transplanted to the main field. Before transplanting, the field was ploughed well and ridges were made 60cm apart. 4 plots each with size of 1.8m×1.0 m were made. Plants were transplanted at 20 cm spacing on the ridges. Planting was done with intact ball of earth without polythene cover. Each plot containing 25 plants, 10 plants for each treatment and three plants tagged for taking observations. There were eight treatments as given below.

PGPR treatments (Cf 7, Te 1, Av 30) + biocontrol agent (Cf 60) in Macrophomina infested soil. (Individual and Combinations)

T1 – Cf 7 + Cf 60
T2 – Te 1 + Cf 60
T3 – Av 30 + Cf 60
T4 – Cf 7 + Te 1+ Cf 60
T5 – Te 1 + Av 30 + Cf 60
T6 – Cf 7 + Av 30 + Cf 60
T7 – Cf 7 + Te 1 + Av 30+ Cf 60
T8 – Uninoculated control (only pathogen)

Cf 7 = *Pantoea agglomerans*, Te 1= *Pseudomonas putida*, Av 30 = *Pseudomonas* spp., Cf60= *Bacillus subtilis*

A second dose of inoculum was added 1 week after transplanting. When only one organism was used for inoculation, it was inoculated on one side of the plant close to root by making a hole in the soil and subsequently closing it. When two or three organisms were used for inoculation, they were inoculated on either side of plant and close to the root system. Irrigation was given once in 3-4 days.

Observations were made for plant growth parameters at different growth stages of growth period of (60, 90, 150 and 180) plant height, number of branches and at harvest, yield parameters like weight of tubers, length and number of tubers, fresh and dry weight and total biomass were recorded.

Disease index

The disease index was computed by development of disease symptoms was observed and recorded. Percent disease index (PDI) was determined by using the formula:

$$\text{\% Disease Index} = \frac{\text{No.of plants showing disease symptoms}}{\text{Total number of plants}} \times 100$$

Plant parameters studied

Three plants in each treatment were randomly selected for taking observations and average was worked out for all the parameters. Plant height (cm), Number of branches per plant, Dry matter production (g/plant), Number of tubers per plant, Length (cm) of tubers, Diameter (mm) of tubers, Fresh and Dry weight (g/plant) of tubers etc.

Statistical analysis

The data obtained in the present investigations for various parameters in the experiments were subjected to ANOVA analysis for a completely randomized design for *in vitro* studies. The experiments were designed as completely randomized design and the data was recorded in triplicate for the selected parameters and subjected to ANOVA (analysis of variance) in accordance with the experimental using SPSS statistical software to quantify and evaluate the sources of significance variances among the treatments and comparisons of treatment means were accomplished by least significance difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the

Table.2. Efficacy of PGPR strains on growth parameters and disease incidence in Coleus under wilt sick soil field conditions

PGPR strain treatments	Tubers/ plant	Tubers			Shoot weight (gm)		Total biomass (gm)		Percent disease incidence (PDI)	% of decrease over control
		Length (cm)	Fresh wt (gm)	Dry wt (gm)	Fresh	Dry	Fresh	Dry		
Cf 7 + Cf 60	3.3	3.3	5.83	1.16	24.33	3.33	30.16	4.49	29.5	62.18
Te 1 + Cf 60	4.0	4.3	5.53	1.46	27.33	3.33	32.86	4.79	26.6	65.90
Av 30 + Cf 60	4.3	6.1	6.30	1.60	28.33	3.93	34.63	5.53	23.0	70.52
Cf 7 + Te 1 + Cf 60	4.6	6.0	7.56	1.76	32.33	4.50	39.89	6.26	20.8	73.34
Cf 7 + Av 30 + Cf 60	5.0	6.3	8.50	2.43	36.00	4.66	44.50	7.09	18.0	76.93
Te 1 + Av 30 + Cf 60	5.6	6.6	9.36	2.73	34.66	5.36	44.02	8.09	16.46	78.90
Cf 7+Te 1+Av 30+Cf 60	6.0	7.5	10.83	3.20	38.66	6.10	49.49	9.30	13.33	82.81
M. phaseolina (Pathogen)	2.6	3.1	5.30	1.10	20.33	3.06	25.63	4.16	78.0	-

DAP- Days after planting; No. Number; Diam- Diameters

rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. PGPR influence the plant growth in different ways by producing phytohormones like IAA and gibberellins, siderophore production, phosphate solubilization, synthesis of antibiotics, enzymes and/or antifungal compounds [18,19]. In the present study, isolation of bacterial cultures from the rhizosphere soil samples of different medicinal and aromatic plants *viz., Coleus forskohlii, Withania somnifera, Ocimum sanctum, Andrographis paniculata, Mentha spicata, Aloe vera, Tagetes erecta, Artemisia vulgaris, Acorus calamus* and *Mimosa pudica.* In the rhizosphere of all medicinal plants, microbial population was more and these bacterial strains possessed multiple PGP activities. Karthikeyan *et al*.[20] isolated rhizosphere, non-rhizosphere and diazotrophic microbial populations from commercially grown *Coleus forskohlii, Aloe vera, Ocimum sanctum* and *Cartharanthus roseus* in Tamil Nadu.

Biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzyme, hydrozen cyanide and siderophore or through competition for nutrient and space can significantly improve plant health and promote growth by increasing of seedling emergence, vigor and yield. Antibiotics produced by *Pseudomonas* sp.[21], *Bacillus* sp.[22] play an important role in the biological control of plant diseases. Seed and soil treatments with biocontrol agents *Bacillus megaterium, Bacillus subtilis* and *Pseudomonas fluorescens* significantly reduced chickpea *Fusarium* wilt disease intensity and increased chickpea seed yield [23]. In the present study antagonistic potential of the isolates was concluded and validated by restriction of pathogen growth by showing zone of inhibition towards the antagonists in dual culture plate assay compared to the control. An isolate Cf 60(*Bacillus* sp.) showed maximum antagonism (52.22%).

Efficacy of PGPR strains (Cf 7, Te 1, Av 30, Cf 60) on growth parameters and disease incidence in Coleus under wilt sick soil field conditions were evaluated. The experiments were designed as completely randomized design and the data was recorded in triplicate for the selected parameters and subjected to ANOVA (analysis of variance) in accordance with the experimental using SPSS statistical software to quantify and evaluate the sources of significance variances among the treatments and comparisons of treatment means were accomplished by least significance difference (LSD) test at 5% level of significance.

Bioinoculants containing Cf 7, Te 1, Av 30 and Cf 60 effectively reduced the disease incidence and root-rot infestation in Coleus than untreated control in field conditions. The field experiments conducted in the present investigation also revealed that the four PGPR strains (Cf 7, Te 1, Cf 60, Av 30) were shown to have plant growth promoting activity under field conditions in a short growing season area, and provided increases in root number, root weight, shoot weight and total biomass of Coleus plants. Similar type of results reported by Santhosh Dharma *et al*.; Boby and Bagyaraj and Singh *et al.*[24,10, 25] on inoculation of bio-inoculants (*T. viride, G. fasciculatum, G. mosseae* and *P. fluorescens*) significantly increased the forskolin content of the Coleus roots.

Biological control is high on the list of potential alternative tactics. In Coleus, lowest disease incidence of 13.33% (82.81% reduction over control) was recorded in Cf 7+Te 1+Av 30+Cf 60 followed by 16.46 % of Te 1+Av 30+Cf 60 (78.90%), 18 % of Cf 7 + Av 30 + Cf 60 (76.93%), 20.8 of Cf 7+Te 1+Cf 60 (73.34%), 23.0% of Av 30+Cf 60 (70.52%), 26.6% of Te 1+Cf 60 (65.90), and 29.5 of Cf 7+ Cf 60 (62.18%). Highest disease incidence of 78.0 % was recorded in untreated plants (control) (Table.1). In general due to PGPR treatment there was reduction of disease incidence in Coleus which varied from 78.0% to 29.5 % reduction (PDI) over control respectively. More disease severity was observed in only pathogen (control) treated plants without PGPR, followed by Cf 7+Cf 60 (29.5%), Te 1+Cf 60 (26.6%), Av 30+Cf 60 (23.0%), Cf 7+Te 1+Cf 60 (20.8%), Cf 7+Av 30+Cf 60 (18.0%), Te 1+Av 30+Cf 60 (16.46%) and Cf 7+Te 1+Av 30+Cf 60 (13.33%) (Table.2) *i.e*., in all treatments with PGPR applications, the expression of symptoms was less and disease severity was less. Management of insect pests and plant diseases by different *Pseudomonas* strains through different formulations have been reported by many workers [26,27,28]. A similar study conducted by Jonathan *et al*. [29,30] also indicated that the rhizobacteira, viz. *P. fluorescens* and *Bacillus* spp. were reported to induce

profuse root development in banana, tomato and betel vine and reduced *Meloidogyne incognita* population. Boby and Bagyaraj[10] also observed the reduction of root rot incidence in *Coleus forskohlii* by microbial inoculants.

Thus, the bacterial isolates from the rhizosphere of medicinal and aromatic plants were able to solubilize phosphorus, produce hydrolytic enzymes, phytohormones and have multiple PGP activities respectively. These characteristics confirm that these rhizosphere microorganisms are PGP microbes. These microbes can also increase the growth of whole plant, vigor index (VI) and alkaloid content of various medicinal plants viz., *Catharanthus roseus* (Karthikeyan 2010)[31], *Coleus forskohlii* [10](Boby and Bagyaraj, 2003) and *Begonia malabarica* (Thangavel Selvaraj *et al*., 2008)[32]. These isolates can be used as bioinoculum and can be exploited for the synthesis of numerous metabolites which can be used commercially. In field trials selected PGPR's have influence over the environment of specific active principle of *Coleus forskohlii.* In the present study, increase in plant height, number of branches, fresh and dry weight, total biomass of the plant as well as less disease severity were observed due to PGPR treatments compare to control (only pathogen). The expression of symptoms and protection of the plants from infection of Coleus depended on PGPR strains. Even in individual or combinations of PGPR strains. Cf 7+Te 1+Av 30+Cf 60 recorded maximum plant height, number of branches, more fresh and dry weight, total biomass with least disease severity.

CONCLUSION

In conclusion the results of this study suggest that simultaneous screening of rhizobacteria for growth and yield promotion under pot and field experiment is a good tool to select effective PGPR for biofertilizer development biotechnology. Selected PGPR strains *Pantoea agglomerans* (Cf 7), *Pseudomonas putida* (Te 1), *Bacillus subtilis* (Cf 60) and *Pseudomonas* sp. (Av 30) exhibited good PGP activities, and in addition *Bacillus subtilis* (Cf 60) strongly inhibited the growth of *M. phaseolina.* It enhanced growth parameters of Coleus and also suppressed root rot disease. It showed excellent root colonization ability. These attributes of *B. subtilis* verifies it as a potent biocontrol agent against *M. phaseolina.* The bioefficacy studies conducted in field experiment indicated the efficacy of the PGPR strains in enhancing the plant growth parameters as they exhibited higher antagonistic activity against pathogen individually and also in combinations. Soil microbial populations are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural systems and natural ecosystems. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low-input biotechnology, to help sustainable, environmentally-friendly, agro-technological practices. Much research is addressed at improving understanding of the diversity, dynamics, and significance of rhizosphere microbial populations and their cooperative activities.

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REFERENCES

1. Kamalakannan A, Mohan L, Valluvaparidasan V, Mareeswari P, Karuppiah R. First report of *Macrophomina* root rot (*Macrophomina phaseolina*) on medicinal coleus (*Coleus forskohii*) in India. *Journal of Plant Pathology* 2006; 55: 302.
2. Chen Y, Mei R, Lu S, Liu L, Kloepper JW. The use of yield increasing bacteria (YIB) as plant growth promoting rhizobacteria in Chinese agriculture 1996; In: *Management of Soil Borne Diseases*, Eds. Utkhede, R.S. and Gupta, V.K., Kalyani Publishers, New Delhi, pp.165-184.
3. Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R. PGPR-induced defense responses in the tea plant against blister blight disease. *Crop Protection* 2007; 26:556-565.
4. Cattelan AJ, Hartel PG, Fuhrmann JJ. Screening for plant growth promoting rhizobacteria to promote early soybean growth. *Soil Science Society of American Journal* 1999; 63: 1670-1680.
5. Whipps JM. Microbial interactions and biocontrol the rhizosphere. *Journal of Experimental Botany* 2001; 52: 487-511.
6. Glick BR. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 1995; 41(2): 109-117.
7. Sturz AV, Christie BR. Beneficial microbial allelopathies in the root zone: The management of soil quality and plant disease with rhizobacteria. *Soil Tillage Research*2003; 72: 107-123.
8. Tuzun S, Kuc J. Persistence of induced systemic resistence to blue mold in tobacco plants derived *via* tissue culture. *Phytopathology* 1987; 77: 1032-1035.
9. Karthikeyan B, Abdul Jaleel C, Azooz MM. Individual and combined effects of *Azospirillum brasilense* and *Pseudomonas fluorescens* on biomass yield and ajmalicine production in *catharanthus roseus. Acdaemic Plant Science* 2009; 2(2): 69-73.
10. Boby VU, Bagyaraj DJ. Biological control of root-rot of *Coleus forskohlii* Briq. using microbial inoculants. *World Journal of Microbiology & Biotechnology* 2003; 19: 175-180.
11. Mallesh SB, Yashodha R, Hegde AN, Mokashi PU, Krishnaraj. Bioefficacy of rhizobacteria on root knot/wilt complex in coleus and ashwagandha. *Karnataka Journal of Agricultural Science* 2009; 22(5): 1116-1120.
12. Pulla Reddy Akkim, Nagendran K, Vanitha S, Karthikeyan G, Raguchander T. Fluorescent Pseudomonads mediated disease management of *Macrophomina phaseolina* inciting *Coleus forskohlii*

(Briq.), a root rot pathogen. *African Journal of Research* 2013; 8(38): 4787-4797.
13. Vanitha S, Ramjegathesh R. Bio Control Potential of *Pseudomonas fluorescens* against coleus root rot disease. *Journal of Plant Pathology* 2014; 5:216.
14. Malleswari D, Bagyanarayana G. Plant growth-promoting activities and molecular characterization of rhizobacterial strains isolated from medicinal and aromatic plants *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 2013; 6(6): 30-37 .
15. Aneja KR. Experiments in microbiology plant pathology tissue culture and Mushroom production technology, 3rd edn. New Age International Publishers 2001; pp. 192-195.
16. Paul NC, Kim WK, Woo SK, Park MS, Yu SH. Fungal endophytes in roots of Aralia species and their antifungal activity. *Journal of Plant pathology* 2007; 23(4): 287-294.
17. Rabindran R, Vidyasekaran P. Development of a formulation of *Pseudomonas fluorescens* PfALR2 for management of rice sheath blight. *Crop Protection* 1996; 15: 715-721.
18. Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* 2006; 36: 1-9.
19. Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R. Rhizobacteria based bio-formulations for the management of fruit rot infection in chillies. 2004; *Crop Protection*. 23: 835-843.
20. Karthikeyan N, Prasanna R, Lata, Kaushik BD. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *European Journal of Soil Biology* 2007; 43: 23-30.
21. Thomashow LS, Weller DM. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici. Journal of Bacteriology* 1988; 170: 3499-3508.
22. Silo-Suh LA, Lethbridge BJ, Raffle, SJ, He H, Clardy J, Handelsman J. Biological activities of two fungistic antibiotics produced by *Bacillus cereus* UW 85. *Applied Environmental Microbiology* 1994; 60: 2023-2030.
23. Landa BB, Navas-Cortes JA, Jimenez-Diaz RM. Integrated management of *Fusarium* wilt of chickpea with sowing date, host resistance and biological control. *Phytopathology* 2004; 94: 946-960.
24. Santhosh Dharma, Laxminarayan Hegde, Rokhade AK, Patil CP, Kulkarni MS. Effect of bioincoculant organisms on growth and yield of *Coleus forskohlii* Briq.- an endangered medicinal plant. *Myccorrhiza News* 2006; 18(2). 15-17.
25. Singh Rakshapal, Paramaeswarn TN, Prakasa Rao EVS, Puttanna K, Kalra Alok, Srinivas KVNS, Bagyaraj DJ, Divya S. Effect of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on root-rot/wilt, growth and yield of *Coleus forskohlii*. *Biocontrol Science Technology* 2009; 19 (8): 835-841.
26. Zhender GW, Yao C, Murphy JF, Sikora ER, Kloepper JW, Schuster DJ, Polston JE. Microbe induced resistance against pathogens and herbivores: evidence of effectiveness in agriculture. In induced plant defenses against pathogens and herbivores. 1999; *Biochemistry, Ecology and Agriculture*. APS Press, St Paul, MN. Edited by Agarwal AA, Tuzun S, Bent, E, 33.
27. Raupach GS, Kloepper JW. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 1998; 88: 1158-1164.
28. Vivekananthan R, Ravi M, Ramanathan A, Samiyappan R. Lytic enzymes induced by *Pseudomonas fluorescens* and other biocontrol organisms mediate defence against the anthracnose pathogen in mango. *World Journal of Microbiology and Biotechnology* 2004; 20: 235-244.
29. Jonathan EI, Basker KR, Abdel-Alim FF, Vrain, TC, Dickson DW. Biological control of *Meloidogyne incognita* on tomato and banana with rhizobacteria, *Actinomycetes* and *Pasteuria penetrans. Nematropica* 2000; 30: 231-240.
30. Jonathan EI, Cannayne I, Samiyappan R. Field application of biocontrol agents for the management of spiral nematode, *Helicotylenchus multicinctus* in banana. *Nematologia Mediterranea* 2004; 32: 169-173.