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Fluorescent Pseudomonads mediated disease management of *Macrophomina phaseolina* inciting *Coleus forskohlii* (Briq.), a root rot pathogen

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Plant growth-promoting rhizobacterial (PGPR) strains were tested for their efficacy against the Coleus root rot pathogen *Macrophomina phaseolina* under *in vitro*, glasshouse and field conditions. Among the thirty isolates of *P. fluorescens* screened under *in vitro* condition, six isolates viz., Pf1, FP 7, COTP-20, COGP-30, TDK-1 and AH-1 were effective in reducing mycelial growth of the pathogen. The isolate Pf1 showed maximum percent inhibition over control which contributes 58.52% reduction over control, followed by FP 7. Mode of action of effective PGPR isolates was studied by testing the production of indole acetic acid (IAA), siderophore, lytic enzymes, hydrocyanic acid (HCN), volatile metabolites, fluorescein and pycovyanin. Among these six isolates, Pf 1 and FP 7 found to produce all these compounds in higher quantity in comparison with other isolates. Plants treated with talc based formulation of Pf1 (stem cuttings dip + soil application) significantly increased the activity of defence related enzymes *viz.*, peroxidase and poly phenol oxidase in coleus plants followed by FP 7 (Stem cuttings dip + soil application) when compared to untreated control. The plants treated with pre mixture fungicide (Carbendazim + Mancozeb) (stem cuttings dip + soil application) at 0.1% effectively reduced root rot incidence over control followed by Pf1 (stem cuttings dip + soil application) at 0.2% under both pot and field conditions. Plants treated with Pf1 (Stem cuttings dip + Soil application) showed best performance of both growth and yield parameters followed by FP 7 (stem cuttings dip + soil application) under both glass house and field conditions.

Key words: Biocontrol, *Pseudomonas fluorescens*, root rot, *Macrophomina phaseolina,* induced systemic resistance, defence enzymes.

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. Recently occurrence of this disease was serious leading to drastic reduction in tuber yield was reported in some districts of Tamil Nadu. 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,

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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. 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.

Induced resistance has emerged as a potential tool in crop protection practices based on biological control. Induced resistance can be defined as the phenomenon by which plants exhibit increased levels of resistance to a broad spectrum of pathogens by the prior activation of genetically programmed defence pathways. The most extensively studied type of induced resistance is systemic acquired resistance (SAR) (Durrant and Dong, 2004). SAR is expressed locally and systemically after a localised infection by a necrotising pathogen or the application of some chemicals such as benzothiadiazole (BTH) (Conrath et al., 2001, 2006) and is characterised by the accumulation of salicylic acid (SA) and pathogenesis-related (PR) proteins. The colonization of roots with selected plant growth-promoting rhizobacteria (PGPR) (De Vleesschauwer et al., 2006; Saravanakumar et al., 2007) can also lead to a type of systemic resistance, commonly denoted as induced systemic resistance (ISR).

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 asbiocontrol agents against plant diseases (Chen et al., 2000; Saravanakumar et al., 2007). More specifically, the soil-borne fluorescent pseudomonads have received particular attention because of their catabolic versatility, excellent root colonising ability and their capacity to produce a wide range of enzymes and metabolites that favour the plant to withstand varied biotic and abiotic stress conditions (Radjacommare et al., 2004; Saravanakumar and Samiyappan, 2007). 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 fluorescent pseudomonads strains for their ability to promote Coleus plant growth and their effectiveness against root rot disease under glasshouse and field conditions.

MATERIALS AND METHODS

Isolation and collection of PGPR strains

Rhizosphere soil from coleus fields was collected from districts of Salem and Coimbatore. The Fluorescent pseudomonad strains were isolated from the collected soil samples by using the serial dilution technique in Kings' B medium (Peptone 20 g; $MgSO_4$ 1.5 g; K_2HPO_4 1.5 g; Glycerol 10 ml; Agar 20; Distilled water 1l) . The strains of *Pseudomonas* sp. were identified according to the description given in 'Bergey's manual for systematic bacteriology' (Krieg and Holt, 1984). These isolates were designated as follows and were maintained at -80°C with 50% glycerol. Twenty four elite strains of *Pseudomonas* sp. *viz*., PAO-1, PFKO-1, FP 7, PRA-1, MDU-1, PCN-3, PSKK-7, PPOL-3, 30-48-R1, PB-2-79, COGP-20, COGP-30, COTP-20, COPP-36, COKP-46, TDK-1, AH-1, Pf1, PFNL-1, PRA-4, PSA-2, PSA-1, PPOL-1 and MDU-2 were obtained from the Culture Collection, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and used for the study.

Antifungal activity of fluorescent pseudomonads

Fluorescent pseudomonad strains were tested for their antagonistic activity against *Coleus* root rot pathogen, *M. phaseolina*. A mycelial disc (8-mm diameter) from seven days old *M. phaseolina* was placed on one side of the Petri dishes containing an equal amount of KB and PDA medium. *Pseudomonas* cultures were streaked on the medium exactly opposites to the mycelial disc. The plates were incubated at room temperature (28 ± 2°C) for 6 days. Efficiency of the antagonistic organisms against the root rot pathogen was rated based on the inhibition zone observed (Rabindran and Vidyasekaran, 1996). The percent inhibition of growth of the test pathogen was calculated by using the formula

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

where, I = Percent inhibition; C = Growth (cm) in control; T = Growth (cm) in treatment.

The effective isolates from dual plate technique were tested for their actual mode of action.

Mechanism of action of PGPR on plant pathogenic fungi

Indole acetic acid (IAA) production

The production of indole acetic acid (IAA) by *Pseudomonas* isolates was determined by a modified method of Gupta (1999). Bacterial isolates were grown in nutrient broth supplemented with tryptophane (5 mg/ml). Five millilitres of each bacterial culture was centrifuged at 2816 g for 15 min. The supernatant was collected and filtered through filter paper. Two millilitres of supernatant was mixed with two drops of O-phosphoric acid and 4 ml of freshly prepared Solawaski's reagent (50 ml of 35% perchloric acid, 1 ml 0.5% $FeCl_3$). IAA development caused the production of a pink colour, which was measured by reading its absorbance at 530 nm. The level of IAA produced was estimated using a standard IAA graph.

Production of siderophores

Production of siderophores was estimated qualitatively on Chrom-Azurol S agar medium adjusted to pH 6.8 with NaOH (Schwyn and Neilands, 1987). An actively growing culture of each *Pseudomonas* isolate was spotted onto the Chrom-Azurol S plates and incubated for 24 to 48 h at 32°C. The appearance of a bright zone with yellowish fluorescent colour in the dark blue medium was the indication of production of siderophore. Production of siderophore was scored as none, little strong and very strong.

Lytic enzymes assay

Protease production was assayed using skim milk agar. Bacterial cells were spot inoculated and incubated for two days at 28°C. Proteolytic activities were identified by clear zone formation around the cell (Smibert and Krieg, 1994). Bacterial strains were screened for their chitinase activity on minimal salt (MS) chitin agar plates containing 0.1% (w/v) chitin with MS medium (0.5% yeast extract, 0.2% K_2HPO_4, 0.1% KH_2PO_4, 0.07% $MgSO_4.7H_2O$, 0.05% NaCl, 0.05% KCl, 0.01% $CaCl_2$, 1.3% bacto agar [pH 6.8]) and incubated at 30°C for seven days. The halo forming strains on the MS chitin plates were selected as chitin producers.β-1, 3-glucanase activity was estimated on plates containing minimal medium containing laminarin (Dunne et al., 1996) as sole substrates. Activity of β-1, 3-glucanase was determined by the ability of bacteria to grow on laminarin as the sole carbon source.

HCN production

Production of HCN was assessed on Kings' B medium (KB) containing 4.4 g/L of glycine with indicator paper (Whatman no 1 soaked in 0.5% (w/v) picric acid and 2% (w/v) sodium carbonate) and plates incubated at 27°C for 48 to 72 h. Any positive response caused the indicator paper to turn from yellow to cream, light brown, dark brown and brick (Alstrom and Burns, 1989).

Effects of volatile metabolites

The effect of volatile metabolites of fluorescen pseudomonads on growth of *M. phaseolina* was studied by a paired Petri dish technique (Gagne et al., 1991). A fresh 24 h old bacterial culture was uniformly inoculated on King's B agar plates. In another set PDA plates were inoculated at the centre with a six mm fungal disc from a three days old culture. The PDA plate with the fungus (downward facing) was then paired with the Petri dish containing the bacteria (upward facing) and sealed with parafilm. Uninoculated plates paired with PDA plates inoculated with fungus only served as control. The paired plates were incubated at 28°C and fungal colony diameters were measured six days after incubation.

Detection of fluorescein and pyocyanin

Pseudomonas agar F (Casein enzymic hydrolysate, 10 g; protease peptone, 10 g; K_2HPO_4, 1.5 g; $MgSO_4$, 1.5 g; distilled water, 1l) favours the formation of fluorescein whereas *Pseudomonas* agar P (Peptone, 20 g; $MgCl_2$, 1.4 g; K_2SO_4, 10 g; Agar, 15 g; Distilled water, 1l) stimulates the pyocyanin production and reduces fluorescein formation (King et al., 1954). All the six isolates of fluorescent pseudomonads were tested for production of fluorescein and pyocyanin.

Pot culture experiment

A pot culture experiment was laid out in completely randomized design to test the efficacy of *Pseudomonas* strains, that is, Pf1 and FP7 and pre mixture fungicide (Carbendazim + Mancozeb) in controlling the root rot disease, changes in growth promotion and yield parameters of *Coleus*. Potting medium (red soil: Cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 h for two consecutive days. The virulent strain of *M. phaseolina* was mass multiplied in sand maize medium (sand and maize powder at the ratio of 19:1) and incorporated in the soil at the rate of 10 g per kg of soil. The coleus cuttings were planted in the inoculated pots. The different treatments are:

T1: Pf1 stem cuttings dip alone at 0.2%,
T2: FP 7 stem cuttings dip alone at 0.2%,
T3: Pre mixture fungicide (Carbendazim + Mancozeb) stem cuttings dip alone at 0.1%,
T4: Pf1 stem cuttings dip + Soil drenching at 0.2% each,
T5: FP 7 stem cuttings dip + Soil drenching at 0.2% each,
T6: Pre mixture fungicide (Carbendazim + Mancozeb) stem cuttings dip + soil drenching at 0.1% each,
T7: Pf1 soil drenching alone at 0.2%,
T8: FP 7 soil drenching alone at 0.2%,
T9: Pre mixture fungicide (Carbendazim + Mancozeb) soil drenching alone at 0.1%,
T10: Inoculated control.

Treatments were inoculated at monthly interval. Three replications (Three pots per replication) were maintained and pots were arranged in a randomized manner. The root rot incidence of *M. phaseolina* was recorded and expressed as percentage of disease incidence. Plant height, number of branches and bacterial population were recorded at monthly interval until harvesting. Harvesting was done after 140 days of transplanting. After harvesting different parameters *viz*., tuber length, tuber weight/plant and number of tubers/plant were recorded.

Assay of defence enzymes

Plant leaf tissues were collected at different time intervals (0, 24, 48, 72, 96, 164 h after pathogen inoculation). Four plants were sampled from each replication of the treatment separately and were maintained for biochemical analysis. Leaf samples were homogenised with liquid nitrogen in a pre-chilled mortar and pestle. One gram of leaf sample was homogenised with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. The supernatant was used as a crude enzyme extract for assaying peroxidase (PO) (Hammerschmidt and Kuc, 1982) and polyphenol oxidase (PPO) (Mayer et al., 1965).

Native polyacrylamide gel electrophoresis analysis

The isoform profiles of PO and PPO were studied by discontinuous native polyacrylamide gel electrophoresis (PAGE). The protein extract was prepared by homogenising 1 g of leaf sample in 2 ml of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 16,000 g for 20 min at 4°C. The protein content of the sample was determined (Bradford, 1976) and samples (50 µg protein) were loaded into 8% polyacrylamide gels (Sigma, USA). After electrophoresis, PO isoforms were visualised by soaking the gels in staining solution containing 0.05% benzidine (Sigma Aldrich, Mumbai, India) and 0.03% H_2O_2 in acetate buffer (20 mM, pH 4.2) (Nadolny and Sequeira, 1980). For assessing the PPO isoform profiles, the gels were equilibrated for 30 min in 0.1% p-phenylene diamine, followed by the addition of 10 mM catechol in the same buffer (Jayaraman et al., 1987).

Field experiment

Two field trials were conducted at Periyanayakapalem, Coimbatore (district) and Attur, Salem (district) Tamil Nadu to test the efficacy of *Pseudomonas* isolates (Pf1 and FP 7) and pre mixture fungicide (Carbendazim + Mancozeb) (Carbendazim + Mancozeb) in controlling the root rot disease, growth and yield parameters of coleus. A randomized block design was used in the experiment. Treatments, replications and observations taken were similar as described in pot culture experiment.

Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared by Duncan's multiple range test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines.

RESULTS AND DISCUSSION

Isolation and collection of PGPR strains

Six isolates were isolated from rhizosphere soil from coleus fields from districts of Salem and Coimbatore, Tamil Nadu. Twenty four strains of *Pseudomonas* sp. were obtained from the culture collection, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Antifungal activity of fluorescent pseudomonads

In recent years there has been much success in biological control of soil borne diseases with the use of antagonistic Fluorescent pseudomonads (Weller and Cook, 1986; Vidhyasekaran and Muthamilan, 1999). Several strains of *P. fluorescens*, *P. putida*, *P. cepacia* and *P. aeruginosa* have been successfully used for biological control of plant diseases (Anderson and Guerra, 1985; Rabindran and Vidhyasekaran, 1996). In the present study result revealed that among thirty isolates tested, six isolates were effective in reducing mycelia growth of the pathogen. The isolate Pf1 showed maximum % inhibition over control 58.52% followed by FP 7 which showed 54.44% inhibition over control. The isolate AH-1 showed 39.13% inhibition over control which is the lowest among six isolates (Table 1). Similarly Harish kumar et al. (2010) isolated *P. fluorescens* LPK2 from disease suppressive soil of tomato rhizosphere which strongly inhibited the growth of *Fusarium udum.*

Mechanism of action of PGPR on plant pathogenic fungi

IAA production

Fluorescent pseudomonads produce plant growth promoters such as gibberellins, cytokinins and IAA, which either directly or indirectly modulate the plant growth and development. This highest production may induce plant growth promotion thereby it may indirectly reduces disease incidence. The present study revealed that all the six isolates grown in culture medium containing soyflour as tryptophan source produced IAA. The highest concentration of IAA was obtained from *Pseudomonas* strain Pf1 (36 µg ml^{-1}) followed by FP 7 (33 µg ml^{-1}) and TDK-1 (32 µg ml^{-1}) (Table 2). Patten and Glick (2002) reported that bacterial IAA from *P. putida* played a major role in the development of host plant root system. Ekta and Naveen kumar (2010) first time reported the direct role of bacterial IAA in suppression of charcoal rot disease of chickpea by the development of roots system, providing nutrients and support to the plants infected by *M. phaseolina*.

Production of siderophores

Siderophore mediated iron deprivation of deleterious microorganisms has been considered as one of the important mechanism by which fluorescent pseudomonads exert their antagonistic activity and plant growth promotion (Scher and Baker, 1982). The results of the present study revealed that fluorescent pseudomonad isolates *viz*., Pf1, FP 7 and TDK-1 showed strong production of siderophores and remaining isolates showed medium production. Estimation of siderophore by spectrophotometer revealed that *Pseudomonas* isolate Pf1 produced the maximum quantity of siderophore (44 µM ml^{-1}) followed by strains TDK-1 (38 µM ml^{-1}), FP 7 (32 µM ml^{-1}) (Table 2). Several authors studied the role of siderophores in disease control against *R. solani*, *F. solani* and *S. rolfsii* (Yeole and Dube, 2000), *F. oxysporum* f.sp*. raphani* (Leeman et al., 1996) and *M. phaseolina* (Meena et al., 2001).

Lytic enzymes assay

Production of lytic enzymes *viz*., chitinase and β-1, 3 glucanase by non pathogenic micro organisms play an important role in the biological control of plant pathogenic fungi (Viswanathan and Samiyappan, 2000). In the present study proteolytic activity was found in all six isolates. Chitinase activity was found only in isolates of Pf1, FP 7 and TDK-1 and β-1, 3-glucanase activity was found in Pf1, FP 7, TDK-1 and AH-1 (Table 2). The production of enzymes includes chitinase, cellulose, β-1, 3-glucanase, protease; lipase canlyse some fungal cells (Muleta et al., 2007) and suppress deleterious rhizobacteria. Chitinase and β-1, 3-glucanases are the key enzymes associated with the decomposition of the fungal hyphal wall. In the present study, chitinase and β -1, 3-glucanase involved in degradation of fungal cell walls since chitin and β -1, 3-glucan are the major components of most fungal cell walls.

HCN production and volatile metabolites

The production of volatile cyanide is very common among the rhizosphere pseudomonads (Bakker and Schippers, 1987; Dowling and O'Gara, 1994). In the present study both qualitative and quantitative estimation revealed that Pf1 and TDK-1 showed strong production of HCN (Table 2). The involvement of HCN in the suppression of

Table 1. Screening of PGPR against mycelial growth of *M. phaseolina* under *in vitro* condition.

S/N	PGPR isolates	Mycelial growth (cm)	Mycelial growth inhibition (cm)	Percent inhibition over control*
1	PFKO-1	7.4	1.6	17.78^{k} (24.95)
2	FP 7	4.1	4.9	54.44^{b} (47.55)
3	PRA-1	6.1	2.9	32.22^{f} (34.57)
4	MDU-1	6.77	2.23	24.78^{hi} (29.84)
5	PCN-3	8.8	0.2	2.22^{q} (8.53)
6	PSKK-7	6.9	2.1	23.33^{ij} (28.88)
7	PPOL-3	7	2	22.22^{j} (28.11)
8	30-48-R1	8.64	0.36	4.00^{p} (11.53)
9	PB-2-79	8.2	0.8	8.89^{n} (17.32)
10	COGP-30	5.1	3.9	43.33^{d} (41.17)
11	COGP-20	8.15	0.85	9.44^{n} (17.88)
12	COTP-20	4.47	4.53	50.33^{c} (45.17)
13	COPP-36	5.9	3.1	34.44^{f} (35.85)
14	COKP-46	6.4	2.6	28.89^{g} (32.49)
15	TDK-1	4.55	4.45	49.44^{c} (44.68)
16	AH-1	5.5	3.5	38.89^{e} (38.56)
17	Pf1	3.73	5.27	58.56^{a} (49.94)
18	PFNL-1	8.7	0.3	3.33^{p} (10.52)
19	PRA-4	8.23	0.77	8.56^{no} (17.02)
20	PSA-2	7.65	1.35	15.00^{l} (22.81)
21	PSA-1	6.57	2.43	27.00^{gh} (31.32)
22	PPOL-1	8.35	0.65	7.22^{o} (15.60)
23	PSA-3	6.55	2.45	27.22^{gh} (31.45)
24	MDU-2	6.5	2.5	27.78^{g} (31.82)
25	GD-1	7.5	1.5	16.67^{kl} (24.10)
26	GD-2	8.9	0.1	1.11^{r} (6.11)
27	SVN-1	9	0	0.00^{s} (0.00)
28	SVN-2	8	1	11.11^{m} (19.48)
29	SLM-1	7.5	1.5	16.67^{kl} (24.10)
30	SLM-2	5.9	3.1	34.44^{f} (35.93)
31	Control	9	-	0.00^{s} (0.00)

*Values are mean of four replications; values in parentheses are arcsine transformed; Means followed by a same letter are not significantly different at the 5% level by DMRT.

pathogen was reported by several workers in different hosts (Meena et al., 2001; Sandeep kumar et al., 2009; Nina et al., 2011). Among the six isolates of *Pseudomonas* the isolate Pf1 showed maximum % inhibition over control (78.88%) due to volatile metabolites against *M. phaseolina* followed by FP 7 and AH-1 inhibited mycelia growth 74.16 and 69.99% respectively (Table 3). Inhibition of mycelial growth of the fungus by the volatile metabolites produced by the *P. fluorescens* isolates has been studied by several authors (Sarangi et al., 2010; Meena et al., 2001).

Detection of fluorescein and pyocyanin

In the present study only Pf1 and FP 7 were showed production of fluorescein and pyocyanin production (Table 2). A marine isolate of Fluorescent *Pseudomonas* sp. having the ability to produce the pyoverdine has been found to inhibit the growth of *A. niger* under *in vitro* conditions (Manwar et al., 2004). Similar results were obtained by Rachid and Ahmed (2005) reported the specific production of fluorescein and pyocyanin by the *Pseudomonas* bacterium. This showed the importance of fluorescein and pyocyanin production in the control of deleterious fungi by fluorescent pseudomonads.

Evaluation of PGPR on the management of coleus root rots disease

Pot culture experiment

In the present study among the different treatments given

Table 2. Production of indole acetic acid (IAA), siderophore, HCN Lytic enzymes, fluorescein and pyocyanin by fluorescent pseudomonads.

S/N	PGPR isolates	IAA	Siderophore		HCN production		Lytic enzymes			Fluorescein	Pyocyanin
		IAA (µg/ml of culture filtrate)*	Siderophore production	Siderophore (µM/ml of culture filtrate)*	HCN	HCN (µg/10^8 cfu/ml)*	Protease	Chitinase	β-1, 3-glucanase		
1	Pf1	36.00^{a}	+++	44.00^{a}	+++	74.25^{a}	P	P	P	+++	+++
2	FP 7	33.00^{b}	+++	32.00^{c}	++	40.00^{b}	P	P	P	+++	+++
3	COTP- 20	29.00^{de}	++	26.00^{d}	+	12.00^{c}	P	N	N	-	-
4	COGP -30	27.00^{e}	++	25.00^{d}	-	6.25^{d}	P	N	N	-	-
5	TDK-1	32.00^{bc}	+++	38.00^{b}	+++	75.50^{a}	P	P	P	-	-
6	AH-1	30.00^{cd}	++	26.00^{d}	-	8.00^{d}	P	N	P	-	-

+, Low production; ++, medium production; +++, strong production; -, no production; P, positive reaction; N, negative reaction; *Values are mean of four replications
Means followed by a same letter are not significantly different at the 5% level by DMRT.

Table 3. *In vitro* screening of volatile metabolites produced by PGPR against mycelial growth of *M. phaseolina.*

S/N	PGPR isolate	Mycelial growth(cm)	Mycelial growth inhibition (cm)	Percent inhibition over control*
1	Pf1	1.90	7.10	78.88^{a} (62.66)
2	FP 7	2.33	6.67	74.16^{b} (59.46)
3	COTP-20	3.40	5.60	62.22^{d} (52.08)
4	COGP-30	4.73	4.27	47.49^{f} (43.56)
5	TDK-1	3.75	5.25	58.32^{e} (49.80)
6	AH-1	2.70	6.30	69.99^{c} (56.80)
7	Control	9.00	-	-

*Values are mean of four replications; Values in parentheses are arcsine transformed; means followed by a same letter are not significantly different at the 5% level by DMRT**.**

pre mixture fungicide (Carbendazim + Mancozeb) (stem cuttings dip + soil application) was relatively more effective and recorded maximum reduction of root rot incidence (73.52%) when compared to other treatments. The next best treatment was Pf1 (stem cuttings dip + soil application) which showed 70.19% disease reduction over control (Table 4). This study was also supported by Karthikeyan et al. (2009) in which pre-inoculation application of blackgram plants with the strains of *P. fluorescens viz.*, Pf1 and CHAO were found to reduce ULCV infection significantly. All growth parameters *viz.*, shoot length, root length and number of branches/plant were more in pots treated with both Pf1 (stem cuttings dip + soil application) and FP 7 (stem cuttings dip + soil application) compared to other treatments. These findings coincide with those of other scientists, Minaxi (2010) in which seed bacterization with these plant growth promoting rhizobacteria (PGPR) showed a significant increase in seed germination, shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, leaf area and rhizosphere colonization in moong bean. Yield parameters *viz.,* number of tubers/plant, tuber length, tuber diameter and tuber yield were more in pots treated with Pf1 (stem cuttings dip +

Table 4. Effect of PGPR on the Coleus root rot disease and plant growth parameters under pot culture.

S/N	Treatment	Percent disease incidence*	% disease reduction over control*	Shoot length* (cm)	Root length* (cm)	Number of branches/plant*	Tuber yield* (g/plant)
1	Pf1 (SCD alone) at 0.2%	40.20^{c} (39.35)	50.49	40.03^{c}	23.37^{d}	21.33^{c}	341.30^{g}
2	FP 7 (SCD alone) at 0.2%	44.30^{b} (41.73)	45.45	39.55^{c}	22.13^{d}	21.33^{c}	319.00^{h}
3	Pre mixture fungicide (Carbendazim + Mancozeb) (SCD alone) at 0.1%	35.20^{d} (36.39)	56.65	37.37^{c}	20.27^{e}	19.33^{e}	300.70^{i}
4	Pf1 (SCD + SA) at 0.2% each	24.20^{i} (29.49)	70.19	63.33^{a}	33.70^{a}	23.33^{a}	501.00^{a}
5	FP 7 (SCD + SA) at 0.2% each	25.50^{h} (30.35)	68.60	62.43^{a}	31.27^{b}	23.33^{a}	480.30^{b}
6	Pre mixture fungicide (Carbendazim + Mancozeb)(SCD+SA) at 0.1% each	21.50^{j} (27.63)	73.52	58.50^{b}	30.27^{bc}	22.33^{b}	441.70^{c}
7	Pf1 (SA alone) at 0.2%	29.20^{f} (32.73)	64.03	61.13^{ab}	30.23^{bc}	23.00^{ab}	402.00^{d}
8	FP 7 (SA alone) at 0.2%	30.10^{e} (33.27)	62.93	58.43^{b}	29.47^{c}	21.33^{c}	381.00^{e}
9	Pre mixture fungicide (Carbendazim + Mancozeb) (SA alone) at 0.1%	27.00^{g} (31.31)	66.74	58.13^{b}	29.30^{c}	20.33^{d}	362.30^{f}
10	Inoculated control	81.20^{a} (64.31)	-	30.00^{d}	16.57^{f}	10.33^{f}	280.00^{j}

SCD, Stem cuttings dip; SA, soil application; Values in parentheses are arcsine transformed; *values are mean of three replications; means followed by a same letter are not significantly different at the 5% level by DMRT.

soil application) compared to other treatments (Table 4). This was supported by Minaxi (2010) in which bacterization of seeds of moong bean with pseudomonads has been reported as a potential method for enhancing yield parameters such as pods, number of seeds, and grain yield per plant also enhanced significantly in comparison to control.

Changes in the peroxidase (PO) and polyphenol oxidase (PPO) activity in Coleus plants treated with biocontrol agents

The biotic and abiotic inducers bring about induced systemic resistance (ISR) through fortifying the physical and mechanical strength of cell wall as well as changing physiological and biochemical reaction of host leading to synthesis of defense chemicals against challenge inoculation of pathogens. Defense reaction occurs due to accumulation of peroxidse, polyphenol oxidase, PR proteins, phenylalanine ammonia lyase, phenolics, callose, lignin and phytoalexins. In the present study maximum PO and PPO activity was observed in plants treated with Pf1 (stem cuttings dip + soil application) (Figures 1 and 2). The activity of the enzyme increased up to 5th day and declined thereafter. The next best treatment was FP 7 (Stem cuttings dip + Soil application). The control (healthy plant) recorded lesser PO and PPO activity. Similarly Karthikeyan et al. (2009) reported that soil and foliar application of *P. fluorescens* (Pf1) induced the accumulation of phenolics and enhanced the activities of peroxidase, phenylalanine ammonia lyase and polyphenol oxidase in black gram against *Urdbean leaf crinkle virus* (ULCV) in blackgram. Also, Mathiyazhagan et al. (2009) reported that application of *Pseudomonas* strains BSCBE4, PA23 and ENPF1 increased the defense related enzymes such as peroxidase, polyphenol oxidase, chitinase and β -1, 3 glucanase in *P. amaranthus* up to ten days after challenge inoculation with *C. cassicola.* Native gel electrophoretic analysis revealed that challenge inoculation of pathogen with PA23 induced both peroxidase and polyphnol oxidase isoforms. Similarly in the present study also many isoforms of PO were induced upon treatment with PGPR in the coleus plant (Figure 3).

Field experiment

Same trend was found as in pot culture experiment regarding all parameters a little

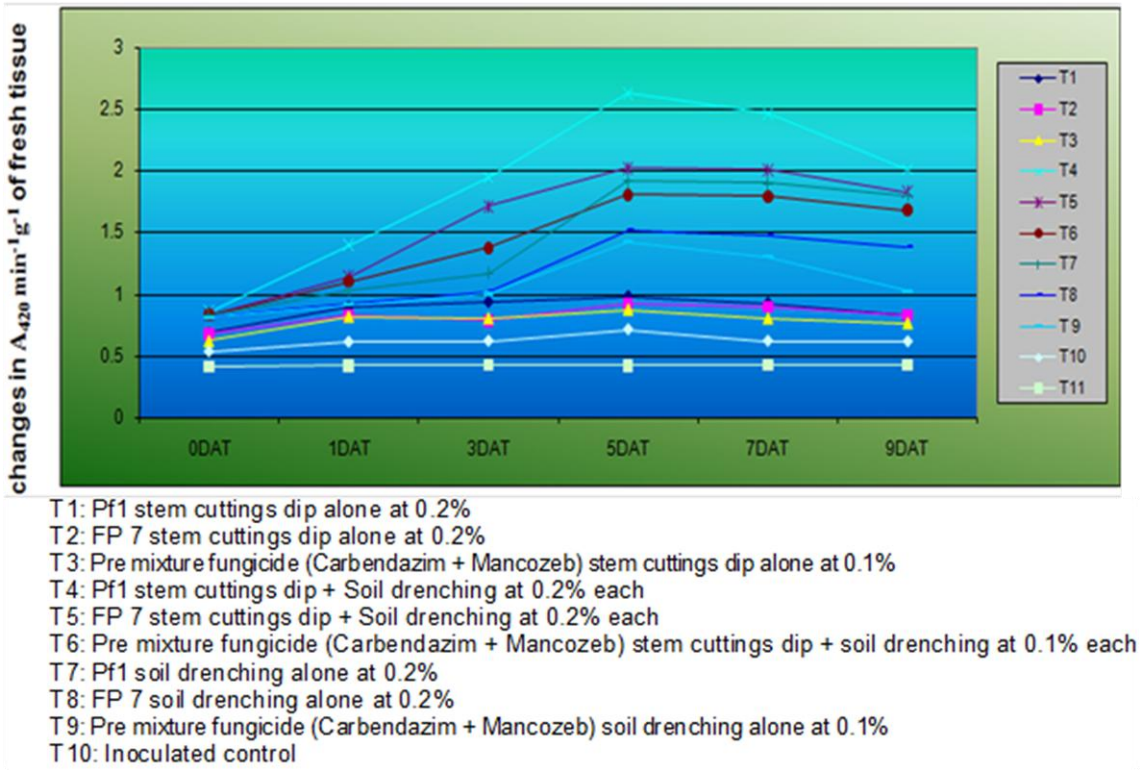

Figure 1. Induction of peroxidase (PO) activity in coleus plants treated with PGPR and fungicide against M. phaseolina under glass house condiction.

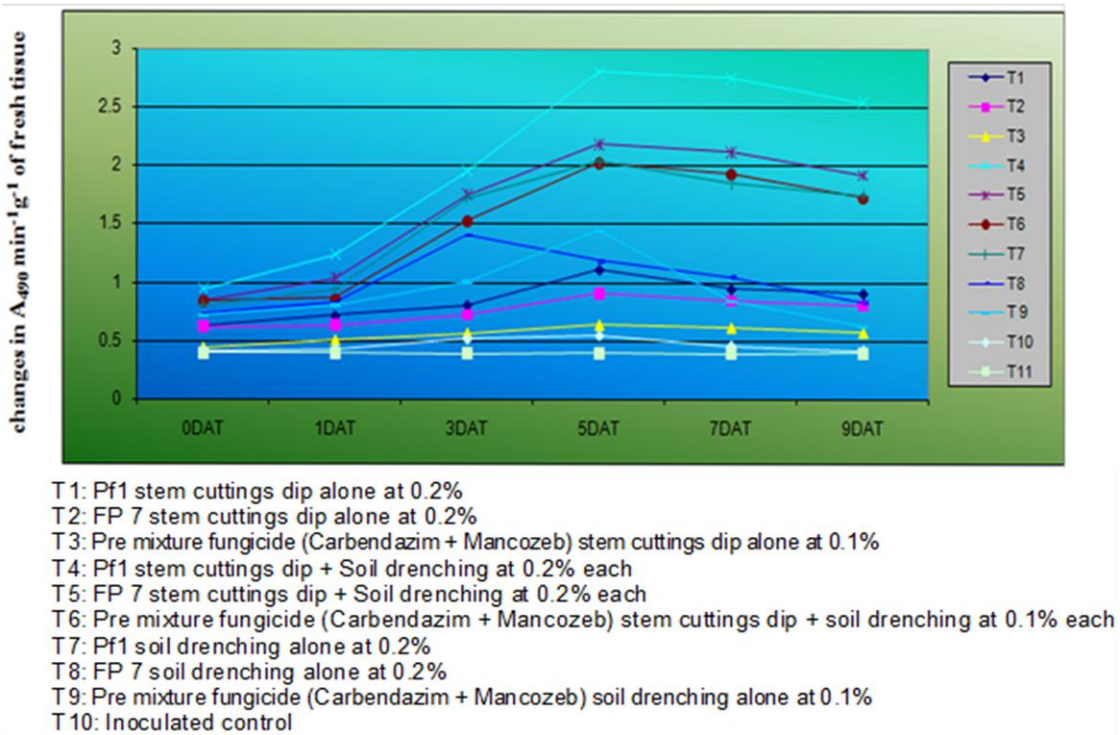

Figure 2. Induction of polyphenol oxidase (PPO) activity in coleus plants treated with PDPR and fungicide against *M. phaseolina* under glass house condition.

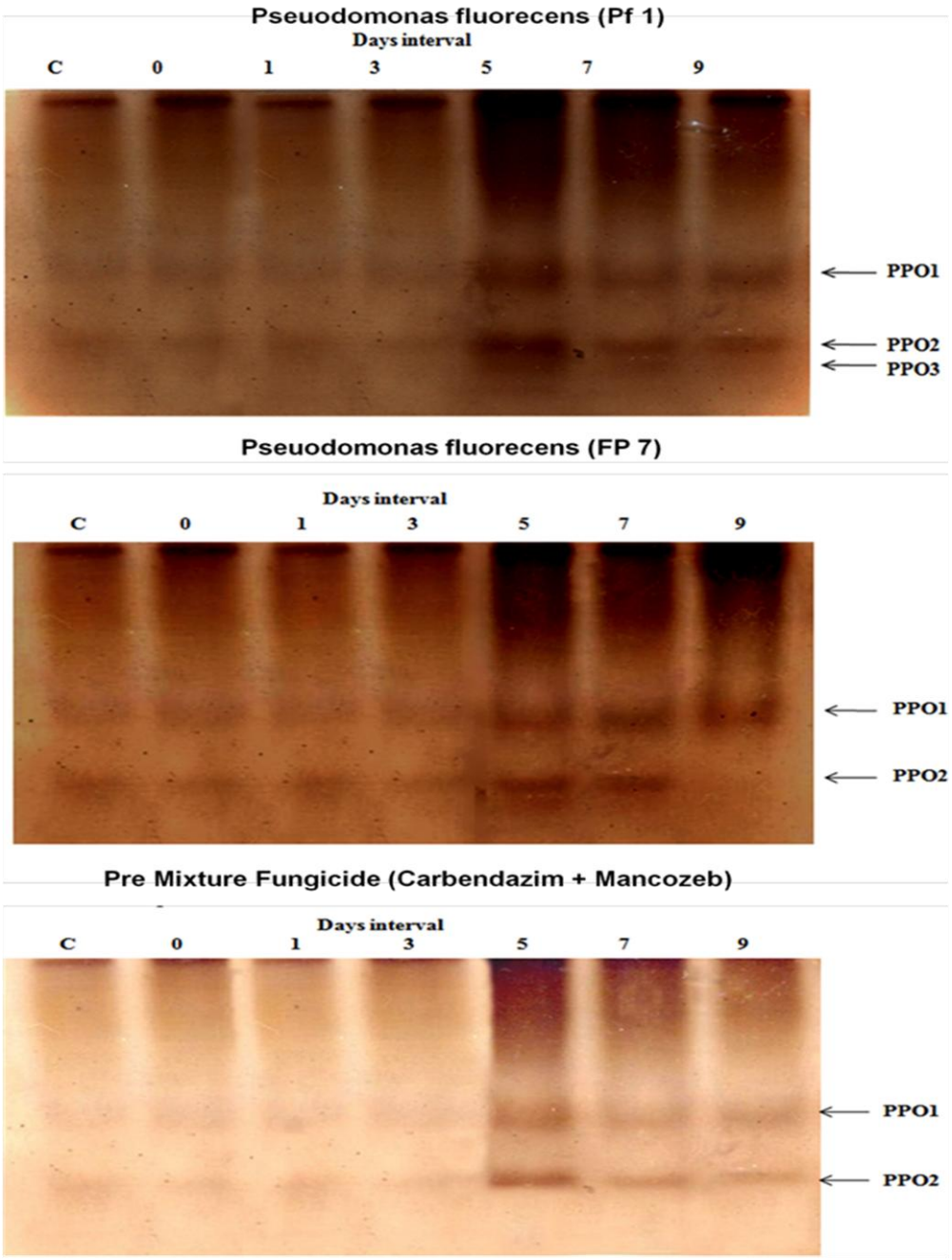

Figure 3. Native PAGE analysis of polyphenol oxidase isoforms in coleus leaf extract treat with Ps 1, FP7 and preixture fungicide (Carbendazim + mancozeb against *M. phaseolina*).

change in values was found in field experiment. *P. fluorescens* has been reputed as an effective biocontrol agent for different fungal pathogens (Meyer et al*.,* 1992; Buysens et al*.,* 1996; Singh et al*.,* 2006). These results supported the present study in which Pf1 (stem cuttings dip + soil application) recorded a reduced root rot infection over control next to the fungicide (stem cuttings dip + soil application). All growth parameters *viz*., shoot length, root length and number of branches/plant were more in plots treated with Pf1 (stem cuttings dip + soil application) compared to other treatments (Table 5). The plots treated with Pf1 (Stem cuttings dip + Soil application) showed shoot length of 61.8 cm, root length of 31.9 cm, branches of 21.4 per plant. Whereas, control treatment showed poor performance with respect to all growth parameters. *P. fluorescens* isolate Pf1 showed the maximum inhibition of mycelial growth of *P. aphanidermatum* and increased plant growth promotion

Table 5. Effect of PGPR on the Coleus root rot disease and plant growth parameters under field conditions

S/N	Treatment	Percent disease incidence*	Percent disease reduction over control*	Shoot length* (cm)	Root length* (cm)	Number of branches/plant*	Tuber yield* (t/ha)
1	Pf1 (SCD alone)) at 0.2%	21.60^{b} (27.70)	71.11	38.00^{f}	21.10^{f}	19.40^{d}	8.03^{d}
2	FP 7 (SCD alone)) at 0.2%	23.23^{d} (28.82)	68.91	37.00^{g}	20.60^{g}	19.00^{e}	7.66^{e}
3	Pre mixture fungicide (Carbendazim + Mancozeb) (SCD alone) at 0.1%	14.13^{ef} (22.08)	81.08	33.86^{h}	18.80^{h}	17.30^{f}	6.93^{f}
4	Pf1 (SCD +SA) at 0.2% each	12.43^{g} (20.65)	83.35	61.80^{a}	31.90^{a}	21.40^{a}	10.10^{a}
5	FP 7 (SCD +SA) at 0.2% each	14.53^{ed} (22.41)	80.55	60.86^{b}	29.70^{b}	21.00^{b}	9.50^{b}
6	Pre mixture fungicide (Carbendazim + Mancozeb) (SCD +SA) at 0.2% each	11.33^{g} (19.67)	84.83	56.50^{d}	28.80^{c}	20.30^{c}	9.20^{c}
7	Pf1 (SA alone) at 0.2%	16.23^{cd} (23.76)	78.28	59.00^{c}	28.00^{d}	21.00^{b}	9.76^{b}
8	FP 7 (SA alone) at 0.2%	16.67^{c} (24.10)	77.69	56.70^{d}	26.80^{e}	19.40^{d}	9.20^{c}
9	Pre mixture fungicide (Carbendazim + Mancozeb) (SA alone) at 0.1%	12.56^{fg} (20.76)	83.18	55.00^{e}	27.20^{e}	18.70^{e}	9.06^{c}
10	Inoculated control	74.86^{a} (59.96)	-	27.90^{i}	14.70^{i}	8.70^{g}	5.50^{g}

SCD, Stem cuttings dip; SA, soil application; *values are mean of three replications; values in parentheses are arcsine transformed; means followed by a same letter are not significantly different at the 5% level by DMRT.

in tomato and hot pepper (Ramamoorthy et al., 2002). The plots treated with Pf1 (Stem cuttings dip + Soil application) showed tuber number of 8.33 per plant, tuber length of 28.63 cm, tuber diameter of 2.73 cm and tuber yield of 10.1 t/ha. In all yield parameters Pf1 (stem cuttings dip + soil application) showed best performance followed by FP 7 (stem cuttings dip + soil application) and the control treatment showed poor performance with respect to all yield parameters (Table 5). Higher yield with the use of bioagent is in the agreement with the observation made in higher seedling establishment and higher yield (Raguchander et al., 1997; Das et al., 2008; Sharma et al., 1999).

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