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Management of cowpea root-rot caused by *Macrophomina phaseolina* (Tassi) Goid. using plant growth promoting rhizobacteria

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ABSRACT: **Five different isolates of *Pseudomonas fluorescens* Migula were isolated from cowpea rhizosphere region and identified by biochemical tests. These strains were screened against *Macrophomina phaseolina* (Tassi) Goid. the causal organism of cowpea root-rot and the results revealed that SVPF2 isolate recorded maximum inhibition of mycelial growth against control. The mechanism of PGPR *viz.* iron-chelating agent (siderophore) and volatiles (HCN) production tests were studied and SVPF2 isolate positively reacted for siderophore and HCN production. The talc-based formulation of SVPF2 was prepared and the bioefficacy was tested under green house conditions. The seed and soil application of talc-based formulation of SVPF2 significantly reduced the root-rot incidence. The growth parameter *viz.* germination percentage and the vigour index were also increased in SVPF2 treated seeds.**

KEY WORDS: Cowpea, *Pseudomonas fluorescens*, root-rot, rhizosphere, siderophore

INTRODUCTION

Cowpea is mostly grown as a kharif crop suitable for rice-fallows in Orissa, Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. The important fungal diseases of cowpea are root-rot (*Macrophomina phaseolina*), Cercospora leaf spot (*Cercospora canescens* Ell. &Mart.), brown rust (*Uromyces phaseoli* typica Arth.) and powdery mildew (*Erysiphe polygoni.* DC.) (Ali *et al.*, 2000).

Root- rot of cowpea caused by *M. phaseolina* is widely distributed in tropical and sub-tropical countries and is more devastating pathogen right from the establishment of the crop (Then *et al.*, 1991). Chemical control measures with broad-spectrum fungicides create imbalances in the microbial community, which may be unfavourable for the activity of the beneficial organisms and also lead to the development of resistant strains. Use of antagonistic organism against *Macrophomina* root-rot has been well documented (Raguchander *et al.*, 1997). Hetrotrophic rhizobacteria of *Pseudomonas fluorescens* types have been successfully used for biological control of several plant pathogens (Ganesan and Gnanamanickam, 1987). In this investigation, attempts were made to

test the antagonistic activity of plant growth promoting bacteria (PGPR) *viz. Pseudomonas fluorescens* and its mechanisms for cowpea root-rot management.

MATERIALS AND METHODS

Isolation of pathogen and PGPR

Macrophomina phaseolina was isolated from infected cowpea roots using potato dextrose agar (PDA) medium. The fungus was multiplied in sand + maize medium and the pathogenicity of the fungus was tested on susceptible variety CV, Co 4 (Riker and Riker, 1936).

The pot mixture consisting of red soil, sand and farmyard manure at equal proportion was prepared and filled in 15 cm^2 diameter pots (500 g) and sterilized at 1.5 kg/cm^2 for 2 hours. Two-week-old inoculum multiplied on sand maize medium was mixed with sterilized pot mixture (1:20 W/W). Uninoculated control and suitable replications were maintained. Pot mixture with inoculum was moistened and seeds of cowpea were surface sterilized with 0.1 per cent mercuric chloride. Known number of seeds was sown in pots. Seed germination was observed up to 20 DAS and per cent disease incidence was calculated based on the seedlings showing root-rot symptoms. From the infected seedlings re-isolation of the pathogen was made and original isolates were compared.

Native cowpea rhizobacterial *Pseudomonas fluorescens* isolates were isolated from samples obtained from different parts of Tamil Nadu state. One gram of rhizosphere soil adhering to root surface was collected and transferred to a 250 ml conical flask containing 100 ml of sterile water. After thorough shaking for 15 minutes in a shaker, different dilutions were prepared. One ml of each 10^{-5} and 10^{-6} dilution was pipetted out and poured into the sterile Petri - dishes. Later King's medium B (KB) (King *et al.*, 1954) was poured rotated and incubated at room temperature (28 ± 2°C) for 24 hours. After 24 hours of incubation, the bacterial growth was purified by the dilution plate technique (Waksman, 1952). The bacterial culture was maintained in King's B broth (KB) in 30 per cent (v/v) glycerol at -80°C.

Characterization of the different cultures of antagonistic bacteria was done according to the methods recommended in the laboratory guide for identification of plant pathogenic bacteria published by the American Phytopathological Society (Schaad, 1992). The biochemical tests, *viz.* Levan formation, Gelatin liquefaction and utilisation of L-arbinose, D-galactose, propylene glycol, ethanol and saccharat. For each test, 24-48 hours old cultures were used.

Screening of antagonistic bacteria under *in vitro* condition

The antifungal efficacy of *Pseudomonas fluorescens* isolates was tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. A mycelial disc (9mm diam.) of the pathogen, *viz. M. phaseolina* was placed at one end of the plate and the bacterial antagonists were streaked at the periphery of the Petri-dish just opposite to the mycelial disc of the pathogen. The plates were incubated at 28±2 °C. The mycelial growth of the pathogen and inhibition zone was measured after 72h of incubation.

Siderophores production by antagonist

Production of siderophores by *P. fluorescens* was assayed by plate assay method as described by Schwyn and Neilands (1987). The tertiary complex chromeazurol S (CAS) served as an indicator. To prepare one liter of the blue agar, 60.5 mg CAS was dissolved in 50 ml water and mixed with 10 ml Fe^{3+} solution (1 mM $FeCl_3.6H_2O$ in 10 mM HCl) and HDTMA dissolved in 40 ml water was added by constantly stirring. Forty eight-hour-old culture of fluorescent pseudomonads was streaked onto the succinate medium (Succinic acid, 4 g; K_2HPO_4, 3g; $(NH_4)_2SO_4.7H_2O$, 0.2 g; distilled water, 1000 ml, pH 7.0) amended with the indicator and incubated for three days.

Formation of bright zone with yellowish fluorescent color in the dark-blue colored medium was the indication of production of siderophore. Production of siderophore was scored as none, little, strong and very strong.

Testing of antagonistic bacteria for the production hydrogen cyanide

Production of HCN was determined using a modification of the procedure of Miller and Higgins (1970). Bacteria were grown on Tryptic-soy-agar (TSA) (animal peptone 15.0 g, soy peptone 5.0 g, sodium chloride 5.0 g, glycine 4.4 g, distilled water-1000ml). Filter paper discs soaked in picric acid solution (2.5 g of picric acid, 12.5 g of sodium carbonate, and 1000ml of distilled water) were placed in the lid of each Petri-plate. Dishes were sealed with parafilm and incubated at 28°C for 48 hours. A change from yellow to light brown, brown or reddish brown of the discs was recorded as an indication of weak, moderate or strong production of HCN.

Preparation of talc-based formulation of biocontrol agents

A loopful of *P. fluorescens* was inoculated into the King's B broth and incubated in a rotary shaker at 150 rpm for 72 hours at room temperature (28 ± 2 °C). After 72 hours of incubation, the broth containing 9 x 10^8 cfu/ml was used for the preparation of talc-based formulation. To 400 ml of bacterial suspension, one kg of the talc powder (sodium ammonium silicate), calcium carbonate 15 g (to adjust the pH to neutral) and carboxy methyl cellulose (CMC) 10 g (as adhesive) were mixed under sterile condition following the method described by Vidhyasekaran and Muthamilan (1995). The product was shade dried to reduce the moisture content to 20 per cent and then packed in polypropylene bags and sealed. At the time of application the population of bacterium in talc formulation was checked to 2.5 to 3 x 10^8 cfu/g.

Greenhouse studies

Cowpea seeds were treated with talc based formulation of SVPF2 at 4g/kg seed and shade dried for 2 hours. Twenty-five gram of the formulated product (2.5kg talc based formulation mixed with 50 kg of farmyard manure) was given as soil application/pot at 30 days after sowing (DAS). Carbendazim was used as standard check fungicide. It was applied as seed treatment @2gm/Kg of seed and also as soil drench @0.05 per cent at 30 DAS. Eight seed were sown pot with three replication for each treatment. All treatments were replicated three times in factorial completely randomized design (CRD).

RESULTS AND DISCUSSION

The pathogenicity test results revealed that the pathogen isolated from infected cowpea root sample is *M. phaseolina*. The results of bio-chemical test for characterization of PGPR inferred that the isolates are *P. fluorescens*.

Isolates of *P. fluorescens* were tested for their efficacy against *M. phaseolina* growth. Varied degree of mycelial growth inhibition was observed. The isolates SVPF2 had higher inhibitory effect (43.92 %) followed by other *Pseudomonas* isolates (Table 1). Gasoni *et al.* (1998) reported that radish seed treatment with *Bacillus cereus* and *P. fluorescens* in peat/vermiculite/clay formulation effectively controlled *Rhizoctonia* damping-off in greenhouse studies. Combination of *P. fluorescens* strains *viz.* Pf1 and FP7 has given effective control of rice sheath blight disease when compared to strain applied individually (Nandakumar, 1998).

All the bacterial isolates produced siderophore in CAS plate assay method. But all isolates except SVPF3 produced yellow colour in blue colored medium. Siderophore produced by *Pseudomonas* spp. and other rhizobacterial organism (*Bacillus, Enterobacter*) have been used in the biological control of damping-off of cotton caused by *Pythium ultimum* (Laha *et al.*, 1992); root-rot of wheat caused by *Pythium* sp. (Beacker and Cook, 1998), potato seed piece decay by *Erwinia carotovora* (Xu and Gross, 1986), stem-rot of peanut caused by *Rhizoctonia solani* and *Sclerotium rolfsii* (Ganesan and Gnanamanickam, 1987). Among the different antagonistic bacteria tested, the intensity of HCN production was strong in *P. fluorescens* isolate SVPF2 followed by *P. fluorescens* isolate SVPF1 and CPPF1 (Table 2). Defago *et al.* (1990) highlighted that cyanide produced by *P. fluorescens* strain CHAO played

Table 1. Growth inhibition of *M. phaseolina* by *P. fluorescens* isolates

Isolate	Mycelial growth of *M. phaseolinia* (mm)	Mycelial growth (% reduction over control)	Inhibition zone (mm)
SVPF 1	54.21[e]	36.57	10.05[b]
SVPF 2	41.00[f]	51.41	12.58[a]
SVPF3	47.51[e]	43.92	9.54[c]
CPPF1	43.42[d]	49.18	9.34[c]
CPPF2	59.00[b]	30.23	8.48[d]
Carbendazim	1.00[g]	98.82	-
Control	85.45[a]	-	-

In a column, means followed by a common letter are not significantly different at the 5 per cent levels by DMRT.

role in the suppression of take all (*Gauemannomyces gramnis* var. *tritici*) and root-rot (*R. solani*) of wheat. The number of running hyphae of *G. graminis* var. *tritici* was reduced by this strain. Production of HCN by *P. fluorescens* strain W-11-3 was found to inhibit the mycelial growth of *Pythium in vitro* (Weststeijn, 1990).

Table 2. Siderophore and HCN production by *P. fluorescens*

Isolate	Siderophore production	HCN production
SVPF 1	+	+
SVPF 2	+	++
SVPF3	-	-
CPPF1	+	+
CPPF2	+	-

Siderophore production: + Positive, -Negative
HCN production: (++) Higher intensity, (+) Low intensity

Standardization of mode of application of bacterial antagonists indicated that seed treatment and soil application were highly effective in inhibiting root-rot incidence rather than individual application of antagonists either through seed treatment or soil application. Among the bacterial antagonists tested, delivery of SVPF2 as seed treatment and soil application recorded the lowest root-rot incidence of 26.42 per cent. It was followed by the application of CPPF1 both as seed treatment and soil application. The chemical carbendazim (0.1 %) recorded 23.26 per cent disease. The highest disease incidence was recorded in the inoculated control (55.81 %) (Table 3). Vidhyasekaran and Muthamilan (1995) reported that the chickpea wilt (*R. solani*) was effectively managed by bacterial antagonist *P. fluorescens*. This was further reported by Raguchander *et al.* (1995) that the *P. fluorescens* (Pf1) was effective in reducing the *Fusarial* wilt disease in of the banana under field condition.

The studies showed that the PGPR is capable of controlling the cowpea root-rot. Amongst different PGPR tried, seed treatment and soil application of SVPF2 suitable for the management of cowpea root-rot under pathogen (*M.*

Table 3. Effect of *P. fluorescens* on the incidence of cowpea root-rot

Isolate	Per cent disease incidence	Root length (cm)	Shoot length (cm)	Germination (%)
SVPF2(ST)	29.31^{e}(32.78)	19.32^{g}	52.21^{h}	94.21^{g}(76.07)
SVPF2 (SA)	28.52^{d}(32.27)	18.45^{e}	48.21^{e}	92.31^{ef}(73.90)
SVPF2 (ST+SA)	26.42^{b}(30.93)	22.38^{j}	55.72^{j}	97.52^{h}(80.93)
CPPF1 (ST)	33.23^{h}(35.20)	17.42^{d}	49.42^{g}	93.24^{f}(74.92)
CPPF1 (SA)	32.14^{g}(34.53)	16.84^{b}	47.53^{d}	91.52^{de}(73.06)
CPPF1 (ST+SA)	27.46^{c}(31.60)	19.85^{h}	52.61^{i}	94.52^{g}(76.46)
Carbendazim (ST)	31.21^{f}(33.96)	18.72^{f}	46.52^{c}	89.26^{c}(70.86)
Carbendazim (SD)	36.41^{i}(37.11)	17.25^{c}	43.24^{a}	85.0^{b}(68.40)
Carbendazim (ST+SD)	23.26^{a}(28.83)	20.13^{i}	48.68^{f}	91.21^{d}(72.75)
Control	55.81^{j}(48.33)	15.42^{a}	45.32^{b}	82.31^{a}(65.12)

ST-seed treatment, SA- soil application, SD- soil drenching

CD for root length at 5 per cent level: 0.0899

CD for shoot length at 5 per cent level: 0.0170

Values are mean of three replications. In a column, means followed by a common letter are not significantly different at the 5 per cent levels by DMRT.

Values in parentheses are arcsine-transformed values.

phaseolina) inoculated soil in greenhouse condition. However, field evaluation is necessary to determine its efficacy under natural ecosystem.

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