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Biocontrol Potentiality of Native Microbial Isolates Against Collar rot Disease of Crossandra

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ABSTRACT

In the present study, we have isolated nine fungi and three bacteria from the rhizosphere of Crossandra by serial dilution method. Of various mycoflora, *Trichoderma viride* (T_1), *Trichoderma* spp (T_2, T_3 and T_4), *Penicillium* spp, *Rhizopus* spp, *Aspergillus flavus* and *A. niger* and three rhizobacterial isolates including *Pseudomonas* spp were obtained. In dual culture studies, *Trichoderma viride* (T_1) was highly effective in inhibiting mycelial growth of *Sclerotium rolfsii*. upto 70% and sclerotial production by 91% followed by *Trichoderma* species (T_2). *Pseudomonas* spp was also effective among rhizobacterial isolates with mycelial and sclerotial inhibition upto 43.1% and 71% respectively.

Key words : Biocontrol, Crossandra, Invitro, Rhizosphere.

Crossandra (*Crossandra infundibuliformis* (L.) Nees) is a major revenue generating ornamental crop of India. In Andhra Pradesh, the crop acerage is to an extent of 719 ha with production of 503 tonnes during 2003. Crossandra cultivation is profitable, to farmer however the crop production is hampered by several production constraints, of which soil-borne diseases play a major havoc. Mainly, Crossandra cultivation in our country is plagued by collar rot disease causing significant yield losses ranging about 40 to 50% (Harinath Naidu, 2000) which is caused by *Sclerotium rolfsii* Sacc. Presently, the pathogen is managed through application of chemical fungicides in various crops (Anitha Chowdary, 1997) and their usage is having several concerns. Among different alternative methods of managing the collar rot disease of Crossandra, biological control is a viable option that can be effective on a long term basis.

Trichoderma spp. are abundantly found in the crop rhizosphere and contribute to disease control of various soil-borne pathogens through various mechanisms. Growth promotion due to *Trichoderma* spp. is also reported in several crop species including ornamentals (Manoranjitham *et al*., 1999). Among bacterial bioagents, plant growth-promoting rhizobacteria (PGPR) were widely employed in controlling several plant pathogens besides enhancing crop yields (Pathak *et al*., 2004). The present experiment was conducted to identify potential fungal and bacterial antagonists that can be used in managing the collar rot disease as an alternative or supplement the existing chemical control practices.

MATERIAL AND METHODS

Isolation and Maintenance of *Sclerotium rolfsii*

Collar rot affected crossandra plants were collected from different crop growing areas of chittoor district of Andhra Pradesh and the pathogen was isolated using tissue segment method (Rangaswami and Mahadevan, 1999). The pathogen was identified as *Sclerotium rolfsii* based on its mycelial and sclerotial characters (Barnett and Hunter, 1972). The isolated pathogen was maintained on PDA at 28±2^0 C. Pathogenicity was proved prior to taking up the in vitro efficacy tests with fungal and bacterial antagonists.

Isolation, Identification and maintenance of native fungal and bacterial antagaonists

Serial dilution technique (Johnson and Curl, 1972) was used to isolate fungal and bacterial antagonist from Crossandra rhizosphere. Composite soil sample collected from rhizosphere of healthy plants and collar rot infected crossandra plants was shade dried and then used for serial dilution. Antagonistic mycoflora were isolated on rose bengal agar medium by using a dilution of 10^{-4}. Antagonistic bacteria were isolated on soil extract agar medium by using a dilution of 10^{-6}. One ml of soil suspension was poured into sterilized petriplates, then the melted and cooled media was poured. Then the plates were incubated at 28±2°C for the development of colonies. Isolations were also carried out on potato dextrose agar for fungi/ and on nutrient agar for bacteria simultaneously. Three days old colonies of mycoflora were picked up and purified by single hyphal tip method, while one day old colonies of

bacteria were picked up and purified by streaking. Rhizosphere mycoflora were identified based on mycological keys, cultural characters and conidial morphology described by Barnett and Hunter (1972) whereas rhizosphere bacteria were identified based on Bergey's manual of determinative Bacteriology (Holt *et al.,* 2000). Mycoflora were maintained by periodical transfer on PDA. Purified and identified rhizobacterial isolates were grown on nutrient agar medium.

In vitro antagonistic studies

Fungal bioagents

Antagonists were screened against the pathogen *in vitro* by using dual culture technique (Morton and Stroube, 1955). Twenty ml of sterilized PDA was plated in 9 cm petriplates and 6 mm diameter disc of actively growing antagonistic fungus was placed at one end of plate over the PDA.

Bacterial bioagents

For testing rhizobacterial isolates, strains were streaked onto nutrient agar and checked for purity after incubation for 24 h at at 28 ± 2^0C. A screening assay was conducted on PDA by adopting dual culture plate technique (Gupta *et al*., 2001) against *S. rolfsii*. Antagonistic bacterium, a 4 cm line was streaked at one side of plate and on the opposite of the antagonist a 6mm diameter mycelial disc of *S.rolfsii* was placed and plates were incubated at 28 ± 2^0C for 7 days. Observations were taken on radial growth and sclerotial population of *S.rolfsii* after 10 days of incubation in treatment and control and percent inhibition was calculated using the following formula.

$$I = \frac{100\ (C-T)}{C}$$

Where I = Inhibition of mycelial growth of test pathogen (%), C = Growth of pathogen in the control plate (mm) and T = growth of pathogen in plates challenged with rhizobacterial isolate / fungal isolate (mm). The width of the inhibition zone between rhizobacteria/fungal isolate and pathogen was measured after 7 days.

RESULTS AND DISCUSSION

In the present study, isolations of microflora from rhizosphere of Crossandra yielded nine mycoflora. Of these mycoflora, the isolates belong to *Trichoderma viride*, three *Trichoderma* spp, collar rot pathogen (*Sclerotium rolfsii*), *Aspergillus flavus*, *A. niger*, *Penicillium* sp. and *Rhizopus* spp. Three rhizobacterial isolates were obtained and effective isolate obtained from in vitro dual culture assays was characterized and identified as *Pseudomonas* sp.

In dual culture studies with fungal biocontrol agents, highest inhibition of mycelial growth of *S. rolfsii* was obtained with *T. viride* (T_1) (69.8%), and is significantly superior over other mycoflora (Table 1). This is followed by other *Trichoderma* sp. with per cent inhibitions ranging from 61.9 (T_2) to 56.8 (T_4). Among other mycoflora, *A. niger* recorded an inhibition per cent of 51.4 %, followed by *Penicillium* sp. (44.6%) and *A. flavus* (36.4%). Least inhibition of test pathogen was recorded with *Rhizopus* sp. (34%). Production of sclerotial bodies was also inhibited by these mycolflora. Among different mycoflora, *T. viride* (T_1) inhibited sclerotial production of *S. rolfsii* up to 90.9%, and this fungal antagonist is significantly superior over other mycoflora. However, when compared to other *Trichoderma* sp.., *A. flavus* recorded comparatively more inhibition of sclerotial production (82.5%) which was followed by *Penicillium* sp. (74.8%). Other *Trichoderma* sp. (T2, T3 and T4) inhibited sclerotial population of *S. rolfsii* in the range of 58.7 (T2) to 65.2 % (T4). Inhibition of sclerotial production was also effective with *Rhizopus* sp. (56.6%) and *A. niger* (54.5%) (Table 1).

Among the three rhizobacterial isolates screened against *S. rolfsii*, highest inhibition of mycelial growth was recorded with *Pseudomonas* sp. (B1) (43.1%) which is significantly superior over others (Table 2). This is followed by B3 isolate with inhibition per cent of 34.9 and B2 isolate (32.9%). Inhibition of sclerotial production was also highest with *Pseudomonas* sp. (B1) and was up to 71%. For the other rhizobacterial isolates, the inhibition of sclerotial production was up to 43.6% (Table 2).

In the present study , all the *Trichoderma* sp. were found to be highly effective among the rhizosphere mycoflora (Table 1). With regard to bacterial antagonist, *Pseudomonas* sp. (B1) was significantly superior over others (Table 2). However, of both fungal and bacterial antagonists, *T. viride* (T_1) was significantly superior over others with highest inhibition of mycelial growth and sclerotial population of *S. rolfsii*. Pranab Dutta and Das (2002) reported that *T.harzianum* ,*T.viride* and *T.koningii* inhibited sclerotial population of *S.rolfsii* of tomato to an extent of 94.2,86.8 and 84.1 per cent respectively.

In the present investigation, coiling of *Trichoderma* isolate-1(T_1) around *S.rolfsii,* and lysis was observed. The reduction in the quantum of hyphal mass of *S.rolfsii* due to lysis by T_1 isolate might have resulted in subsequent reduction in

Table 1. *In vitro* efficacy of rhizosphere mycoflora against *Sclerotium rolfsii*.

Antagonist	*S. rolfsii*			
	Radial growth * (mm)	Inhibition of mycelial growth (%)	Population of Sclerotia	Inhibition in population of sclerotia (%) *
Aspergillus flavus	54.0	36.5 (37.1)	38.3	82.5 (65.3)
Aspergillus niger	41.3	51.4 (45.8)	100.0	54.5 (47.6)
Penicillium sp.	47.0	44.7 (41.9)	55.3	74.8 (59.9)
Rhizopus sp.	56.0	34.1 (35.7)	95.3	56.6 (48.8)
Trichoderma viride (T_1)	25.6	69.8 (56.6)	20.0	90.9 (72.3)
Trichoderma sp. (T_2)	32.3	61.9 (51.9)	90.6	58.7 (50.0)
Trichoderma sp. - (T_3)	35.0	58.8 (50.1)	82.3	62.5 (52.2)
Trichoderma sp.- 4 (T_4)	36.6	56.8 (48.9)	76.3	65.2 (53.9)
Control	85.0	-	220.0	-
CD at 5%	-	1.00	-	1.11

Figures in parentheses are angular transformed values

Table 2. *In vitro* efficacy of rhizobacterial isolates against *Sclerotium rolfsii*.

Antagonist	Radial growth * (mm)	Inhibition of mycelial growth (%)	Population of Sclerotia	Inhibition of population of Sclerotia (%)*
Pseudomonas sp. (B_1)	48.3	43.1 (41.0)	63.6	71.0 (57.4)
Rhizobacterial isolate (B_2)	57.0	32.9 (35.0)	124.0	43.6 (41.3)
Rhizobacterial isolate (B_3)	55.3	34.9 (36.2)	137.6	37.4 (37.6)
Control	85.0	-	220.0	-
CD at 5%	-	0.73	-	1.06

* Mean of 3 replications

Figures in parentheses are angular transformed values

sclerotial population. This is in accordance with Rajeev Pant and Mukhopadhyay (2001). While lysis of *S.rolfsii* by *Trichoderma* spp. was reported by Kajal Kumar and Chitreswar Sen (2000). *Similarily* Uma Maheswari *et al.,* (2002) reported that *P. fluorescens* inhibited maximum mycelial growth (67.22%) and sclerotial population (86%) *S.rolfsii* of Jasmine.

Identification of fungal and bacterial antagonists with superior inhibiting abilities of *S. rolfsii* is a key step in biocontrol of collar rot disease. From the above investigation potential native antagonists *Trichoderma viride* (T_1) and *Pseudomonas* sp.(B_1)were found to be superior in inhibiting *S. rolfsii* in invitro conditions. However, greenhouse studies has to be investigated for further ascertaining the bio-efficacy of these fungal and bacterial isolates for their final application at field level in managing the collar rot disease of Crossandra.

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