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Isolation, Identification and Evaluation of Native Antagonists Micro flora from Stem Rot infected Rice plants against *Sclerotium oryzae*.

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ABSTRACT
The mycoflora and bacteria were isolated from rhizosphere soil associated with diseased rice plants during the survey on Martin medium and soil extract agar medium, respectively. Mycoflora viz., Aspergillus flavus, A. niger, Cladosporium, Trichoderma viride isolate-1 and 2 while bacterial isolates viz., Pseudomonas fluorescens(BI-1), isolate–2 (BI-2), isolate-3 (BI-3), isolate-4 (BI-4), isolate-5 (BI-5) were found to be antagonistic to test pathogen S. oryzae. The detected mycoflora and bacterial isolates were further screened following dual culture technique and the results indicated that among mycoflora screened, T. viride (T1) was found to have most potential antagonistic effect with maximum inhibition (75.3 %) of test pathogen. Similarly among antagonistic bacterial isolates screened P. fluorescens (BI-1) was found to be highly effective in inhibiting the test pathogen by 77.2 per cent. These potential biocontrol agents can be exploited as an integrated approach in the management of stem rot of rice.
The compatibility studies between T. viride (T1) and P. fluorescens (BI-1) following dual culture technique under in vitro conditions indicated that the per cent inhibition of T. viride (T1) by P. fluorescens was 5.0 per cent, while no inhibition was observed in the growth of P. fluorescens.
***Key words**: Stem rot, Antagonists, Rice, Sclerotium oryzae, Microflora*

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INTRODUCTION

"Rice is life" aptly describes the importance of rice in food and nutritional security particularly for Asian countries. The crop is prone to the attack of many diseases caused by fungi, bacteria and viruses. Of which, stem rot disease caused by *Sclerotium oryzae* hither to considered as a minor disease is now prevalent in most popular cultivars of rice causing considerable loss in quality and quantity of the produce. In Telangana and Andhra Pradesh the disease is reported from rice growing areas of East Godavari, West Godavari, Khammam and Warangal districts. The yield losses upto a maximum of 80 per cent in different rice cultivars has been reported by several workers form varied agro climatic regions in India and aboard (1). Continuous and contiguous cultivation of rice during different seasons under high dosages of nitrogenous fertilizers and prevalence of many graminaceous weed flora in rice fields which serve as collateral host of *S. oryzae* and lack of proper irrigation and drainage facilities have progressively aggravated the stem rot disease in recent years. Although control of stem rot through fungicides examined, induction of host resistance through antagonists has not received attention. Hence the present study was undertaken to record disease incidence in various rice growing areas and the effect of antagonist microflora against *S.oryzae*.

MATERIALS AND METHODS

Isolation of native microflora from rhizosphere of diseased rice plants

Rhizosphere of healthy and diseased samples collected from Khammam and Warangal districts were isolated by following serial dilution technique (2). Composite soil sample (50 g) was collected from

rhizosphere of healthy plants and stem rot infected rice plants. The soil was shade dried and then used for serial dilution.

To get 10^{-1} dilution, 10 g of this soil was dissolved in 100 ml of sterile distilled water, from this 1 ml of soil suspension was taken and added to 9 ml of sterile distilled water to get 10^{-2} dilution. This was repeated until a dilution of 10^{-6} was obtained.

Rhizosphere mycoflora were isolated on rose bengal agar medium by using a dilution of 10^{-4} and bacteria were isolated on soil extract agar medium by using dilution of 10^{-6}. One ml of final dilution of soil suspension was poured into sterilized Petriplates and then the melted and cooled media was poured. Plates were rotated gently on the laminar air flow bench to get uniform distribution of soil suspension in the medium. Then the plates were incubated at 28 ± 2°C and observed at frequent intervals for the development of colonies.

Three days old colonies of mycoflora were picked up and purified by single hyphal tip method whereas, one day old colonies of bacteria were picked up and purified by streak plate method.

Identification of native rhizosphere microflora and their maintenance

Rhizosphere mycoflora were identified based on mycological keys as described by (3). Rhizosphere bacteria were identified based on Bergey's Manual of Determinative Bacteriology (4). Mycoflora were maintained by periodical transfer onto PDA, whereas bacteria were maintained by periodical transfer onto nutrient agar medium.

In vitro* evaluation of efficacy of microflora against *Sclerotium oryzae

The efficacy of rhizosphere mycoflora and bacteria was determined by dual culture technique. Among the rhizosphere mycoflora, the potential fungus against *Sclerotium oryzae* was selected for further studies.

***In vitro* compatibility between potential native antagonistic fungus and bacterium**

Compatibility / incompatibility between potential native antagonistic fungus and bacterium were determined using dual culture technique. Three days old mycelial disc of 6 mm diameter antagonistic fungus was placed at one corner of PDA plated Petri plate and on the opposite side antagonistic bacterium was streaked and incubated at 28 ± 2°C for 6 days.

RESULTS

ISOLATION AND IDENTIFICATION OF THE PATHOGEN

The fungus was isolated on to PDA medium from the stem rot infected rice stems showing the typical symptoms of stem rot. Pathogenicity of the fungus isolated was proved by using susceptible rice cultivar MTU-3626.

On PDA medium the fungus formed distinct white colonies with abundant globose, white sclerotia from the fourth day onwards. Sclerotia that were formed in the culture were initially white in colour later turned to reddish brown and finally they became dark brown to almost black with spherical shape measuring 418 (185-685) x 355 (165-545) µm in size.

Based on the morphological and colony characteristics described by (3) the causal organism was identified as *Sclerotium oryzae* Catt.,

Identification of native rhizosphere mycoflora and bacteria

Mycoflora and bacteria were identified based on colony and morphological characters (3 and Bergey's Manual of Determinative Bacteriology). Five fungi *viz*., *Aspergillus flavus*, *A. niger*, *Cladosporium* sp., *Trichoderma* sp. isolate-1 and *Trichoderma* sp. isolate-2 were isolated and five bacterial isolates *viz*., *Pseudomonas fluorescens* (BI-1) Bacterial isolate-2 (BI-2), Bacterial isolate-3 (BI-3), Bacterial isolate-4 (BI-4) and Bacterial isolate-5 (BI-5) were detected during isolation. The observations regarding the number of colonies per 10 g soil (Table 1).

In vitro* evaluation of all rhizosphere microflora against *Sclerotium oryzae

Out of five fungal isolates tested *T. viride* (T1) was found to be significantly superior in inhibiting the mycelial growth of *Sclerotium oryzae* by 75.3 per cent with a mean radial growth of 22.2 mm while the *Cladosporium* sp. was found to be least effective by inhibiting the pathogen to the extent of 29.2 per cent only. The percentage inhibition by other were in between 36.3 to 60.2 per cent (Fig. 1).

Similarly among the five bacterial isolates tested for their antagonistic activity, *P. fluorescens* (BI-1) was significantly superior in inhibiting the growth of *Sclerotium oryzae* by 77.2 per cent with a mean radial growth of 20.4 mm of pathogen as against 90.0 mm in control. The percentage inhibition by other bacterial isolates were in between 1.8 to 8.3 per cent (Fig. 2).

Among the microflora tested, *Trichoderma viride* (T1) and *Pseudomonas fluorescens* (BI-1) inhibited the mycelial growth of *Sclerotium oryzae* to a maximum extent and the same were used as a potential native antagonists fungus against the test pathogen for further studies.

In the present investigation, among the bacterial isolates, *Pseudomonas fluorescens* (BI-1) was found to be more effective against *S. oryzae* as it inhibited mycelial growth to an extent of 77.2 per cent.

Compatibility studies between potential native antagonistics *in vitro*

Compatibility between *Trichoderma viride* (T1) and *Pseudomonas fluorescens* (BI-1) was evaluated by using dual culture technique. *P. fluorescens* (BI-1) showed five per cent inhibition of growth of *T. viride* (T1). The bacterial isolate -1 (BI-1) growth was not inhibited by *T. viride* (T1) (Table 2).

DISSCUSSION

Isolation and evaluation of native rhizospheric micro flora against *S.oryzae*

Biocontrol involves harnessing disease suppressive microorganisms to improve plant health. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment (6). Biocontrol of soil borne diseases is particularly complex because these sclerotial diseases occur in the dynamic environment at interface of root and soil grown as the rhizosphere, which is defined as the region surrounding a root that is affected by it. The rhizosphere is typified by rapid change, intense microbial activity, and high populations of bacteria compared with non-rhizosphere soil. Plants release metabolically active cells from their roots and deposit as 20 per cent of carbon allocated to roots in the rhizosphere, suggesting a highly evolved relationship between the plant and rhizosphere microorganisms.

The rhizosphere is also described as the first line defence for roots against attack by soil borne pathogenic fungi (7). Therefore it is an excellent opportunity to exploit the benefits of the soil microflora for the management of soil borne plant pathogens.

In the present investigation all the isolates of fungi *viz., Aspergillus flavus*, *A. niger*, *Cladosporium* and *Trichoderma viride* isolate-1, *Trichoderma* sp. isolate-2 showed inhibition of the test pathogen. However, *Trichoderma* spp. isolates were found to be more inhibitory to *Sclerotium oryzae* with a maximum inhibition by *Trichoderma viride* isolate-1 (T1) (75.3%) followed by *Trichoderma* isolate-2 (T2) (60.2%), *Aspergillus niger* (40.7%), *Aspergillus flavus* (36.3%) and *Cladosporium* Sp. (29.2%).

The antagonistic activity of *Trichoderma* spp. might be due to production of diffusible antibiotics which are detrimental to *Sclerotium oryzae* (8). The dual culture studies conducted by (9) revealed that *T. viride* was effectively inhibiting by 68.0 per cent growth of *Sclerotium hydrophilum* followed by *T. koningii* (63.1%), *T. harzianum* (55.5%) and *T. reseii* (55.3%). (10) reported maximum reduction (72.2%) of mycelial growth by *Trichoderma viride* among the soil mycoflora isolated against *Sclerotium rolfsii* in dual culture. (11) also reported that *Trichoderma harzianum* showed 73.4 per cent reduction in colony diameter of *Sclerotium rolfsii* of groundnut in dual culture technique.

According to (12) *T. viride* (local strain) inhibited maximum mycelial growth of *Sclerotium rolfsii* causing tomato color rot and was significantly better than *T. harzianum* and *Gliocladium virens*. Both the species of *Trichoderma* have already been reported to be effective against *Sclerotium rolfsii* causing sclerotial diseases in oilseeds and vegetables crops (13 and 14). (15) reported mycoparasitism (penetration and infection) of *Trichoderma* spp. against *Sclerotium rolfsii*, where chlamydospores were abundantly produced in contrast to conidia with in the infected fungal sclerotia.

In the present investigation the two *Trichoderma* isolates (T1, T2) caused drastic reduction in the mycelial growth. Decreased mycelial growth of the pathogen in the presence of antagonist will naturally lead to production of sclerotia of smaller size. The smaller size of sclerotia indicates less amount of reserve food material stored in them. Sclerotia may not attain maturity when produced in the presence of antagonists. These two factors might have contributed to reduction in sclerotial germination and number of hyphae produced by the *Sclerotium* during germination. Thus *Trichoderma* fungi are well known for their antagonism against several soil phytopathogens. Their biocontrol activity is mainly attributable to various anti-microbial/ antagonistic compounds they produce, in addition to their aggressive mode of growth and physiology. Full exploitation of the biocontrol agent like *Trichoderma* spp. could easily provide growth enhancement of domestic plants, green house plants and agricultural crops.

The present finding is in agreement with the studies conducted by 16 and 17. They found that *P. aeruginosa, B. subtilis* and *B. pumulus* strains were effective against stem rot of rice pathogen *Sclerotium oryzae*.

In the present investigation, among the bacterial isolates, *Pseudomonas fluorescens* (BI-1) was found to be more effective against *Sclerotium oryzae* as it inhibited mycelial growth to an extent of 77.2 per cent. Fluorescent pseudomonads also play an important role in biological control of plant pathogens. Fluorescent pseudomonads dominate in the rhizosphere and posses several properties that have made them as biocontrol agents (18). The mechanisms by which bacteria affect the plants involve the production of diverse metabolites including siderophores, hydrocyanic acid (HCN), phytohormones and the other associated activities including phosphate solubilization and root colonization resulting in plant growth promotion (19). *Pseudomonas fluorescens* inhibited maximum mycelial growth by 67.2 per cent

and sclerotial population of *Sclerotium rolfsii* of jasmine by 86.0 per cent in dual culture studies under *in vitro* conditions (20). (21) reported that *P. fluorescens* showed significant reduction in the mycelial growth and sclerotial production of *Sclerotium rolfsii* of potato under *in vitro* conditions.

Compatibility Studies

The compatibility between *Trichoderma viride* isolate-1 (T1) and *Pseudomonas fluorescens* (BI-1) was determined by dual culture technique. *Pseudomonas fluorescens* (BI-1) showed five per cent inhibition of growth of *Trichoderma viride* isolate -1 (T1). The bacterial growth was not inhibited by *Trichoderma viride* isolate -1 (T1). (22) tested the compatibility of *Trichoderma* isolate-1 (T1) with *Pseudomonas* sp. (BI-1) by dual culture technique and found that both were compatible showing just five percent inhibition in growth of *Trichoderma* sp., by *Pseudomonas* sp., (BI-1).

Table 1: Rhizosphere mycoflora and bacteria isolated from rhizosphere of rice samples of Khammam and Warangal districts.

Sl.No.	Rhizosphere Mycoflora/Bacteria	Cfu x 10^{5*}
	I. Mycoflora	
1.	*Aspergillus flavus*	27
2.	*Aspergillus niger*	12
3.	*Cladosporium* sp.	15
4.	*Trichoderma* sp. isolate-1(T1)	21
5.	*Trichoderma* sp. isolate-2 (T2)	12
	II. Bacteria	
1.	*Pseudomonas fluorescens* (BI-1)	67
2.	Bacterial isolate–2 (BI-2)	72
3.	Bacterial isolate–3 (BI-3)	64
4.	Bacterial isolate–4 (BI-4)	89
5.	Bacterial isolate–5 (BI-5)	102

* Mean of 10 plates

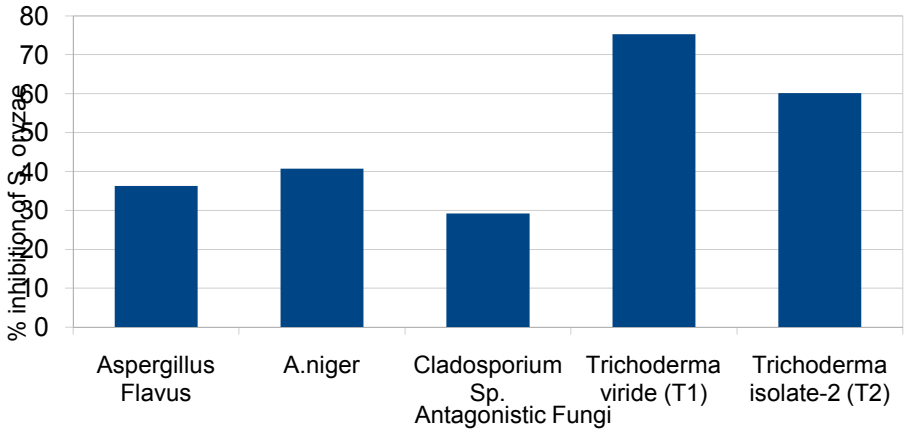

Fig. 1: *In vitro* evaluation of efficacy of antagonistic mycoflora against *Sclerotium oryzae* (Dual culture technique)

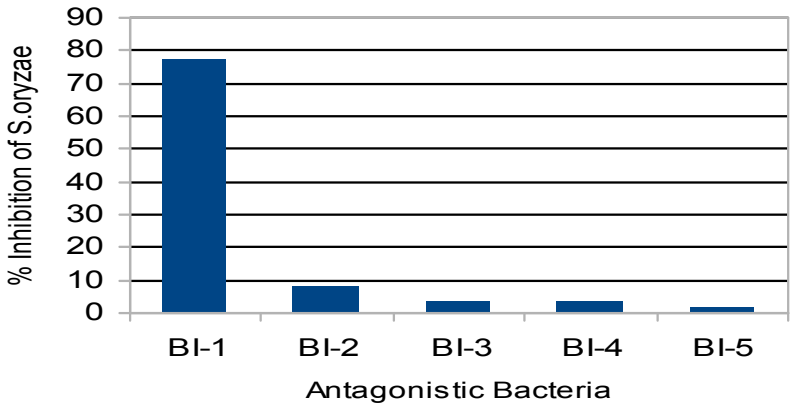

Fig. 2: *In vitro* evaluation of efficacy of antagonistic bacteria against *Sclerotium oryzae* (Dual culture technique)Table 2 :Compatibility between *Trichoderma viride* (T1) and *Pseudomonas fluorescens* (BI-1)

Treatment	Growth of *Pseudomonas fluorescens* (BI-1) (mm)	Growth of *Trichoderma viride* (T1) (mm)	Per cent inhibition of growth *Pseudomonas fluorescens* (BI-1)	*Trichoderma viride* (T1)
Control (T1) *Trichoderma viride*	-	85.0	-	-
Control (*Pseudomonas fluorescens* (BI-1)	90.0	-	-	-
BI-1+ T1	90.0	85.0	0.0	5.0

All the figures are means of 4 replications

REFERENCES

1. Vyas, R. K. and Mathur Kusm. (2002). Indian Phytopathology, 55 : 451-457.
2. Johnson, L. F. and Curl, E. A. (1977). Burgess Publishing Company, Minnea Polis, pp.247
3. Barnett, H. L. and Barry, B. Hunter. (1972). Burgess Publishing Company, Minnesota.
4. Holt, J. G., Kreeg, N. R., Sneath, P. H., Stanley, J. T. and Williams, S. T. (2000). Bergey's Manual of Determinative Bacteriology. Lippincott Williams and Wilkins, Maryland, USA
5. Dennis, C. and Webster, J. (1971). Transactions of British Mycological Society, 57 : 41-48
6. Handelsman, Jo. and Eric, V. Stabb. (1996). The Plant Cell, Vol. 8 : 1855-1869
7. Weller, D. M. (1988). Annual Review of Phytopathology, 26 : 379-407
8. Upadhyay, J. P. and Mukhopadhyay, A. N. (1983). Indian Journal of mycology and Plant Pathology, 13 : 232-233
9. Hemanthu, (2006). Thesis Studies on stem rot of Rice M. Sc (Ag) thesis submitted to Acharya N G Ranga Agricultural University, Rajendranagar.
10. Sonali. and Gupta, A. K. (2004). Journal of Mycology and Plant Pathology, 34 : 637-641
11. Pushpavati, B. and Chandrasekhara, Rao. (1999). Indian Journal of Plant Protection, 26 : 149-154
12. Banyal, D. K., Mankotia, V. and Sugha, S. K. (2008). Journal of Plant Pathology, vol.32 (2) : 164-167
13. Das, B. C., Dutta, P., Devi, G. and Dutta, P. (2000). Journal of Agricultural Science Society North East India, 13 : 101-103.
14. Dutta, P., Das, B. and Dutta, P. (2002). Indian Phytopathology, 55 : 235-237
15. Henis, Y., Adams, P. B., Lewis, J. A. and Papavizas, G. C. (1983). Phytopathology, 73 : 1043-1046
16. Rosales, A. M., Vantomme, R., Swings, J., Deley, J. and Mew, T. W. (1993). Journal of Phytopathology, 138, 189-208
17. Laha, G. S. (2009). DRR Rajendranagar, pp. :191-198
18. Johri, B. N., Rao,C. V. S. and Goel, R. (1997). Jodhpur Science Publishers, pp. 193
19. Shivani Bhatia., Dubey, R. C. and Maheswari, D. K. (2005). Indian Phytopathology, 58 :17-24
20. Umamaheswari, M. P., Muthuswamy, M. and Alice, D. (2002). Journal of Biological Control, 16 : 135-140
21. Narasimha Rao, S., Anahosur, K. H. and Srikanth Kulkarni. (2004). Journal of Mycology and Plant Pathology
22. Arunasri, P. (2003). Thesis submitted to Acharya N. G. Ranga Agricultural University, Hyderabad, Andhra Pradesh.

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