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IN VITRO EVALUATION OF FUNGICIDES AND TWO SPECIES OF *TRICHODERMA* AGAINST *SAROCLADIUM ORYZAE* CAUSING SHEATH ROT OF PADDY (*ORYZA SATIVA* L.)

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ABSTRACT

Sheath rot of paddy caused by *Sarocladium oryzae* is an important disease of paddy inflicting heavy losses. The present investigation was carried out to test the efficacy of fungicides and two species of *Trichoderma* inhibiting the pathogen *in vitro*. Fungicides viz. Carbendazim (Bavistin 50% wp), Captaf (Captan-50 wp), Mancozeb (Dithane-75), Copperoxychloride (Blitox -50wp), Ethanol and Methanol were tested by poisoned food technique against Paddy fruit pathogen (*S.oryzae*) in PDA medium. All the fungicides were significantly proved effective. Among them, Carbendazim at 0.20% showed 85.2% inhibition of the mycelial growth of the pathogen. Among the two species of *Trichoderma* tested, *T.harzianum* was found to be most effective with 96% inhibition followed by *T. viride* 86 % inhibition respectively over control after 7th days of incubation.

KEYWORDS: Paddy, *Sarocladium oryzae*, Fungicide, Trichoderma spp.

INTRODUCTION

Rice, the staple food of nearly half of the humanity is mainly grown and consumed in Asian countries such as India, China, Japan, Indonesia, Thailand, Pakistan, Bangladesh, North and South Koreas, Myanmar, Philippines, Sri Lanka etc. It is constantly subjected to several fungal, bacterial, viral and nematode diseases. Sheath rot, caused by *Sarocladium oryzae* (Sawada) Gams. and Hawksw. has gained the status of a major disease of rice and yield loss

varies from 9.6 to 85%. The fungus is detected frequently during routine seed health testing and causes empty grain production (Kulwanth and Mathur, 1992) and glume discolouration (Sachan and Agarwal, 1995) and also seed discoloration (Reddy *et al.*, 2000). Seeds from infected panicles became discoloured and sterile (Mew and Gonzales, 2002).

Fungicides are playing pivotal role in reducing crop losses. The application of chemical fungicides over a long period may result in plant pathogenic fungi developing resistance (Benítez *et al*., 2004, Agrios, 2005; Kim and Hwang, 2007). When this happens the chemical fungicides become ineffective and other fungicides must be used for effective disease control. The use of microorganisms as biological control agents to control plant disease is a potentially powerful alternative method (Kulkarni *et al*., 2007). Because of their rich diversity, complexity of interactions and numerous metabolic pathways, microbes are an amazing resource for biological activity (Emmert and Handelsman, 1999; Alabouvette *et al*., 2006; Tejesvi *et al*., 2007; Mitchell *et al*., 2008; Raghukumar, 2008). Over the past 30 years, microorganisms have been described, characterized, and tested for their use as biocontrol agents against diseases caused by soil borne plant pathogens. Biocontrol agents and especially antagonistic fungi have been used to control plant diseases with 90% of applications being formulated using different strains of *Trichoderma* e.g. *T. harzianum, T. virens, T. viride* (Benítez *et al*., 2004). Therefore, the experiment was undertaken to find out the effective fungicides and with two species of *Trichoderma* in controlling Sheath rot disease in paddy.

MATERIALS AND METHODS

In the present study, paddy fields was located in four (Naduvoor, Mariyamman Kovil, Papanasam and Vallam) different villages of Thanjavur district, Tamilnadu during the study period 2010-2011.

Collections of sheath rot diseased samples

During the crop season above said areas were surveyed. Diseased plants showing sheath rot symptom were collected from different locations. The samples were kept in clean polythene bag and each sample was marked clearly to show details of the location and variety. The samples were brought to the laboratory for microscopic examination, isolation, purification and pathogenicity test.

Isolation of soil fungi

Dilution plate technique

Dilution plate technique was used for the isolation of fungi from soil sample as described by Warcup (1950; 1960). 10g of soil from each sample was weighed separately and then dissolved in 100ml of distilled water. The flasks were shaken thoroughly in order to get uniform distribution of the soil. The soil suspensions were diluted in 10 fold increment from 10^2 to 10^4. One ml of the diluted sample was plated onto potato dextrose agar medium (PDA). Before pouring the medium into petridish it was added with 1% streptomycin. The plates were incubated at room temperature (28±2°C) for 7 days. Three replicates were maintained for each sample. After three to four days of incubation, the colonies growing on PDA plates, with different morphology were counted and purified on PDA medium separately. A portion of the growing edge of each colony was picked up with the help of a pair of sterile needles and mounted on a clean glass slide with lactophenol cotton blue (Hi-media). The slide was observed under microscope and microphotographs of the individual fungal species were also taken using Nikon Microphotograph Microscope (Japan).

Identification

The isolated fungal organisms were identified with the help of standard manuals such as Manual of Soil fungi (Gillman, 1957).

Pathogenicity test

To test pathogenicity of phytopathogen, rice plants of cultivar IR36 were grown in the greenhouse. At booting, the panicle-emerging stage, tillers were inoculated with *S. oryzae* following the standard grain inoculum technique (Sakthivel and Gnanamanickam, 1987). A total of 100 tillers from 1 plant were inoculated with isolate of *S. oryzae*. After 14 days, sheath rot disease occur (International Rice Research Institute, Philippines, 1988).

***In vitro* effect of different fungicides**

The comparative toxicity of fungicides on the growth of the fungus under *in vitro* condition was evaluated by poisoned food technique (Nene, 1971). Fungicides like Carbendazim (Bavistin 50 wp), Captaf (Captan -50 wp), Copper oxychloride (Blitox-50 wp), Mancozeb (Dithane M-45), Ethanol and Methanol at different concentration (0.1, 0.15 and 0.2 percent) were used for *in vitro* assay. The fungicides were incorporated into the sterilized PDA medium. The sterilized petriplates containing amended medium were inoculated with 7 mm disc of freshly prepared culture of the test fungus and incubated at 28± 1°C for 7 days. The

efficacy of fungicides was expressed as percent of radial growth over control, which was calculated by using the formula (Vincent, 1947).

I= C-T/CX100

Where,

I= Percent inhibition over control

C= Radial growth in control

T= Radial growth in treatments.

In vitro antagonism of the two species of *Trichoderma*

In vitro antagonism of the two species of *Trichoderma* against *Sarocladium oryzae* was tested by dual culture technique on PDA medium (Dhingra and Sinclair, 1985). Control was maintained without pathogen. All the plates were incubated at room temperature (28±1˚C). Each experiment was replicated three times. Observation on mycelial growth of the pathogen was recorded after 7 days of incubation. The percent inhibition over control was calculated.

RESULT AND DISCUSSION

Pathogenicity test

After 14 days, infected tillers were observed for sheath rot infection. Sheath rot-infected panicles were harvested at maturity. To confirm the *S. oryzae* infection, seeds were placed onto PDA plates. An abundant whitish powdery growth were observed (Plate I).

The results revealed (Table 1) that all the fungicides having different concentration significantly inhibited the mycelial growth of *Sarocladium oryzae*. It was observed that fungicides tested, Carbendazim was found most effective at (@ 0.2%) caused highest reduction of mycelial growth 85.2 percent followed by Mancozeb 70.4 percent (@ 0.2%); while Captan 84 percent (@ 0.2%), Copper oxychloride 67.1 percent at the same

concentration over control as ethanol and methanol. The findings of the present investigation are well supported by the findings of (Sabalpara , *et al*.2009., Muneeshwar *et al*.2012., Hossain *et al*. 2013) who reported that Bavistin (Carbendazim) as effective fungicide against *Phomopsis vexans.*

Table 1: *In vitro* evaluation of fungicides against *Sarocladium oryzae.*

S. No	Fungicide	Percentage inhibition of mycelia growth			
		% concentration			Mean
		0.10	0.15	0.20	
1.	Carbendazim	83.5	85	85.2	84.4
2.	Captan	81.1	82	84	82.3
3.	Mancozeb	60	69.3	70.4	66.7
4.	Copper oxychloride	53.4	59.4	67.1	59.9
5.	Ethanol(control)	-	-	-	-
6.	Methanol(control)	-	-	-	-

Effect of two species of *Trichoderma* on mycelial growth of *Sarocladium oryzae*

Antagonistic fungi viz. *Trichoderma harzianum* and *T.viride* were isolated from the soil samples of brinjal rhizosphere. The identification was confirmed according to the identification key (Rifai, 1969) based on the branching of conidiophores, shape of phialides, emergence and shape of phialospores. The two fungal cultures isolated from soil were found to have inhibitory effect on the mycelial growth of the pathogen (Table 2) and data showed that degree of inhibition was maximum with *T.harzianum* (96%) followed by *T. viride* (86%) after 7th days of incubation. *T. harzianum* was found highly effective in comparison to *T. viride*. The present findings are found in agreement with the works of Jadeja (2003).

Table 2: Effect of two species of *Trichoderma* on mycelia growth of *Sarocladium oryzae*

S. NO.	Treatment	% inhibition of mycelia growth
1.	*Trichoderma harzianum*	96%
2.	*Trichoderma viride*	86%

CONCLUSION

Sheath rot of rice caused by *Sarocladium oryzae* (Sawada) should be kept under control with minimum possible loss through effective biocontrol measure than chemical control. So, reliance only on Mycocide and search for more effective new rice cultivars/ germplasm for the management of sheath rot disease has to be continued, which would help in finding the stable and effective management strategy in the country.

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