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INVITRO ASSESSMENT OF ANTAGONISTIC ACTIVITY OF *TRICHODERMA* SP. AGAINST *SAROCLADIUM ORYZAE* CAUSING SHEATH ROT DISEASE IN PADDY

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ABSTRACT: Invitro screening using the dual culture technique was undertaken to assess the potential of seven *Trichoderma* species. *Trichoderma viride, Trichoderma lignorum, T.album, T.hamatum, T.harizanum, T.glaucum* and *T.koeningi* as biological control agents against Sheath rot fungus *Sarocladium oryzae*. The test organisms were isolated from the paddy field where the disease occurred. Results revealed that all the test antagonists effectively checked the growth of the pathogen. The test antagonists grow faster than the pathogen and produced inhibition zones thereby limiting the growth of the pathogen. In solid medium, *Trichoderma harizanum* was the most antagonistic organism under the conditions of this study. The culture filtrates of the test fungi also inhibited the growth of *Sarocladium oryzae* with *Trichoderma harizanum* showing the highest percentage inhibition (79%) *and T.viride* and *T.lignorum* (78%). *T.harizanum* culture filtrate showed the highest percentage growth inhibition at 15% concentration in *Sarocladium oryzae* while *T.viride* and *T.lignorum* filtrates showed inhibition,at 25% respectively.

Key words: Invitro, antagonism, *Trichoderma harizanum*, inhibition.

Rice (*Oryza sativa*) is the staple food of the people in the eastern, southern and south eastern parts of India. The crop is prone to various biological constraints; of which disease markedly affect the crop production. Sheath rot disease in rice is caused by *Sarocladium oryzae*. It is one of the serious soil borne pathogen having a broad host range and as saprophyte in the pre-colonized dead host tissues. The management of the pathogen is a major problem among the agricultural community.

The saprophytic growth and activity of the pathogen vary depending upon the environmental and soil condition. The differences in the saprophytic activities of variations in the cellulolysis rate of the organisms as suggested by Garrett, (1956). Though Garrett is pioneer in the studies on various aspects of saprophytic ability of the pathogen in soil, the conditions that inhibit the saprophytism of the pathogen may be exploited for biological control in several ways. The toxic metabolite produced by the initial fungal colonies of natural substrate may act to slow or present invasion by other species (Ambikapathy *et al*., 1994). *Trichoderma* sp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. Several fungal cell wall degrading enzymes, amongst them chitinase and glucanase, which seem to play an important role in the antagonistic action of *Trichoderma* against a wide range of fungal pathogens (Kucuk and Kivanc, 2008). The antagonistic activities of *Trichoderma harzianum* (Rifai,1969) against several pathogenic fungi have been reported by many workers (Henis and Chet,1975., Backman and Rodrigues-Kabana,1975, Hadar *et al*,1979, Elad *et al*,1980) The present study aimed to find out the efficiency of *Trichoderma* sp. against Sheath rot pathogen.

MATERIALS METHOD

Organisms

Sheath rot fungus (*Sarocladium oryzae*) was isolated from paddy field of Thanjavur district. Antagonistic fungus (*Trichoderma viridae, T.lignorum, T.album, T.harizanum, T.hamatum* and *T.koenigi*) were taken from TNAU, Coimbatore. Both these cultures of pathogens and the antagonist were maintained on Potato dextrose Agar.

TEST OF ANTAGONISM INVITRO

Dual culture technique:

Inhibition of pathogen growth by these test antagonists was carried out on Potato dextrose Agar medium using the dual culture technique. Five millimeter diameter mycelia plugs of each test antagonist were placed at the periphery of three different culture plates and incubated for 3 days at 28±2ºC (Evans *et al*., 2003). After three days each plate was doubly-inoculated with another 5mm diameter mycelia plug of the pathogen placed 5cm from the test antagonist. The dual culture plates were incubated for additional 9 days at 28±2ºC. In the control experiment, the test antagonists were replaced with sterile agar plugs. The growth of the pathogen in both the test and control experiments were recorded. Data were obtained for the Percentage inhibition of radial growth (100x(R1-R2)/R1) where R1= radial growth of the pathogen in control and R2= radial growth of the pathogen in dual culture with antagonist) and the width of the zone of inhibition (ZI)(measured as the smallest distance between the colonies in the dual culture plate) (Royse and Ries, 1978, Garrett, 1956). Five types of interaction grades as proposed by Skidmore and Dickinson (1976) have been used. Results were recorded in the (Table 1& Plate I).

CULTURE FILTRATE ASSAY

One hundred milliliters (100ml) of potato dextrose broth (PDB) were dispensed into separate 250-Erlenmeyer flasks and inoculated with 5mm-diameter discs from the edge of 7 day old cultures of the test antagonists maintained on PDA. Each flask was inoculated with three discs and the set up incubated at 28±2ºC for 7 days. Culture filtrates were harvested by filtering through Whatman No.1 filter papers and finally through Millipore filter (0.45µm) to obtain sterile culture filterate. The culture filtrate was adjusted to pH 5.6 by using 0.1N HCl or 0.1N NaOH before use. Different concentrations viz., 5, 10, 15, 20 and 25 % of the culture filtrate were mixed with cooled Potato Dextrose Agar before plating. The medium devoid of culture filtrate served as control. Petridishes were inoculated separately with a 9mm agar disc of the tested pathogens, cut from actively growing colony of 5 days old culture, and incubated at 28±2ºC. The radial growth of tested pathogens was measured after 24 hours intervals.

RESULTS AND DISCUSSION

Antagonism in culture:

Results showed that all the fungi tested in this study exhibited antagonistic activities against sheath rot fungi *Sarocladium oryzae.* Radial growth of the pathogen was considerably hindered by all the test antagonists under the conditions of this study. *T. harizanum* was the most antagonistic and inhibited the radial growth of the pathogen most while *T.album* was the least antagonist.

Table 1: Colony interaction between *Sarocladium oryzae* and Seven different strains of *Trichoderma* sp. in dual culture experiment(mm).

Growth response of the antagonistic and test fungi	*T.viride*	*T.lignorum*	*T.album*	*T.harzianum*	*T.hamatum*	*T.glaucum*	*T.koeningi*
Colony growth of the pathogen towards antagonist (mm)	12	13	14	5	9	9	13
Colony growth of the pathogen away from the antagonist (mm)	13	13	14	5	10	10	12
% growth of the pathogen in the zone of the interaction	33	28	24	72	50	50	28
Colony growth of the antagonist in control i.e., growth towards the centre of the plate in the absence of the pathogen	78	78	72	79	77	76	77
Colony growth of the antagonist towards the pathogen (mm)	34	33	33	36	35	34	30
Colony growth of the antagonist away from the pathogen (mm)	25	24	23	30	23	24	25
% growth inhibition in the zone of inhibition	56	58	54	55	55	55	61

Growth of *Sarocladium oryzae* towards the centre of the plates in the absence of any antagonistic fungus (control) was 18mm measurement was taken into 96 hours.

Plate 1: Colony interaction between *Sarocladium oryzae* and Seven different strains of *Trichoderma* sp. in dual culture experiment

Effects of culture filtrate on antagonism:

Culture filtrates of the fungi tested in this study showed inhibitory effect on the growth of the pathogen. Growth inhibition was found to increase with the period of incubation (Table 2). *T.harizanum* culture filtrate showed maximum percentage of inhibition at 15% while *T.Viride* and *T.lignorum* filtrates produced 25% inhibition, respectively.

Table 2: Effect on incubation period of Antagonists culture on growth of pathogen in Potato dextrose agar medium(mm).

% of inhibition	*T.viride*	*T.lignorum*	*T.album*	*T.harzianum*	*T.hamatum*	*T.glaucum*	*T.koeningi*
5	20	21	30	13	25	20	25
10	18	18.5	25.2	12	25	20	25
15	15	15.3	22	12	23	19	23
20	12.5	12.5	19.5	10	22	15	22
25	10	10.5	19	10	22	15	22

% of growth inhibition of the antagonist in the zone of interaction

DISCUSSION

Previous studies have demonstrated that before mycelia of fungi interact, *Trichoderma* sp. produces low quantities of extracellular exochitinases (Kullnig *et al*.,2000., Brunner *et al*., 2003). The diffusion of these enzymes dissolves cell fragments of host cells. These cell fragments in turn induce the production of further enzymes and trigger a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma* sp. (Zeininger *et al*., 1999). In the present, invitro studies have demonstrated that due to chemotropism hyphae of *Trichoderma harzianum* can grow and branch directly towards the host.

The idea of a sustainable agricultural practice and environmental protection enhances the importance of biocontrol. The adoption of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining worldwide recognition. One of the key elements of such sustainable agriculture is the application of biocontrol agents. *Trichoderma* sp. is antagonistic by nature with rich resource and a broad action scope. The present study addresses the effective control mechanisms of *Trichoderma harzianum* against the sheath rot pathogens. The observation is similar to the findings of Tronsmo and Dennis (1977) and Okigbo and Ikediagwu (2000) in their investigation on the effects of *T.viride* on post harvest Botrytis rot of strawberry and yam rot, respectively.

Panneerselvam and Saravanamuthu (1996) had reported that antagonistic interaction of some soil fungi namely, *Aspergillus candidus, A.flavus, A.fumigatus, A.nidulans, A.niger, A.sulphureus, A.terreus, A.variecolor, Gliocladium sp. Penicillium citrinum, P.fumiculosum and Trichoderma viride* against *Sarocladium oryzae* was studied. The maximum percentage inhibition of growth with *S.oryzae* with *Trichoderma viride*, followed by our study *Trichoderma* sp. used for antagonist against *Sarocladium oryzae*.

Tondje *et al,*(2007) had reported that *Trichoderma asperellum* isolates controlled cocoa black pod caused by *Phytophthora megakarya* in Cameroon. Many beneficial fungi and bacteria that occur naturally and associated with coca were reported to show potential as antagonists of major cocoa pathogens (Bong *et al*,. 2000., Samuel and Habber, 2003).

Our, result explains that significant success in biocontrol is achieved under invitro conditions. Even though more research is needed to understand the antagonistic mechanism, improvement of strains and development of supplementary products of biocontrol agent for restraint of pathogens. Thus, it is noticeable that a microbial biocontrol agent offers harmless to the animals and human beings, cheaper than chemicals and highly effective.There is no risk of the pathogens develop resistance, fungicide residues in food and ground water.

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