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Biological control of paddy brown spot caused by *Bipolaris Oryzae* (Breda de Haan)

Manimegalai V., *Ambikapathy.V and Panneerselvam.A

P.G. and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu (India)

ABSTRACT

The antibiotic potentially of the soil fungi was studied in paddy field . The test pathogen Bipolaris oryzae varied in its sensitivity to the metabolic growth products of antagonisitic fungi. Invitro screening using the dual culture technique was undertaken to assess the potential of nine fungal species such as Aspergillus niger, A. fumigates, A .flavus A. sulphureus , A. terreus, Penicillium chrysogenum, P. janthinellum, Trichoderma viride and T. harzianum as biological control agents against Brown spot fungus Bipolaris oryzae. 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 has been discussed.

Keywords: *Bipolaris oryzae*, Antagonism, *Trichoderma harzianum*, Biological control.

INTRODUCTION

Rice (*Oryza sativa L.*) is among the most important cereals in world. On the basis of economic value. FAO [1] forecast of global paddy production has been lifted by nearly 10 million tones to 678 million tones, but still the second highest production on record. One of the main reasons for the low productivity is the entamic presence of seed borne brown spot disease caused by *Bipolaris oryzae* .

Brown spot is one of the important rice diseases in the world. It can be a serious disease causing a considerable yield loss. Where, it affects the quality and the number of grains per panicle and reduces the kernel weight[2].

Bipolaris oryzae is classified in the subdivision Deuteromycotina (imperfect fungi), class Deuteromycetes, order Moniliales, and family Dematiaceae and is the causal agent of brown spot disease of rice.

Biological control is an innovative, cost effective and ecofriendly approach. *Trichoderma spp* is known for its mycoparasitic and antagonistic mechanism for the control of fungal disease. They are termed bioagent that is capable of combating a number of fungal diseases of plant crops.

Trichoderma are present in all soil and they are the most cultural fungi. *Trichoderma* species are strongly antagonistic to other phytopathogenic fungi. They produce hydrolytic enzymes which are believed to play an important role in the parasitism of phytopathogenic fungi. 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. [3]

The objective of this study was to evaluate the efficacy of existing biocontrol strains *T.harzianum* for controlling paddy disease brown spot under *in vitro* conditions.

MATERIALS AND METHODS

Organisms

Brown spot fungus (*Bipolaris oryzae*) was isolated from paddy field of Thanjavur district. Both these cultures of pathogens and the antagonist were maintained on Potato dextrose Agar (PDA) medium for further investigations.

Test of antagonism *invitro*

Dual culture technique:

The test pathogen, B*ipolaris oryzae* and soil fungi viz ., *Aspergillus flavus, A . fumigatus, A. terreus, A. niger, A. sulphures, Penicillium chrysogenum, P. janthenillum, T. harzianum, and T. viride* were maintained on PDA medium. Inhibition of pathogen growth by these test antagonists was carried out on Potato dextrose Agar medium using the dual culture techniques 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 [4]. 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. Colony interaction study was performed by dual culture method [5]. The growth inhibition in the colony of the test pathogen and the antagonistic fungi was calculated the results were recorded in the (Table- 1).

$$\text{Percentage inhibition of growth} = \frac{r - r1}{r} \times 100$$

r = growth of the fungus was measured from the center of the colony towards the center of the plate in the absence of antagonistic fungus.

r1= growth of the fungus was measured from the center of the colony towards the antagonistic fungus.

The colony interaction between the test pathogen and the soil fungi were assessed following the model proposed by Porter, (1924) [6] and Dickinson and Broadman, (1971) [7]. Five type of interactions grade as proposed by Skidmore and Dickinson, (1976) [5] have been used.

They are as follows.
1. Mutual intermingling growth without any macroscopic sights of interaction – Grade 1
2. Mutual intermingling growth where the growth of the fungus is ceased, and being over grown by the opposed fungus-Grade2.
3. Intermingling growth where the fungus under observation is growing into the opposed fungus either above (or) below – Grade 3.
4. Sight inhibition of both the interacting fungi with narrow demarcation line (1-2 mm)-Grade4.
5. Mutual inhibition of growth at a distance of >2mm–Grade 5.

RESULTS AND DISCUSSION

Antagonism in culture:
Trichoderma are present in all soil and they are the most cultural fungi. *Trichoderma* species are strongly antagonistic to other phytopathogenic fungi. They produce hydrolytic enzymes which are believed to play an important role in the parasitism of phytopathogenic fungi

Results showed that all the fungi tested in this study exhibited antagonistic activities against brown spot fungus *Bipolaris* oryzae. Radial growth of the pathogen was considerably hindered by all the test antagonists under the conditions of this study. *T. harzianum* was the most antagonistic and inhibited the radial growth of the pathogen most while *A. flavus* was the least antagonist.

Trichoderma harzianum was the most antagonistic organism under the conditions of this study. The dual culture of the test fungi also inhibited the growth of *Bipolaris oryzae* with *Trichoderma harzianum* showing the highest percentage inhibition (71.2%) and *T.viride(67.9%)* and *Penicillium chrysogenum* (64.2%) , *P. janthinellum* (60.7%), *A . sulphures* (64.3%), *A. niger* (57.1%), A. *fumigatus* (46.4%), The mycelium of *T.viride,* and *T.harzianum* were found growing over the pathogen (Table.1).

The antagonistic properties of different species of *Aspergillus, Pencillium and Trichoderma* against different pathogens have also been reported Panneerselvam *et al.*,(1999)[8] Ambikapathy *et al.*, (1994).[9] and Madhanraj *et al.*, (2010) [10] . In the present, invitro studies have demonstrated that due to the host. However, this biocontrol agent demonstrates a powerful antagonistic behaviour in the control of rice diseases brown spot. It can therefore be concluded that *Trichoderma harzianum* is an effective biological control agent.

Antibiotic interaction between soil fungi and *Bipolaris oryzae*
The types of interactions of the pathogen with soil fungi were as follows:

T.harizanum	Grade 1
T. viride	Grade 2
P. chrysogenum and A.sulphures	Grade 3
P. janthinellum	Grade 4
A .niger	Grade 5

Table 1: colony interaction between *Bipolaris oryzae* and soil fungi in dual culture experiments

S. No	Growth response of the antagonist and the test fungus	*Antagonistic fungi tested (mm)*								
		A.fla	***A.fum***	***A.sul***	***A.nig***	***A.ter***	***P.jan***	***P.chr***	***T.vir***	***T.har***
1	Colony growth of the pathogen towards antagonist (mm)	18	15	10	12	16	11	10	9	8
2	Colony growth of the pathogen away from the antagonist (mm)	27	24	15	13	25	15	14	10	20
3	% growth inhibition of the pathogen In the zone of the interaction (mm)	35.7	46.4	64.3	57.1	42.9	60.7	64.2	67.9	71.4
4	Colony growth of the antagonist in Control i.e. Growth towards the centre of the plate in the absence of the pathogen (mm)	32	28	24	35	33	30	25	28	30
5	Colony growth of the antagonist towards the pathogen (mm)	11	12	16	15	10	12	16	18	20
6	Colony growth of the antagonist away from the pathogen (mm)	20	19	35	23	27	25	34	43	41
7	% growth of inhibition in the zone of interaction	60.7	57.14	42.9	46.4	64.3	57.0	42.9	35.8	28.6

Growth of *Bipolaris oryzae* towards the centre of the plates in the absence of any antagonistic fungus (control)was 28 mm measurement was taken into 72 hours.

A.fla - Aspergillus flavus, A. fum - Aspergillus fumigatus , A.nig - Aspergillus niger, A.sul - Aspergillus sulphureus, A.ter– Aspergillus terrus ,P. chry - Penicillium chrysogenum, P.jan- Penicillium janthinellum , T.har - Trichoderma harzianum, T.vir - Trichoderma viride

DISCUSSION

Antagonism has been defined [11] as "to include any activity of one organism which in some way adversely affects another growing in association with it" it includes antibiosis, competition and exploitation. Antibiosis is generally recognized as the principal mechanism of interference competition by which fungi may exclude other organisms from resources potentially available to each other.

The interfungal interaction studies were observed in dual culture experiment .[12] . The mutual intermingling growth of *Bipolaris oryzae* with the soil fungi without any zone of inhibition indicate the failure of the production of antibiotics either by the pathogen (or) by the antagonist. The formation of zone of inhibition is an indication for the production of antibiotic substances either by the pathogen against antagonistic fungi (or) vice versa.

Among the antagonistic microorganisms *Trichoderma harzianum, T.viride* and *Aspergillus luchuensis* have proved their effectiveness. The effectiveness of *Trichoderma* in biocontrol could be attributed to the production of chitinase [13].

The observation is similar to the findings of Tronsmo *et al.*,(1977).[14] and. Kullnig *et al.*, (2000).[15] in their investigation on the effects of *T.viride* on post harvest Botrytis rot of strawberry and yam rot, respectively Panneerselvam *et al.*,(2011) [16] had reported that antagonistic interaction of some soil fungi *and Trichoderma viride* against *Sarocladium oryzae* was studied.

The maximum percentage inhibition of growth with *Trichoderma harzianum* against *Biplaris oryzae*, followed by our study .

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.

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