The biological control of paddy disease brown spot (*Bipolaris oryzae*) by using *Trichoderma viride in vitro* condition

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ABSTRACT

One of the most serious rice diseases in the rice sector is fungal disease infection. Plant diseases need to be controlled to maintain the quality and abundance of food supply. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and fungicides. A more balanced, cost effective and eco-friendly approach can be adopted by rice farmers. Biological control is an innovative, cost effective and ecofriendly approach. *Trichoderma viride* 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. It is a soil borne free living fungus that grows abundantly and produce millions of minute green coloured spores. The principle of *T. viride* is to use as a biological control agent against fungal disease in rice. *T. viride* was isolated from agricultural soils and the *in vitro* method was used to control rice disease, brown spot. The objective of this study was to evaluate the efficacy of existing biocontrol strains *T.viride* for controlling paddy disease brown spot under *in vitro* conditions.

Key words: *Trichoderma viride, Bipolaris oryzae,* biological control

INTRODUCTION

Rice is of vital importance to Guyana as a staple in the diet of the Guyanese people. It is estimated that approximately 20% of the population depends directly and indirectly on the rice industry for their livelihoods (GRDB News letter, 2008). One of the most serious rice diseases in the rice sector is the fungal disease infection. It results in poor production, poor quality, poor milling returns and reduced income. This has a negative impact on the livelihood of farmers. In Guyana, fungal disease control is achieved through the use of fungicides such as benlate, and fuzi-one etc. which is hazardous and toxic to both people and domestic animals. This leads to environmental pollution. Therefore, a more balanced, cost effective and eco-friendly approach must be implemented and adopted by rice farmers. Biological control is an innovative, cost effective and ecofriendly approach. Use of natural enemies to control disease is termed biological control. Biological control is an alternative to the use of chemical pesticides. Biological fungicides may act to suppress the population of the pathogenic organisms through competition with pathogneic organisms. Stimulated plant growth, which may allow plants to quickly outgrow any pathogen effects, or damage the pathogen by means of toxins produced (Cook, 2000; Gilreath, 2002). Biocontrol agents are derived from natural materials such as animals, plants, bacteria, fungi and certain minerals. Fungus exhibiting mycoparasitic behaviour eliminates the threat of synthetic fungicides (EPA website, 2008). With the knowledge of the adverse effects of synthetic fungicides worldwide, attention is rapidly, being shifted to non-synthetic, safer alternatives. *Trichoderma viride* is known for its mycoparasitic and antagonistic mechanism for the control of fungal disease. *Trichoderma harzianum* is a fungal biocontrol agent that attacks a range of pathogenic fungi. *Trichoderma harzianum* alone or in combination with other *Trichoderma* species can be used in the biological control of several plant diseases (Papavizas, 1985: Chet, 1987; Samuels, 1996). They could be environmentally safe alternatives to synthetic fungicide. Although *Trichoderm* spp is ubiquitous, the type of fungus in the soil can affect growth, proliferation and effectiveness as biocontrol agent. Because, soil ecology is complex, and with year–to-year fluctuations in climate conditions, treatments with fungicides are often inconsistent. This experiment to test the mycoparasitic effect of *Trichoderma viride* against the rice disease (brown spot).

MATERIALS AND METHODS

The isolate of bio control agent of *T. viride* and pathogen of *Bipolaris oryzae* used in this study were isolated from rice field soil and infected rice plants respectively. Pathogen collected from infected rice leaves were thoroughly washed with tap water. They were surface sterilized with 0.01% Mercuric Chloride for 3-4 minutes followed by two rinses in sterile distilled water. Washed samples were placed on potato dextrose agar (PDA) (Difco laboratories, Detroit, MI,USA) and incubated at room temperature for 5 days. Grown fungus was isolated on

new PDA plates, purified and identified according to Domsch *et al.* (1980). Pathogenicity was confirmed for *B.oryzae* in the test with rice a spore suspension (6 x 10^4 mycelial fragments/ml) which was prepared by blending 12 days old culture of B. *oryzae* grown on PDA at room temperature with sterile distilled water and filtering the suspension through cheesecloth. Conidial densities in the suspension were deter mined by use of a hemocytometer under a light micro scope. The pathogens were transferred to PDA slant and kept at 4°C for further experiment. The strain of biocontrol agent *T.viride* was used in this study. *Trichoderma viride* was isolated form rice field using standard serial dilution methods. Isolated *T. viride* was maintained on PDA and used on preparation of the strain for inoculum. The *T. viride* was transferred to PDA slant and kept at 4°C for further experiments. The experiment were carried out in laboratory conditions (*in vitro*), Johns Science center, University of Guyana, Berbice campus, Guyana, South America.

Selection of the medium, temperature and pH

The present studies PAD, PDYEA and OMA were used for selection of medium to growth of pathogen and biocontrol agent. The present studies were used the pH range from 4.5, 5.5, 6.5, and 7.5 and temperature were used 25°C, 28°C, 31°C and 34°C for growth of pathogen and biocontrol agent, which is determined which pH and temperature more suitable growth of pathogen and biocontrol agent.

Antagonistic test (dual culture techniques) and Radial Measurement of pathogen

Plates with PDA were simultaneously inoculated with 9mm discs of the pathogens as well as the biocontrol agent (T.*viride*) near the periphery at diametrically opposite points. The inoculated plates were kept at room temperature for growth. The radial measurements were taken of both the pathogen and biocontrol agent at a 48 hours interval (Huang and Hoes, 1976). The result was tabulated. Pathogens radial measurement was taken against biological control agent by using standard scaling method.

RESULT AND DISCUSSION

Test pathogen

Bipolaris oryzae are fungal pathogens that caused brown spot on rice. The pathogen was collected from the infected rice leaves in the location–Black Bush Polder Guyana, South America. The information in Table 1 illustrates the growth of *Trichoderma harzianum* on three different media – potato dextrose agar, potato dextrose yeast extract agar and oatmeal agar.

Table 1. Selection of Medium for cultures

Organisms	PDA (cm)*	PDYEA (cm)*	OMA (cm)*
Bipolaris oryzae	8.5	8.6	7.3
Trichoderrma viride	8.5	8.2	7.2

* Mean value

The result shown significant growth of *T. harzianum* on PDA which is indicated by 8.5 cm followed PDYEA 8.2 cm and OMA 7.2cm.

Table 2. Growth of pathogen and biocontrol different in temperature

Parameters	*B.oryzae* (mg)	*T.viride* (mg)
Temperature (°C)		
25	1.0	1.2
28	2.8	2.9
31	6.0	7.0
34	7.6	8.9
pH		
4.5	0.9	0.8
5.5	2.8	2.7
6.5	3.5	4.9
7.5	6.8	7.8

The result shown Table 2 significant growth and variation of pathogen and biological control agent in different temperature. The pathogen growth showing in different temperature from 25°C to 34°C, the dry weight of mycelium 1.0 mg to 7.6 mg in respectively. The biocontrol agent growth showing in different temperature from 25°C to 34°C, the dry weight of mycelium 1.2 mg to 8.9 mg in respectively. The result shown Table 2 significant growth and variation of pathogen and biological control agent in different pH. The pathogen growth showing in different pH from 4.5 to7.5, the dry weight of mycelium 0.9 mg to 6.8 mg respectively. The biocontrol agent growth showing in different pH from 4.5 to 7.5, the dry weight of mycelium 0.8 mg to 7.8. mg in respectively. The antagonistic growth of the pathogen *Bipolaris oryzae* against *Trichoderma harzianum* shows that growth is maximum in third day (4.0 cm) and diminished on sixth day (1.7 cm) and ninth day (0.6 mm).

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. Thus, in this experiment *Trichoderma viride* was tested for its antagonistic behavior against pathogen (*Bipolaris oryzae).* However, this biocontrol agent demonstrates a powerful antagonistic behaviour in the control of rice diseases brown spot. It can therefore be concluded that *Trichoderma viride* is an effective biological control agent.

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