Comparative study of biological agents, *Trichoderma harzianum* and *Trichoderma viride* for controlling brown spot disease in rice

S. Gomathinayagam[1*], Sherena Amela Persaud[1] and M. Rekha[2],

ABSTRACT

Biological control is an innovative, cost effective and eco-friendly approach for control of rice diseases. Brown spot *Trichoderma sp.* is known for its mycoparasitic and antagonistic mechanism for the control of wide range of fungal disease in all types of crops. *Trichoderma* sp. is an ecofriendly organism that does not cause any harmful and side effect on human beings and domestic animals when handled. This research is based on the principle of biological control of fungal diseases infection in rice plants by the use of *T. harzianum* and *T. viride.* Cultures of *T. harzianum* and *T.viride* were collected and isolated from agricultural soil and were used in laboratory condition for the control of rice diseases of brown spot. The present study used *T. harzianum* and *T. viride* as biocontrol agents to control rice disease of brown spot, investigating the effectiveness of the control agents. Satisfactory results were obtained in both biocontrol agents against rice pathogen *Bipolaris oryza* .

Key words: Antagonistic activity, *Bipolarize oryzae*, *in vitro*, microbial fungicide, *Trichoderma harzianum, T. viride*

INTRODUCTION

The cultivation of rice was first introduced in Guyana by Laurens Storm Van Gravesande, a Dutch Governor who resided in Essequibo in 18th century. Since that historic time, rice cultivation in Guyana has increased gradually over the past years. Rice planting in Guyana, however, has been plagued by a variety of pathogens, which are responsible for great losses. One of the most common diseases affecting rice presently is known as brown spot disease. In Guyana, brown spot disease in rice is more prevalent especially Black Bush Polder Region 6. Severe symptoms of the disease were observed in rice variety G98-135. Hence attempts were made for biological control of rice disease with *Trichoderma* sp.

Although there are many strains of *Trichoderma spp.* this project seeks to discover the beneficial qualities of using *Trichoderma harzianum* and *T. viride* in controlling brown spot disease in rice cultivation practices in Guyana and also to determine which of the two is a better biological control agent. *T. harzianum* is a fungus that is used for controlling other fungal causing diseases such as *Fusarium spp, Rhizoctonia spp,* etc, on cereals, fruits, plantation crops, etc. *T. harzianum* can be applied as a foliar, soil or seed treatment. *Trichoderma* species including *T. harzianum* colonize and penetrate plant root tissues. At this point, it commences a series of changes, both morphological and biochemical which cause an enhancement of the plant's defenses leading to Induced Systemic Resistance (ISR) in the plant. Studies have shown that there was a 30% increase in seedling emergence in plants inoculated with *Trichoderma* spp. In the present study different species of *Trichoderma* were tested for its efficacy against brown spot of rice.

MATERIALS AND METHODS

General Methods

Glassware was first soaked in chromic acid cleaning solution (10% potassium dichromate solution in 25% sulphuric acid) for a few hours and washed thoroughly in tap water. After a second wash in detergent solution, they were again washed thoroughly in tap water, rinsed in distilled water and air dried. Distilled water from Kilburn metal distillation unit was used for preparation of media. Double distilled water from an all glass Pyrex still was routinely used for other experimental purposes. Glassware, media and buffers were sterilized at 15 Ib pressure for 20 min.

Media

In the present study, Potato Dextrose Agar (PDA), Potato Dextrose Yeast Extract Agar (PDYEA) and Oat meal Agar (OMA) were used for characterization of *T. harzianum, T. viride* and *B. oryzae.*

Test pathogen

The test pathogen *B. oryzae* causing brown leaf spot were chosen as test pathogen. The phytopathogenic strain of *B. oryzae* were collected from infected paddy leaves (variety G98-135) in from Lesbeholden, Black Bush Polder (Region 6)

Guyana. Infected leaves were sterilized in mercuric chloride (0.01%) and placed onto PDA, to which streptomycin (50 µg/ mL) was added to suppress bacterial growth and was incubated at 20 °C for 3 days. Hundred agar blocks containing germlings of single conidia were picked up with a sterile needle under microscopic observation, transferred individually to PDA slants and incubated till sporulation. Antimicrobial agent *T. harzianum* and *T. viride* from 100g of soil were collected from Lesbeholden, Black Bush Polder (Region 6) in Guyana. 100 mL PDA was prepared and poured into four sterilized Petri plates. 10 g of the soil was taken and crushed using a mortar and pestle. One gram of the soil was then sprinkled in the medium containing Petri plates and left undisturbed for the growth of *Trichoderma* sp. After six days, 100 mL PDA was prepared and poured into four petri plates of which two were labeled A and B. After one week, the cultures were well grown and the physical and the morphological (identification of spores and conidia) features were taken into account. *T. harzianum* and *T. viride* were successfully identified. Sub cultures of *T. harzianum* abd *T. viride* were poured into the Petri plates labeled A (vertical line) and B (Horizondal line) respectively. This procedure was followed to obtain pure culture of the biological control agents.

Growth of the culture on different solid media

Hundred milliliter of PDA, PDYEA and OMA medium was prepared, poured into nine sterilized petri plates with 50 mg of antibiotic, Ampicillin in sterile conditions and one of well grown mycelium disc each of the cultures were inoculated into the Petri plates respectively. After three days the growth rate of the cultures in each medium was measured calculated and tabulated.

Antagonistic test

Simultaneously inoculated with 0.9 cm diameter of disc of *B. oryzae* and *T. harzianum and T.viride* were placed at opposite ends in PDA containing with 50 mg of Ampicillin Petri plates for antagonistic test. The main purpose of this method was to check the radial growth of the pathogen and biocontrol agent in order to determine whether the biocontrol agent has the capability to override and suppress the growth and development of the pathogen (Huang and Hoes, 1976). Using radial measurement method the growth of the cultures each day up to 10 days was measured.

Growth of the cultures in different pH

Two thousand and four hundred milliliter of PDB was prepared and poured into 24 conical flasks. One mL NHCl solution was used to lower the PDB pH to obtain a pH of 4.5, 5.5 and 6.5 and I N NaoH solution was used to obtain a pH of 7.5 of PDB. Then three discs of grown mycelium of each culture were inoculated in PDB to measure the mass weight. After six days, four conical flasks from each culture was taken, shaken and then filtered with the help of sterile cease clothe. The filtrate of fresh weight was measured and filtrate was placed in a hot air oven at 120°C for an hour and then the dry weight was measured and tabulated.

Growth of the cultures in different temperature

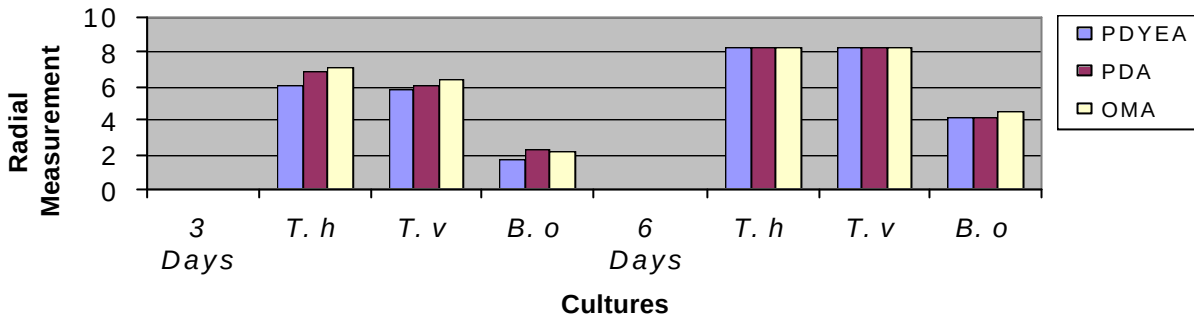
Two thousand and four hundred milliliter of PDB was prepared and poured into 24 conical flasks. Then three discs of grown mycelium of each culture were inoculated in PDB to measure the mass weight. The inoculated conical flasks were kept in different temperature such as 25°C in ice box, 28°C in air condition 31°C in water bath and 34°C in incubator. After six days, four conical flasks from each culture were taken, shaken and then filtered with the help of sterile cease clothe. The filtrate of fresh weight was measured and filtrate was placed in a hot air oven at 120°C for an hour and then the dry weight was measured and tabulated.

Pot experiment

Randomized Complete Block Design (RCBD) with three treatments (including control) replicated four times was used to conduct this experiment in the John's Science Center located in Berbice Campus. Twelve pots with a measurement of 61.5 cm in diameter and 14.1 cm in height were selected and pots were filled with clay loamy soil (pH 6.7). Then healthy and matured paddy seeds, G98-135, were selected and placed in the sun to dry for 2 days and soaked for 1 day. The soaked paddy seeds were removed from water and left for 3 days in a warm condition for germination. Well germinated paddy (15 to 20) seeds was sown in each of the pot and left for growth for six weeks.

After six weeks, *B. oryzae* spores suspension was prepared from well grown *B. oryzae* mother plate, which was mixed with 300 mL sterile distilled water and poured into a hand sprayer. The sprayed spore suspension was let into rice plants in each of the pots, which were covered with a poly bag. After two weeks, the average sizes of the brown spots symptoms on the rice leaves were measured. The *T. harzanium* and *T. viride* spores suspension was prepared from well grown mother petri plates, mixed with 300 ml sterile distilled water and was poured in different hand sprayers. *T. harzanium and T. viride* spore suspension was sprayed on all T1 and T2 pots respectively while T3 was devoid of spore suspension and the pot acted as a control for the experiment. After two weeks, infected rice leaves from five plants randomly from each pot were recorded and the size of fifteen spots from each of the five plants from each pot was measured and the average size were recorded.

Figure 1. Growth of *T. harzianum* (T.h), *T. viride* (T.V.) and *B. oryzae* (B.v) in different media of potato dextrose agar (PDA), potato dextrose yeast extract agar (PDYEA) and oat meal agar (OMA).



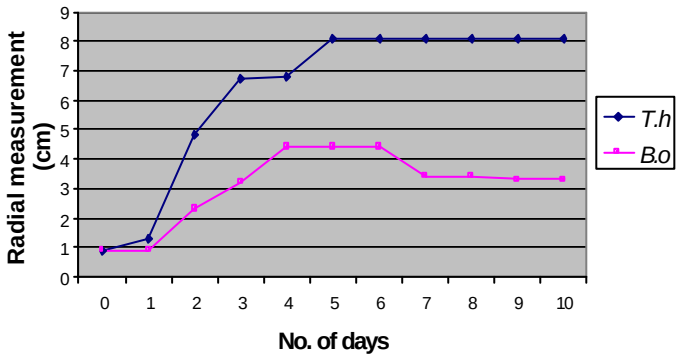
RESULTS AND DISCUSSION

Growth of cultures *T. harzianum*, *T. viride* and *B. oryzae* in different media on PDA, PDYEA and OMA, after three days rapid growth was observed in *T. harzianum* and *T. viride*, after six days *T. harzianum* and *T. viride* completed covered whole in Petri plate, however *B. oryzae* takes a long time to completely cover the full Petri plate (Fig. 1).

Also, with references to the pictures (1-3) taken, even though there was rapid growth observed on PDYEA, taking into consideration the light yellow discoloration on Petri plate with *T. harzianum* and *T. viride* and creamy discoloration on Petri plate with *B. oryzae*, only vegetative growth was observed. Vegetative spores also called imperfect stage of development, do not multiply and produce low quantity and quality of enzymes which are necessary for the defense mechanism in order to enable the microorganisms survive and establish themselves (Elad *et al*., 2007). On the other hand, Petri plate labeled 2 (PDA) and 3 (OMA) high percent of reproductive growth was observed.

Reproductive spores also called perfect stage of development, have the ability to multiply and produce high quantity and quality of enzymes needed for survival of the microorganisms

Figure 2a. Shows antagonistic test between *T. harzianum* (T.h) and *B. oryzae* (B.o).



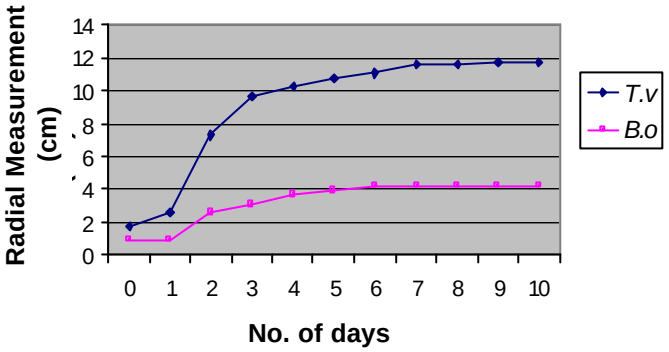
(Webster and Gunnell, 1992). In conclusion, PDA and OMA are media better suited for the growth and development of *T. harzianum*, *T. viride* and *B. oryzae*. At day 5, *T. harzianum* completely overrides the pathogen *B. oryzae* and 7 day because of secretion of lytic enzyme by the *T. harzianum* (Fig. 2a), development was ceased and the pathogen started to inhibited growth rate, finally completely destroyed pathogen *B. oryze*. The antagonistic test between *T. viride* and *B. oryzae* shows that growth rate of *T. viride* progress at a faster rate in contrast to *B. oryzae* (Fig. 2b). When compared to the activities of *T. harzianum* (Fig. 2a), *T. viride* did not override the pathogen within the 10 days that the experiment was conducted. However, on day 9, because of secretion of gliotoxin lytic enzymes by *T.viride* to control the growth of *B. oryzae* is not up to the expectation. Approximately 50 % of growth accords after 6 days except for *T. viride*. After 9 days, maximum growth was at pH 5.5 for *T. harzianum*, 6.5 for *T. viride* and 4.5 for *B. oryzae* (Fig. 3). After 9 days, maximum growth was recorded at 25°C for *T. harzianum*, 34°C for *T. viride* and 28°C for *B. oryzae* (Fig. 4).

Treatment 1 shows the less number of leaves infected while treatment 3 recorded most number of infected leaves. Although, the graph shows vase different between treatment 1 and 3 and 2 and 3, because of high level of variation among the treatments, there was no significant difference among the treatment means (Fig. 5a). Similarly, Fig. 5b shows that treatment 2 showed less number of infected spots while treatment 3 showed high levels of infected spots. When average means from each treatment was compared, no significant difference was reported between treatment 1 and 2. However, significant differences were reported between treatment 1 and 3 and 2 and 3.

CONCLUSION

T. harzanium and *T. viride* are potential biological control agents and after comparative studies, it can be concluded

Figure 2b. The antagonistic test between *T. viride* and *B. oryzae*



that there are significant differences between *T. harzanium* and *T. viride* in controlling brown spot disease in rice plants.

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Figure 3. Growth of *T. harzianum* (T.h), *T. viride* (T.v) and *B. oryzae* (B.o) in different pH such as 4.5, 5.5, 6.5 and 7.5.

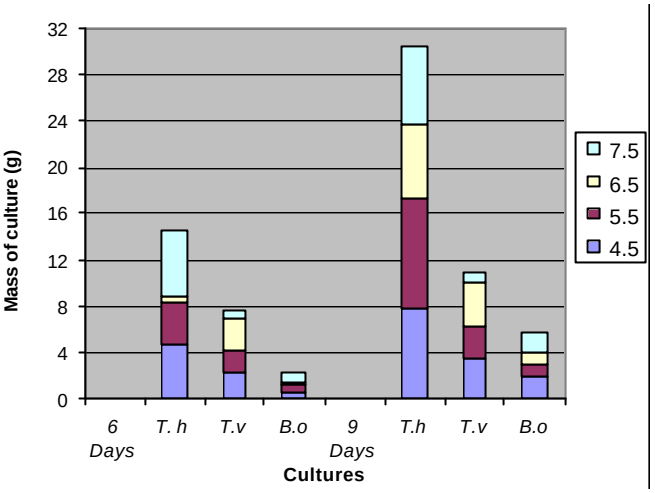


Figure 4. Growth of *T. harzianum* (T.h), *T. viride* (T.v) and *B. oryzae* (B.o) cultures in different temperatures such as 25°C, 28°C, 31°C and 34°C.

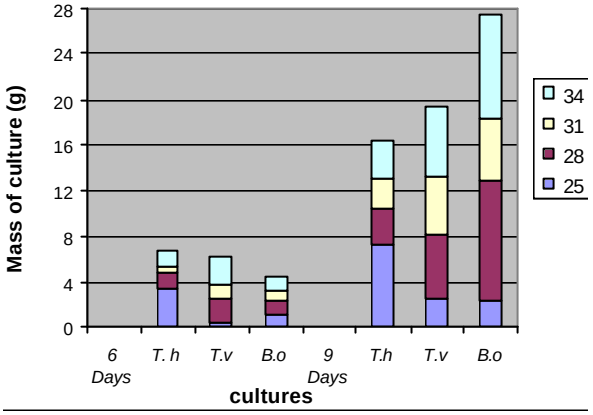


Figure 5a. Number of infected leaves from different treatments.

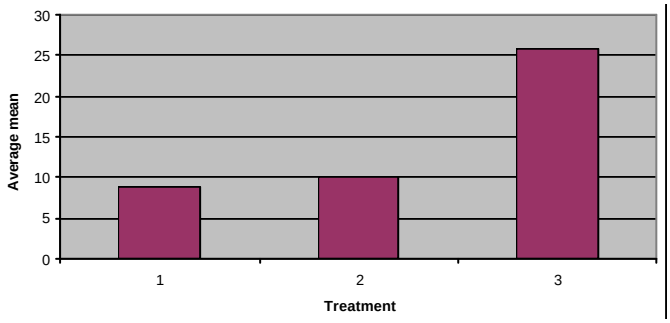
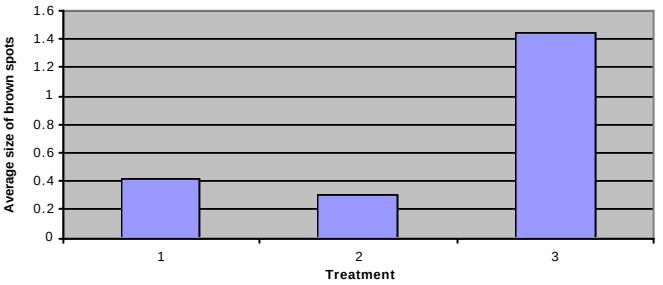


Figure 5b. Illustrates average size of brown spots from the different treatments.



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S. Gomathinayagam[1*], Sherena Amela Persaud[1], M. Rekha[2] and V. Shanmugaiah[3]

[1]Faculty of Agriculture and Forestry, University of Guyana, Berbice Campus, Tain, Guyana, South America

[2] Department of Biotechnology, Kalasalingam University, Krishnankovil, Tamil Nadu, India.

[3] Department of Microbial Biotechnology, Madurai Kamaraj University, Madurai, Tamil Nadu.

*E-mail: drgoms@rediffmail.com

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