CARIBBEAN FOOD CROPS SOCIETY

45

Forty Fifth

Annual Meeting 2009

Frigate Bay
Federation of St. Kitts and Nevis

Vol. XLV
Number 2

PROCEEDINGS

OF THE

45th ANNUAL MEETING

Caribbean Food Crops Society
45th Annual Meeting
July 12 to 17, 2009

St. Kitts Marriot Resort and Royal Beach Casino
Federation of St. Kitts and Nevis

"Reality and Potential of Food Security and Agricultural Diversification in Small Island Developing States"

"Realidad y Potencial de la Seguridad Alimentaria y la Diversificación Agrícola en Pequeños Estados Insulares en Desarollo"

"Securité alimentaire et diversification agricole dans les petits états insulaires en développement: realizations et perspectives"

Edited by Wanda I. Lugo and Wilfredo Colón

Published by the Caribbean Food Crops Society

ISSN 95-07-0410

Copies of this publication may be obtained from:

Secretariat, CFCS
P.O. Box 40108
San Juan, Puerto Rico 00940

Proceedings of the Caribbean Food Crops Society. 45: 79-86. 2009

BIOLOGICAL CONTROL OF RICE DISEASE (BLAST) BY USING *TRICHODERMA VIRIDE* IN LABORATORY CONDITIONS

S. Gomathinayagam[1], M. Rekha[2], S. Sakthivel Murugan[3] and R.C. Jagessar[4]. [1]Faculty of Agriculture and Forestry, University of Guyana, Berbice Campus, Guyana, South America.[2] Department of Biotechnology, PSR Engineering College, Sivakasi, Tamil Nadu, India.[3] Department of Chemistry, Indian Public School, Erode, Tamil Nadu, India. [4] Department of Chemistry, University of Guyana, Faculty of Natural Sciences, Turkeyen Campus, South America. Email: drgoms@rediffmail.com

ABSTRACT: The most common biological control agents of the genus *Trichoderma* have been reported as strains of *T. virens, T. harzianum*, and *T. viride*. Since *Trichoderma* biological control agent use different mechanisms of biocontrol, it is important to explore the synergistic effects expressed by different genotypes for their practical use in agriculture (Hemosa et al., 2000). *Trichoderma* species have been investigated as biological control agents for over 70 years, but it is only recently that strains have become commercially available. This finding is largely a result of the change in public attitude towards the use of chemical pesticides and fumigants such as methyl bromide. *Trichoderma viride* is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which includes competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds (Harman et al., 1993). Rice disease blast is caused by *Pyricularia oryzae*. Blast disease initial symptoms are white to gray-green lesions or spots with darkened borders produced on all parts of shoot; older lesions are elliptical or spindle-shaped and whitish gray with necrotic borders. Lesions are wide in the centre and pointed toward either end; lesions may enlarge and coalesce to kill entire leaves. The objective of this study is to control blast disease in rice. The study is to be conducted in the Faculty of Agriculture and Forestry, University of Guyana, at Berbice Campus, Science Centre, John, from February 2009 to May 2009. The experiments are designed in a completely random block design with three replicates. Parameters to be observed are selection of medium, temperature, pH, and measurement of disease index and disease severity randomly. The studies are to show the potential of *T. viride* for control of blast disease in rice.

Keywords**:** Blast, Biological control, *Pyricularia oryzae, Trichoderma viride*, Laboratory conditions

INTRODUCTION

Rice is of vital importance to Guyana and is a staple in the diet of the Guyanese people. It is estimated that approximately 20% of the population depend directly and indirectly on the rice industry for their livelihoods (GRDB letter, 2008). One of the most serious rice diseases in the rice sector is fungal disease infection. It results in poor production, poor quality, poor milling returns and reduced income. This fungal infection 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, which are hazardous and toxic to both people and domestic animals. This toxicity 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 eco-friendly approach. Use of natural enemies to control disease is termed biological control. 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).

With the knowledge of the adverse effects of synthetic fungicides worldwide, attention is rapidly shifting to non-synthetic, safer alternatives. *Trichoderma viride* is known for its mycoparasitic and antagonistic mechanism for the control of fungal disease. They could be environmentally safe alternatives to synthetic fungicide. However, in Guyana no attempt has been made to use the above-mentioned fungus in the control of paddy disease. Thus, in this experiment we propose to test the mycoparasitic effect of *Trichoderma viride* on paddy disease (blast).

Trichoderma spp. are fungi that are present in substantial numbers in nearly all agricultural soils and in other environments such as decaying wood (Harman et al., 1993). They grow tropically towards hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by a process called mycoparasitism (Harman et al., 1993). This process limits growth and activity of plant pathogenic fungi. The antifungal abilities of these beneficial microbes have been known since the 1930s and there have been extensive efforts to use them for plant disease control. The soil fungus *Trichoderma harzianum* has shown to act as a mycoparasite against a range of economically important aerial- and soil-borne pathogens. (Carsolio et al., 1994)

Microscopic observation suggests that *Trichoderma harzianum* produces and excretes mycolytic enzymes which lysis cell wall at point of interaction (Carsolio et al., 1994). The 1,3-ß-glucanases, chitinases and protease produced extracellularly by *Trichoderma harzianum* play an important role in biocontrol (Carsolio et al., 1994). A preparation of *Trichoderma harzianum* was sprayed on cucumber plants in greenhouses in order to control fruit and stem grey mould (*Botrytis cinerea*) and resulted in a 90% control (Elad and Kapat, 1999).

This biocontrol agent (BCA) controls the foliar pathogens *Botrytis cinerea*, *Pseuperonospora cubensis*, *Sclerotinia sclerotiorum* and *Sphaerotheca fusca* (syn. *S. fuliginea*) in cucumber under commercial greenhouse conditions (Elad, 2000).

Trichoderma harzianum
Scientific classification

Kingdom:	Fungi
Division:	Ascomycota
Subdivision:	Pezizomycotina
Class:	Sordariomycetes
Order:	Hypocreales
Family:	Hypocreaceae
Genus:	*Trichoderma*
Species:	*T. viride*

Trichoderma viride is a saprophytic fungus which is used as a biological control agent against a wide range of economically important aerial- and soil-borne plant pathogens. The mycoparasitic activity of *Trichoderma* spp. may be due to antibiosis, competition, production of cell wall-degrading enzymes, or a combination of these antagonistic activities.

Trichoderma viride is an antagonist of soil-borne fungal pathogens. The antagonistic process starts with physical contact. *Trichoderma viride* secretes a number of cell wall-degrading enzymes and antibiotics. These cell wall-degrading enzymes include ß-1,3-glucanase, chitinases and proteinases. These enzymes and antibiotics provide a synergistic effect on their hosts. The enzyme weakens the host cell wall and increases the rate of diffusion of the

antibiotics through the cell wall. Upon physical contact the *T. viride* coils around its host where it proceeds with invasive growth. The host hyphae collapse because of loss of turgor pressure.

Blast

Blast is a foliar disease attacking above-ground plant parts. Blast is often considered the most serious rice disease because it spreads rapidly and is highly destructive under favorable conditions. It may infect plants at any stage of growth. Depending on the part of the plant affected, the disease is often called leaf blast, rotten neck or panicle blast. Two phases of the disease are most pronounced: leaf blast, which occurs from infection between the seedling and maximum tillering stages, and panicle blast, which occurs from infection after flowering.

Leaf blast stunts the plants and reduces the number of mature panicles, the weight of individual grains, and the weight and quality of brown rice.

Causal Agent

Blast is caused by the fungus *Pyricularia oryzae (Magnporethera grisae).* The conidia of *Pyricularia oryzae* germinate in four hours and invade the host cells in eight to 10 hours when conditions are favorable. Lesions begin to appear in four days and a crop of spores is produced in six to seven days. A large lesion produces 2,000 to 6,000 conidia each night for about two weeks. A small lesion produces 50 to 300 conidia for about five to seven days. Most conidia are released at night in the presence of dew or rain. The disease cycle is short, and most damage is caused by secondary infections.

Symptoms

The fungus produces spots or lesions on leaves, nodes, panicles, collars of the flag leaves and the grains. Leaf lesions are elliptical and somewhat tapered and pointed at both ends and run parallel to the long axis of the leaf or stem. The center of the spot is usually gray or whitish, and the margin brown or reddish-brown. Both the shape and colour of the spots vary, however, depending on the environmental conditions, age of spots and varietal susceptibility.

The blast fungus frequently attacks the node at the base of the panicle and the branches of the panicle. Most damage occurs when the fungus spreads to the area below the seed head of the plant, causing it to break off (rotten neck). Otherwise, the disease prevents the maturation of the rice grains (panicle blast). If the panicle is attacked early in its development, the grain on the lower portion on the panicle may be blank, giving the head a bleached whitish color, thus giving the term “blasted” head or rice “blast”. If the node at the base of the panicle is infected, the panicle breaks causing the “rotten neck” condition (Ou, 1973).

Spread of the Disease

Blast generally occurs scattered through the field. One feature of *Pyricularia oryzae* is that it generates spores, called conidia or conidiospores. Spores of the fungus are produced in great abundance on blast lesions and become airborne and easily disseminated by wind and splashing rain.

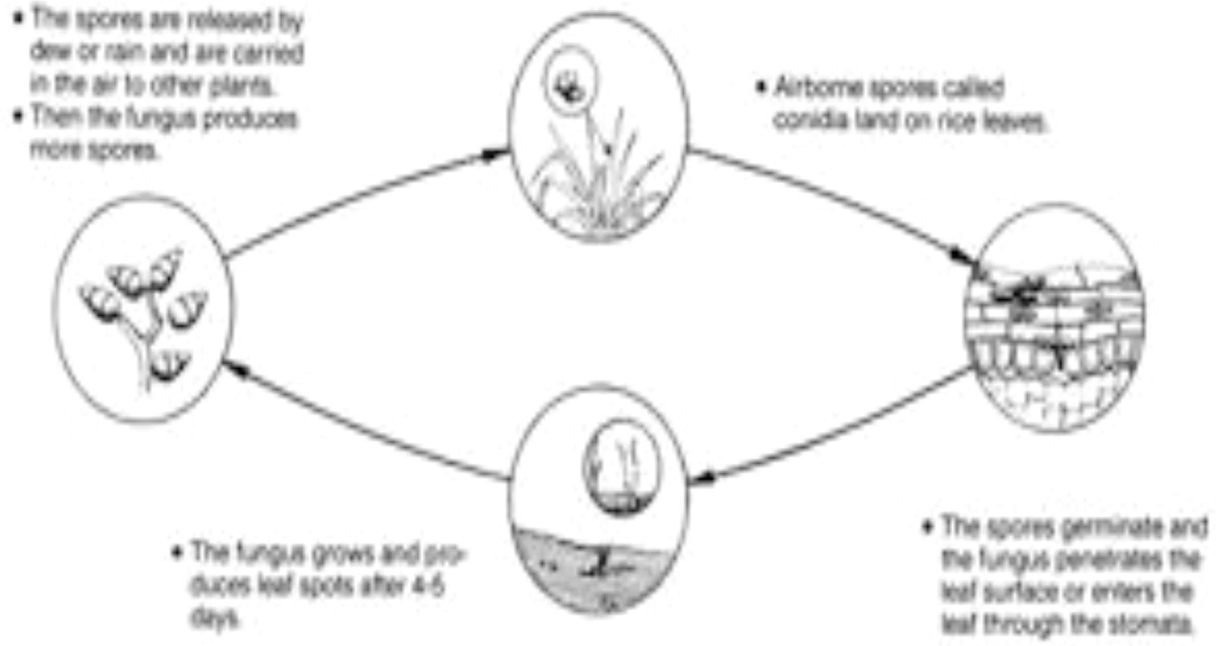


Figure 1. The life cycle of blast disease (Suparyono et al., 2003).

MATERIALS AND METHODS

Selection and sterilization of Glassware

Selection and sterilization of glassware was done for the purpose of making the appropriate media for the culturing and growth of the pathogens, *Pyricularia oryzae* and *Trichoderma harzianum*. The glassware was thoroughly cleaned by soaking in chromic acid for 12 hours and then rinsed repeatedly with distilled water. Petri plates were swabbed with methylated spirit and placed in an oven at a temperature of 120º for 20 minutes and then placed under the lamina flow for another 20 minutes.

Selection of the medium for the growth of the pathogens and *Trichoderma viride*

The following media were selected; 500 ml of each medium was prepared and used for these studies.

Potato Dextrose Agar (PDA)
Potato Dextrose Yeast Extract Agar (PAYEA)
Oatmeal Agar (OMA)

Collection of pathogens and soil sample

Pathogens were collected from infected rice plants in Black Bush Polder (Region 6) Guyana, South America.

Collection of soil sample

1. Five areas were identified randomly in the rice field, the four corners and the centre of the field.
2. About 3 cm^3 of the top soil was removed and 1 g of soil sample was taken from the identified areas in the field.
3. The soil sample was air dried, ground into a powder form and mixed thoroughly to make a composite sample for the isolation of *Trichoderma viride*.
4. The above soil samples were taken from the rice field in Black Bush Polder (Region 6) Guyana, South America.

Isolation and characterization of *Trichoderma viride* from soil

Standared serial dilution method

Seven 9 ml sterile water blanks were labeled as 1, 2, 3, 4, 5, 6 and 7, and sterile Petri dishes as 10^{-2},10^{-4}, 10^{-6}, 0.1 g of finely pulverized, air-dried soil was added into number one water blank to make a 1:10 dilution (10^{-1}). The dilution was vigorously shaken to obtain a uniform suspension of microorganisms. One milliliter of suspension was transferred from test tube numbered 1 into water blank numbered 2 with a sterile pipette under aseptic conditions to make 1:100 (10^{-2}) dilution, which was shaken well. Another dilution was prepared 1:1000 (10^{-3}) by pipetting 1 ml of suspension into water blank numbered 3, using a fresh sterile pipette, and then shaken. Further dilutions were made 10^{-4} to 10^{-7} by pipetting 1 ml of the suspension into water blanks (4, 5, 6, and 7) as prepared above. One milliliter of aliquots from each diluted water blank was transferred into each sterile cool medium Petri dish for inoculation. All plates were incubated at 25º C for three to six days.

Surface sterilization of infected paddy leaves

The infected rice plant was washed under running tap water. The infected areas were cut into small pieces and placed in sterile water for five minutes, then transferred to 0.1% mercury chloride for three to four minutes. It was then rinsed with sterile water to remove the mercury chloride.

Isolation of pathogens

Fifty milligrams of antibiotic was added to100 ml potato dextrose agar and shaken to distribute evenly. The potato dextrose agar was poured into the six Petri plates under the laminar flow and allowed to solidify. Three pieces of sterilized infected rice leaf were inoculated with the medium. Pathogen growth was observed over a six-day period.

Identification of the pathogens

Samples from the "mother" plates were taken and slides were prepared to view under the microscope for the identification of pathogens, according to text book of Compendium Soil Fungi (K. H. Domsch et al., 1980)

Single spore isolation using water agar method

Hindered milliliter water was placed to boil and 2 g of agar was stirred into boiling water, Water agar was poured into sterile Petri plates and allowed to solidify. Pathogen spores were

collected from mother plate and suspended in liquid water agar medium and then poured into sterile Petri plates for solidification. Petri plates were placed in an inverted position to observe under the microscope.

Antagonistic test (Dual Culture Techniques) (Huang and Hoes, 1976)

Plates with PDA were simultaneously inoculated with 9-mm discs of the pathogens as well as with the antagonist near the periphery at diametrically opposite points. Periodic observations were made at different intervals on the radial growth of antagonist and pathogens.

Radial measurement of pathogen

Pathogen radial measurement was taken against biological control agent (*Trichoderma viride)* by using standard scaling method.

RESULTS AND DISCUSSION

Pyricularia oryzae is a fungal pathogen that causes blast on paddy. These pathogens were collected from the infected rice plants in the location of Black Bush Polder (Region 6) Guyana, South America (Table 1).

Table 1. Test pathogens

S.No	Organism	Place of collection	Disease
1	*Pyricularia oryzae*	Black Bush Polder (Region 6), Guyana, South America	Blast
2	*Trichoderma viride*	Black Bush Polder (Region 6), Guyana, South America	Nil

Table 2: Selection of media for test cultures

Cultures	PDA (cm)*	PDYEA (cm)*	OMA (cm)*
Trichoderma viride	8.5	8.2	7.2
Pyricularia oryzae	8.5	8.6	7.3

* Mean value

Table 2 illustrates the growth of *Trichoderma viride and Pyricularia oryzae* on three different media –potato dextrose agar, potato dextrose yeast extract agar, and oatmeal agar. From the results obtained it can be seen that there is significant growth of cultures *Trichoderma viride* and *Pyricularia oryzae* on PDA, indicated by 8.5 cm, followed by PDYEA 8.2 cm, and by OMA 7.2 cm, and by 8.5 PDA, by 8.6 PDYEA, and by 7.3 OMA, respectively.

Table 3: Growth of cultures with different pH

Cultures	pH (mycelial weight mg)*			
	4.5	5.5	6.5	7.5
Trichoderma viride	0.6	1.7	2.9	3.9
Pyricularia oryzae	0.9	1.7	2.8	3.4

* Mean value

Table 3 illustrates the growth of *Trichoderma viride* and *Pyricularia oryzae* on different pH (4.5, 5.5, 6.5 and 7.5). From the results obtained it can be seen that there is significant growth of cultures of *Trichoderma viride* on pH 7.5, which is indicated by 3.9 mg; followed by pH 6.5 with 2.9 mg; pH 5.5 with 1.7 mg; and pH 4.5 with 0.6; and of *Pyricularia oryzae* with 3.4 mg in pH 7.5; 2.8 mg in pH 6.5; 1.7 mg in pH 5.5; and 0.9 mg in pH 4.5.

Table 4: Growth of cultures in different temperature

Cultures	Temperature (mycelial weight mg)*			
	25° C	28° C	31° C	34° C
Trichoderma viride	1.5	2.9	4.6	4.3
Pyricularia oryzae	1.2	2.8	3.9	3.7

* Mean value

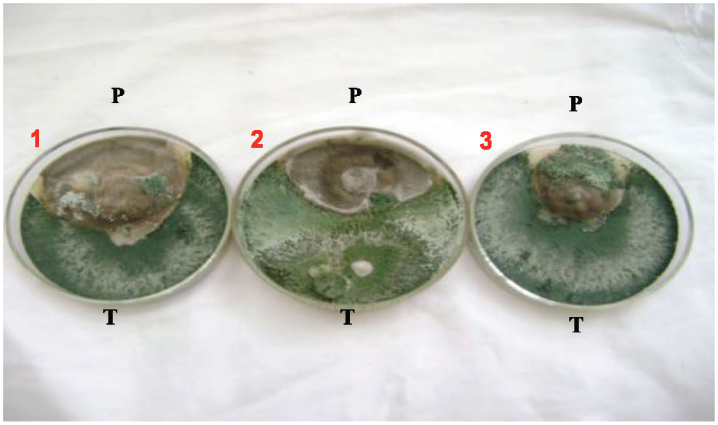
Table 4 illustrates the growth of *Trichoderma viride* and *Pyricularia oryzae* at different temperatures (25° C, 28° C, 31° C and 34° C). From the results obtained, it can be seen that there is significant growth of cultures of *Trichoderma viride* at 31° C, indicated by 4.6 mg; at 34° C, 4.3 mg; at 28° C, 2.9 mg; and at 25° C, 1.5 mg; and of *Pyricularia oryzae* with 3.9 mg at 31° C; 3.7 mg at 34° C; 2.8 mg at 28° C, and 1.2 mg at 25° C.

Table 5: Radial measurement of pathogen against *Trichoderma viride*

Culture	Pathogen	Growth in cm*
Trichoderma viride	*Pyricularia oryzae*	3.5

*Mean value

Fig 1. Antagonistic activity of *Trichoderma viride* against pathogen (*Pyricularia oryzae*)



T- *Trichoderma viride*
P- *Pyricularia oryzae*

1. Three days culture
2. Six days culture
3. Nine days culture

The photograph (Figure 1) shows the antagonistic growth of the biocontrol agent *Trichoderma viride* against pathogen *Pyricularia oryzae*. The biocontrol agent overrides the pathogen and releases lystic enzymes over the pathogen and inhibits the growth of pathogen and eventually completely kills the pathogen.

CONCLUSION

Trichoderma viride demonstrates a powerful antagonistic behavior in the control of the rice disease blast pathogen. It can be concluded that *Trichoderma viride* is an effective biological control agent for rice blast disease.

RECOMMENDATION

This research is in the preliminary stage; hence field study is encouraged. Therefore, relevant research agricultural institutions are encouraged to carry out similar and more extensive research in this area to combat fungal infection not only in rice but also in other agricultural crops. *Trichoderma viride* should be used as part of an integrated approach in the control of plant diseases since it demonstrates an antagonistic behavior.

REFERENCES

Carsolio, C.A., B. Gutierrez, M. Jimenez, V. Montagu, and H. Estrella. 1994. Characterization of ech-42, a *Trichoderma harzianum* endochitinase gen expressed during mycoparasitism. Proc. Nath. Acad. Sci. USA. 91: 10903-10907.

Domsch, K.H., W. Gams, and T.H. Anderson. 1980. Compendium of Soil Fungi. Vol. 1. Academic Press (London) Ltd. 24/28, Oval Road, London NWI, 795-809

Elad, Y. and A. Kapat. 1999. The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea.* Eur. J. Plant Pathol. 105: 177-189

Elad, Y. 2000. Botrytis: Biology, Pathology and Control: Kluwer Academic Publisher, Printed in The Netherlands, 181-194

Guyana Rice Development Board (GRDB) newsletter, 2008, 25-29

Harman, D.E., C.K. Hayes, M. Lorito, R.M. Broadway, A. Dipietro, C. Peterbauer, and A. Tronsmo. 1993. Chitinolytic enzymes of *Trichoderma harzianum*: purification of chitobioidase and endocitinase. Phytopathology 83: 313-318.

Haran, S., H. Schickler, and I. Chet. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum.* Microbiology 142:2321-2331.

Hermosa, M.R., I. Grondona, E.A. Iturriaga, J.M. Díaz-Minguez, C. Castroc, E. Monte, and I. García-Acha. 2000. Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. Appl. Environ. Microbiology, 66: 1890-1898.

Kubicek, C.P. and G.E. Harman. 1998. *Trichoderma and Gliocladium. Vol. 1. Basic Biology, Taxonomy and Genetics*, Taylor & Francis, London. 278 pg.

Ou, S.H. 1973. Hand book of rice disease in the tropic. International Rice Research Institute, Los Baños, Laguna, Philippines. Press New York.

Roco, A., and L.M. Pérez. 2001. "*In vitro* Bicontrol activity of *Trichoderma harzianum* on *Alternaria alternata* in the presence of growth regulators", Universidad Católica de Valparaíso, Journal Vol.4 No.2, 345-8.

Suparyono, J.L.A. Catindig, and I.P. Ona. 2003. Rice Doctor, International Rice Research Institute, Philippines, 1-4. www.eps.edu.com.