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Full Length Research Paper

Antifungal studies of *trichoderma ghanense* (ng t.162), *trichoderma longibrachiatum* (NG T.167) and *trichoderma asperellum* (NG T.163) for biocontrol of rice blast.

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Studies on the potential of three *Trichoderma* spp. (*ghanense, longibrachiatrud* and *asperellum*) as biological control agents of rice blast (*Magnaporthe grisea)* pathogen populations were undertaken in Ibadan, southwest Nigeria during 2009 wet season. Two blast susceptible varieties of Rice seed used in the study were selected because they are widely cultivated by farmers in southwest Nigeria. Rapid colonization growth of the *Trichoderma* broth were observed at four different dilutions(1:1,1:2,1:3,1:4) at lowest concentration of 20ml/250ml of water the inhibition of *Magnaporthe grisea* was significant on the field and in the laboratory. The results of all these experiments showed that each of the *Trichoderma* species is a potential biological control agent of rice blast. However, the best result was obtained when sprayed before blast infection (that is, applied 21 days after planting) and at the initial stages of blast manifestation.

Keywords: Three *Trichoderma spp. ghanense, longibrachiatrud and asperellum*, biological control agents, rice blast,

INTRODUCTION

The terms "biological control" and its abbreviated synonym "biocontrol" have been used in different fields of biology, to describe the use of live or microbial pathogens in suppressing populations of different pest insects. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations. In both fields, the organism that suppresses the pest or pathogen is referred to as the Biological Control Agent (BCA). More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources (Te Beest et al., 2007).

Trichoderma species are free-living fungi that are common in soil and root ecosystems. Discoveries show that they are parasite, of other fungi. *Trichoderma* is one of the major fungal preparations of many microbes that are commercially available as biological control agents to protect plants against other fungi. The mechanisms used by *Trichoderma* include the following: The mechanisms used by bio control agents, specifically also by *Trichoderma* include the production of chitinase (Sivan and Chet, 1989) and production of AFMs (antifungal metabolites), such as Phl (2,4-diacetyl phloroglucinol) (Keel et al., 1990; Pierson et al., 1995; Bangera and Thomashow, 1996; Chin-A-Woeng et al., 1998; Delaney et al., 2001), *zwittermycin* (Stohl et al., 1996), pyoluteorin (Nowak-Thompson et al., 1999), and pyrrolnitrin (Kirner et al., 1998).

Rice blast

The objective of this study therefore is to evaluate the efficacy of *Trichoderma ghanense* (NG T.162), *Trichoderma longibrachiatum* (NG T.167) and *Trichoderma asperellum* (NG T.163) as effective and efficient biocontrol agents. Also the determination of the appropriate dose/concentration of *Trichoderma* spp. for biocontrol of rice blast is done

MATERIALS AND METHODS

This study was carried out in the Nematology Laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Isolation and purification of rice blast fungus used in this study was obtained from AfricaRice, Ibadan, while *Trichoderma* spp. used were collected from CABI, UK, by IITA, Nigeria. (Plate 3).

Tryptone Soy broth.(21 grammes) was dissolved in 700ml of sterile distilled water and dispensed in 20ml quantities into 10 test tubes, after which 2 loopful of pure *Trichoderma strains* were inoculated into the test tubes and incubated for 5 days(plate 4) , uninoculated tubes served as control. Blast infected leaves were cut and dipped in 10% sodium hypochlorate for surface sterilization, rinsed in sterile distilled water and blot-dried by using sterile blotting paper, after which they were placed in Potatoes Dextrose Agar (P.D.A.) incubated at 28^0C, for 5 days .

In Vitro determination of inhibitory activities of crude extracts of *Trichoderma* spp. on rice blast pathogen

One to four -fold serial dilutions of crude extract of *Trichoderma. spp* were prepared with sterile distilled water assayed by agar well – diffusion method against blast pathogens and incubated at 28^0C for 21 days. Experiment was in triplicates.

For dilutions (1.1, 1.2, 1.3, 1.4) of each *T. spps* was prepared 0.5mm cock borer was used.

Agar-well diffusion method

Agar was poured into Petri dishes. After solidification test strains were inoculated in the media separately. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5mm).The extract compound was introduced into the well and plates were incubated all samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition. The metabolite of the three *Trichoderma spps* were measured using spectrophotometer at 630 nanometer wavelength

Detection of Fungicidal/Fungistatic Activity (*In vitro*)

The inhibition zones of *Trichoderma spp* against *M. oryzae* were cut using cork borer (size5mm), transferred to uninoculated PDA plates and incubated for 7 days at 26^0–28^0C.

Determination of Shelf Life or Stability of Active Crude extracts:

The stability of active crude extracts in soluble state was investigated. Samples of *Trichoderma spp* were prepared in tubes, kept at room temperature for 28 days and tested using agar well- diffusion method for anti *M. oryzae* activity at 7 days intervals. After 28 days at room temperature biodegradation/decomposition set-in which resulted in bad/foul odour? This became unbearable to withstand, during handling.

Absorbance of crude extracts of *Trichoderma ghanense*, *Trichoderma longibrachiatum* and *Trichoderma asperellum* were 0.104,0.78 and 0.73 at 630 wavelength, while *Trichoderma asperellum* had lowest value at 10^{-4} dilution factor Table 4 and Figure 1 below

Determination of thermal inactivation point (TIP)

One ml of each soluble *Trichoderma spp.* Crude extract was exposed to the following temperatures 30^0, 40^0, 50^0, 60^0, 70^0, 80^0, 90^0C for 10 minutes and later cooled in ice. The effects of these temperatures on bioactivity were monitored by agar well-diffusion method, while control incubation of untreated sample was at 29^0C.

RESULTS AND DISCUSSION

Colony spores 5320, 4560 and 5020 in 0.2ml were obtained from *Trichoderma ghanense*, *Trichoderma longibrachiatum* and *Trichoderma asperellum* respectively.

The Crude extracts of the three *Trichoderma. Spp*. showed strong inhibitory activities on rice Blast pathogens for all the dilutions. This is in accordance with the work of Harman, 2006 where strains of *Trichoderma harzianum*, *viride* and *hamatum* have been developed as biocontrol agents against fungal diseases of plants. This present work demonstrates that each of these *Trichoderma ghanense*, *Trichoderma longibrachiatum* and *Trichoderma asperellum* can serve same purpose.

Table I: In *vitro* inhibition growth rates (mm) of *Trichoderma spp*. on rice blast pathogens

Trichoderma spps	**(mm diameter)**		
	1	2	3
A	8.0	7.0	6.0
B	8.0	8.0	8.0
C	9.0	9.0	8.0

Table 2: The average result Triplicate of one to four fold dilutions of *Trichoderma ghanense*, *Trichoderma longibrachiatum* and *Trichoderma asperellum*

Dilution Factors	**Zones of Inhibition (mm diameters)**		
	1	2	3
10-1	7.8	7.6	7.4
10-2	7.0	7.6	8.7
10-3	7.8	7.5	7.2
10-4	8.1	9.0	7.0

Table 3: Shelf life/stability of active crude at room temperature

DAYS	**A**	**B**	**C**
14	+ve	+ve	+ve
28	+ve	+ve	+ve
42	+ve	+ve	+ve

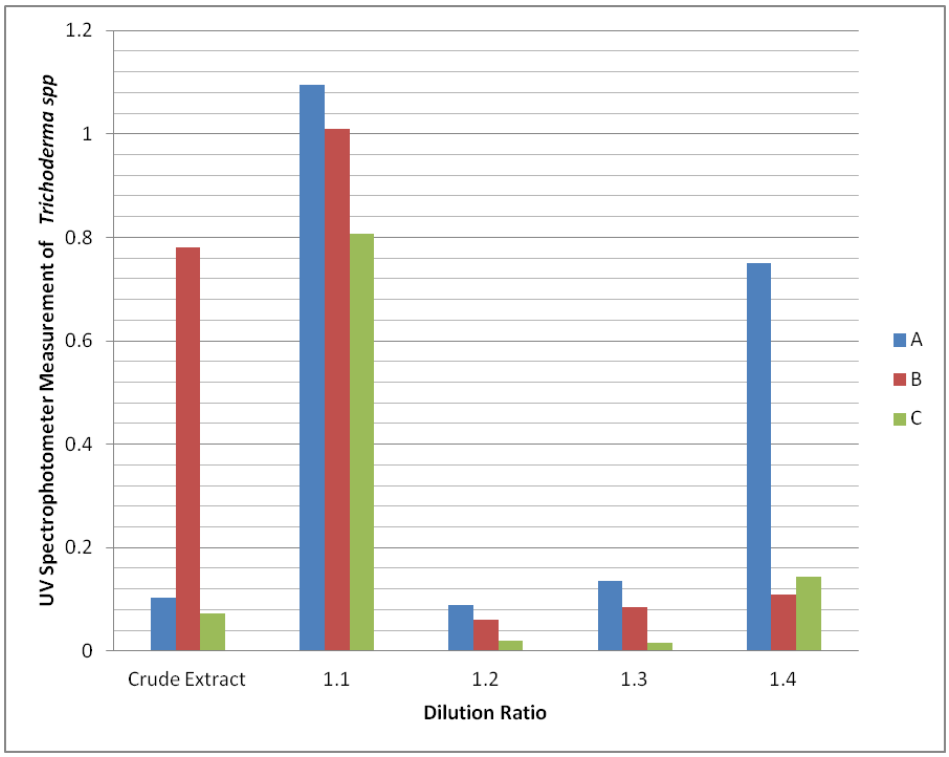
+ve = bioactive
-ve = non-active

Key:
A= *Trichoderma ghanense*,
B=*Trichoderma longibrachiatum*
C= *Trichoderma asperellum*

Table 4: UV Spectrophotometer results of *Trichoderma. Spp.*

	Crude	1:1	1:2	1:3	1:4
A	0.104	1.095	0.089	0.135	0.75
B	0.78	1.009	0.060	0.084	0.110
C	0.073	0.806	0.021	0.016	0.143

630 wavelength nanometer
- 0.04 absorbance
A = log (% T./100)
% Transmission concentration



Keys:

A= *Trichoderma ghanense*,
B=*Trichoderma longibrachiatum*
C= *Trichoderma asperellum*

Table 5: Thermal Inactivation point of *Trichoderma ghanense*, *Trichoderma longibrachiatum* and *Trichoderma asperellum*

Temp ^{o}C	A	B	C
20	+ve	+ve	+ve
30	+ve	+ve	+ve
40	+ve	+ve	+ve
50	+ve	+ve	+ve
60	-ve	-ve	-ve
70	-ve	-ve	-ve
80	-ve	-ve	-ve
90	-ve	-ve	-ve

+ve = bioactive
-ve = non-active

Keys:
A= *Trichoderma ghanense*,
B=*Trichoderma longibrachiatum*
C= *Trichoderma asperellum*

Shelf life or stability of active crude

The three *Trichoderma. spp*. showed biological activities when kept at room temperatures for 42 days, but after 28 days decomposition began which resulted in bad/ foul odour which became difficult to withstand during handling. This confirms the work of Sivan and Chet,1989 that some species of *Trichoderma* produce foul odour.

Thermal inactivation point

Each of these *Trichoderma. spp*. showed biological

activities and viability at temperatures ranging between 20-40 $^{0C.}$ This is a good indication of the stability during storage and temperature increase that result during transportation especially in rural areas where access to electricity or refrigeration is not available. *Trichoderma. spp*. were not viable even after they were cooled in ice-cubes, which suggest that all the spore died when exposed to higher temperatures.

Fungicidal/fungistatic activities

When 0.5mm blocks of inhibition zones of *Trichoderma. spps* were transferred to fresh P.DA plate against *M. oryzae* (blast)and incubated for 7 days at 28^{0}C, It was observed that, *Trichoderma. spp*. grew faster than *M. oryzae*, visually and microscopically. It was observed that *M. oryzae* did not grow in the presence of *Trichoderma spp*, this suggests fungicidal property on the part of *Trichoderma spp.*

Rapid growth of the three *Trichoderma* spp. colonized *M. oryzae* surface and substrate completely overgrew on the coming of the pathogen. There is production of some pigments. The *Trichoderma* spp. isolates reduced the pathogen colony growth in laboratory experiments and prevent disease in screen house. These results suggest that metabolites (antibiotics and hydrohps enzymes) of *Trichoderma*, are very important in the prevention of disease severity. This confirms that competent strains of *Trichoderma* spp. can completely colonize surface for a few weeks or months and protect plant from invading pathogenic fungi (Thrane et al., 2000; Harman, 2006). In future, it will be possible to select the confirmed *Trichoderma* strains/isolates with high biocontrol capacity and the preparation of more effective formulations to combat plants pathogens.

CONCLUSION AND RECOMMENDATIONS

These Results of *in-vitro* studies of the potential of *Trichoderma spp.* as a biocontrol agent of rice blast (*Magnapothe Oryzae*) in upland rice suggests its adoption and its recommendation for use on this rice ecology.

The mean temperature throughout the experiment was 27^{oC} and the relative humidity was 69 %, which show that, and even at higher temperatures, these *Trichoderma spp.* will still be very active against *M. Oryzae*.

Metabolite and spore formulation of these *Trichoderma spp*, the dilution of 20mls of crude sample of *Trichoderma spp.* in 250ml of sterile distilled water used in this study, notwithstanding, these *Trichoderma spp*. can still be very active in 500ml of S.D.W. and the activity may remain the same. The presence of antifungal metabolite against *M. Oryzae* in all these *Trichoderma spp* highlight their candidacies for further investigation in biological control of the world-wide destructive rice Blast disease.

The result of this finding can form basis for production of *Trichoderma spp.* isolate in vials against rice blast.

There can also be production of resistant transgenic – plants with recombinant DNA having antifungal genes cloned from biologically active *Trichoderma spp.* isolates which would lead to environmentally safer measures in plant-fungal disease management.

The preparation of any of these *Trichoderma spp.* in vials will be one of the cheapest biological methods controlling and preventing rice blast, if developed, because it is water soluble, and vials of any of these preparation, diluted in water and sprayed on plant will be affordable to low income farmers in Nigeria and parts of world.

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