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Full Length Research Paper

Interactions between environmental factors and *Magnaporthe grisea* in the management of rice blast disease

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Studies on the potential of *Trichoderma* .(*ghanense, longibrachiatum* and *asperellum*)as biological control agents of rice blast (*Magnaporthe grisea)* pathogen populations were undertaken in Ibadan, southwest Nigeria during 2009 wet season. Widely cultivated susceptible (cisadine and wita4) rice were used for this study. The influence of rainfall, wind speed, temperature, evaporation, radiation and relative humidity on the interaction between *Trichoderma spp*. and blast fungal disease were also measured. Treatments, Experimental design, data collected at lowest concentration of 20ml each crude of *T. spps*/250ml, the inhibition of blast fungi was significant on the field, 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: *Trichoderma* spp. blast, abiotic factors, biological control agent.

INTRODUCTION

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts (Harman et al., 2004) and many of them have been used as biocontrol agents.

Trichoderma is one of the major preparations of many microbes that are commercially available as biological control agents to protect plants against fungi. 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 is widespread in tropical West Africa. Incidences of the disease were first reported in the 1930s when the disease was known to affect seedling in Sierra Leone but was of negligible importance then. However, with the expansion and intensification of rice cultivation, it is now the most serious and destructive fungal disease of rice in the West African sub-region (Notteghem and Baudin, 1981). Upland/hydromorphic rice constitutes more than 60% of the area cultivated to rice in West Africa. Rice blast is most common and destructive disease in the upland environments and it is particularly so in newly cleared areas (Raymundo and Fomba, 1979; Thrane et al., 2000). Upland rice fields are usually contiguous parcels interspersed with bushes which serve as windbreaks thus promoting longer retention of water droplets from rain, drizzle and dew.

This study investigated the performance of three *Trichoderma* species as biocontrol agents in rice blast and the influence of abiotic factors on the relationship.

MATERIALS AND METHODS

Experiments were carried out under screen house conditions in the Nematology Laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The rice blast fungus inoculum used in this study was obtained from AfricaRice, field, screen house and Laboratory in Cotonu and Ibadan, while *Trichoderma* spp. used were collected from CABI, UK.

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 , uninoculated tubes served as control.

Isolation of the pathogen

Blast infected leaves were cut and dipped in 1% sodium hypochlorate for surface sterilization, rinsed in sterile distilled water and blot-dried by using sterile blotting paper, after which they were placed in Potato Dextrose Agar and incubated at 28^0C, for 5 days .

Preparation of inoculums: 20 ml of each *Trichoderma spp* cultured in test tubes were dissolved in 250 ml of sterile distilled water, and fine-sprayed on rice seedlings two weeks and six weeks after planting

The blast screen house has been designed in a way that blast innoculum have been centered around the field, few weeks prior to planting of the two varieties. This simply means that blast infestation already existed in the screen house before the two rice varieties were brought and planted .The two rice varieties were planted 3-4cm below soil surface as three replications, two entries and five treatments as follows;

- Pathogen plus *Trichoderma asperellum*
- Pathogen that is, infested rice, treated with *Trichoderma longibrachiatum*, before manifestation and after disease manifestation.
- Infested rice treated with *Trichoderma ghanense* before and after manifestation of disease.
- Infested rice (blast infested plants)
- The control (untreated plants).

The *Trichoderma spps* were fine sprayed on the rice plants, before the disease manifested, (that is, preventive) and after the disease manifested. First treatment was two weeks after planting and the second was six weeks after planting.

Disease rating was scored in a scale of 0-9 using IRRI rating scale (IRRI, 1996), where -

0 No lesion observed
1 Small brown specks of pin-point size or larger brown specks without sporulating center
2 Small roundish to slightly elongated, necrotic gray spot, 1-2mm diameter
3 Lesion type as above but significant number of lesion on upper leaves.
4 Typical susceptible blast lesions 3mm or longer, infecting less than4% of leave area.
5 Typical blast lesion infecting 4-10% of leaf area.
6 Typical blast lesions infecting11-25% of leaf area
7 Typical blast lesions infecting 26-50% of leaf area.
8 Typical blast lesions infecting 51-75% of the leaf area and many leaves dead
9 More than 75% leaf area affected.

This study was done during the planting season (August-December) measurement of rainfall wind speed, temperature, relative humidity; evaporation and radiation were taken using facilities of I.I.T.A. weather station.

RESULTS AND DISCUSSION

Efficacy results of *Trichoderma ghanense*, *Trichoderma longibrachiatum* and *Trichoderma asperellum* can be seen on figures I and 2, that is, untreated and treated rice plants

All the *Trichoderma* showed good activity/potential in prevention and control of rice blast on the field, while it is believed that the blast inoculums that were already established on the blast field must have infested the rice test entries the combination of the application of each *Trichoderma spp* before and after blast incidence gave better results-symptoms free observation.

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 et al., 2004). 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.

Rainfall during this studies, August to December ,range between 14mm – 61mm, wind speed range between2.4 -5.0 km/hr, temperature range from 23.3 - 30.0^0C. In addition, relative humidity range between 61 - 76 percent, evaporation was between 4.4-5.6mm while radiation range from 7.1 -10.2 mg/m2/day.

These environmental conditions did not in anyway influence the performance of these *Trichoderma. spp*., particularly temperature, wind speed and relative humidity (Ou, 1985; Greorgopoles and Ziogas, 1992), while the conditions favour infection and disease development of Blast fungus. . These microclimatic conditions of the relative humidity (79%) and the minimum ambient temperatures (21-23^{0c}) promote

Table 1: The efficacy results of *Trichoderma spps* on the field

Plots	entries	treatments	observed lesions	six weeks T	observed lesions
1	20	A	0	A	0
2	20	B	0	B	0
3	20	C	0	C	0
4	20	sdw	0	sdw	3
5	20	untreated	0	untreated	3

Table 2

Plots	entries	treatment	lesions	six weeks treat	lesions	eight weeks lesions
1	20	-	0	A	3	0
2	20	-	0	B	3	0
3	20	-	0	C	3	0
4	20	-	0	sdw	3	0
5	20	-	0	untreated	3	0

Source of scale: IRRI Standard Evaluation System for Rice
Keys:
A= *Trichoderma ghanense*,
B=*Trichoderma longibrachiatum*
C= *Trichoderma asperellum*
Sdw = sterile distilled water

Figure I: Untreated (rice blast plants) with *Trichoderma spp*.

Figure 2: Treated plant with *Trichoderma spp*

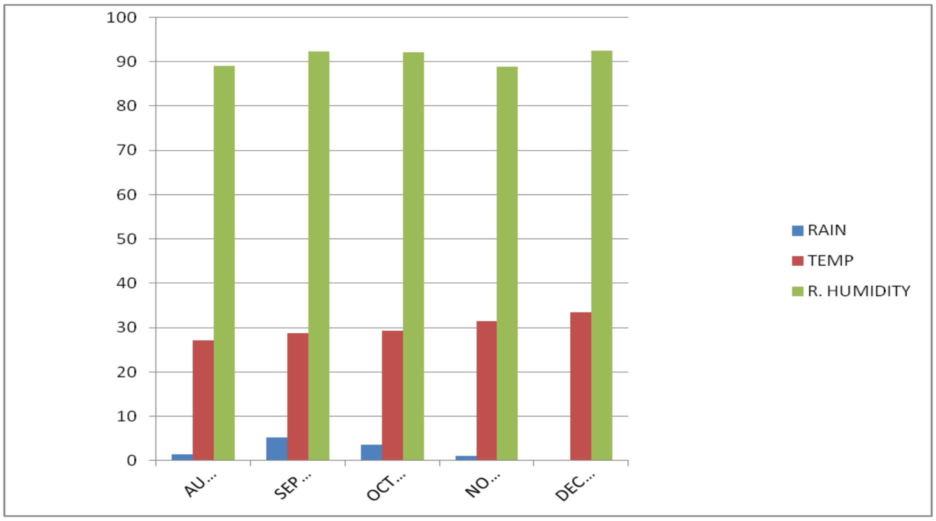

Figure 2: Rainfall, temperature and relative humidity of study site taken from August- December 2009

successful germination of fungal spores and subsequent invasion depending upon host resistance/susceptibility (Ou, 1985).

These Results of in- vitro and In-vivo 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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