Effect of different *Trichoderma asperellum* formulations on management of sheath blight of rice

ABSTRACT

Trichoderma spp. were known to have antagonistic activity against against many soil borne diseases. In this experiment we have prepared five different formulations of *Trichoderma asperellum* and stored them in normal temperature. Later the efficacy of all the formulations were checked against sheath blight disease of rice both *in-vitro* and *in-vivo.* Among formulations the F5 [*Trichoderma* grown in potato dextrose broth (500ml) + Talc(500g)] treated plants showed very good result in managing sheath blight of rice by enhancing the plant height, total number of filled grains and root length. All the formulations performed significantly better than untreated control plants. The F5 treated plants were also recorded to have less disease incidence with increased yield as compared to other formulations.

Keywords: *Trichoderma* formulation, rice, sheath blight

1. INTRODUCTION

Trichoderma spp. is found in a wide variety of environments, including plant material, wood, soil and rotting vegetation. The majority of *Trichoderma* species have enormous economic significance since they are a source of antibiotics, enzymes, plant growth promoters, xenobiotic degraders, and the majority of commercial bio fungicides (Ozbay and Newman, 2004). Even though sclerotial diseases like *Sclerotium rolfsii, Sclerotinia sclerotiorum* and *Rhizoctonia solani* can be controlled chemically to some extent, it is not a cost-effective or environmentally friendly solution. Sheath blight is a serious disease that impacts rice production in India and is regarded as the most commercially important rice disease in the world. *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatophorus* cucumeris), a fungal pathogen of rice, causes the disease. In cases of severe infection of the leaf sheath and leaf blade, yield loss of 30-40% has been documented. It is a significant soil-borne plant pathogen that lives by forming sclerotia. *Rhizoctonia* moves slowly due to the lack of spores and survives in difficult environments by generating sclerotia or dormant mycelia, which serve as the primary inoculum. Secondary infections occur when hyphae migrate upward towards uninfected plant sections, causing new lesions and sclerotia on leaf sheaths to complete the disease cycle (Singh *et al.,* 2012). The introduction of biological control agents is a substitute and effective technique to control these infections (Harman *et al.,* 1994, 2004). Increasing the use of biological control agents is the only approach that shows any promise in the current agricultural landscape for managing diseases without upsetting the delicate balance of harmful and useful components of the environment and ecosystem. Biological control can be accomplished in two ways: either by introducing biocontrol agents into an ecosystem or by adopting methods that encourage the population growth of biocontrol organisms in their natural environment. The most effective strategy is probably a hybrid of the two. Remarkable developments have been made in this field in recent years. Due to their ability to generate substantial rhizosphere population densities on the emerging root system and suppress diseases of treated plants, *Trichoderma* spp. have garnered important attention among various fungal and bacterial biocontrol agents (Rai and Tiweri, 2016). This can be inferred from the fact that a number of companies throughout the world are engaged in the production of microbe-based biopesticides (Fravel, 2005). The most significant barrier to biological control in the Weld is a lack of understanding on the mass manufacture and distribution of biocontrol agents (Papavizas, 1985). An important condition for the successful implementation of bioagent, regardless of the organism employed, is the creation of microbial biomass with large population densities and high levels of viability and vigour (Kumar *et al*., 2014). Formulation of biological control agents is contingent upon the production and preservation of biomass (Adekunle *et al.,* 2001). In our experiment we have formulated different *Trichoderma* formulations and checked their efficacy against sheath blight of rice both under *in-vitro* and *in-vivo* conditions.

2. MATERIALS AND METHODS

2.1 Isolation and characterisation of *Trichoderma* spp:

Trichoderma spp. was collected and isolated from farmlands of OUAT, Bhubaneswar through serial dilution method by growing them in TSM (*Trichoderma* selective medium). All the experiments were performed in the Department of Plant Pathology ,OUAT, Bhubaneswar. Molecular identification has been by extracting DNA from fungal samples using ITS primers and then all the four DNA were sent for sequencing to HKP scientific, Patia, Bhubaneswar.

2.2 Development of bio formulation and their ingredients:

0.2 percent carboxyl methyl cellulose (CMC) was incorporated into each formulation during the production of wettable powder-based formulations. In each liquid-based formulation, 0.2% carboxyl methyl cellulose, and 1.0% tween 80 were appropriately combined. WP (wettable powder) based formulations were carefully dried in the shade at room temperature and ground into fine powder before being stored in airtight polyethene bags and liquid formulations in glass vials and kept for one month

List 1 : Bio formulation and their ingredients

Sl No.	Formulation name	Ingredients
1	F1	*Trichoderma* filtrate (300ml)+ Dextrin (700g)
2	F2	*Trichoderma* filtrate (500ml)+ Paraffin oil (500 ml)
3	F3	*Trichoderma* filtrate(500ml)+ Talc powder (500g)
4	F4	*Trichoderma* spore suspension collected from sorghum grain as substrate (500ml)+ Paraffin oil (500 ml)
5	F5	*Trichoderma* grown in Potato dextrose broth (500ml) + Talc(500g)

2.3 Antagonistic activity of different formulations against *Rhizoctonia solani*

After one month antagonistic activity of all the five formulations were check by growing them in PDA (potato dextrose agar) plates against *Rhizoctonia solani* by following dual culture method. Radial growth of *Rhizoctonia* isolates was recorded and per cent inhibition of pathogen growth was calculated.

2.4 Artificial inoculation of *Rhizoctonia solani*

The isolates were artificially inoculated by inserting the sclerotia beneath the leaf sheath.The inoculated sheath was immediately wrapped in aluminium foil. Following inoculation, regular observations were undertaken for the emergence and development of symptoms. When lesions first developed. The aluminium foil was removed after 4-5 days. Lesions were measured 8 days after inoculation. The disease was induced in both treated and untreated plants.

2.4 Effect of *Trichoderma* formulations against *Rhizoctonia solani* in pot culture experiment:

Effect of *Trichoderma* formulations against sheath blight in pot culture experiment were performed by growing rice plants in individual pots and they are compared with the control untreated plant. Different morphological data such as Plant height (cm), Days to active tillering, Total numbers of grains/panicle, Number of filled grains/panicle, Root length (cm) were recorded. Percent disease incidence and yield attributes were also recorded. Percent disease incidence was calculated by using 0-9 point scale for scoring of disease severity given by IRRI.

Statistical analysis

Statistical analysis was performed using the OPSTAT software package created by the Department of Statistics at CCS Haryana Agricultural University. An one-way Analysis of Variance was used to calculate the Crucial Difference (CD) at the 5% level of significance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Isolation and characterisation of *Trichoderma* spp:

After sequencing of DNA the isolate was identified as *Trichoderma asperellum.* The NCBI accession number of the organism is OM721716.

3.2 Antagonistic activity of different formulations against *Rhizoctonia solani*

Among the five formulations the F5 formulation showed the highest level of antagonism (33.76) against followed by F4, F2,F1 and F3 respectively. Seema and Devki (2012) also evalutated the efficacy of fungal bio- agents which are of *Trichoderma harzianum* &*Trichoderma virens* under in vitro condition against *Rhizoctonia solani* and obtained an inhibition upto 37% by *Trichoderma harzianum* and 40% by *Trichoderma viride.*

Table 1: Antagonism of different *Trichoderma* formulations

Formulations	Inhibition percentage (%) against *Rhizoctonia solani*
F1	10.29 (18.71)*
F2	11.76 (20.06)*
F3	8.82 (17.28)*
F4	14.17 (22.55)*
F5	30.88 (33.76)*
Control	0
SEm (±)	2.474
C.D.(p≤0.05)	7.213

3.3 Effect of formulations on morphological characters of rice:

The study revealed a considerable increase in plant height in treated plots compared to control plants. There was some difference among the treated plants, indicating the performance of the isolates utilised. F5 formulation of *Trichoderma asperellum* treated plants has the tallest plant, measuring 78.50 cm followed by F4,F2, F1 and F3 respectively. The untreated control plants recorded the lowest plant height (73.25cm). According to Chauhan *et al*. (1999), water scarcity and disease in rice caused a considerable drop in plant height. In the current study, the increase in plant height over the control indicates that *Trichoderma* alleviated disease stress condition.

The number of days to active tillering in rice plants was likewise altered by treatment of *Trichoderma* formulation with a considerable reduction in time taken to active tillering. The treated plants began active tillering substantially sooner than the control pots that were not treated. The use of *Trichoderma* formulations had a substantial impact on the total number of grains per panicle as well as the number of filled grains per panicle. T5 formulation produced the highest number of total grains, as well as the highest number of filled grains, with a fertility percentage of 83%.

Formulations	Plant height (cm)	Days to active tillering (days)	Total numbers of grains/panicle	Number of filled grains/panicle	Fertility percentage (%)	Root length (cm)
F1	77.25	71.33	183.00	144.67	79.05	15.33
F2	77.50	72.33	183.33	148.00	80.73	15.00

F3	76.25	72.67	180.33	141.67	78.56	13.33
F4	76.50	71.33	183.67	155.00	82.21	16.33
F5	78.50	71.00	219.67	178.00	83.00	16.67
Control Plant	73.25	78.33	155.67	98.67	63.66	11.67
SEm (±)	0.309	0.418	1.285	0.959		0.378
C.D.(p≤0.05)	0.914	1.28	3.935	2.939		1.158

Table 2: Morphological characters of rice plant as influenced by treatment of *Trichoderma* formulations

The data collected on root length under the influence of various *Trichoderma* treatments in the current investigation shows an increase in root length. Compared to the untreated control, all treatments produced longer roots. The F5 formulation treated rice plants resulted in the longest root length (16.67 cm) followed by F4 (16.33cm). The elements that affected yield performance, particularly in conditions of restricted water and diseased condition, were root size and architecture. According to Arora *et al*. (1992), the application of *Trichoderma* strains to plants increased root length and increased water accumulation, which increased plant resilience to both biotic and abiotic stress. This result is supported by findings of the present experiment that all the *Trichoderma* formulation treated plants had longer roots than untreated control plants under drought condition.

3.4 Effect of *Trichoderma* formulations on incidence of disease and yield:

All the treated palnts were significantly better than the control where no *Trichoderma* formulation was used. The *Trichoderma* formulation F5 recorded least incidence of disease (22%) and yielded highest amount of grain (36.33 g per plant) and the highest disease incidence and less yield were recorded in which recorded 28.67% disease incidence and yielded only 25 g per plant. Although the untreated control pant recoded the highest disease incidence (38.67%) and lowest yield (16.67).

By examining the effectiveness of several isolates of Trichodema spp. against Rhizoctonia solani, Naeimi *et al.,*(2011) also demonstrated variance in *Trichoderma* spp. efficacy. They also noted disease incidence in *Trichoderma*-treated plants ranging from 19.34 to 29.70%, which appears to be consistent with the current findings. Khan and Sinha (2007) also found similar findings by using *Trichoderma* spp., where rice sheath blight incidence and disease severity were reduced. They saw a 24.6% decrease in the severity of the disease and a 21% rise in the amount of grain produced per plant. Both Das *et al.* (1998) and Denish and Webster (1971) showed that *Trichoderma harzianum* is effective at lessening the severity of sheath blight and increasing grain yield. Along with lowering the severity of the disease, the data from our experiment also showed an increase in yield over control. As a result, the results of the current experiment support those of earlier researchers.

Table 3: Incidence of disease and yield as influenced by *Trichoderma asperellum* formulations

Formulations	**PDI (%)**	**Yield (gm/plant)**
F1	25.33	30.00
F2	28.00	28.00
F3	28.67	25.00
F4	23.33	31.67
F5	22.00	36.33
Control Plant	38.67	16.67
SEm (±)	0.344	0.577
C.D.(p≤0.05)	1.054	1.768

4. CONCLUSION

Synthetic fertilisers and pesticides have greatly increased crop yield, but their widespread usage has resulted in environmental issues such as soil salinity, pathogen resistance and other issues. Green technology, particularly microbial applications, may provide superior alternatives to chemicals. In comparison to chemical pesticides, the popularisation of biopesticides has been extremely sluggish. At the end it may be concluded that the application of *Trichoderma* formulations can be considered as a safer way for managing diseases which modifies the morphological characters of the plant to adapt to the situation.

REFERENCES

1. Ozbay N, Newman SE. Biological control with *Trichoderma* spp. with emphasis on T. harzianum. Pakistan Journal of Biological Sciences. 2004;7(4):478-84.
2. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nature reviews microbiology. 2004 Jan 1;2(1):43-56
3. Harman GE, Jin X, Stasz TE, Peruzzotti GP, Leopold AC, Taylor AG, inventors; Cornell Research Foundation Inc, assignee. Method of increasing the percentage of viable dried spores of a fungus. United States patent US 5,288,634. 1994 Feb 22.
4. Rai D, Tewari AK. Shelf life studies of different formulations based on *Trichoderma* harzianum (Th14). Annals of Biological Research. 2016;7(7):1-5.
5. Fravel DR. Commercialization and implementation of biocontrol. Annu. Rev. Phytopathol.. 2005 Jul 28;43:337-59.
6. Papavizas GC. *Trichoderma* and Gliocladium: biology, ecology, and potential for biocontrol. Annual review of phytopathology. 1985 Sep;23(1):23-54.
7. Adekunle AT, Cardwell KF, Florini DA, Ikotun T. Seed treatment with *Trichoderma* species for control of damping-off of cowpea caused by Macrophomina phaseolina. Biocontrol Science and Technology. 2001 Aug 1;11(4):449-57.
8. Singh HK, Singh UD. Evaluation of vertical and horizontal spread of sheath blight in rice varieties for resistance against Rhizoctonia solani. International Journal of Agriculture, Environment and Biotechnology. 2012;5(4):367-72.
9. Seema M, Devaki NS. In vitro evaluation of biological control agents against Rhizoctonia solani. Journal of Agricultural Technology. 2012;8(1):233-40.
10. Chauhan JS, Moya TB, Singh RK, Singh CV. Influence of soil moisture stress during reproductive stage on physiological parameters and grain yield in upland rice. Oryza. 1999;36(2):130-5.
11. Arora DK, Elander RP, Mukherji KG. Fungal biotechnology handbook of applied mycology. Dekker. Nueva York. 1992;4:270-4.
12. Naeimi S, Okhovvat SM, Javan-Nikkhah M, Vágvölgyi C, Khosravi V, Kredics L. Biological control of Rhizoctonia solani AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. Phytopathologia Mediterranea. 2010 Dec 1;49(3):287-300.
13. SINHA A, KHAN AA. Screening of *Trichoderma* spp. against Rhizoctonia solani the causal agent of rice sheath blight.
14. Das BC, Khairuzzaman AS, Bora LC. Biological seed teatment for management of sheath blight of rice. Journal of Mycology and Plant Pathology (India). 1998.
15. Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma*: II. Production of volatile antibiotics. Transactions of the British Mycological Society. 1971 Jan 1;57(1):41-IN4.
16. Kumar S, Thakur M, Rani A. *Trichoderma*: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. African journal of agricultural