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, bio control agents, morphological characters, *Trichoderma asperellum*

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 [1]. 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 [17]. 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 [8]. The introduction of biological control agents is a substitute and effective technique to control these infections [2, 3]. 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 agent 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 [4]. Bio control agents (BCAs) can be found in the genus *Trichoderma*, and most of the time, these BCAs are put to use in agricultural biocontrol because of their adaptability and versatility [20]. The benefits of the Trichoderma-plant interaction include not only an increase in biomass and overall nutrition, but also defence against a number of phytopathogens, either directly by acting as a mycoparasite over the pathogen and competing for nutrients, or indirectly by inducing the plant defence system [21]. This can be inferred from the fact that a number of companies throughout the world are engaged in the production of microbe-based

biopesticides [5]. The most significant barrier to biological control in the world is a lack of understanding on the mass manufacture and distribution of biocontrol agents [6]. 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 [16]. Formulation of biological control agents is contingent upon the production and preservation of biomass [7]. 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 from crop growing farmlands of OUAT, Bhubaneswar by collecting soil in randomize manner in the polythene bags by using spade. After that the *Trichoderma* was isolated by serial dilution method by taking dilution upto 10^8 and growing them in TSM (*Trichoderma* selective medium). All the experiments were performed in the Department of Plant Pathology, OUAT, and Bhubaneswar.

Molecular identification has been done by extracting DNA from fungal samples by CTAB method using ITS primers. Two primers, ITS1- (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5TCCTCCGCTTATTGATATGC-3) were used in the PCR in a 25ul reaction volume where 1 µ ll of DNA, 1 µll dNTP, 2.5 µll HIIPCR buffer, and 0.5 µll PFE polymerase were used. Thermocycler (BIORAD, USA) was configured with the following PCR parameters: initial denaturation at 95°C for 3 minutes, primer annealing at 60°C for 40 seconds, chain extension, and final extension, each at 72°C for 1 minute and 10 minutes. The amplicons were observed under UV with Ethidium bromide as a staining agent, and the PCR amplification products were verified and characterised by gel electrophoresis in a 1XTAE agarose gel at 70V for 1hr. 100bp ladder was used as reference standard molecular weight marker [18].

PCR purification kit and PROMEGA GEL kit were used to purify the PCR generated DNA product. By observing under UV light, the desired DNA fragments acquired during electrophoresis were cut from the gel with a sterile knife. The membrane binding solution was added to the agarose slice containing DNA, which had been heated to 70°C to melt the gel. Via the creation of minicolumns, the DNA was retrieved from the gel slice and purified. Moreover, the eluted fragment was stored at –20 °C for sequencing. All the DNA samples were sent for sequencing to HKP scientific, Patia, Bhubaneswar. A commercial service provider used an ABI 3730l DNA analyzer equipped with 96 capillary arrays to carry out the sequencing using the Sanger sequencing method. Using the Clustal tool, the sequence assembly and alignment were carried out. The sequence data has been submitted in the NCBI database to get the accession number.

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2.2 Development of bio formulation and their ingredients:

F1: 100 ml of potato dextrose broth (PDB) were inoculated with *Trichoderma asperellum*, which was then left to grow for 10 days at 22°C. The cultures were filtered via 0.22 mm millipore filters after incubation, and the aliquots were then taken and mixed with 300ml sterile distilled water where the CFU count was $2x10^6$. The the solution was mixed with 700g dextrin to make the final formulation.

F2: 100 ml of PDB were inoculated with *Trichoderma asperellum*, which was then left to grow for 10 days at 22°C. The cultures were filtered via 0.22 mm millipore filters after incubation, and the aliquots were then taken and mixed with 500ml sterile distilled water where the CFU count was maintained @ $2x10^6$. The the solution was mixed with 500g talc powder to make the final formulation.

F3: 100 ml of PDB were inoculated with *Trichoderma asperellum*, which was then left to grow for 10 days at 22°C. The cultures were filtered via 0.22 mm millipore filters after incubation, and the aliquots were then

taken and mixed with 500ml sterile distilled water where the CFU count was maintained @ $2x10^6$. The the solution was mixed with 500ml paraffin oil to make the final formulation.

F4: *Trichoderma asperellum*, were grown in sorghum grains for 10 days at 22°C after that the 500g gram sorghum grains were grounded to powder form and mixed with 500ml paraffin oil to make the final formulation.

F5: *Trichoderma asperellum*, were grown in potato dextrose broth for 10 days at 22°C after that the 500ml potato dextrose broth were mixed with 500g talc powder to make the final formulation.

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

Table 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* grown in sorghum grain as substrate (500g)+ 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 [15].Radial growth of *Rhizoctonia* isolates was recorded and per cent inhibition of pathogen growth was calculated.

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

Twenty days old seedlings grown in nursery were transplanted and inoculated with different *Trichoderma asperellum* formulations @ 2×10^6 CFU by using seedling root dip treatment method. Effect of *Trichoderma* formulations against sheath blight in pot culture experiment were performed by growing rice plants in individual pot 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 [19]

Table 2:0-9 point scale for Scoring of disease severity given by IRRI

Scores	Description
0	No infection
1	Vertical spread of the lesions up to 20 per cent of plant height
3	Vertical spread of the lesions up to 21-30 per cent of plant height
5	Vertical spread of the lesions up to 31-45 per cent of plant height

7	Vertical spread of the lesions up to 46-65 per cent of plant height
9	Vertical spread of the lesions up to 66-100 per cent of plant height

2.5 Artificial inoculation of *Rhizoctonia solani*

The concentration *Rhizoctonia solani* used for inoculation were 2 x 10^6 CFU. The isolates were artificially inoculated 30 days after transplanting to individual pot 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. The aluminium foil was removed after 4-5 days after development of lesions. Lesions were measured 8 days after inoculation. The disease was induced in both treated and untreated plants.

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). All the percentage data were angular transformed.

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*

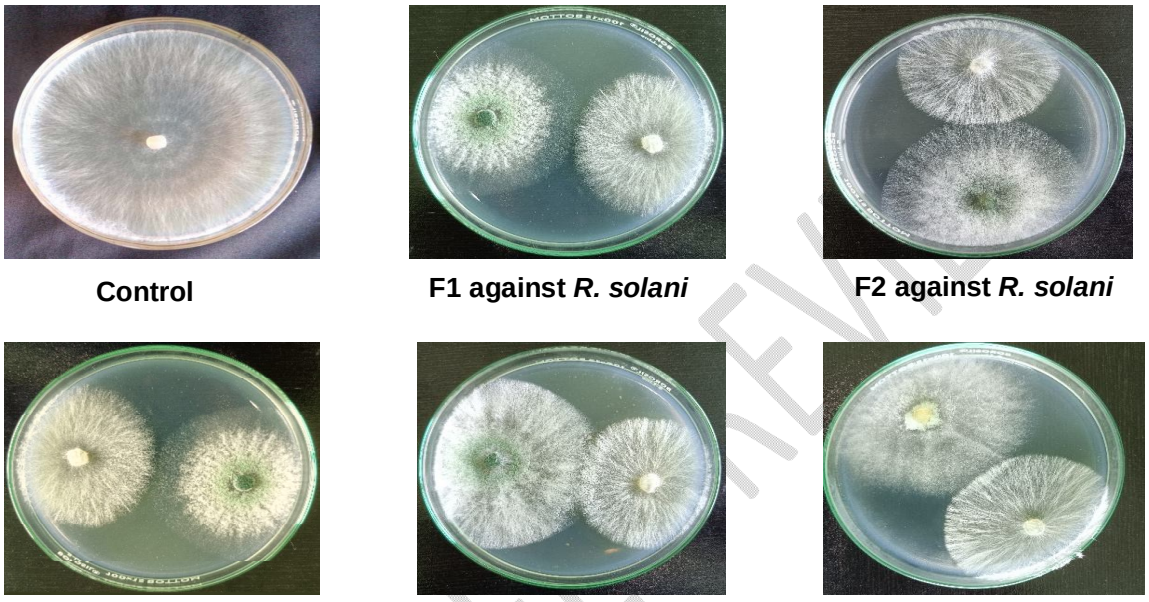
Among the five formulations the F5 formulation showed the highest level of antagonism (36.36%) against the target pathogen followed by F4, F2, F1 and F3 respectively after 7 days (Table 3). The difference in Inhibition percentage among the treatments was significantly different from each other. Seema and Devki [9] also evaluated 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 3: Antagonism of different *Trichoderma* formulations against *Rhizoctonia solani*

Formulations	Inhibition percentage (%) against *Rhizoctonia solani*		
	After 3 Days	After 5 Days	After 7 Days
F1	5.17 (13.13)*	10.20 (18.62)*	15.73 (23.36)*
F2	5.23 (13.21)*	11.10 (19.45)*	16.23 (23.75)*
F3	4.87 (12.73)*	9.03 (17.48)*	13.37 (21.44)*
F4	5.80 (13.93)*	13.73 (21.74)*	17.23 (24.517)*
F5	23.27 (28.83)*	29.33 (32.78)*	35.17 (36.36)*
Control	0	0	0
SEm (±)	0.079	0.077	0.12

C.D.(p≤0.05)	0.252	0.247	0.375

NB: The values given in ()* are transformed by using angular transformation



Control | **F1 against *R. solani*** | **F2 against *R. solani***

F3 against *R. solani* | **F4 against *R. solani*** | **F5 against *R. solani***

Fig 1: Radial growth of different *Trichoderma* formulations against *Rhizoctonia solani* after 5 days.

3.3 Effect of different *Trichoderma asperellum* 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 (Table 4). However the difference of plant height between all the treatments including control plants were statistically significant except F1 and F2. The untreated control plants recorded the lowest plant height (73.25cm). According to Chauhan *et al*. [10] 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% (Table 4). The data found on the number of filled grains in all treatments were statistically significant.

Table 4: The effects of *Trichoderma* formulations on the morphological characteristics of rice plants

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

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 (Table 4). 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*. [11] 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. Although all the treatments were statistically significant with control plants, both F4, F5 and both F1, F2 were statistically on par.

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

All the treated plants were significantly better than the control where no *Trichoderma* formulation was used. The *Trichoderma* formulation F5 recorded least incidence of disease (28.19%) after 24 of inoculation and yielded highest amount of grain (36.33 g per plant) [Table 5]. Although the untreated control pant recoded the highest disease incidence (42.11%) and lowest yield (16.67 g per plant). The difference in percent disease incidence (PDI) among all the treatments were statistically significant.

By examining the effectiveness of several isolates of *Trichodema* spp. against *Rhizoctonia solani*, Naeimi *et al.* [12] 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. [13] 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.* [14] and Dennish and Webster [15] 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 5: Incidence of disease and yield as influenced by *Trichoderma asperellum* formulations

Formulations	**PDI (%) after 8 days of inoculation**	**PDI (%) after 16 days of inoculation**	**PDI (%) after 24 days of inoculation**	**Yield (gm/plant)**
F1	16.67 (24.083)*	25.00 (29.99)*	28.67 (32.36)*	30.00
F2	19.33 (26.072)*	27.67 (31.72)*	31.33 (34.03)*	28.00

F3	21.00 (27.26)*	29.00 (32.57)*	31.67 (34.23)*	25.00
F4	13.33 (21.41)*	21.67 (27.73)*	26.67 (31.08)*	31.67
F5	13.67 (21.67)*	21.00 (27.26)*	22.33 (28.19)*	36.33
Control Plant	30.00 (33.20)*	38.67 (38.43)*	45.00 (42.11)*	16.67
SEm (±)	0.418	0.378	0.238	0.577
C.D.(p≤0.05)	1.302	1.178	0.74	1.768

NB: The values given in ()* are transformed by using angular transformation

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.

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