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Management of sheath blight of rice (*Oryza sativa*) under *in-vitro* condition with indigenous *Trichoderma* spp.

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Abstract
Rice is a monocotyledonous annual grass belonging to the family Gramineae and the genus *Oryza*. It includes 20 wild species and two cultivated species: *Oryza sativa* (grown throughout the world) and *Oryza glaberrima* (grown only in Africa). Total yield loss in rice due to rice diseases in the world is 10-25%. In India, total yield loss due to diseases in rice is 35%, in which blast costs 25% loss, sheath blight 20%, BLB 10%, tungro and other diseases 45%, *Rhizoctonia solani* was isolated from sheath blight diseased rice plants and Koch's postulate was established for pathogenicity and then purified and maintained for further study. The results of presented in this investigation were conducted under laboratory and field conditions. In the present study we isolated *Rhizoctonia solani* from the rice variety Rajendra Kasturi plant showing symptom of sheath blight from BAU farm Sabour, Bhagalpur. Morphological and Cultural characterization of *R. solani* was studied by visual observation of mycelia and sclerotia colour and its size. Hyphal width ranged from 4.75 µm to 7.43 µm and sclerotial colour changed from brown, light or dark brown, and black brown. The diameter of sclerotia ranged from 1.13-2.03 mm. Mycelia of isolated pathogen were light brown during early growth and produced large amounts of aerial hyphae throughout the growth cycle. Sclerotia were tan when young, generally dark brown when mature, and up to 1.5 mm in diameter with clumps up to 5 mm in diameter. Some sclerotia clumps were found on the agar surface but most clumps and individual sclerotia were embedded in the agar. Our observations are in complete with the findings of the numerous workers. Isolated the *Rhizoctonia solani* from infected plant and studied its morphological or cultural characteristic as sclerotia colour was light brown, brown, dark brown and deep dark brown.
Disease incidence was found 42.82% in Rhizoctonia solani inoculated pots. Seed treatment with different treatments of Trichoderma @ 10g/kg seed found highly effective and managing the disease by 50.49 - 60%. Among all the treatments, T10 [combined seed application of *Trichoderma asperellum* (Tvb1) and *Trichoderma hamatum* (Thg) with 5g each and foliar application with 107conidia /ml each at 5 days before pathogen inoculation (5 DBPI) as prophylactic and 5 days after pathogen inoculation (5 DAPI) as curative spray] was found to be the most effective treatment managing the disease by 71.69% which was at par with propiconazole treated plants.
In all the treatments number of Rhizoctonia solani sclerotia decreased significantly from 18.67 to 2.33 sclerotia per plant. It was interestingly found that decreased number of sclerotia in T10 was at par with chemical treatment T11 (Propiconazole 25 EC seed treatment 0.1% + propiconazole foliar spray 0.1%) with 2.67 sclerotia/plant in compression with pathogen (R. solani) inoculated control with 18.67 sclerotia / plant.

Keywords: *Rhizoctonia solani*, *Oryza sativa*, Rajendra Kasturi, pot evaluation, *Trichoderma* spp.

Introduction
Rice (*Oryza sativa* L.) is the most widely cultivated food crop and is being cultivated in 114 countries over the world. The majority of the rice (90%) is being produced in Asia with China and India being the major producers (IRRI, 2008). The other major rice producing countries includes Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil and Japan. It is one of the most important food crops of India in terms of area, production and consumer preference. India is the second largest producer and consumer of rice in the world. Rice production in India crossed the mark of 104.32 million MT in 2016-17 accounting for 22.81 per cent of global production. In Bihar rice occupied 32.55 lakh hectares with total production of 80.74 lakh tonnes during 2016-2017. The average yield in terms of paddy in Bihar is 25.0 quintal / acre (Directorate of Economics and Statistics 2016-17).
Rice grain is a composite of carbohydrate, protein, fat, fiber, and other significant nutritive constituents (Qian *et al.* 2010) [19]. Globally, more than 3 billion people uses rice as staple food and it accounts for 50 to 80% of their daily calorie intake (Delseny *et al.*2001) [3]. Over the next 20 years it is expected that demand of rice will grow by 2.5% per year (Hobbs, 2001) [8].

Ultimately, the challenge is to provide food for the increasing population and to ensure food security. However, its production and productivity is affected because more than half of the rice area about 55% is rain fed and 80% of the rain fed rice area is distributed in Eastern India, making its cultivation vulnerable to the vagaries of monsoon.

Rice crop production suffers from a number of fungal, bacterial, viral and nematode diseases, which renders estimated annual yield and quality losses of 8 to 10 percent. In India the major diseases causing significant losses in yield were blast, brown spot and stem rot. Later, the disease situation has completely changed consequent to the introduction of new technologies and the diseases like bacterial leaf blight, tungro, sheath blight and sheath rot have gained the importance posing a threat to the rice crop in certain areas of the country (Singh *et al.*2014) [26].

Among the fungal diseases, sheath blight incited by *Rhizoctonia solani* (Shahjahan *et al.* 1986; Singh *et al.*2014) [22], 26] is gaining importance due to widespread occurrence in almost all rice growing areas of the world, in India and other countries *viz.*, USA causing a yield loss as high as 50% (Lee and Rush, 1983) [13] Bangladesh with yield loss of 14-17.3% (Shahjahan *et al.* 1986) [22]. China with yield loss of 10-15% (Xie *et al.* 2008) [29] and many other south eastern countries with yield loss of 10-30% and may reach up to 50% during prevalent years. The other species of *Rhizoctonia* that contribute to this disease are *Rhizoctonia sativae* and *Rhizoctonia oryzae sativae.* But since there is no report regarding the two mentioned pathogen in India so our concern is only *Rhizoctonia solani.*

The occurrence of sheath blight in rice caused by *Rhizoctonia solani* was first time described by Miyake (1910) [14] in Japan and subsequently its occurrence on rice has been reported from almost all rice growing countries of the world in Asia, Africa and America (Kozaka, 1975 [11] Ou, 1985 [17] Shahjahan *et al.*1986) [22]. In India sheath blight caused by *Rhizoctonia solani* Kuhn is becoming one of the important diseases of rice. The disease has become widespread in Andhra Pradesh, Kerala, Orissa, Bihar Tamil Nadu, West Bengal, U.P. and Uttarakhand and has caused serious yield loss (Prasad and Kumar, 2011). However, losses due to this disease vary from one area to another and from year to year depending on the environmental conditions and the genetic makeup of the host cultivars and the pathogen, *R. solani* can caused 45% loss depending on the plant growth stage, the disease onset and under favorable conditions around the world (Kumar *et al.* 2009) [12]. At present most of promising varieties were found to be susceptible to this disease. No resistant cultivar is available for practical use and intensive rice cultivation practices offer a favourable condition for disease development. So until the resistant cultivars are evolved, it is considered imperative that the disease should be kept under control with minimum possible loss through effective control measures. Disease symptoms appeared as circular, oblong or ellipsoid, greenish-grey water-soaked spots (about 1cm long) and later converted into lesions. These lesions enlarge and become oblong and irregular in outline becomes grey white center and brown margins. In later stage sclerotia develops as round, brown to dark brown in colour with 1-5 mm in diameter.

The various rice management styles used on different farms, plus patterns of rice variety and geographic regions, often determine the type and seriousness of particular disease. Impacts of this disease on rice production have gain momentum and the losses due to this disease have to be tackled. However various ways of management are available to control diseases. All diseases including sheath blight is being controlled by chemicals for a long time, but due to hazardous nature of chemical and increasing adverse environmental conditions these chemicals are not performing well with increased negative effects. Therefore, scientists are giving emphasis towards biological control to reduce the chemical uses as well as to increase sustainability. Among the biological control agents, *Trichoderma* is one of the most efficient antagonists of pathogens having highest adaptability towards adverse environmental conditions such as high temperature salinity and other characters. *Trichoderma* spp. present in nearly all types of soil and other diverse habitats. This genus comprises large number of fungal species like *T. asperellum*, *T. atroviride*, *T. harzianum*, *T. hamatum*, *T. koningii*, *T. virens* and *T. viride* that are widely used for bio-control of plant diseases incited by fungal pathogens. In addition, it is effective to increase plant growth and development (Mukhopadhyay and Mukherjee 1996 Harman and Bjorkmann, 1998; Hjeljord and Tronsmo, 1998; Singh *et al.*2006) [6, 7, 15].

Several advantages of using biological control agents have been reported by different workers such as Eco-friendly (Gaur *et al.*2005 [5] and Bohra *et al.*2006) [1] Effective in managing diseases caused by soil-borne plant pathogens which cannot be easily controlled by chemicals (Howell, 2003) [9] Ease of multiplying antagonists with less cost of production (Gaur *et al.*2005 and Das *et al.*2006) [2, 5] Growth promoting effect (Das *et al.* 2006 and Pan and Bhagat, 2007), [2, 18] Long lasting effective disease management (Howell, 2003 and Sarojini *et al.* 2007) [9, 21].

Material and Methods

The laboratory experiments and field experiments were carried out in the Department of Plant Pathology, BAU Sabour and BAU Sabour farm, respectively. The detailed account of the materials and methodology adopted for laboratory and field experiments are described below:

General method of sterilization and preparation of media

Cleaning and sterilization of glasswares

All glassware's such as Petri plates, test tubes, conical flasks, beaker, pipettes etc. were cleaned through chromic solution (Sulphuric acid 300ml, Potassium dichromate 80g and distilled water 400ml) then thoroughly washed in running water. All the glassware's were air dried overnight and sterilized in hot air oven at 180ºC for two hours. Inoculating needles, cork borer and forceps were sterilized first by dipping in spirit then sterilized it in red hot under flame of spirit lamp.

Preparation of media

Potato Dextrose Agar media was used to study test pathogen cultural and physiological studies. The constituents of media used during investigation were as follows:

Potato (peeled): 200 g
Dextrose/sucrose: 20 g
Agar-agar: 20 g
Distilled water: 1 L

Two hundred gram of peeled potatoes were cut into small pieces and boiled in distilled water and the extract was cooled by filtering through muslin cloth. Dextrose 20 g and agar 20 g of each were dissolved in potato extract and the final volume was makeup to 1000 ml with distilled water and sterilized at 15 p.s.i for 20 min and preserved it in refrigerator for further

use. The media was amended with 0.001% streptomycin just before use to control bacterial contamination.

Isolation of sheath blight pathogen
Rice plants showing characteristic symptoms of sheath blight were collected from B.A.U farm, BAU Sabour. Sheath blight samples were thoroughly washed in running tap water and cut into small pieces of 3 mm size along with the lesion having half healthy and half diseased tissue. The pieces were surface sterilized with mercuric chloride solution (0.1%). The samples were subsequently washed thrice with sterile distilled water to eliminate excess mercuric chloride and then transferred on PDA medium in petri dishes and incubated at 28 °C in BOD. The cultures of the pathogen were obtained by single hyphal tip method and maintained on PDA slants throughout the investigation. Regular transfer of hyphal tip pure culture of the pathogen was done during the period of investigation for maintenance of the pathogen

Purification and maintenance of the pathogen
The fungus was purified by hyphal tip method. The culture obtained by this method will be maintained on slants having PDA. The sub culturing of axenic culture will be done at an interval of fifteen days on fresh PDA slants and also preserved in refrigerator at 4 °C for further studies. The pathogen was identified on the basis of cultural and morphological characteristics (Singh *et al*., 2014) [26].

Procurement of *Trichoderma* spp.
Trichoderma asperellum (Tvb1), Procured from Department of Plant Pathology BAC Sabour; *Trichoderma hamatum* (Thg), Procured from Department of Plant Pathology BAC Sabour; *Trichoderma viride* (TC) (isolated from commercial formulation 'BIODERMA', Biotech International Ltd.).

Pot evaluation of *Trichoderma* spp. against sheath blight of rice
Layout
Pot experiments were conducted at Department of Plant Pathology, Bihar Agricultural College, Sabour.
Design: CRBD
Replication: 03
Treatment: 12
No. of plants per pot: 01
Variety: Rajendra Kasturi
Trichoderma asperellum (Tvb1), Procured from Department of Plant Pathology BAC Sabour; *Trichoderma hamatum* (Thg), Procured from Department of Plant Pathology BAC Sabour; *Trichoderma viride* (TC) (isolated from commercial formulation 'BIODERMA', Biotech International Ltd.); ST= Seed treatment, FS = Foliar spray.

Preparation of pot culture
Nine-inch diameter and height earthen pots were taken and filled with sterilized soil mixture (soil and compost, 2:1)

Pathogen inoculation
Barley seeds were inoculated aseptically with *Rhizoctonia solani* pathogen and allowed to over grew. Inoculation of rice plant were done by placing pathogen inoculated barley seed covered with mycelia at the centre of each hill above water level (Sudhakar, 1996) [27] and Inoculation of the pathogen were done at 62 DAS as suggested by de Franca *et al*. 2015 [4].

***Trichoderma* spp. treatment**
Seed treatment: Seed treatment with 10 g/kg seed of *Trichoderma* formulation were done before sowing and in Foliar spray: One prophylactic spray 57 DAS and another curative spray 67 DAS were done

Observations recorded
a. Disease severity were recorded at 30 days after pathogen inoculation as relative lesion height (RLH) (IRRI, 1996). Percentage RLH were calculated as follows:
RLH (%) = 100 x highest point a lesion occurred (cm) / plant height(cm).
The percentage RLH were converted in to Disease score as recommended by IRRI, 1996.
b. Number of sclerotia / replicate produced in rice phallosphere by *Rhizoctonia solani* were calculated.
c. Disease incidence: Percent Disease incidence was observed and calculated as recommended by Naeimi *et al*., 2010 [16]
% Disease incidence = No. of infected tillers/Total No. of tillers/hill x 100
d. % Reduction in disease incidence = No. of infected tillers in control - No. of infected tillers in treatment / No. of infected tillers in control x 100 (Naeimi *et al*., 2010) [16].
e. Growth and yield: No. of tillers/hill and grain yield/plant was observed at harvest.

Results and Discussion
The results of present investigation conducted on the "Management of sheath blight (*Rhizoctonia solani* Kuhn) of rice with indigenous *Trichoderma* spp." under laboratory and field conditions are presented in this chapter.

Isolation, purification and maintenance of *Rhizoctonia solani* causing sheath blight of rice.
Isolation
The pathogen of *R. solani* was isolated from diseased plant of rice variety Rajendra kasturi showing sheath blight symptoms. The isolation was done according to Miyake (1910) [14]. After the isolation is done, cultural and morphological characters were studied. The pathogenicity test was done then purified and maintained for further study.

Symptomatology
Symptoms of the disease usually appeared when plants were in the late-tillering or early internodes elongation stages. The disease was favoured by highly humid and warm temperatures. Small, water-soaked spots first appeared on the leaf sheath within three inches above the water line. These spots enlarged rapidly under favourable conditions, become longer up and down on the plant than they widen, and had greyish-white centres with a tan-to-brown margin. The disease progressed up the plant caused white-to-gray lesions on the leaves. Lesions were as much as three-fourths of an inch long and involved the entire width of the leaf. As the disease progressed and lesions coalesced, areas of diseased rice become 1/2 to 3 feet in diameter and in some places developed throughout the rice canopy. Lodging occurred in the severely diseased plants, and growth of the mycelium was seen on the affected parts of the plant under humid conditions. Sclerotia produced loosely externally on the sheaths or between the sheath and culm (Fig. 1).

The Pathogen, *Rhizoctonia solani*

Cultural and morphological characteristics

In the present study, compound microscope studies revealed that isolates of *R. solani* characteristically having hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction which is of immense taxonomical importance. It was an obvious observation for the mycelial branching at right angles as a known feature of *R.* solani. Hyphal width ranged from 4.75 µm to 7.43 µm and sclerotia colour changed from brown, light/dark brown, black brown, chocolate brown. The diameter of sclerotia ranged from 1.13-2.03 mm and formation of sclerotia was observed in the Petri dish (Fig. 2). Mycelia of isolated pathogen were light brown during early growth and produced large amounts of aerial hyphae throughout the growth cycle. As cultures aged, their colour darkened and most was very dark brown after 21 days. Concentric rings formed on all cultures by day three but rings tended to disappear as cultures matured and darkened. By day six, sclerotia formed near the edge of the petri dishes. However, after 21 days of growth, sclerotia were scattered randomly about the agar surface as well as in the agar. Individual sclerotia were tan when young, generally dark brown when mature, and up to 1.5 mm in diameter with clumps up to 5 mm in diameter. Some sclerotia clumps were found on the agar surface but most clumps and individual sclerotia were embedded in the agar (Fig 2).

Pathogenicity test

The isolated pathogen, *R solani* was tested for Koch's postulates. After artificial inoculation in the form of sclerotia, the inoculated plants were kept for disease development. Regular observations were made for recording gradual development of symptoms. Symptoms appeared about 7 days after inoculation. The sheath blight showed water-soaked spots first appear on the leaf sheath within 3 inches above the water line. The organism was re-isolated from the infected portion of the sheath of the inoculated plants by the methods already stated earlier. Then their cultural and morphological characters were compared with those of original one, which was found similar. Thus, satisfying the Koch's postulates and the pathogenicity of the fungus *Rhizoctonia solani* was established on rice plant var. Rajendra Kasturi (Fig. 3).

Fig 1: Symptoms of sheath blight of rice caused by *Rhizoctonia solani* under pot condition

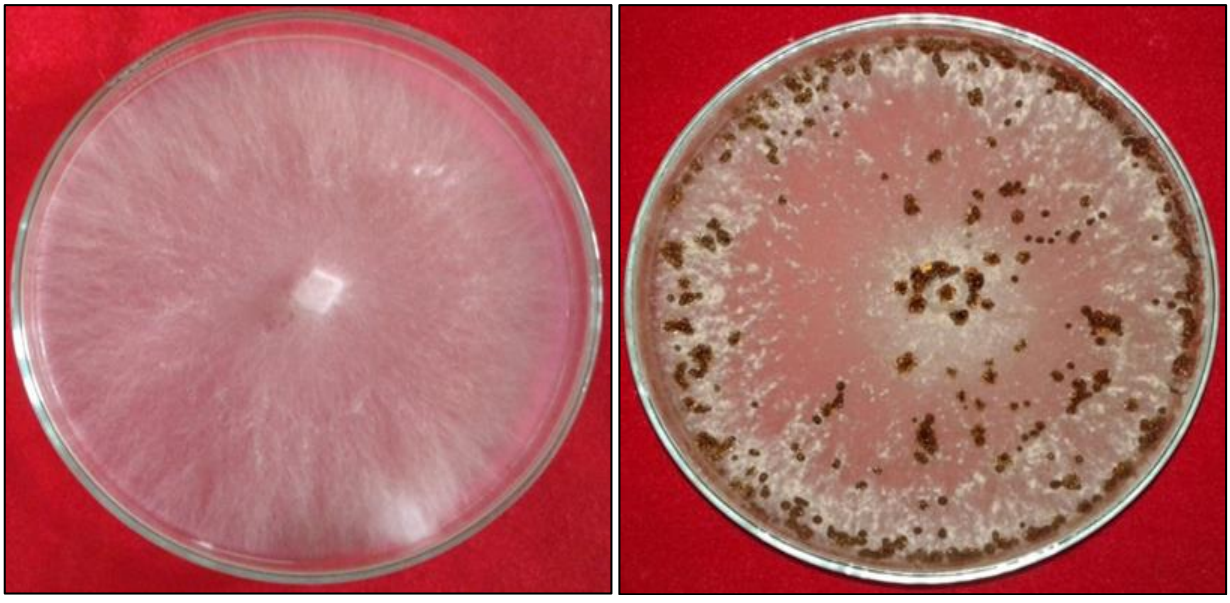

Fig 2: Mycelia (a) and sclerotia (b) of *Rhizoctonia solani* isolated and maintained for study

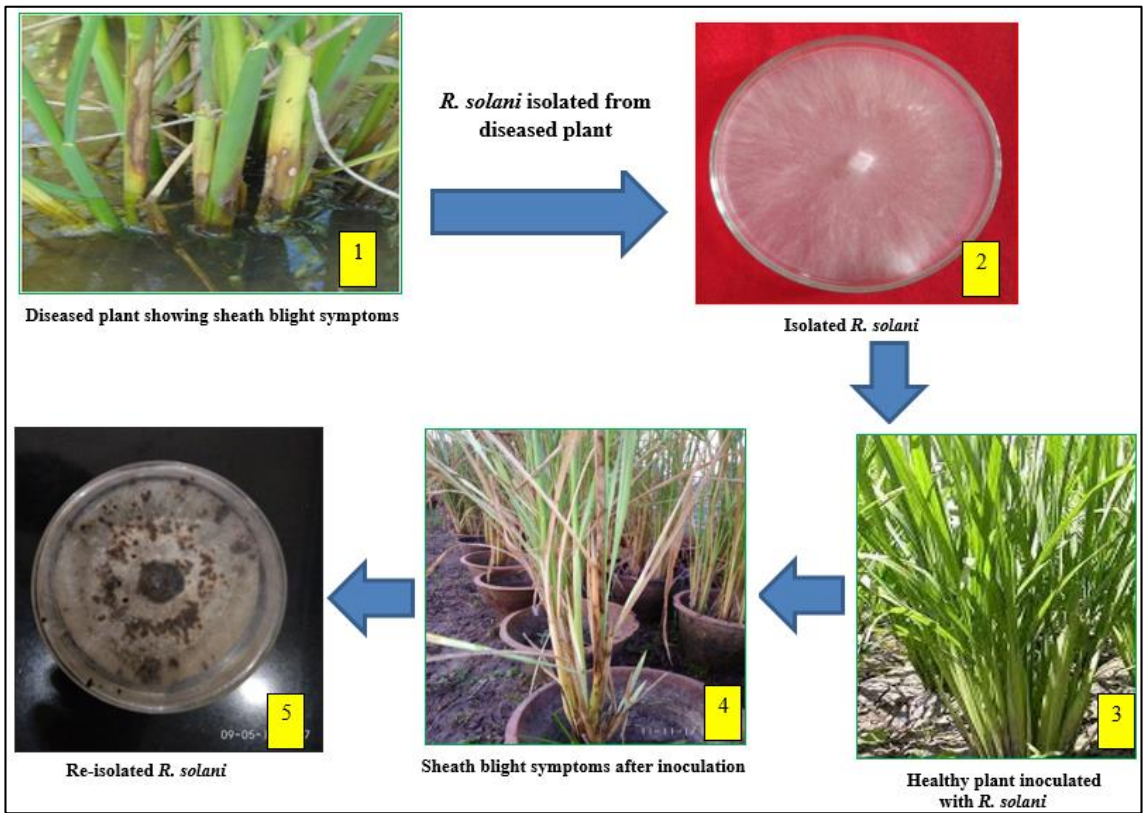

Fig 3: Pathogenicity of *Rhizoctonia solani*

Purification

After the pathogenicity test, the pathogen, *R. solani* was purified on PDA from hyphal tip method and maintained for further study at 4°C.

Pot evaluation of *Trichoderma* spp. against sheath blight of rice

Disease severity

Pot experiment was conducted to find out the most effective bio control agent against sheath blight of rice. The result depicted in table 3, showed disease severity from 0.00 – 71.10%. Maximum disease severity was found in T12 [inoculated control with *Rhizoctonia solani*] i.e. 71.10% whereas minimum disease severity was found in T6 (un-inoculated control) i.e. 0.00% which was followed by, T11 [(Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] i.e. 18.47%, T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] i.e. 21.67%, T8 [(Thg (S.T) +Thg (F.S, 10^7 conidia/ml)] i.e. 23.54%. Among different treatments of bio agents T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] i.e. 21.67% was found most effective in reducing disease severity which was at par with T8 [(Thg (S.T) + Thg (F.S, 10^7 conidia/ml)] i.e. 23.54%. Whereas in case of two treatments of fungicides T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] i.e. 18.47% was found to be the best.

Disease incidence

Disease incidence ranged from 0.00-42.82%. Maximum disease incidence was found in T12 [inoculated control with *Rhizoctonia solani*] i.e 42.82% whereas minimum disease incidence was found in T6 (un-inoculated control) with 0.00% which was followed by T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] with 10.20%, T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] with 12.13%, T7 [Tvb1(S.T) +Tvb1 (F.S, 10^7 conidia/ml)] i.e. 14.23%. Whereas in case of two treatments of fungicides T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] i.e. 10.20% disease incidence was found to the best. Among different treatments of bio agents, T10 [(Tvb1+Thg) S.T + (Tvb1 + Thg) FS (10^7 conidia/ml)] i.e. 12.13% was found the most effective in reducing disease incidence which was at par with T7 [(Tvb1 (S.T) +Tvb1 (F.S, 10^7 conidia/ml)] i.e. 14.23% (Table 3).

Percent reduction in disease incidence

The data for efficacy of different treatments of *Trichoderma* spp. percent reduction in disease incidence is presented in Table 3, showed that reduction in disease incidence (%) ranged from 100% - 0.00%. Maximum disease incidence in percent was found in T6 (un-inoculated control) i.e. 100.00% whereas minimum % reduction in disease incidence was found in T12 [inoculated control with *R solani*] i.e. 0.00% which was followed by T3 [TC seed treatment (10 g/kg seed)] i.e. 50.49%, T1 [Tvb1 Seed treatment (10 g/kg seed)] i.e. 56.40%. Among different treatments of bio agents, T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] i.e. 71.69% was found to be the most effective in reducing disease incidence which was followed by T7 [(Tvb1 (S.T) +Tvb1 (F.S, 10^7 conidia/ml)] i.e. 66.27%. Whereas in case of two treatments of fungicides T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%] i.e. 73.80% reduction was found to be the best.

Number of sclerotia

The result of pot experiment is dipicted in table 3, showing number of sclerotia / plant which was ranged from 0.00 - 18.67. Maximum number of sclerotia / plant was found in T12 [inoculated control with *R. solani*] i.e 18.67 whereas minimum number of sclerotia / plant was found in T6 (un-inoculated control) i.e. 0.00 which was followed by T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S,

0.1%)] i.e. 2.33, T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] with 2.67. In case of two treatments of fungicides T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] i.e. 2.33 was found to be the best. Among different treatments of bio agents, T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] with 2.67 sclerotia/ plant was found most effective in reducing number of sclerotia / plant which was at par with T7 [Tvb1 (S.T) +Tvb1 (F.S, 10^7 conidia/ml)] i.e. 2.68.

Each value is mean of three replicates. Different superscripted alphabets followed by number in each column are significantly different at 5% level of significance according to DMRT. Values in the parenthesis are angular transformed value.

Grain yield

In the pot experiment, grain yield (g) per plant was observed to find out the most effective bio control agent against sheath blight of rice caused by *R. solani.* The result presented in table 3, showed that grain yield (g/plant) ranged from 6.33-10.68. Maximum grain yield (g/plant) was found in T6 (un-inoculated control) with 10.68 g/plant, whereas minimum grain yield (g/plant) was found in T12 (inoculated control with *R. solani*) i.e. 6.33 whereas in case of two treatments of fungicides T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] i.e. 10.63 was found to be the best. Among different treatments of bio agents T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] i.e. 10.53 was found to be the most effective in increasing grain yield (g/plant) which was at par with T9 [(TC (S.T) +TC (F.S, 10^7 conidia/ml)] i.e. 9.88 g/plant.

Table 1: Treatments details of pot experiments

T1	Tvb1 Seed treatment (10 g/kg seed)
T2	Thg Seed treatment (10 g/kg seed)
T3	TC seed treatment (10 g/kg seed)
T4	(Tvb1+Thg) Seed treatment (5 g + 5g /kg seed)
T5	Propiconazole 25 EC seed treatment (0.1%)
T6	Un-inoculated
T7	Tvb1(S.T) +Tvb1 (F.S, 10^7 conidia/ml)
T8	Thg (S.T) +Thg (F.S, 10^7 conidia/ml)
T9	TC(S.T) +TC (F.S, 10^7 conidia/ml)
T10	(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)
T11	Propiconazole 25 EC seed treatment (0.1%) + Propiconazole foliar spray (0.1%)
T12	Inoculated pathogen

R_1	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
R_2	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
R_3	T_1	T_3	T_4	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}

Table 2: Disease score (0-9) based on RLH (IRRI, 1996):

0	No infection
1	Lesion limited to lower 20% of the plant height
3	20-30%
5	31-45%
7	46- 65%
9	More than 65%

Table 3: Efficacy of different treatments of *Trichoderma* spp. on sheath blight severity and incidence of rice in pot culture.

Treatments	Disease severity RLH (%)	Score (0 9)	Disease incidence (%)	Reduction in disease incidence (%)		No. of sclerotia / plant	Grain yield (g/plant)
T1	34.08 ^{b}	5.00 ^{b}	18.67 ^{c}	56.40 ^{g}	(48.68)	4.00 ^{c}	9.06c
T2	30.74 ^{c}	4.33 ^{b}	17.24 ^{c}	59.74 ^{f}	(50.61)	5.00 ^{c}	8.25d
T3	28.24 ^{c}	4.33 ^{b}	21.20 ^{b}	50.49 ^{h}	(45.28)	6.67 ^{b}	7.45e
T4	37.82 ^{b}	5.00 ^{b}	17.13 ^{c}	60.00 ^{e}	(50.76)	4.67 ^{c}	8.26d
T5	35.56 ^{b}	5.00 ^{b}	16.30 ^{d}	61.93 ^{e}	(51.90)	3.67 ^{d}	9.57b
T6	0.00 ^{f}	0.00 ^{e}	0.00 ^{g}	100.00^{a}	(90.00)	0.00 ^{e}	10.68a
T7	26.23 ^{c}	3.67 ^{c}	14.23 ^{e}	66.27 ^{d}	(54.80)	2.33 ^{d}	9.68b
T8	23.54 ^{d}	2.33 ^{d}	14.87 ^{d}	65.77 ^{d}	(53.89)	3.33 ^{d}	9.75b
T9	31.02 ^{c}	4.33 ^{b}	16.20 ^{d}	62.17 ^{e}	(52.04)	4.00 ^{c}	9.88b
T10	21.67 ^{de}	1.33 ^{de}	12.13 ^{ef}	71.69 ^{b}	(57.84)	2.67 ^{de}	10.53a
T11	18.47 ^{e}	1.00 ^{e}	10.20 ^{f}	73.80 ^{b}	(58.05)	2.33 ^{e}	10.63a
T12	71.10 ^{a}	9.00 ^{a}	42.82 ^{a}	0.00 ^{i}	(0.00)	18.67 ^{a}	6.33
SEm (±)	2.680	0.390	2.89	3.33		0.77	
CV (%)	9.67	18.94	4.10	5.36		7.37	
LSD ($P \leq 0.05$)	4.89	1.23	2.03	2.12		1.32	

T1= Tvb1 Seed treatment (10 g/kg seed)	T5=Propiconazole 25 EC seed treatment (0.1%)	T9=TC (S.T) +TC (F.S, 10^7 conidia/ml)
T2=Thg Seed treatment (10 g/kg seed)	T6 (Un-inoculated control)	T10=(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)
T3=TC seed treatment (10 g/kg seed)	T7=Tvb1(S.T) +Tvb1 (F.S, 10^7 conidia/ml)	T11=Propiconazole 25 EC seed treatment (0.1%) + Propiconazole (F.S, 0.1%)
T4=(Tvb1+Thg) Seed treatment (5 g + 5g /kg seed)	T8=Thg (S.T) +Thg (F.S, 10^7 conidia/ml)	T12=Inoculated control

Discussion

The results of present investigation conducted on the **"**Management of sheath blight (*Rhizoctonia solani* Kuhn) of rice with indigenous *Trichoderma* spp.**"** under laboratory and field conditions are discussed in this chapter.

Isolation, purification and maintenance of *Rhizoctonia solani* causing sheath blight of rice

In the present study we isolated *Rhizoctonia solani* from the rice var Rajendra Kasturi plant showing symptom of sheath blight from BAU farm Sabour, Bhagalpur. Morphological and Cultural characterization of *R solani* was studied by visual observation of mycelia and sclerotia colour and its size. Hyphal width ranged from 4.75 µm to 7.43 µm and sclerotial colour changed from brown, light or dark brown, and black brown. The diameter of sclerotia ranged from 1.13- 2.03 mm. Mycelia of isolated pathogen were light brown during early growth and produced large amounts of aerial hyphae throughout the growth cycle. Sclerotia were tan when young, generally dark brown when mature, and up to 1.5 mm in

diameter with clumps up to 5 mm in diameter. Some sclerotia clumps were found on the agar surface but most clumps and individual sclerotia were embedded in the agar.

Our observations are in competing with the findings of the numerous workers. Gopireddy *et al***,** (2017) isolated the *Rhizoctonia solani* from infected plant and studied its morphological or cultural characteristic as sclerotia colour was light brown, brown, dark brown and deep dark brown. The hyphal width varied from 5.05 µm to 7.98 µm, and also observed by Reinking (1918) [20]. that initially the young hyphae are colourless but become yellow and ultimately brown with age, 8-12 µm in diameter with a septum near each hyphal branch and a slight constriction at the branch which tend to branch at right angles as observed in this study. The shape of the sclerotia is roughly spherical or somewhat flattened and irregular. Young sclerotia are composed of compact masses of hyphal cells about 5 µm wide, the cell wall 0.9 µm thick.

Pot evaluation of *Trichoderma* spp. against sheath blight of rice

Disease severity

The results of investigation found that maximum disease severity was found in T12 (inoculated control with *Rhizoctonia solani*) i.e. 71.10%, whereas minimum disease severity was found in T6 (un-inoculated control) i.e. 0.00%. In this study maximum disease control was observed with T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] *i.e* 69.52% and it was at par with chemical treatment, T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] *i.e* 74.02%.

Our observations are in accordance with the finding of the numerous workers. Surilirajan and Kandhari (2005) [28] found to access the disease severity and percentage disease incidence (PDI) parameters. Out of treatments, *T. viride* (TV-3235) + Propiconazole (0.1%) spray showed maximum reduction in sheath blight severity and percent disease incidence over the control. Franca *et al*. (2015) [4] also found that the efficiency of *Trichoderma asperellum* and fungicides against sheath blight of rice and found all treatments reduced sheath blight progression rate with reduced disease severity by 78.96%.

Disease incidence

The results of this study found that maximum disease incidence was observed in T12 (inoculated control with *Rhizoctonia solani*) i.e 42.82%, whereas minimum disease incidence was found in T6 (un-inoculated control) i.e 0.00%. Our observations are in accordance with the findings of the numerous workers. Singh and Sinha (2009) [23] reported that the disease incidence of rice sheath blight ranged from 45.01% to 40.42% in affected soil. Kannaiyan and Prasad (1978) [10] reported decreased disease incidence had highest in *Trichoderma* treated plants with 85.87% and lowest in without *Trichoderma* treated plants.

Grain yield

The results of investigation found that maximum grain yield (g/plant) was found in T6 (uninoculated control) i.e 10.68 g/plant. The inoculated of *R solani* only reduced the yield of rice from 10.68 (g/plant) to 6.33 (g/plant). Maximum yield was observed with highest disease control in T10 [(Tvb1+Thg) S.T + (Tvb1+Thg) FS (10^7 conidia/ml)] i.e. 10.53 (g/plant) which was at par with chemial control T11 [Propiconazole seed treatment (0.1%) + Propiconazole (F.S, 0.1%)] i.e 10.63 (g/plant). Our observations are in accordance with the finding of the numerous workers. Franca *et al*. (2015) [4] observed the efficiency of a biocontrol agent, *Trichoderma asperellum*, and fungicides increased yield by 41%. Prasad and Kumar (2011) evaluated isolates of *Trichoderma* spp. isolated from rhizosphere soil against sheath blight disease. The inoculation of pathogen and foliar spray of bioagent was done at 30 DAT and 60 DAT was found highly effective against sheath blight pathogen *R. solani* under the pot *conditions*. It was found most effective incidence and increasing grain yield. In our study two spray one prophylactic spray at 57 DAS (5 days before pathogen inoclation) and second curative spray at 67 DAS (5 days after pathogen inoculation) with *Trichoderma* spp. was found the most effective controlling the disease among all treatments and at par with propiconazone treatment.

Summary

The summary and conclusions of present investigation conducted on the "Management of sheath blight (*Rhizoctonia solani* Kuhn) of rice with indigenous *Trichoderma* spp." under laboratory and pot conditions are presented here.

Rice (*oryza sativa)* crop belongs to the family Poaceace having genus *Oryza* consisting 24 species among them only two species are grown widely. One of the 'big three' cereals, is the principal food for 60% of the worlds' people. Rice grain is a composite of carbohydrate, protein, fat, fiber and other significant nutritive constituents. It is grown under a wide range of climatic and soil conditions and grows well in clay loamy soil.

There are several constraints to the world production of rice. The crop suffers from different diseases like fungal, bacterial, viral and physiological disorders in the country. Among fungal diseases sheath blight is economically most important one. Sheath blight is caused by *Rhizoctonia solani* is the major and devastating disease of rice in India.

The rice cv. Rajendra Kasturi exhibiting characteristic symptom of sheath blight infected sheath portion sample was collected in paper bags from the rice field. The fungus was isolated and purified by hyphal tip method. The sub culturing of axenic culture was done at an interval of fifteen days on fresh PDA slants and also preserved in refrigerator at 4°C.

The biological control agents *Trichodema asperellum* (Tvb1) and *T. hamatum* (Thg) were procured from the Deptt. of Plant Pathology, BAC, Bihar Agricultural University, Sabour, Bhagalpur, Bihar and *T. viride* (TC) was isolated from the commercial formulation of BIODERMA, Biotech International Ltd.

Pot culture test was conducted in the Department of Plant Pathology, Bihar Agricultural College, BAU, Sabour. Disease severity, Number of sclerotia / replicate, Disease incidence, Growth and yield were recorded. Data was analyzed statistically. Inoculation of *Rhizoctonia solani* Kuhn caused the disease showing typical symptoms of sheath blight and it was quantified in the form of percentage relative lesion height (%RLH) with 71.1% or 9 (at 0-9 scale). Disease incidence was found 42.82% in *Rhizoctonia solani* inoculated pots.

Seed treatment with 10g/kg seed with different treatments of *Trichoderma* found highly effective and managing the disease by 5.49% - 71.69%. Among all the treatments, T10 [combined seed application of *Trichoderma asperellum* (Tvb1) and *Trichoderma hamatum* (Thg) with 5g each and foliar application with 10^7conidia /ml each at 5 days before pathogen inoculation (5 DBPI) as prophylactic and 5 days after pathogen inoculation (5 DAPI) as curative spray] was

found to be the most effective treatment managing the disease by 71.67% which was at par with propiconazole (Propiconazole 25 EC seed treatment 0.1%+ Propiconazole foliar spray 0.1%) treated plants. In all the treatments, number of *Rhizoctonia solani* sclerotia decreases significantly from 18.67 to 2.33 sclerotia per plant.

It was interestingly found that decreased number of sclerotia in *Trichoderma* treated, T10 [S.T(Tvb1 @5g/kg seed+Thg @5g/kg seed) + F. S (Tvb1+Thg @ 10^7 conidia/ml)] with 2.67 sclerotia/plant was at par with chemical treatment, T11 (Propiconazole 25 EC seed treatment 0.1%+ Propiconazole foliar spray 0.1%) with 2.33 sclerotia/plant in compression with pathogen (*R. solani*) inoculated control with 18.67 sclerotia / plant. Maximum disease incidence was found in *R. solani* inoculated plants with 42.82%, whereas no disease incidence was found in un-inouclated control.

Conclusion

The fungus, *Rhizoctonia solani* isolated from rice field of BAU farm was found virulent caused typical symptoms of sheath blight in rice var. Rajendra Kasturi. Under pot culture assay, inoculation of *Rhizoctonia solani* Kuhn caused the disease showing typical symptom of sheath blight with 71.1% severity (% RLH) or 9 (at 0-9 scale) and 42.82% disease incidence. In pot culture test, among all the treatments, combined seed application of *Trichoderma asperellum* (Tvb1) and *Trichoderma hamatum* (Thg) with 5g/kg seed each and foliar application with 10^7conidia /ml each at 5 days before pathogen inoculation (57 DAS) as prophylactic and 5 days after pathogen inoculation (67 DAS) as curative spray was found to be the most effective treatment managing the disease by 71.67% which was at par with Propiconazole (Propiconazole 25 EC seed treatment 0.1%+ Propiconazole foliar spray 0.1%) treated plants. In all the treatments of pot assay, number of *Rhizoctonia solani* sclerotia decreased significantly from 18.67 to 2.33 sclerotia per plant. Decreased number of sclerotia (2.67/plant) in the treatment [having combined seed application of *Trichoderma asperellum* (Tvb1) and *Trichoderma hamatum* (Thg) with 5g/kg seed each and foliar application with 10^7conidia /ml each at 5 days before pathogen inoculation (57 DAS) as prophylactic and 5 days after pathogen inoculation (67 DAS) as curative spray] was at par with 2.33 (sclerotia/plant) in chemical treated plants [with Propiconazole 25 EC seed treatment 0.1% + Propiconazole foliar spray 0.1%] in compression with pathogen (*R. solani*) inoculated control with 18.67 sclerotia / plant.

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