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Evaluation of fungicide, bio-agents and screening for the resistance against sheath blight disease of rice

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Abstract
Rice is one of the most important food crop among all the cereals which provide stable diet over all the rice growing countries and world. Asia is a largest producer of rice in cereal crops. Chhattisgarh is known as "bowl of rice" in India, which is covered 70% of net shown area. After Blast, sheath blight is the second destructive disease of rice which causes economic loss in rice growing areas. Under lowland growing condition yield loss ranges between 5 to 10% in tropical Asia. Sheath blight of rice is a necrotrophic pathogen and due to their wide host range nature only application of fungicides provides effective management of disease under field conditions throughout the world. All the tested fungicides were observed effective in preventing the fungal growth under *in vitro* condition. The Bio-efficacy studies of *Pseudomonas fluorescens*, *Trichoderma viride* (T14 and IRRI2) and *Trichoderma harzianum* (94a) for management of sheath blight of rice were showed significant reduction in the disease severity over control. Decrease in disease development over control with or without fungicide combination. All the tested genotypes, fourteen were showed resistance against sheath blight disease of rice under the field evaluation.

Keywords: Evaluation, fungicides, bio-agents, bio-efficacy, screening, resistance

Introduction
Rice is one of the most important food crop among all the cereals which provide stable diet over all the rice growing countries and world. Asia is a largest producer of rice in cereal crops. Except Antarctica, rice is cultivated in all the continents over an area about more than 150 million hectares, but highest production of rice takes place in Asia. In the world rice provides is about twenty percent of the world's diet followed by wheat and maize. Rice sheath blight, caused by the *Rhizoctonia solani* Kühn is the second destructive disease of rice which causes economic loss in rice growing areas after Blast (Groth, 2005; Zhou and Jo, 2014) [8, 15]. Infection caused by this pathogen is via soil borne sclerotia that floating on water surface which is come to the contact with the rice culms under flooded paddy conditions, initial infection starts at the waterline were germination of sclerotia is occur after that germinating hypha come to the contact with stem and to the canopy, usually development of symptoms occurs during tillering and heading stage. 5–6-week-old sheaths are highly susceptible to disease. Worldwide, the most common and effective practice which is followed by the farmers for the management of sheath blight under field conditions is application of fungicides. However, it is very essential to evaluate the new fungicides for the management of sheath blight because long time use of single cause development of resistance against fungicides by pathogens is commonly observed. If the fungicide molecules used judiciously in a proper way, chemical control can provide long way for effective management of the disease. Chemical molecules induce long term resistance against large number of plant diseases. Fungicides are hazardous for humans and environment which limits the regular use of fungicides for disease management under field condition. To reduced the adverse effect of fungicides on nature, use of bio-control agents are the most potent tool which provide long term disease management under field condition. Host resistance is the important tool for the eco-friendly and long-term management of plant diseases under field conditions. Development of resistance varieties is the best approach which do not required any extra cost for the disease management and it is safe for nature. Several accessions of wild germplasm identified as moderate resistance to sheath blight in the recent past. However, it is still not clear how many sheath blight resistant loci present in the wild species of rice. In recent years, many QTLs are identified which confer resistance to sheath blight, in which most of the QTLs showed small effects and few are have utilization potential.

Various sources of resistance have been found in many rice wild species, other plant species and microorganisms.

Materials and Methods

Collection of disease samples, isolation, purification and mass multiplication of *Rhizoctonia solani*

Diseased Sample collection

The disease samples were collected from the rice plants which naturally infected with sheath blight from fields of farms of different places of experiment area of Plant Pathology, IGKV, Raipur, during *Kharif* 2018.

Isolation of pathogen

Sheath blight pathogen *R. solani* is isolated from naturally infected rice plants collected from the field. the plant leaves contain disease symptoms are cut into five-centimeter small pieces with the help of sterilized blade after that the sample were washed with distilled water to remove debris from the sample and dried with the help of blotter paper to remove excess moisture. Cut samples are surface sterilized with 1% of sodium hypochlorite 0.01% mercuric chloride and washed 3 times with sterile distilled water to remove traces of the chemical. the dried plant samples are then transferred in pathogen isolation media containing, modified Ko & Hora medium, alkaline water agar or Potato Dextrose Agar (PDA) medium and incubated at 28±2 ºC under BOD incubator. when the fungal mycelium growth observed on the medium the hyphal tip of the mycelium cut with the help of sterile needle and transferred into freshly prepared Potato Dextrose Agar medium.

Purification

For the purification of the pathogen alkaline water agar medium or PDA medium addition with antibacterial antibiotic at 300 ppm were used to avoid the bacterial contamination. Small piece of a mycelium of pathogen are cut with cork borer and transferred into freshly prepared PDA medium and incubated at 28±2 ºC in BOD incubator for favor the growth of fungal growth. Desired number of subculturing were made if the further purification is required.

Pathogenicity test

Typha bits inoculation method was followed to establish the disease under field condition for Pathogenicity Test on 45 to 55 days old rice plant of cultivar Swarna. Fully colonized typha bits (2-3 pieces) are inserted into the hills of each plant just above the 5-7 cm height of the water line and each hill were tied with a rubber band to maintain the high humidity at inoculated area which developed microclimate for the fungal growth. The inoculated plants were regularly observed for disease development. to develop the disease under field condition the rice plants at 45 to 55 days old are preferred. For the Pathogenicity Test 50 plants of each variety like Swarna, TN-1 and Kranti were inoculated. Inoculated plants were regularly observed for disease development and re-isolation of pathogen was done to confirms the Koch's postulates. The disease measurement was taken by following standard evaluation system (SES), IRRI (2002) at 30 days after pathogen inoculation.

Mass multiplication of inoculums

R. solani were mass multiplied in autoclaved stem pieces of typha cut into 2 to 3 inches size, soaked in 1% dextrose and peptone solution. Small disk of fungal mycelium from 2 days old culture plate of *R. solani* was used for inoculation of autoclaved typha flasks after the pathogen inoculation the flasks were incubated in incubator for 8-10 days to promote the fungal growth. After the fully coverage of fungal growth on typha the inoculum is used for artificial inoculation of rice plants under field conditions (Bhaktavatsalam *et al.*, 1978) [2].

Inoculation

To develop the artificial disease under field conditions, the typha bits inoculation method was followed and all the rice plants were inoculated at 45 to 55 days after transplanting at maximum tillering stage. 2-3 fully colonized typha pieces with fungal growth and sclerotia was used for the inoculation. The fungal inoculum was placed into central region of hills after that plants are tied with rubber band which provide favorable conditions like moisture and humidity for the development of microclimate at inoculation site. The plants are inoculated just above the water surface at 5 to 7 cm height.

Influence of different fungicides concentrations on germination of *R. solani sclerotia*

Under *in vitro* condition, 5 fungicides (WCPL6060, Hexaconazole 5% SC, Propiconazole 25% EC, BAS 750 02 F 400g/L SC and Validamycin 3% L) evaluated against *R. solani* at 2 different concentrations (500 ppm and 1000 ppm) by poison food technique. Chemical name and trade name are given below.

Table 1: Chemical name and trade name of fungicides

S. No.	Technical name	Trade name
1	WCPL 6060	New fungicide
2	Hexaconazole 5% SC	Contaf 5E
3	Propiconazole 25% EC	Tilt
4	BAS 750 02 F 400g/L SC	New fungicide
5	Validamycin 3% L	VALID PRO
6	Control	-

For the experiment, fungal culture was grown by using PDA for one week. Freshly prepared PDA media melted and desired quantity of fungicides were added in the melted media to meet tested concentration. Fungicide added media used for evaluation of fungicides against pathogen and control maintained without addition of chemical. At the time of pouring, 300 ppm antibiotic added in medium to avoid bacterial contamination. A mycelium disk of 5 mm size from freshly prepared fungal culture was taken and put on the center of petri-plate containing poisoned media and incubated in incubator at 28 ºC and for 3 days. Three replications are maintained for each treatment. After 3 days, diameter of the fungal colony were measured when control plate fully covered with fungal growth. Observations were recorded and percent inhibition was calculated by using the formula of Vincent (1947) [13].

Where,

$$\text{Percent inhibition} = \frac{C - T}{C} \text{ X } 100$$

I= Percent inhibition
C= Mycelial growth in control
T= Mycelial growth in treatment

Bio-efficacy of bio-agents against sheath blight of rice under field conditions

The Bio-efficacy studies of Fluorescent *Pseudomonas*, *Trichoderma viride* (T14 and IRRI2) and *Trichoderma harzianum* (94a) for management of sheath blight of rice field experiment were conducted at the experimental field of the Department of Plant Pathology situated in the Research farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur, (C.G.) during *Kharif* 2018. Three randomized replicates per treatment were used with a minimum plot size of 75X100cm. The package of practice of crop was according to normal practical standards. Total 25 hills are selected per plot which are 45 days old and inoculated with 2-3 typha stem bits. Bio-agents were sprayed after 2-3 days of inoculation when initial lesion shows. The plant height, lesion height and disease score were recorded at 20 days after spraying from randomly selected plants.

Table 2: Bio-efficacy of bio-agents against sheath blight of rice with their doses

S. No.	Treatments	Dose ml/ha
1	Fluorescent *Pseudomonas* (P5)	10 ml
2	Fluorescent *Pseudomonas* (P8)	10 ml
3	Fluorescent *Pseudomonas* (P10)	10 ml
4	*T. viride* (IRRI2)	10 ml
5	*T. viride* (T14)	10 ml
6	*T. harzianum* (94a)	10 ml
7	Fluorescent *Pseudomonas* (P5) + Thifluzamide 24% SC	10 ml + 1.5 ml
8	Fluorescent *Pseudomonas* (P8) + Thifluzamide 24% SC	10ml + 1.5 ml
9	Fluorescent *Pseudomonas* (P10) +Thifluzamide 24% SC	10ml + 1.5 ml
10	*T. viride* (IRRI2) + Thifluzamide 24% SC	10ml + 1.5 ml
11	*T. viride* (T14) + Thifluzamide 24% SC	10ml + 1.5 ml
12	*T. harzianum* (94a) + Thifluzamide 24% SC	10ml + 1.5 ml
13	Thifluzamide 24% SC	1.5 ml
14	Control	-

Observations

$$\text{Disease Severity} = \frac{\text{Total lesion lenght}}{\text{Total Sheath lenght}} \text{X } 100$$

$$\text{PDI} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of plants assessed x maximum rating}} \text{X } 100$$

Screening of previous years sheath blight resistant material through artificial inoculation

The study was conducted under irrigated conditions in bunded rice field during *Summer* 2019. 17 rice entries were grown in research field plant pathology department, I. G. K. V., Raipur i.e., S/B-2761-77, S/B-2762-83, S/B-2763-121, S/B-2774-20, S/B-2777-4, S/B-2779-108, S/B-2780-144, S/B-2784-149, S/B-2782-370, S/B-2784-402, S/B-2786-855, S/B-NSN-1-77, S/B-BREEDING-4, S/B-G/P-108, S/B-G/P-855, S/B-NSN-2-298, S/B-SIET-1-42.

The rice entries were shown in a nursery bed by direct sowing in simple 3 rows design with a spacing of 20 cm from row to row and 15 cm from plant-to-plant distance a single row of check Swarna was taken. Placement of seed was done approximately at a distance of 2 to 3 cm. Fertilizer was used @ N120: P50: K 0 kg/ha. Half quantity of N and Full quantity of P are applied as a basal dose and rest quantity of N are applied in two split doses. The environment was kept aseptic to ensure that the seedlings were disease and contaminant-free.

***Rhizoctonia solani* inoculum preparation**

R. solani were mass multiplied in autoclaved stem pieces of typha cut into 2 to 3 inches size, soaked in 1% dextrose and peptone solution. small disk of fungal mycelium from 2 days old culture plate of *R. solani* was used for inoculation of autoclaved typha flasks after the pathogen inoculation the flasks were incubated in incubator for 8-10 days to promote the fungal growth. after the fully coverage of fungal growth on typha the inoculum is used for artificial inoculation of rice plants under field conditions (Bhaktavatsalam *et al.*, 1978) [2].

Method of inoculation

To develop the artificial disease under field conditions, the typha bits inoculation method was followed and all the rice plants were inoculated at 45 to 55 days after transplanting at maximum tillering stage. 2-3 fully colonized typha pieces with fungal growth and sclerotia was used for the inoculation. The fungal inoculum was placed into central region of hills after that plants are tied with rubber band which provide favorable conditions like moisture and humidity for the development of microclimate at inoculation site. The plants are inoculated just above the water surface at 5 to 7 cm height. All the genotypes were screened for disease severity. Each hill were observed for disease development in the form of lesion height and plant height and disease severity was calculated as standard evaluation system (SES), IRRI (2002). Observations were taken on 30 days after inoculation and graded as per 0-9 SES scale. The scale was as follows:

The disease development would be recorded in each variety and Percent Disease severity and Percent Disease Index will be calculated as:

$$\text{Disease Severity} = \frac{\text{Total lesion lenght}}{\text{Total Sheath lenght}} \text{X } 100$$

Table 3: Measurement of the disease 0-9 SES scale developed by IRRI (2002)

Score	Description
0	No disease
1	Vertical spread of lesion up to 20 percent plant height
3	Vertical spread of lesion up to 21-30 percent plant height
5	Vertical spread of lesion up to 31-45 percent plant height
7	Vertical spread of lesion up to 46-65 percent plant height
9	Vertical spread of lesion up to 66-100 percent plant height

Results

Influence of different fungicides concentrations on germination of *R. solani* sclerotia

Under *in vitro*, five fungicides were tested for their efficacy against germination of sclerotia of *R. solani*. No germination of sclerotia were found in case of Hexaconazole 5% SC, Propiconazole 25% EC and WCPL 6060. 18 mm, 5 mm and 25.3 mm, 8.3 mm growth recorded at the concentration 500 ppm, 1000 ppm in case of BAS 750 02 F 400g/L SC and Validamycin 3% L respectively (Table 4).

Table 4: Influence of different fungicides concentrations on germination of *R. solani* sclerotia

S. No.	Fungicides	Radial growth (mm) 4 DAI Concentration (ppm)	
		500 ppm	1000 ppm
1	WCPL6060	0	0
2	BAS 750 02 F 400g/L SC	18	5
3	Hexaconazole 5% SC	0	0
4	Propiconazole 25% EC	0	0
5	Validamycin 3% L	25.3	8.3
6	Control	51	54

Bio-efficacy of bio-agents against sheath blight of rice under field conditions

The great interest in *Trichoderma* as bio-protectants is due to the need for alternatives to chemical plant protection. Chemical fungicides have undesirable effects on the environment and, when used regularly, encourage development of fungicide resistance in pathogens. Replacement of some of the chemical fungicide treatments with biological control agents not only reduces the input of chemicals into agricultural soils but can also result in improved disease control. By using chemical and biological control measures together (integrated control), the duration of active disease control will be extended. Chemical protectants are effective under climatic conditions or levels of disease pressure in which the biological antagonist is less effective, while an active biological control agent can prophylactically colonize wounds or senescing plant tissue.

The Bio-efficacy studies of *Pseudomonas fluorescens*, *Trichoderma viride* (T14 and IRRI2) and *Trichoderma harzianum* (94a) for management of sheath blight of rice (Figure 1 and Table 4) showed after 20 days of sprayed, all the treatments were significantly reducing the disease severity over control. Decrease in disease development over control when only bio-control agents were sprayed ranged from 6.7% to 11.46%. In combination with Thifluzamide 24% SC vertical sheath colonization of preinoculated *R. solani* was decreased.

To affect mycelium of fungi in plant tissue, the combination of fungicide and bio-agent must come with contact with the pathogen inside the plant. Fungitoxic compound can reach vegetative or generative structures of a sensitive pathogen in a plant, and can exert certain effects on pathogen's physiology and morphology are inevitable to occur and therefore will restrict the growth of *R. solani.*

The bio-control agent (sprayed along with the fungicide) must come into contact with the sheath blight pathogen to exert an effect on mycelium of *R. solani* in and on the surface of plant tissue. Because sheath blight pathogen does not produce spores, and lesion multiplication in a rice canopy occurs through the growth of runner hyphae from a (mother) lesion at the surface of rice tissues (leaf sheath of blade). It might be possible that the bio-control agent can restrict the surface runner hyphae. To exert an effect on mycelium of *R. solani* inside the host tissue the bio-control agent has to colonize and become entophytic.

The present observation does not indicate that bio-control agents have exerted any effect to decrease in sheath blight development when the different bio-control agents were applied with Thifluzamide 24% SC (Figure 1 and Table 5). Application technology needs to be fine-tuned to get appropriate effects of potential bio-control agent identified during *in vitro* studies.

Table 5: Bio-efficacy of Bio-agents under field conditions during *Kharif* 2018

S. No.	Treatments	Dose ml/lit	PDI	%decrease over control
1	*P. fluorescens* (P5)	10 ml	57.29 (70.73)	11.46
2	*P. fluorescens* (P8)	10 ml	60.37 (75.55)	6.70
3	*P. fluorescens* (P10)	10 ml	58.71 (72.96)	9.27
4	*T. viride* (IRRI2)	10 ml	61.61 (77.40)	4.79
5	*T. viride* (T14)	10 ml	56.87 (69.99)	12.11
6	*T. harzianum* (94a)	10 ml	57.89 (71.66)	10.53
7	*P. fluorescens* (P5) + Thifluzamide	10 ml + 1.5 ml	36.15 (34.81)	44.13
8	*P. fluorescens* (P8) + Thifluzamide	10ml + 1.5 ml	36.82 (35.94)	43.09
9	*P. fluorescens* (P10) + Thifluzamide	10ml + 1.5 ml	35.93 (34.44)	44.40
10	*T. viride* (IRRI2) + Thifluzamide	10ml + 1.5 ml	36.4 (35.22)	43.74
11	*T. viride* (T14) + Thifluzamide	10ml + 1.5 ml	36.59 (35.55)	43.45
12	*T. harzianum* (94a) + Thifluzamide	10ml + 1.5 ml	35.71 (34.07)	44.81
13	Thifluzamide	1.5 ml	36.8 (35.92)	43.13
14	Control		64.71 (81.67)	
	C.V		4.44	
	C.D (0.05%)		3.58	

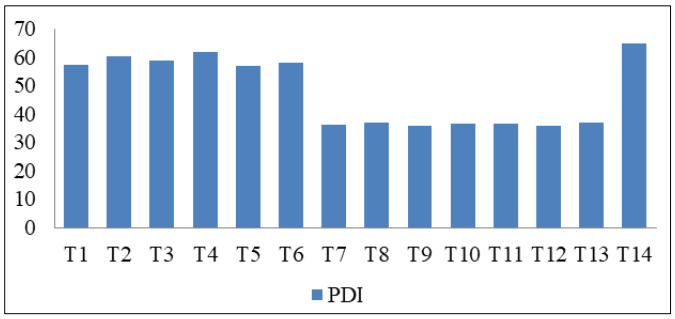

Fig 1: Bio-efficacy of Bio-agents against sheath blight of rice under field conditions during *Kharif* 2018

Screening of previous years sheath blight resistant material through artificial inoculation

Host resistance is the important tool for the eco-friendly and long-term management of plant diseases under field conditions. Development of resistance varieties is the best approach which do not required any extra cost for the disease management and it is safe for nature. Rice germplasm is a critical source for improving varieties for a range of traits as it reflects the genetic diversity (aus, aromatic, indica, temperate japonica and tropical japonica (Glaszmann 1987; Garris *et al.*, 2005) [7, 6] and also captures the diversity of genotypes in terms of morphological traits and duration to maturity (Bio-diversity International *et al.*, 2007). Overall past two decades, several sheath blight resistance quantitative trait loci (QTLs) have been mapped, but consistency across results from different studies has not always been observed (Pinson *et al.*, 2005) [10]. The accurate measurement of sheath blight resistance under field conditions depends on a range of environmental factors, (Eizenga *et al.*, 2002; Yuen and Forbes 2009) [5, 14]. Minimal considerations of pathogen infection and spread or epidemiology, and combined with the complexity of resistance expression, has contributed to slow progress in breeding for sheath blight resistance (Srinivasachary, 2011) [11]. The role of quantification of the physiological resistance and disease escape on overall resistance to sheath blight may help in further identification of resistance source, in breeding programme of rice. In the present investigation we attempt to address the first group of resistance mechanisms following artificial inoculation with typha bit (pre-colonized with *R. solani* mycelium) to derive the components of physiological resistance to rice sheath blight. The seventeen rice entries i.e., S/B-2761-77, S/B-2762-83, S/B-2763-121, S/B-2774-20, S/B-2777-4, S/B-2779-108, S/B-2780-144, S/B-2784-149, S/B-2782-370, S/B-2784-402, S/B-2786-855, S/B-NSN-1-77, S/B-BREEDING-4, S/B-G/P-108, S/B-G/P-855, S/B-NSN-2-298, S/B-SIET-1-42 and Swarna (used as a check variety), were screened for resistance against sheath blight under field conditions during *Rabi* 2019 by artificial inoculation technique. Among all the tested entries of rice, no any entry was resulted immune or highly resistant response (Table 6) to sheath blight of rice. Fourteen entries were recorded as resistant (3-Score) i.e., S/B-2761-77, S/B-2762-83, S/B-2777-4, S/B-2780-144, S/B-2784-149, S/B-2782-370, S/B-2784-402, S/B-2786-855, S/B-NSN-1-77, S/B-G/P-108, S/B-NSN-2-298, S/B-SIET-1-42 and S/B-BREEDING-4 and three as moderately resistant (5-Score) S/B-2774-20, S/B-2779-108 and S/B-G/P-855.

Table 6: Reaction of rice entries for sheath blight Resistant

S. No.	Score	Varietal Reaction	Frequency Distribution	Entries (IET No.)
1	0	Immune	0	Nil
2	1	Highly Resistant	0	Nil
3	3	Resistant	14	S/B-2761-77, S/B-2762-83, S/B-2763-121, S/B-2777-4, S/B 2780 -144, S/B-2784-149, S/B-2782-370, S/B-2784-402, S/B-2786-855, S/B-NSN-1-77, S/B-BREEDING-4, S/B-G/P-855, S/B-NSN-2-298, S/B-SIET-1-42
4	5	Moderately resistant	3	S/B-G/P-855, S/B-2774-20, S/B-2779-108
5	7	Susceptible	0	Nil
6	9	Highly Susceptible	0	Nil
Total Entries: 17				

Several lines of evidence indicate the screening of rice germplasm for sheath blight following artificial inoculation, but no germplasm has been identified to confer complete resistance to sheath blight (Chandra *et al.*, 2016, Tejaswini, 2016) [4, 12]. Bashyal *et al.*, 2017 reported two *Oryza rufipogon viz.*, IC336719 and IC336721 were resistant to sheath blight.

Conclusion

All the tested fungicides found effective against *R. solani* reducing mycelial growth under *in vitro* studies. Application of combination of Fungicide (Thifluzamide 24% SC) + bio-agent (fluorescent *Pseudomonas*/ *Trichoderma* spp.) during the present observation do not indicates that bio-control agents have exerted any effect to decrease in sheath blight development in view of this application technology needs to be fine-tuned to get appropriate effects of potential bio-control agent identified during *in vitro* studies. Following artificial inoculation 14 entries were found resistance to sheath blight infections.

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