Screening of native isolates of Trichoderma spp and *Pseudomonas fluorescens* against *Rhizoctoniasolani* under *in vitro*

ABSTRACT:

Production and inhibitory effect of volatile compounds of Trichoderma isolates and *Pseudomonas fluorescens*were tested by dual culture technique against the *Rhizoctoniasolani*..Trichoderma isolates were isolated from various places of Rajnandgaon, Raipur and Durg district of Chhattisgarh. The study about screening of native isolates of *Trichoderma harzianum*aginst R. solani*in vitro* revealed that all native isolates of *Trichoderma harzianum* were significantly inhibited the mycelial growth of R. solani. The maximum reduction in mycelial growth from R. solani in volatile compounds was recorded in Th-4 followed by Th-1, Th-2, Th-5 and Th-3. The study of native *Pseudomonas fluorescens* on mycelial growth and sclerotial formation of R solani revealed that all Pseudomonas *fluorescens* isolates significantly inhibited the mycelial growth of R. solani. The mycelial growth of R. solani was highly inhibited by Pf-05 isolate (with 7.94mm mycelial growth and 91.18% decrease over control).The study about screening of native bioagents, Trichoderma and Pseudomonas revealed that the bioagent isolates significantly reduced the mycelial growth of R. solani under *in vitro*.

Key Words: Sheath blight of rice, *Rhizoctoniasolani*, native isolates, Trichoderma spp., *Pseudomonas fluorescens,* dual culture technique.

INTRODUCTION:

Sheath blight is one of India's widespread and harmful rice diseases. Rice sheath blight disease is causing significant loss, particularly in areas where high yielding varieties are cultivated. *Rhizoctoniasolani* (Perfect stage-*Thanatephoruscucumeris*) which causes rice sheath blight in both soil and water borne. Miyake (1910) stated that the sheath blight disease was first reported from Japan, and stated that the casual organism was sclerotia irregular. Subsequently this disease was recorded from various global rice-growing regions, and particularly from major rice-growing countries. The presence of sheath blight disease in rice from several parts of India and beyond has been confirmed by workers of different parts of India. Butler made reference to the Indian disease as early as 1918. The presence of this disease has been confirmed by Andhra Pradesh, Assam, Jammu and Kashmir, Kerala, Tamil Nadu (Anonymous, 1971), Orissa and West Bengal (Das, 1970), Madhya Pradesh (Anonymous, 1975; Verma *et al*., 1979).

There are several important reports for the screening of native isolates of Trichoderma spp and *Pseudomonas fluorescens* against *Rhizoctoniasolani.* Kumar and Marimuthu (1994) tested this trichoderma sp. with *Rhizoctoniasolani* in double cultures and found that T. viride was the most

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antagonistic, followed by *T. harzianum* and *T. longibrachiatum*. Das *et al.* (1998) found that *Trichoderma viride* is more effective than *Bacillus subtilis* in reducing rice infection with sheath blight. Glow in the dark pseudomonas additionally carried out the ISR against various fungal, bacterial and viral infections (Maurhofer*et al.*, 1994 and Lin *et al*., 1995). Tang-ZiaBin*et al*. (2002), which were examined on PDA plates, the inhibition rate of 13 (antagonistic mycetes of T. aureoviride) was highest and reached 52.54%.A more sustainable approach for sheath blight management in rice with less reliance on synthetic fungicides, greater use of natural fungicides such as strobilurins and biological agents like Trichoderma spp., Pseudomonas spp., Gliocladium spp., Bacillus spp. to restrict disease (Singh *et al*., 2019).

The losses due to sheath blight disease can be managed through the application of bio control agents in disease management is considered as eco-friendly, without any environmental pollution. The present study was carried out to explore the native isolates of Trichoderma spp and *Pseudomonas fluorescens* against *Rhizoctoniasolani in vitro.*

MATERIAL AND METHODS:

Production and inhibitory effect of volatile compounds of Trichoderma sp.:

Production and inhibitory effect of volatile compounds of Trichoderma isolates were tested against the *Rhizoctoniasolani* by following the procedure of Dennis and Webster (1971b). Trichoderma isolates were isolated from various places of Rajnandgaon, Raipur and Durg district of Chhattisgarch. Bottom of Petri plate containing R. solani culture disc were inoculated on PDA was inverted atop on the bottom of plate containing Trichoderma isolate culture disc on PDA. The assembly was sealed with cello tape. Control plate have R. solani in both, lower and upper plates. This experiment was conducted under *in vitro* condition in completely randomyzed design (CRD) with three replications during kharif 2016. For each replication, five plates were maintained. All the plates were incubated at 28 ±2 ^{0}C in BOD incubator. Mycelial growth and number of sclerotia of *Rhizoctonisolani* was recorded seven days after inoculation.

Mycoparasitism of Trichoderma spp:

The antagonistic potential of the Trichoderma isolates against *Rhizoctoniasolani* was tested by dual culture technique (Dennis and Webster, 1971c). Twenty milliliter of PDA was poured into sterile petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3 to 4 cm gap. One control was maintained where in only test fungus was grown. Three replicated petri plates were maintained for each treatment. The plates were incubated at 28±2°C for 96 hours. (Rajput and Zacharia, 2017). The mycelial growth of the pathogen and antagonist in dual culture and control plates was measured seven days after inoculation.

Isolation of *Trichoderma harzianum*:

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The soil samples randomly collected from rhizosphere soil of rice crop from farmer's fields at Raipur, Durg and Rajnandgaon districts. Ten gram of soil collected from each samples used for isolation of the *Trichoderma harzianum* following the serial dilutions and pour plate method.

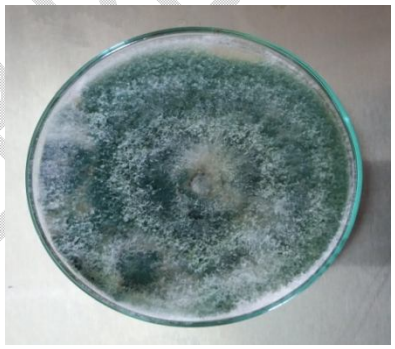
Preparation of the serial dilutions for isolation:

Rhizosphere soil (10 g) was suspended in Erlenmeyer flask with 90 ml sterile distilled water. Suspension on vortex shaker was shaken for 10 minutes. One ml of the suspension from the first dilutions (10^{-1}) was transferred aseptically to a culture tube with 9 ml of sterile water, labelled as (10^{-2}) and the initial suspension was diluted to 100 times. This procedure was repeated till the original sample was diluted to 10^{-8} time (Mew and Rosales, 1986). For *Trichoderma harzianum*, sufficient dilutions in quantity of (1 ml) from the sample were put on Potato Dextrose Agar media to isolate single colonies by pour plate method.

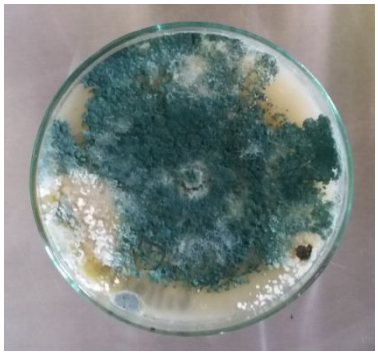
Pour plate method

One ml of soil suspension was applied aseptically from adiquot dilutions (10^{-1} to 10^{-8}). For each petriplate twenty ml of sterilized, melted, and cooled medium were added and poured. For 24 hours, the petriplates were incubated at 28±2°C. Established individual colonies were marked and analyzed separately. For° further purification and isolation the individual colonies were picked up and moved onto the surface of solidified petriplates.. The pure cultures were stored in refrigerator at 4°C.

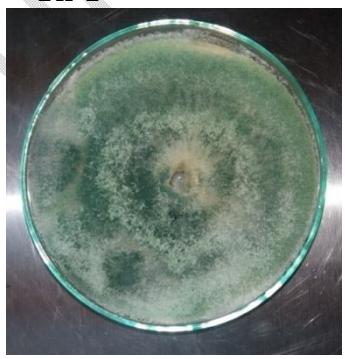
Th-1

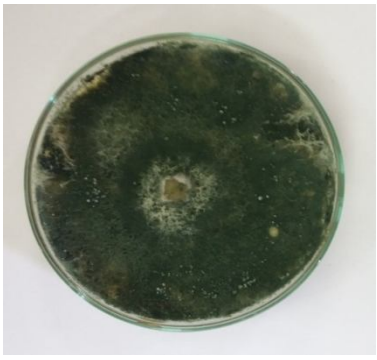


Th-2



Th-3





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Th-4 **Th-5**

Fig.1: *Trichoderma*isolates collected from different locations of Chhattisgarh

Production and inhibitory effect of volatile compounds of Pseudomonas spp.:

Production and inhibitory effect of volatile compounds of Pseudomonas isolates were tested against the *Rhizoctoniasolani*. This experiment was performed in complete randomized design (CRD) under *in vitro*, with three replications during the kharif 2016. Five plates were taken for each replication. All the plates had been incubated in BOD incubator at 28 $\pm 2^0$C. Seven days after inoculation, mycelial growth and number of sclerotia of *Rhizoctonisolani* were recorded.

Antagonismm of *Pseudomonas fluorescence*:

The antagonistic potential of the *Pseudomonas fluorescens* isolates against *Rhizoctoniasolani* was tested by dual culture technique. 20ml of sterile PDA medium was poured into petridishes under aseptic conditions and allowed to solidify. After solidification, 9mm culture disc obtained from the periphery of 10 day old culture of R. solani was inoculated 1.5 cm away from the edge of the petridish. Similarly, two day old bacterial antagonists was streaked (1cm long) at equidistance just opposite to the pathogenic culture. The control plates were inoculated by opposing the two discs of culture of R. solani. Three replicated petri plates were maintained for each treatment. The plates were incubated at 28 $\pm 2^0$C. The growth of the pathogen and antagonist in dual culture and control plates was measured seven days after inoculation (Dannis and Webster, 1971). The per cent inhibition of mycelial growth was calculated by the following formula (Vincent, 1927):

Per cent Inhibition (I) = C-T/C x 100

Where, I is the Inhibition per cent,
C is the radial growth in control
T is the radial growth in Treatment.

Isolation of *Pseudomonas fluorescens*:
The soil samples randomly collected from rhizosphere soil of rice crop from farmers fields at Raipur, Durg and Rajnandgaon districts. Ten gram of soil collected from each samples used for isolation of the *Pseudomonas fluorescens* following the serial dilutions and pour plate method.

Preparation of the serial dilutions for isolation:
Rhizosphere soil (10g) was suspended in 90 ml sterile distilled water in Erlenmeyer flask. The suspension was shaken for 10 minutes on vortex shaker. One ml of the suspension from the first dilutions (10^{-1}) was aseptically transferred to a culture tube having 9 ml of sterile water, marked as (10^{-2}) and thus diluting the original suspension to 100 times. This procedure was repeated till the original sample was diluted to 10^{-8} time (Mew and Rosales, 1986). Appropriate dilutions in quantity of (1 ml) from the sample were placed on Kings B medium (KBM) for *Pseudomonas fluorescens* to isolate single colonies by pour plate method.

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The medium:

King‟s B medium, a selective one (Kings *et al*., 1954) was used for the isolation of *Pseudomonas fluorescens*. The composition of the medium was as follows:

List 1 : composition of the medium

Ingredients	**Quantity**
Protease peptone	20.0 g
Potassium sulphate	1.50 g
Magnesium sulphate	1.50 g
Glycerol	8.00 g
Agar-Agar	20.00 g
Cyclohexamide	75.00 g
Novobiocin	45.00 g
Penicillin	75.00 g
Distilled water	1000.00 g

All three antibiotics namely cyclohexamide, novobiocin and penicillin were dissolved in 3 ml of 90 per cent ethanol and added after autoclaving when medium was cooled to 45C°.

Pour plate method

One ml of soil suspension from aliquot dilutions (10^{-1} to 10^{-8}) were aseptically added. Twenty ml sterilized, melted and cooled medium was added and poured in each petriplate. The petriplates were incubated at 28±2^{o}C for 24 hours.The separate individual colonies with yellow-green and blue white pigments developed were marked and examined under UV light. The individual colonies were picked up with sterilized loop and transferred on to the surface of solidified KMB petriplates by streaking to make a series of parallel, non-overlapping streaks, for further purification and single colony isolation. The petriplates were incubated at 28±2^{o}C for 24 hours.The single colonies developed separately at the last tip of the streaks were transferred to KMB slants to serve as pure culture and designated. The pure cultures were stored in refrigerator at 4°C.

RESULTS AND DISCUSSION:

Screening of native isolates of Trichoderma spp against R. solani*in vitro*:

Under dual culture study :

The present study was undertaken during the kharif 2016 to screen the native isolates of Trichoderma spp against R. solani*in vitro*. Treatments were allocated under completely randomized design (CRD). The data presented in the table no.1 and fig. 2, 3 and 4 showed that in dual culture study, all the native Trichoderma isolates were significantly inhibited the mycelial growth of R. solani over the control. The mycelial growth of R. solani was highly inhibited by the Th-2 (34.66%) with 61.47% decrease over control which is statistically on par with the Th-1 with 35.40mm mycelial growth and 60.66% decrease over control treatment, Th-5 with 35.60mm mycelial growth and 60.22% decrease over control, Th-3 with 36.80mm mycelial growth and 59.11% decrease over control and Th-4 with 41.97mm mycelial growth and 53.70% decrease

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over conrol treatment. The maximum mycelial growth was recorded under control treatment (90.00 mm). The sclerotial production was not observed in all the Trichoderma isolate treated plates. Similar results are in the agreement with Kumar and Marimuthu (1994) revealed that Trichoderma sp. with R. solani in dual-cultures and found that T. viride was the most antagonistic followed by T. harzianum and T. longibrachiatum. Salam (2007) tested that all the isolates of Trichoderma in dual culture inhibited mycelial growth of R. solani and inhibition ranged from 60.37 to 71.48 per cent over control. A clear visible band was formed in the zone of contact between the two fungal growth. The sclerotial production was not recorded in all the Trichoderma isolate treated plates. Chakravarthy and Nagamani (2007) tested the antagonistic potential of twenty-six isolates against R. solani Kuhn and reported that T. harzianum (M10P) and T. aureoviride (TA1) were more aggressive and significantly reduced the formation of sclerotia of R. solani.

Effect of volatile compounds on *R. solani*:Results showed that all Trichoderma isolates secreted volatile compounds significantly reduces the mycelial growth of R. solani (table no. 1 and fig. 4). Among all Trichoderma isolates, the volatile compound of Trichoderma isolate Th-4was found significantly superior to check the mycelial growth of R. solani (19.60mm) with 78.51% decrease over control and found most effective for release of volatile compounds against R. solani. It is followed by Th-1 isolate (with 39.06mm and 56.66% decrease over control), Th-2 (with 40.13mm and 55.55% decrease over control), Th-5 (with 44.80mm and 50.37% decrease over control) and Th-3 (with 55.53mm and 38.51% decrease over control) treatment of volatile compound. The maximum mycelial growth was recorded under control untreated treatment (90.00 mm). Since, Trichoderma sp. known to exhibit the various types of mechanisms when come into contact with other microorganisms especially with soil borne fungal pathogens. Mycoparasitism, release of volatile and non-volatile diffusible compounds (antibiotics) and hydrolytic enzymes are mainly responsible for their effectiveness against other microorganism. In this study, native isolates of *Trichoderma harzianum* were also evaluated for some of the mechanisms like mycoparasitism (dual culture) and release of volatile compounds against R. solani and result showed that isolate Th2 was found best for mycoparasitism and isolate Th-4 for release of volatile compounds. Similar results are in the agreement with Salam (2007) revealed that five isolates of Trichoderma harzianum inhibited the mycelial growth and sclerotial formation of the R. solani by the production of volatile compounds. Similarly, efficacy of various species of Trichoderma against R. solani was also observed by several workers when they evaluated Trichoderma sp. by various ways, supports the present findings (Ogura and Akai 1965; Hashioka and Fukita 1969; Dennis and Webster 1971; Well *et al*. 1972; Elad *et al*. 1982; Kumar and Marimuthu 1994; Cliquet and Scheffer 1996; Dubey 1998; Das *et al.*1998; Das and Hazarika, 2000; Kharakrang*et al*. 2002; Mishra and Sinha, 2002; Nagaraju *et al*. 2002; TangZia Bin *et al*. 2002; Kazempour*et al*. 2003; Meena *et al*. 2003; Rudresh *et al*. 2005 and Chakravarthy and Nagamani, 2007). A more sustainable approach for Sheath blight management in rice with less reliance on synthetic fungicides, greater use of natural fungicides such as strobilurins and

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biological agents like Trichoderma spp., Pseudomonas spp., Gliocladium spp., Bacillus spp. to restrict disease (Singh et al., 2019).

Table 1: Effect of native *Trichoderma* isolates on mycelial growth and sclerotia formation of *R. solani*

***Trichodermaharzianum* isolates**	**Mycelial growth (mm) of *R. solani***	**% decrease over control**	**Volatile compounds (Mycelial growth)**	**% decrease over control**
Th-1	35.40	60.66	39.06	56.66
Th-2	34.66	61.47	40.13	55.55
Th-3	36.80	59.11	55.53	38.51
Th-4	41.97	53.70	19.60	78.51
Th-5	35.60	60.22	44.80	50.37
Control	90.0	-	90.0	-
SE(m)+	**2.50051**		**2.21560**	
CD (5%)	**7.70486**		**6.82694**	

Production and inhibitory effect of volatile compounds of *Trichoderma* isolates tested against *R. solani*. Such antagonism tested by Dual culture technique.

4 plates per replication

After 48 hrs.

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After 72 hrs.

Fig.2.: Effect of native *Trichoderma* isolates on mycelial growth of *R. solani*

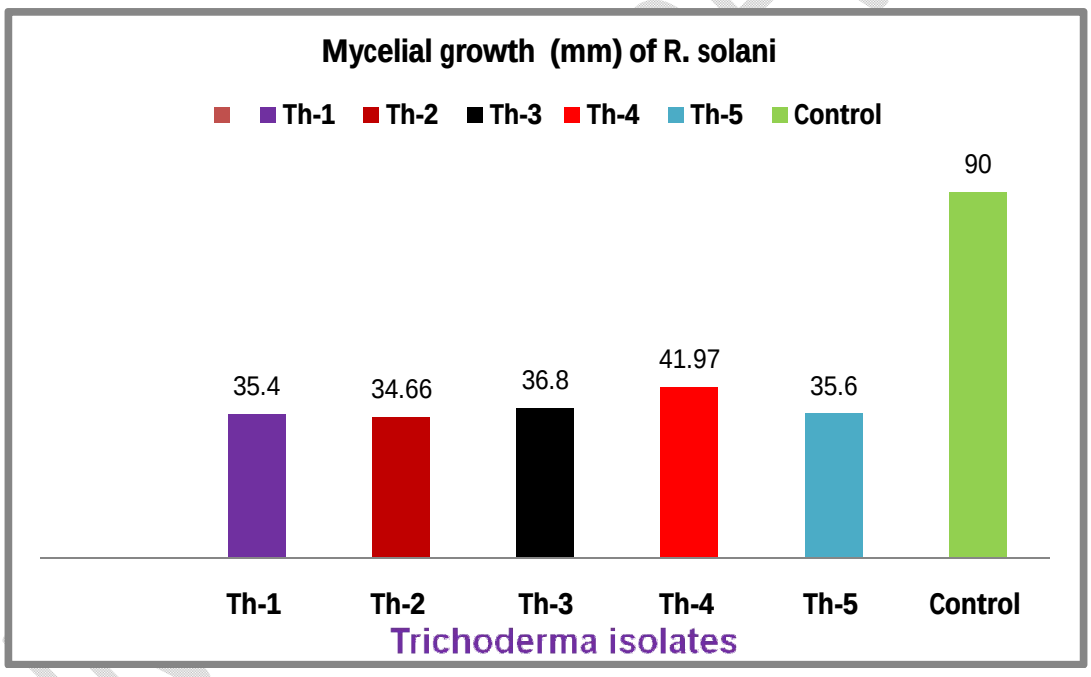

Fig3: Effect of native *Trichoderma* isolates (Dual culture) on mycelial growth of *R. solani*

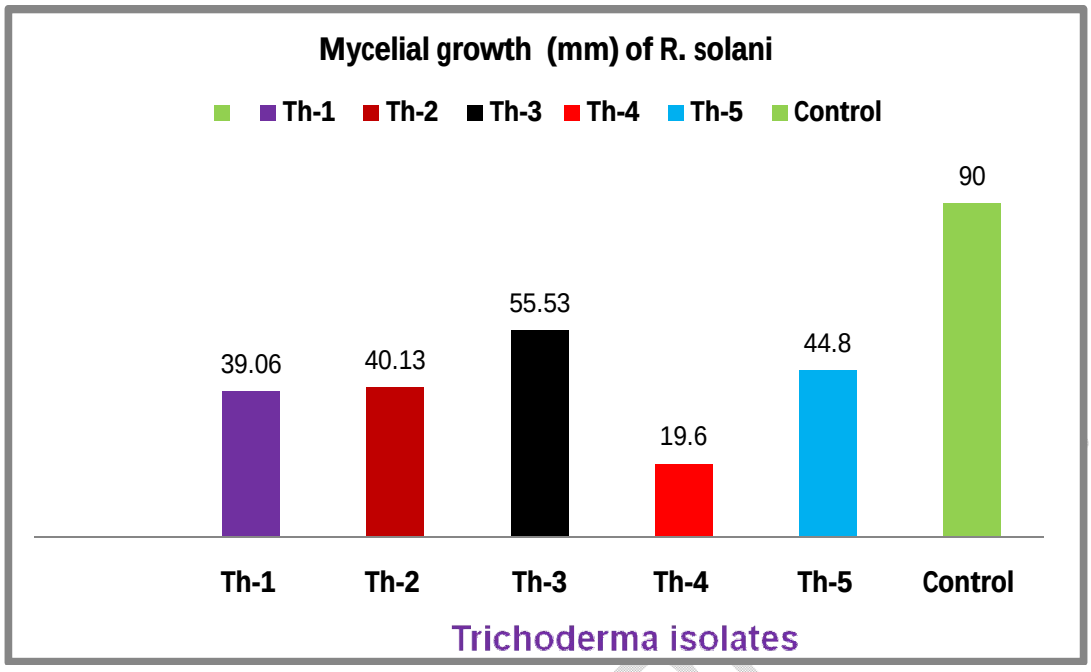

Fig. 4: Effect of native *Trichoderma*isolates (volatile compounds) on mycelial growth of *R. solani*

The study about screening of native isolates of *Trichoderma harzianum*aginst*R. solani* in vitro revealed that all native isolates of *Trichoderma harzianum* were significantly inhibited the mycelial growth of R. solani. The maximum reduction in mycelial growth from R. solani in volatile compounds was recorded in Th-4 followed by Th-1, Th-2, Th-5 and Th-3.

Effect of native *Pseudomonas fluorescens* strains on mycelial growth and sclerotia formation of R. solani

To screen the native isolates of *Pseudomonas fluorescens* against R. solani in vitro, the present study was undertaken during the kharif 2016. Treatments were allocated under completely randomized design (CRD). The data presented in the table no. 2 showed that in dual culture study, all the native Pseudomonas strains were significantly inhibited to the mycelial growth of R. solani over the control. The mycelial growth of R. solani was highly inhibited by the Pf-05 (with 7.94mm mycelial growth and 91.18% decrease over control) and was found on par with the Pf-01 (with 9.54mm mycelial growth and 89.40% decrease over control) which is followed by Pf02 (with 16.67mm mycelial growth and 81.48% decrease over control), Pf-03(with 55.60mm mycelial growth and 39.34% decrease over control) and Pf-04 (with 60.67mm mycelial growth and 32.59% decrease over control). The maximum mycelial growth was recorded under control treatment (90.00 mm). No sclerotial formation was recorded in all the isolates taken in the study. Mishra and Sinha (2000) found two strains of P. fluorescens, viz. PF1 and FP7, inhibited the mycelial growth of sheath blight fungus R. solani and enhanced rice plants seedling vigour and yield under greenhouse and field conditions. Treatment Pseudomonas with rice cv. IR50 led to induction of systematic resistance against R. solani as a result of increase in chitinase and

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peroxidase activity (Nandakumar *et al*. 2001). Singh Rajbir and Sinha (2005) recorded P. fluorescens strains 1 and 5 having an effect on sheath blight, R. solani on rice under glasshouse conditions. They found that higher rate of P. fluorescens i.e., 8 g/l was highly effective in reducing 60 percent of disease severity and 35.6 percent of incidence (and raising grain yield (33.8%) and 1000-grain weight (12.9%).

Table 2: Effect of native *Pseudomonas fluorescens*strains on mycelial growth and sclerotia formation of *R. solani*

***P. fluorescens*strain**	**Mycelial growth (mm) of *R. solani***	**% decrease over control**
P.f 01	9.54	89.40
P.f 02	16.67	81.48
P.f 03	55.6	39.34
P.f 04	60.67	32.59
P.f 05	7.94	91.18
Control	90.0	0.0
SE(m)+	**1.466919**	
CD (5%)	**4.520028**	

The study of native *Pseudomonas fluorescens* on mycelial growth and sclerotial formation of R solani revealed that all *Pseudomonas fluorescens* isolates significantly inhibited the mycelial growth of R. solani. The mycelial growth of R. solani was highly inhibited by Pf-05 isolate (with 7.94mm mycelial growth and 91.18% decrease over control).

The study about screening of native isolates of *Trichoderma harzianum*aginst*R. solani* in vitro revealed that all native isolates of *Trichoderma harzianum* were significantly inhibited the mycelial growth of R. solani. The maximum reduction in mycelial growth from R. solani in volatile compounds was recorded in Th-4 followed by Th-1, Th-2, Th-5 and Th-3. The study of native *Pseudomonas fluorescens* on mycelial growth and sclerotial formation of R solani revealed that all *Pseudomonas fluorescens* isolates significantly inhibited the mycelial growth of R. solani. The mycelial growth of R. solani was highly inhibited by Pf-05 isolate (with 7.94mm mycelial growth and 91.18% decrease over control).

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