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RESEARCH PAPER

Antagonistic activity of native *Trichoderma* spp. and fluorescent Pseudomonads against *Rhizoctonia solani* causing sheath blight in rice crop

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Abstract : Rice (*Oryza sativa* L.) is a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia and Africa. Rice production is often subjected to several biotic and abiotic stresses rice sheath blight is one of the most destructive diseases causing great damage to rice yield and quality worldwide.Twenty rhizospheric soil samples of rice obtained during survey from different regions of Northern Karnataka were used for the isolation of *Trichoderma* spp. and fluorescent Pseudomonads. The antagonistic potential was studied by using dual culture technique. Among twenty *Trichoderma* spp., the highest inhibition (> 50%) was recorded in nine isolates which ranged from 54.20 to 65.10 per cent. The results obtained were highly significant between the different isolates and also over control. Among the twenty fluorescent Pseudomonads, the per cent inhibition was higher (> 50%) in twelve isolates which varied from 51.92 to 81.79 per cent. The results obtained were highly significant between the different isolates and also over control.

Key words: Antagonistic potential, Fluorescent pseudomonads, *Rhizoctonia solani*, Sheath blight, *Trichoderma* spp

Introduction

Rice (*Oryza sativa* L.), as a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia and Africa. Rice was cultivated in the Indian subcontinent from as early as 5,000 BC. It is the agricultural commodity with the third highest worldwide production after sugarcane and maize. It is grown all around the world for its high quality carbohydrates (77-89%), protein, dietary fibre, B vitamins and amino acids.

Rice production is often subjected to several biotic and abiotic stresses. The estimated yield loss is up to 4-50 per cent and is reported depending on the crop stage during infection, disease severity and environmental conditions (Bhukal *et al.,* 2015). Among biotic stresses, diseases are pivotal one. Among the diseases, bacterial blight, sheath blight, blast, sheath rot and false smut are the most important for this region causing economic yield losses. Of these, rice sheath blight is one of the most destructive diseases causing great damage to rice yield and quality worldwide.

Sheath blight (also named as oriental sheath and leaf blight, sheath and leaf blight, snake skin disease and banded blight) of rice was first reported from Japan by Miyake (1910) and recognized by Reinking (1918) in Phillippines. In India, sheath blight was first time reported from Gurdaspur by Paracer and Chahal (1963). In India, sheath blight is known to occur in almost all the rice growing states of the country causing up to 58 per cent loss in yield. The losses in grain yield varied between 10-36 per cent depending upon the stage of crop at the time of occurrence of disease (Roy, 1979).

Initial symptoms of sheath blight appear in the form of circular, oblong or ellipsoid, greenish-grey water soaked spots about 1cm long that occur on leaf sheath near the water level. These lesions enlarge and become oblong and irregular in outline, the centre of which become greyish white with brown margins. Lesions may also appear on any part of sheath and several lesions may unite to encircle the whole Culm. Under humid conditions, the infection may spread to upper leaf sheaths and leaf blades, which ultimately results in rotting of leaf sheath and drying up of the whole leaf. In these conditions, cob web like mycelia spread externally and sclerotia, initially white but turning brown on maturity are produced respectively on diseased plant parts. There are loosely attached and easily dislodge from the plant at maturity. In severe cases, most of the leaves in a plant may be blighted.

The fungus, *Rhizoctonia solani* is a typical soil borne pathogen, which do not produce spores and hence it is identified only from mycelia characteristics. The hyphal cells are multinucleated. It produces white to deep brown mycelium when grown on artificial medium. The hyphae tend to branch at right angles. A septum near each hyphal branch and a slight constriction at the branch are diagnostic. It produced large number of globose sclerotia which initially turn white, late turn brown to purplish brown. Sclerotia serve as a major source of primary inoculums. The teleomorph of *R.solani* is *Thanatephorous cucumeris.*

In recent years, the increasing use of pesticides in agriculture has been the subject of growing concern for both environmentalists and public health authorities. Besides their non-target effects and hazardous to nature, these are becoming more expensive and some are losing their effectiveness due to development of resistant strains. Biological control has emerged as an alternative and most promising means of the management of plant pathogens. Bio-control of *Rhizoctonia* can be achieved by either promoting the native antagonists to reach a density sufficient to suppress pathogens or by introducing alien

antagonists. Antagonism between organisms is common in the ecosystem and is most prevalent among soil microorganisms. Natural interference between beneficial soil microorganisms and plant pathogens results in zone of buffer, there by inhibiting or reducing disease development (Kohl *et al.,* 2011).

Biological control agents are the organisms that interact with the components of disease triangle to manage the disease. Understanding how the bio control agents work can facilitate optimization of control as well as help in screening the more efficient strains of bio control agents. Understanding the mechanisms of biological control of plant diseases through the interactions between bio control agent and pathogen may allow us to manipulate the soil environment to create conditions conducive for successful bio control or to improve bio control strategies. Mechanism of some bio control agents are now understood in detail (Zhang *et al.,* 2002). Understanding the mechanism of action of a bio control agent, may also improve the consistency of control either by improving the mechanism or by using the bio control agents under conditions where it is predicted to be more successful.

Among the several antagonists, species of *Trichoderma* and *Pseudomonas fluorescens* have been reported to be promising in minimising sheath blight of rice.

Material and methods

Isolation of pathogen

Sheath blight diseased samples collected during survey were used for the isolation of pathogen. The samples were washed thoroughly with tap water. Small portion of infected parts containing healthy as well as diseased tissues were cut in to 0.5 cm pieces with the help of sterilized scalpel blade. These pieces were then surface sterilized with 1 per cent sodium hypochlorite solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to Petri dishes containing sterilized Potato Dextrose Agar (PDA) and incubated at 28±2 ºC under BOD incubator. The Petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

Isolation and maintenance of *Trichoderma* spp.

Trichoderma spp. was isolated from rhizosphere soil by serial dilution technique (Krassilnikov, 1950) on PDA medium. Ten grams of soil sample was taken and suspended in 90 ml of sterilized distilled water and stirred well to get 1:10 dilution (10^{-1}). One ml from this was transferred to test tube containing 9 ml of sterilized distilled water to get 1:100 (10^{-2}) dilution. Likewise, the dilution of the sample was prepared up to 1:100000 (10^{-5}). One ml of a final dilution of each sample was pipetted out into each sterile Petri plate separately to which a quantity of 15-20 ml of sterilized and molten medium was poured and gently rotated for uniform mixing and the plates were incubated at 28 ± 1^0 C for about 6-10 days. The Petri plates were kept under observation daily for the appearance of *Trichoderma* colonies. The colonies were initially white in color and later turned green. From the isolated plates, among the different colonies, an actively growing colony of *Trichoderma* was selected and plated on PDA medium and plates were incubated at 28 ± 1 ^{0}C for about 4 days. Likewise, twenty isolates of *Trichoderma* spp. were obtained from collected soil samples. For the maintenance, the cultures of *Trichoderma* isolates were sub cultured on PDA slants and allowed to grow at 28°C and such slants were preserved in refrigerator at 4°C and sub cultured once in 30 days.

Isolation and maintenance of fluorescent Pseudomonads

Isolation of fluorescent Pseudomonads were carried out according to the method of Weller and Cook (1983) by Serial dilution technique. Loosely adhering soils from the rhizosphere of healthy plant was taken. Ten grams rhizosphere soil was transferred to 100 ml sterilized double distilled water. The suspensions from all soil samples were serially diluted up to 10^{-7} with three replications for each sample for isolation of fluorescent pseudomonads and hundred micro litres of 10^{-5}, 10^{-6} and 10^{-7} diluted samples were serially diluted and the suspensions were used to isolate fluorescent Pseudomonads bacteria (FPB) on plates containing King's B agar medium (KBM) (King *et al*., 1954) and incubated at 28 ± 2°C. After 48 hours of incubation, all the isolates were checked for fluorescence production under UV light and representative types of colonies were selected and further purified on KBM. Pure culture of isolates was preserved at -80^{0}C after an addition of glycerol to a final concentration of 40 per cent (v/v).

Antagonistic activity of native *Trichoderma* spp. against *R. solani*

Efficacy of *Trichoderma* strains was tested by dual culture method described by Broadbent *et al*. (1971). Twenty ml of PDA was poured in each of the sterilized Petri plate of 90 mm diameter. On solidification dual inoculation was done in each plate using 5 mm disc of *R. solani* at one side and that of test fungi (*Trichoderma* spp.) at other. Petri plates were incubated at 25 ± 1 ^{0}C. Observations on growth of *Trichoderma* over the pathogen were recorded after five days of incubation with the help of plastic scale. The mean of three observations was calculated and expressed in mm. The per cent inhibition over the control was calculated by using the formula (Vincent, 1947) as follows,

Where; $$I = \frac{(C - T)}{C} X\ 100$$

I = Per cent inhibition

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

Antagonistic activity of native fluorescent Pseudomonads against *R. solani*

Antagonistic activity was verified by following dual culture technique (Skidmore and Dickinson, 1976). First, the bacterial isolates were streaked on respective media plates and incubated at respective temperature and time. Loopfull of each bacterial isolate was streaked on the PDA plate at either end and at the centre, 5 mm mycelial disc of test pathogen was placed. Control plate was maintained by placing only pathogen mycelial disc

on the plate without bacteria. The assay plates were incubated at 28 ± 1 ^{0}C for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. For each bacterial isolate three replications were maintained with suitable controls.

The percent growth inhibition over control was calculated by using the formula

Where;

I = Per cent inhibition

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

Results and discussion

The pathogen *R. solani*

The samples of affected sheath showing typical blight symptom collected from different geographic regions were used for isolation of pathogen by standard tissue isolation method. The fungus was obtained in pure form by hyphal tip isolation as explained in material and methods and maintained in pure form on Potato Dextrose Agar (PDA).

Upon inoculation of the *R. solani* on medium, it produced shade of brown hypha, constriction at the point of branching and right angle branching in matured hyphae. The isolate shared typical characteristics of *R. solani* (a) branching at right angle near the distal septum of the cell, (b) formation of a septum in the branch near the point of origin, (c) constriction of the branch at origin. Further it also produced sclerotia which were undifferentiated aggregations of thick-walled cells, small (1-3 mm diameter) irregular-shaped, brown to black structures (Fig. 1).

The results are in agreement with Sunder *et al*. (2003), who reported the production of light brown, brown and dark brown mycelium on the PDA media. The discolorations of the growth media is mainly attributed to the production of pigments by the pathogen. Branching of the mycelium was found near the distal septum of a cell in young and advanced hyphae. In older hyphae, branching may occurred at any place along the cell.

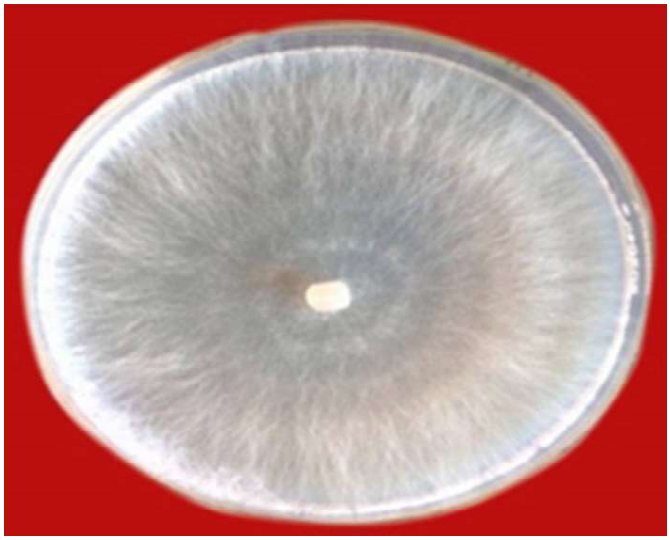
Isolation and maintenance of native *Trichoderma* isolates

Twenty rhizospheric soil samples of rice obtained during survey from different regions of Northern Karnataka were used for the isolation of *Trichoderma* spp.

Native *Trichoderma* spp. were isolated by serial dilution technique on PDA and incubated for 7 days at 25 ± 2 ºC. After incubation, all the twenty isolates showed typical character of greenish fungal colony and produced fungal characters of *Trichoderma* under microscope. Sharma and Singh (2014) isolated thirty isolates of the *Trichoderma* spp. from the rhizosphere soils from districts of Uttarakhand (India) on *Trichoderma* selective medium using serial dilution technique. These *Trichoderma* isolates were maintained in slants as pure cultures and used for further studies.

Isolation and maintenance of native fluorescent Pseudomonads

Isolation of native fluorescent Pseudomonads from rhizosphere soil was carried out by standard procedure of serial



A. Colony on PDA medium

B. In advanced stage production of brown sclerotia

C. Mycelium constriction and right

dilution technique on King's B medium (KBM). Totally twenty isolates were collected from rhizosphere soil representing different districts. After 48 hours of incubation on KBM, all the thirty three isolates showed typical character of fluorescence under UV light. The representative type of colonies were selected and further purified on KBM. Pure culture of all isolates was preserved at -80 ^{0}C after an addition of glycerol to a final concentration of 40 per cent (v/v).

Manjunatha *et al*. (2012) isolated 92 fluorescent pseudomonads from the rhizosphere soil of paddy using specific medium. Similarly, Shivalingaiah and Umesha (2013) isolated ten strains of from the rhizosphere soils of rice growing areas in Karnataka, India. These twenty isolates of fluorescent Pseudomonads were maintained in slants as pure cultures and used for further studies.

Antagonistic activity of *Trichoderma* isolates against *R. solani*

Twenty isolates of *Trichoderma* spp. isolated from healthy rhizosphere soil of rice were screened against *R. solani* for mycelial inhibition by dual culture technique. Zone of inhibition of mycelium (in mm) was recorded and the per cent inhibition was calculated.

The results revealed that per inhibition of mycelial growth varied greatly among the twenty isolates. The highest inhibition (> 50%) was recorded in nine isolates which ranged from 54.20 to 65.10 per cent. The results obtained were highly significant between the different isolates and also over control. Maximum per cent inhibition of 65.10 per cent was observed in TD-14 followed by TD-9 (58.28%) and TD-3 (56.46%), while least inhibition of 41.24 per cent was observed in TD-2 and TD-7 (Table 1 Fig. 2).

Table 1. Antagonistic activity of native isolates of *Trichoderma* spp. against *R. solani*

Isolate	Colony growth (mm)*	Mycelial inhibition (%)*
TD-1	40.33	54.15(47.71)
TD-2	51.67	41.24(40.29)
TD-3	38.33	56.46(48.69)
TD-4	45.67	48.09(43.97)
TD-5	47.67	45.86(42.28)
TD-6	39.67	54.91(48.15)
TD-7	51.67	41.24(40.30)
TD-8	49.33	43.93(41.50)
TD-9	36.67	58.28(50.48)
TD-10	40.33	54.15(47.71)
TD-11	48.33	45.06(42.48)
TD-12	38.67	56.04(49.48)
TD-13	49.33	43.93(41.49)
TD-14	30.67	65.10(53.85)
TD-15	53.33	39.38(37.43)
TD-16	49.33	43.93(41.50)
TD-17	47.00	46.58(43.70)
TD-18	45.00	48.85(44.83)
TD-19	41.33	53.01(45.96)
TD-20	38.67	56.04(49.92)
Control	88.00	0.00(0.00)
S. Em.±	-	0.56
C. D. at 1 %	-	2.15

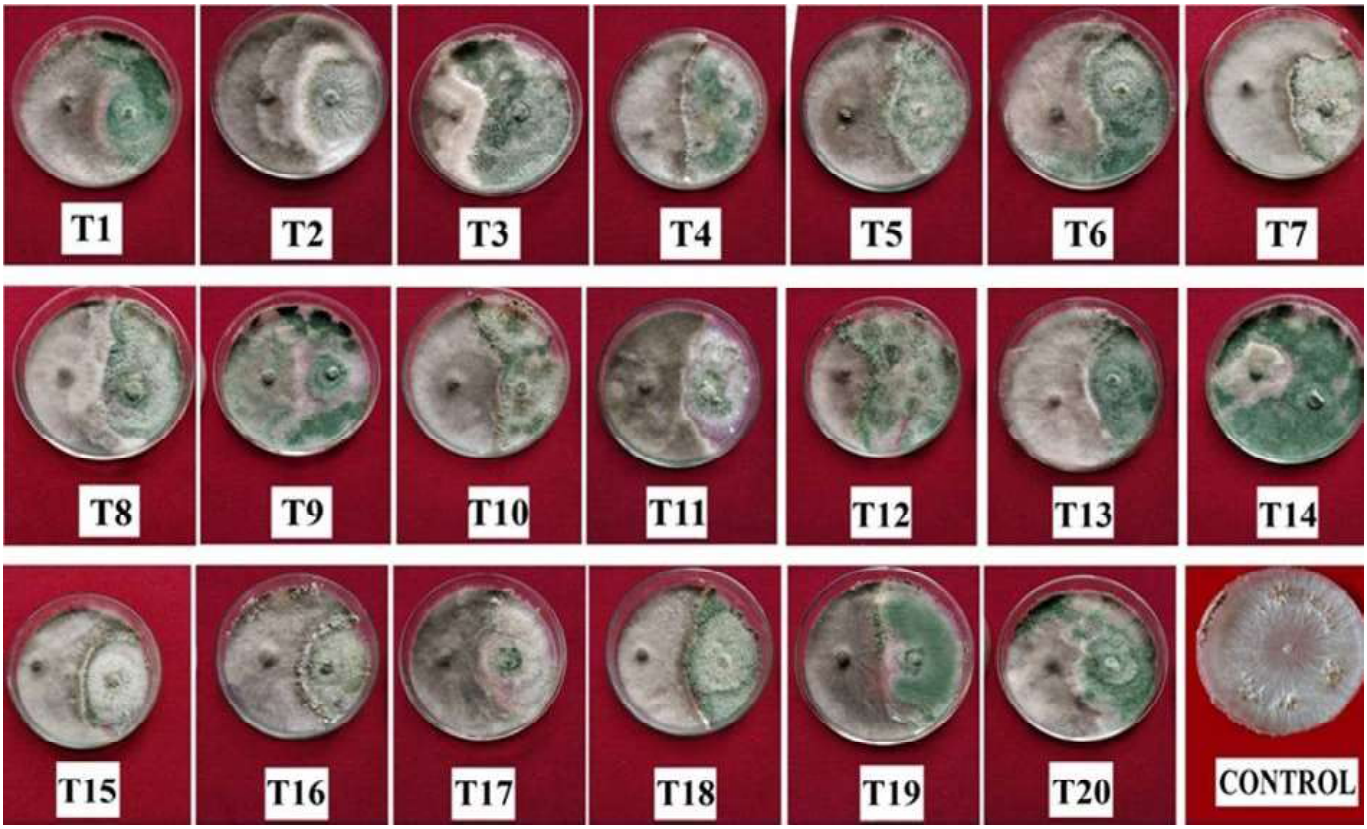


Fig. 2. Antagonistic activity of native isolates of *Trichoderma* spp. against *R. solani*

Similarly, Seema and Devaki (2012) evaluated the efficacy of fungal bio agents *viz, Trichoderma viride* and *Trichoderma harzianum* under *in vitro* conditions against *Rhizoctonia solani* and reported that the percentage inhibition of growth by *T. viride, T. harzianum* on *R. solani* was 70 per cent and 67 per cent, respectively. Similar findings were also made by Kumari *et al*. (2016) who also tested the several isolates of *Trichoderma* against *R. solani*. Result indicated that the antagonistic potential of 26 isolates of *Trichoderma spp.* against *R. solani* were varied which inhibited *R. solani* ranges 33-54%. Among isolates of *Trichoderma*, seven isolates showed strong antagonistic potential which inhibited >50% mycelial growth of *R. solani*, *viz*., RCT1 (53.71%) followed by RCT22 (52.6%), RCT3 (51.85%), RCT7 (51.11%), RCT10 (50.37%), RCT 8 (50%) and RCT14 (50%). Moreover, seventeen (17) isolates were also showed inhibitory but their antagonistic potential <50% of the mycelial growth while two isolates (RCT12 and RCT17) showed <40% mycelial growth. These potential isolates of *Trichoderma* may be further exploited as bio control agent against *R. solani* as well as other Soil borne phytopathogenic fungi.

Antagonistic activity of fluorescent Pseudomonads against *R. solani*

Twenty isolates of fluorescent Pseudomonads isolated from healthy rhizosphere soil of rice were screened against *R. solani* for mycelial inhibition by dual culture technique. Zone of inhibition of mycelium (mm) was recorded and the per cent inhibition was calculated.

The results revealed that per inhibition of mycelial growth varied greatly among the twenty isolates. However, the per cent inhibition was higher (> 50%) in twelve isolates which varied from 51.92 to 81.79 per cent. The results obtained were highly significant between the different isolates and also over control. Maximum per cent inhibition of 81.79 per cent was observed in PF-12 followed by PF-17 (78.38%) and PF-4 (76.45 %). Least inhibition of 18.97 per cent was observed in PF-3 (Table 2; Fig. 3).

Table 2. Antagonistic activity of native isolates of fluorescent Pseudomonads against *R. solani*

Isolate	Colony growth (mm)*	Mycelia inhibition (%)*
PF-1	40.33	54.15(49.71)
PF-2	23.67	73.08(58.74)
PF-3	71.33	18.97(25.45)
PF-4	20.67	76.45(60.99)
PF-5	37.00	57.94(51.94)
PF-6	49.67	43.55(41.62)
PF-7	70.67	19.69(26.66)
PF-8	42.00	52.26(47.62)
PF-9	40.67	53.77(48.72)
PF-10	42.33	51.88(46.74)
PF-11	49.00	44.30(42.38)
PF-12	16.00	81.79(64.75)
PF-13	31.67	63.99(53.12)
PF-14	46.00	47.71(44.35)
PF-15	50.33	42.79(40.18)
PF-16	46.67	46.95(43.04)
PF-17	19.00	78.38(61.58)
PF-18	37.33	57.56(51.71)
PF-19	42.33	51.87(46.74)
PF-20	46.33	47.33(44.66)
Control	88.80	0.00 (0.00)
S.Em.±	-	0.50
C. D. at 1 %	-	1.93

Antagonistic activity of native isolates of fluorescent Pseudomonads spp. against *R. solani*

Vinay *et al.* (2016) investigated thirty indigenous Fluorescent Pseudomonads against *Rhizoctonia solani* under *in vitro* conditions by dual culture method and found out that antagonists effectively suppressed the growth of *R. solani.* Among those isolates, RFP-22 showed the maximum per cent inhibition of mycelium (46.66%) followed by RFP-6 (45.55%). Similarly RFP-19, RFP-3, RFP-21 and RFP-7 have shown 44.00, 44.00, 43.33 and 42.22 per cent mycelia inhibition respectively.

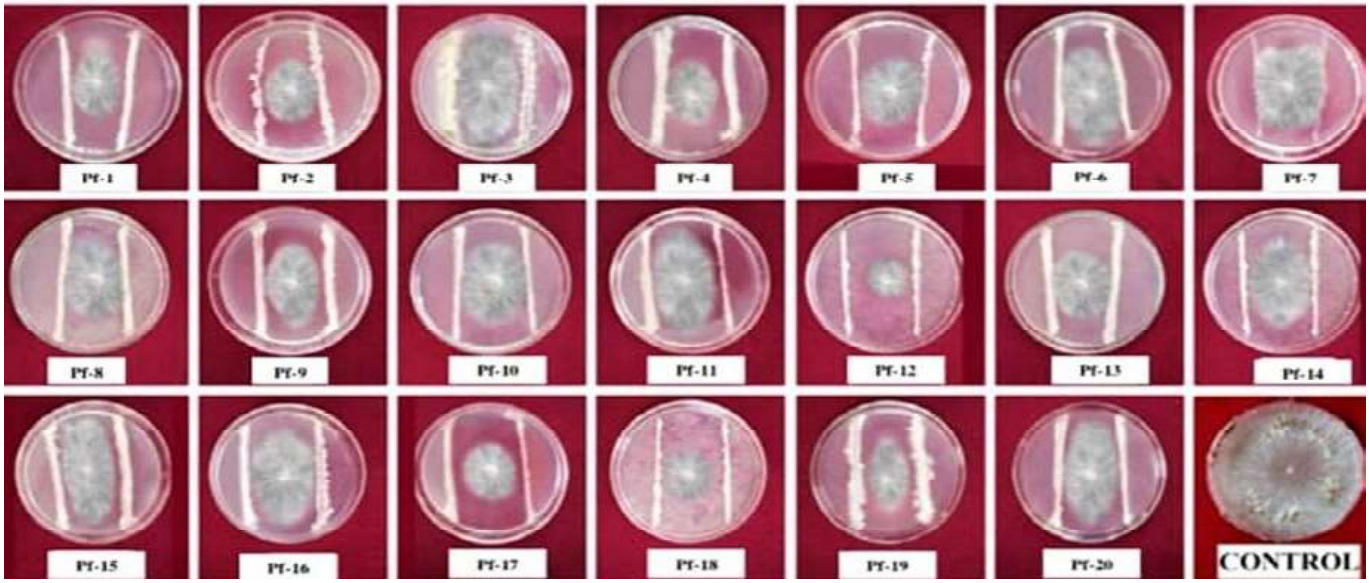


Fig. 3. Antagonistic activity of native isolates of fluorescent Pseudomonads spp. against *R. solani*

The remaining isolates showed the moderate inhibition of *R. solani*. The RFP-9 (16.66%) and RFP-17 (6.66%) have shown the least inhibition of the pathogen.

Conclusions

All twenty isolates of *Trichoderma* spp. inhibited the mycelia growth of *R. solani, among them* per cent inhibition was highest (>50%) in nine isolates. In case fluorescent pseudomonads, all isolates also inhibited the growth of *R. solani,* but twelve isolates were very efficient by recording more than 50% mycelia inhibition of pathogen.

References

Bhukal N, Singh R and Mehta N, 2015, Progression and development of sheath blight of rice in relation to weather variables *Journal of Mycology and PlantPathology*, 45(2): 166-172.

Broadbent P, Baker K F and Water-worth Y, 1971, Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Australian Journal of Biological Sciences*, 24: 925-944.

King E Q, Ward M K and Raney D E, 1954, Two simple media for the demonstration of pyocyanine and fluroscein. *Journalof Laboratory and Clinical Medicine*, 44: 301-307.

Kohl J, Postma J, Nicot P, Ruocco M and Blum B, 2011, Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. *Biological Control,* 57:1-12.

Krassilnikov N A, 1950, Actinomycetes-antagonists and antibiotic substances (in Russian). Academy of Sciences, U.S.S.R., Mascow, Leningrad.

Kumari A, Kumar R, Maurya S and Pandey P K, 2016, Antagonistic potential of Different isolates of *Trichoderm*a against *Rhizoctonia solani*. *European Journal of Experimental Biology*, 6(2): 2248-9215.

Manjunatha H, Naik M K, Patil M B, Lokesha R and Vasudevan S N, 2012, Isolation and characterization of native fluorescent pseudomonads and antagonistic activity against major plant pathogens. *Karnataka Journal of Agricultural Sciences*, 25(3): 346-349.

Miyake I, 1910, Studies uber die pilze der Reispflanze in Japan. *Journal of the College of Agriculture*, 2:237-276.

Paracer C S and Chahal D S, 1963, Sheath blight of rice caused by *Rhizoctonia solani* Kuhn- A new record in India. *Current Science*, 32:328-329.

Reinking O A, 1918, Philippine economic plant diseases. *Philippines Journal Sciences Section*, 13:165-274.

Roy A K, 1979, Susceptibility of rice plants to sheath blight at different stages of growth. *KAVAKA*, 7: 25-26.

Seema M and Devaki N S, 2012, *In vitro* evaluation of biological control agents against *Rhizoctonia solani*. *Journal of Agricultural Science and Technology*, 8: 233-40.

Sharma K K and Singh U S, 2014, Cultural and morphological characterization of rhizospheric isolates of fungal antagonist *Trichoderma*. *Journal of Natural and Applied Science,* 6(2): 451-456.

Shivalingaiah and Umesha S, 2013, *Pseudomonas fluorescens* inhibits the *Xanthomonas oryzae pv. oryzae*, the bacterial leaf blight pathogen in rice. *Canadian Journal of Plant Protection*, 1(5): 147-153.

Skidmore A M and Dickinson C H, 1976, Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Transaction and Journal of British Ceramic Society*, 66: 57-74.

Sunder S, Singh R and Dodan D S, 2003, Standardization of inoculation methods and management of sheath blight of rice. *Indian Journal of Plant Pathology*, 21: 92-96.

Vinay J U, Naik M K, Rangeshwaran R, Chennappa G, Shaikh S and Sohel Sayyed R Z, 2016, Detection of antimicrobial traits in Fluorescent pseudomonads and molecular characterization of an antibiotic pyoluteorin. *Biotechnology*, 6: 227- 238.

Vincent J M, 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159: 850.

Weller D M and Cook, 1983, Colonization of wheat roots by fluorescent Pseudomonads suppressive to take-all. *Phytopathology,* 73:1548-1553.

Zhang S, Moyne A L, Reddy M S and Kloepper J W, 2002, The role of salicylic acid in the induced systemic resistance elicited by plant growth promoting rhizobacteria against blue mould of tobacco. *Biological Control,* 25: 288-96.