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Isolation and Evaluation of Native Soil Bio-agents against *Rhizoctonia solani* Kuhn

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A B S T R A C T

Seven rhizospheric myco-flora (*Trichoderma harzianum, Trichoderma viride, Penicillium* sp., three *Aspergillus* species and one was unknown) and four rhizobacteria (RB1- *Pseudomonas fluorescence*, RB2, RB3 and RB4) isolated from rhizosphere and were evaluated against sheath blight pathogen of rice *Rhizoctonia solani* Kuhn by dual culture technique. Among seven rhizospheric myco-flora *T. harzianum* has been shown highest growth inhibition 55.56% and has been followed by *T. viride* which produced 48.70%. Remaining myco-flora were showed comparatively less growth inhibition. Among rhizobacteria RB1 (*Pseudomonas fluorescence*) and RB2 have produced noticeable amount of growth inhibition compared with others 20.86% and 9.30% respectively.

Introduction

Rhizoctonia solani Kuhn is the acceptable name for the sheath blight of rice fungus. *R. solani* was originally described by Julius Kuhn on potato in 1858 which the most widely documented and the most important destructive species of *Rhizoctonia* (Ogoshi, 1996). In 1963, Pracer and Chahal had first reported and described sheath blight of rice caused by *Rhizoctonia solani* Kuhn from Gurdaspur district of Punjab (Gangopadhyay and Chakrabarti, 1982).Under favourable conditions this disease becomes divesting and

causes severe yield loss in rice. According to Dasgupta (1992), in Asia sheath blight resulting in 8-50% of yield losses, in case of moderate infection it was caused 5-15% yield loss (Annou *et al.,* 2005) and under severe infestation it was leads to loss 30-40% (Kozaka, 1970).

In the market a plenty of chemicals are available for the control of sheath blight disease. But, it is known that lots of hazardous issues related to pesticides like fungicides and bactericides. So that, a head the naturally available beneficial microbes are

known very well, they provide nutrition to plant, enhance the plant immunity by inducing resistant and are act as antagonist to several pathogens by inhibiting growth and development of the pathogen. It is important to study about microbial antagonist of *R. solani* the pathogen of sheath blight of rice. There is several research works have been going on antagonism of beneficial microbe against sheath blight pathogen, which is the most economically important disease of rice. Khan and Sinha (2007) have revealed that, *Trichoderma harzianum* and *T. Viride* was showed best growth inhibition of *R. solani.* Sixty rhizosphere bacterial strains were screened against sheath blight pathogen under *in vitro* condition, out of them, *Pseudomonas fluorescens* (Tu(Pf)19 and Kk(Pf)24) and *Bacillus subtilis* (Tn(Bs)7 and Kk(Bs)19) were showed maximum growth inhibition of the pathogen (Nagendran *et al.,* 2019).

Considering the importance of native bio-agents in the present scenario, the current investigation was done on isolation and morphological identification of rhizospheric myco-flora and rhizobacteria and evaluation of efficacy of their antagonism against *Rhizoctonia solani* by dual culture technique.

Materials and Methods

The experiment on isolation and evaluation of native bio-agents against *Rhizoctonia solani* was conducted under laboratory condition at department of plant pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) -818425, Bihar, India.

Isolation and purification of *Rhizoctonia solani*

Diseased plant leaves were collected and washed in running tap water to remove any attached organic debris and dust particles. These were cut into pieces of 1-2 mm along with healthy tissue with use of sterilized blade. Pieces were dipped in 0.1% mercuric chloride about 30 seconds for the surface sterilization. Finally washed for three times in sterilized water and allowed to blotted dry. Under aseptic condition 2 to 3 pieces were transmitted to each media plate with use of heat sterilized inoculation needle and transferred plates allowed to incubation at 28 ± 1℃. After 24 - 48 hours of incubation active growing tips of hyphae were transferred to other new plates and pure culture was prepared by transferring single sclerotium on to PDA slants.

Soil sample collection

The healthy rice plants from research farm during *Kharif* (2019) were selected randomly and uprooted gently along with rhizospheric soil and dried under shade condition. Dried soil is collected from root surface mixed thoroughly, then removed pebbles and debris from that dried soil and it was sieved to get fine sample.

Isolation and purification of native bio agents

Soil sample collected from rhizosphere subjected to serial dilution. One gram of rhizosphere soil was transferred to 10 ml of sterile water and mixed thoroughly. 1 ml solution was taken from that suspension and mixed to next test tube with 9 ml of sterile water to get 10^{-1} diluted sample. From this dilution 1 ml transferred to another 9 ml sterile water to get 10^{-2} diluted sample. This method was continued till getting 10^{-4} and 10^{-5} diluted sample. From the final dilutions of 10^{-4} and 10^{-5}, 1 ml of each was spread on PDA and Peptone dextrose rose Bengal agar medium to get rhizospheric mycoflora; it was also spread on King's B medium (for *Pseudomonas* isolation) and Nutrient agar

medium to get rhizobacteria. The plates were gently rotated clockwise and anti-clockwise direction for uniform distribution of the soil suspension. The plates were finally allowed to incubation at 28 ± 1 °C.

After 78 hours of inoculation fully grown colonies of fungi were obtained on media. To get pure culture of fungi active growing tips of hyphae were transferred to other new plates. After 28 hours bacterial colonies were formed on media. By using the inoculation loop different bacterial colonies were streaked on the fresh nutrient agar slants to obtain the pure culture. Rhizospheric myco-flora was identified based on their cultural and morphological characters. They were stained on slides with lacto phenol or cotton blue to observe their mycelial branching, conidial structure under microscope.

Screening of native soil bio agents against *Rhizoctonia solani* Kuhn

Antagonistic effect of rhizospheric micro-flora against *Rhizoctonia solani* was done by dual culture method. One side of PDA media containing Petri plates was inoculated with 9 mm disc of 2 to 3 days old culture of pathogen and another side transferred with disc of 5 days old culture of rhizospheric myco-flora; in case of bacteria another side was streaked with loop of 2 days old culture of rhizobacteria. Control plate was inoculated with pathogen without any soil micro-flora. Each treatment has been done with three replications. Inoculated plates were kept in BOD at 28 ± 1 °C. Readings were noted three times with 24 hours intervals and first reading was done at 72 hours after inoculation. By using following formula percentage growth inhibition of *Rhizoctonia solani* was calculated.

Percentage Growth Inhibition $= \frac{(C-T) \times 100}{C}$

Where, C = Radial growth of pathogen (mm) in control plate.

T = Radial growth of pathogen (mm) in dual culture.

Statistical analysis

The data obtained in the experiment was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1967) and Panse and Sukhatme (1985).

Results and Discussion

Identification of rhizospheric micro-flora

Seven rhizospheric myco-flora isolated from soil were identified based on their cultural and morphological characters (Figure I). Serial name for them has been given as RMF (Rhizospheric myco-flora).

RMF 1 (*Trichoderma harzianum*): Initially, colony was white in colour. Fully grown mycelium was thick and fuzzy. It was matured within 5 days and developed bluish green patches of conidia. Microscopic view revealed that, hypha was hyaline and septate. Conidiophores were erected and their branching usually opposite. Conidia were single celled with slightly sphere shape.

RMF 2 (*Trichoderma viride*): Initially, colony was white in colour, later it was appeared like yellowish green due to pigmentation, and was smooth and leathery to naked eye. Hypha was hyaline and septate. Conidia were one celled and bigger than *T. Harzianum.* Phialides were irregular and less crowned. Conidiophores were irregularly branched.

RMF 3 (*Aspergillus* sp.): Colonies on PDA were beige to buff to cinnamom in colour. Yellow pigmentation was observed at lower

side of the Petri plate. Colonies became finely granular with conidial production. Under microscope conidial heads were look like biseriate. Conidiophore was hyaline with soft wall. Conidium was hyaline and sphere shaped.

RMF 4 (P*enicillium* sp.): Colonies were initially white in colour, later become bluish green and were flat, filamentous and velvet in texture. Yellow to orange pigmentation was observed at lower side of the Petri plate. Hyphae were hyaline, branched and septate. Conidia were single celled; circular shaped, appeared in chain, and farmed brush like structures. Conidiophore was erected, branched, septete, and metula was formed on it.

RMF 5 (*Aspergillus* sp.): Colonies on PDA were initially white in colour, round to oval in shape. It quickly became dull greenish and later turned into yellowish green with loose surface. Pale yellow colour was shown on

reverse of the Petri plate.

RMF 6 (Unknown): Initially, small circular and white coloured colonies were formed. Later they turned to light green colour. Mycelium was hyaline. Conidiophore was long and cylindrical.

RMF 7 (*Aspergillus* sp.): Colonies were suede-like to floccose, white to pale yellow with slow to poor condition and were grown very slowly. Conidial heads were short, columnar and unseriate. Conidiophore stipes smooth walled and vesicles were subglobose to flask shaped and conidia globose to sub globose.

Four different rhizobacteria were isolated based on variation in their colony morphology and have been named as RB (Rhizobacteria). Among these, RB 1 is *Pseudomonas fluorescence* which was isolated on King's B medium and has illuminate property under the light (Figure II).

Table.1 Effect of various rhizospheric myco-flora on radial growth and percent growth inhibition of *R. solani* at different time intervals

Rhizospheric mycoflora	72 hours		96 hours		120 hours	
	G	I	G	I	G	I
RMF 1	38.67	46.30	40.00	55.56	40.00	55.56
RMF 2	46.17	35.88	46.17	48.70	46.17	48.70
RMF 3	66.67	7.41	81.17	9.81	81.17	9.81
RMF 4	70.83	1.62	80.33	10.74	80.33	10.74
RMF 5	63.33	12.04	75.67	15.93	75.67	15.93
RMF 6	68.33	5.09	82.67	8.15	82.67	8.15
RMF 7	55.33	23.15	69.00	23.33	69.00	23.33
Control	72.00	0.00	90.00	0.00	90.00	0.00
CD at 5%	1.357		1.054		1.008	
SEm±	0.449		0.349		0.333	
C.V.	1.292		0.855		0.891	

RMF –Rhizospheric myco-flora; G- Radial growth (mm); I- Per cent growth inhibition

Table.2 Effect of various rhizobacteria on radial growth and percent growth inhibition of *R. solani* at different time intervals

Rhizobacteria	72 hours		96 hours		120 hours	
	G	I	G	I	G	I
RB 1	71.23	1.07	71.23	20.86	71.23	20.86
RB 2	72.00	0.00	90.00	0.00	90.00	0.00
RB 3	71.83	0.23	81.63	9.30	81.63	9.30
RB 4	72.00	0.00	90.00	0.00	90.00	0.00
Control	72.00	0.00	90.00	0.00	90.00	0.00
CD at 5%	0.070		0.067		0.067	
SEm±	0.199		0.229		0.229	
C.V.	0.480		0.480		0.480	

RB –Rhizobacteria; G- Radial growth (mm); I- Inhibition per cent

Fig.1 Rhizospheric myco-flora

RMF- Rhizospheric myco-flora, RMF 1 –*Trichoderma harzianum*, RMF 2 –*Trichoderma viride*, RMF 3 – *Aspergillus sp.*, RMF 4 –*Penicillium sp.*, RMF 5 –*Aspergillus sp.*, RMF 6 –Unknown, RMF 7 –*Aspergillus sp.*
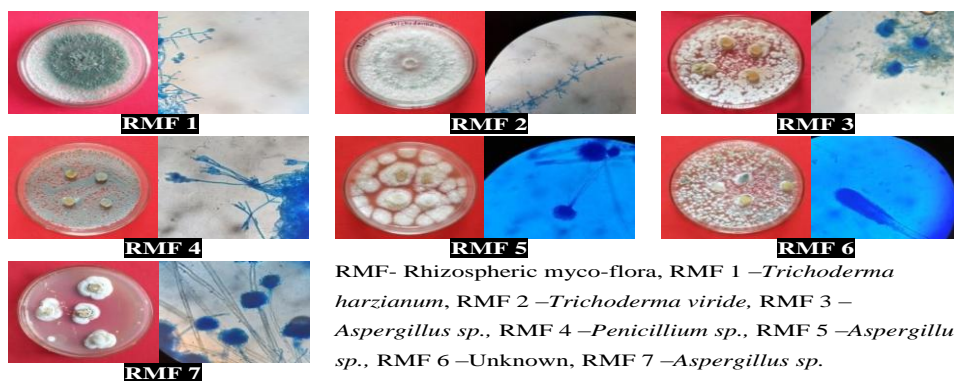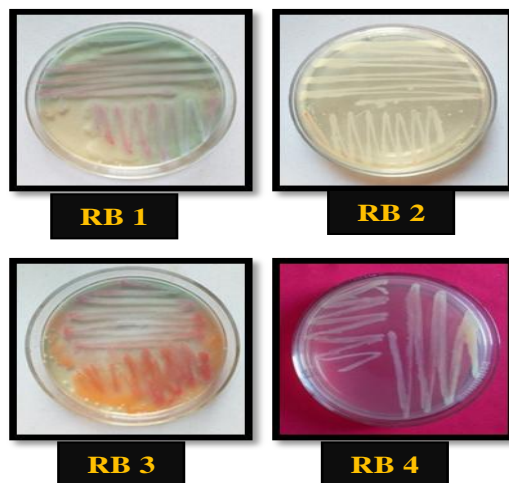
Fig.2 Rhizobacteria

Fig.3 Antagonistic effect of rhizospheric myco-flora on growth of *R. solani* at different time intervals
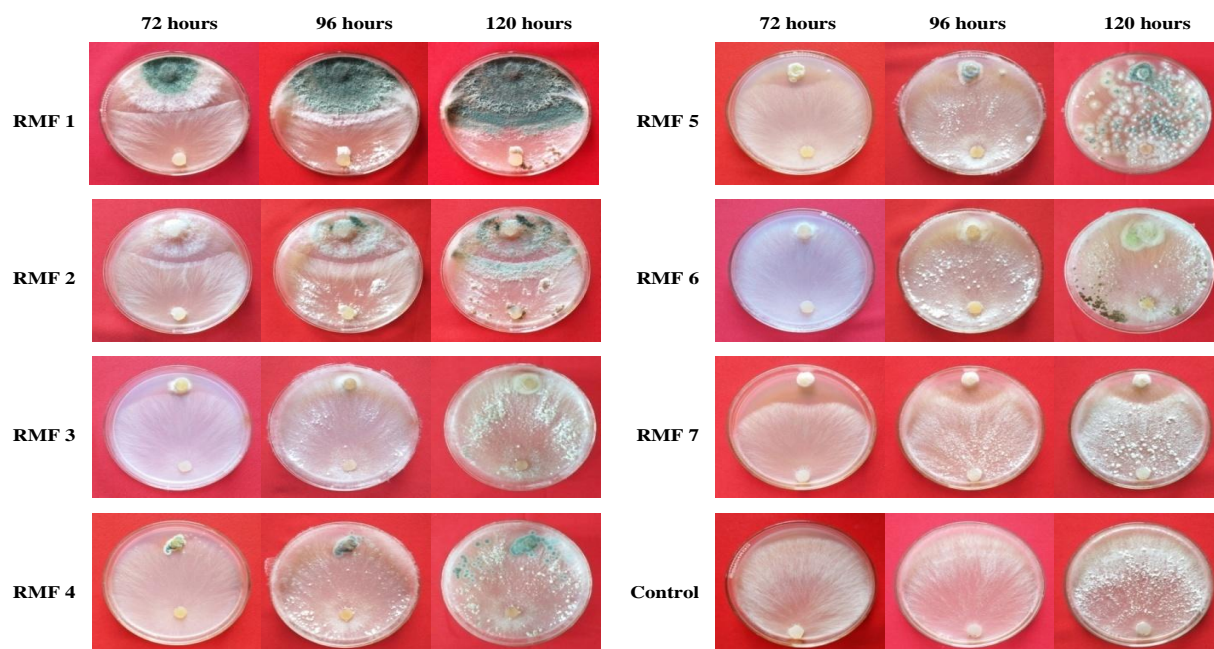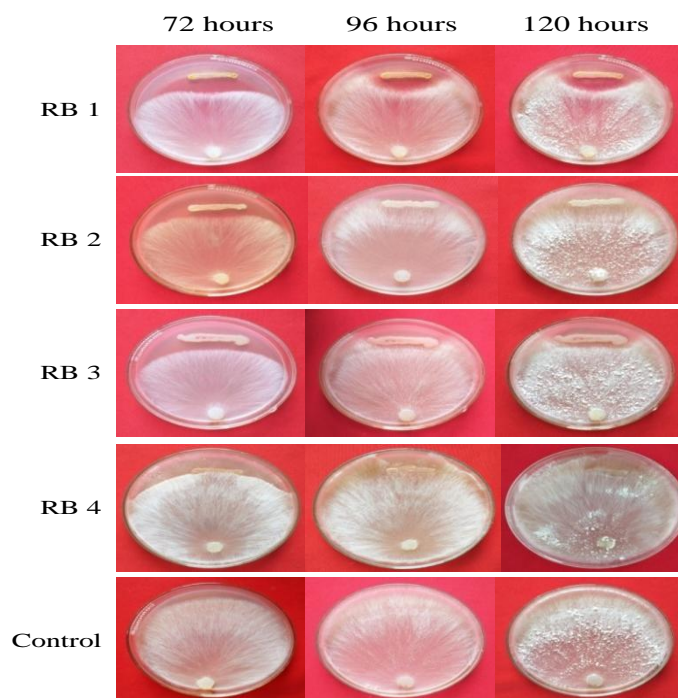

Fig.4 Antagonistic effect of rhizobacteria on growth of *R. solani* at different time intervals

Evaluation of rhizospheric myco-flora against *R. solani*

Seven native mycoflora were evaluated contrary to *R. solani* by dual culture method. Data on radial growth of the *R. solani* has been taken at 72, 96 and 120 hours after inoculation. Readings on the radial growth (G in mm) and per cent growth inhibition (I) have been provided in Table 1 and visualized in Figure III.

Data presented in Table 1 revealed that all rhizospheric myco-flora examined had antagonistic nature against *Rhizoctonia solani*, they were exhibited 1.62% to 46.30%, 8.15% to 55.56% and 9.81% to 55.56% growth inhibition of *R. solani* after 72, 96 and 120 hours of inoculation respectively. Among these, RMF 1 (*Trichoderma harzianum*) had maximum antagonistic effect on pathogen compared to other rhizospheric myco-flora, it produced 46.30%, 55.56% and 55.56% of inhibition after 72, 96 and 120 hours of inoculation respectively.

Next best antagonist was RMF 2 (*T. viride*) which produced 48.7% of growth inhibition after 120 hours of inoculation. Remaining rhizospheric myco-flora were showed less effective against *R. solani,* they were produced less than 25% of growth inhibition after 120 hours after inoculation (RMF 3 (9.81%), RMF 4 (10.74%), RMF 5 (15.93%), RMF 6 (8.15) and RMF 7 (23.33%)).

Ali and Nadarajah (2013) noted that out of the twenty isolates of *Trichoderma*, isolates 2, 6, 7, 8, 20, 13, 17 and 22 were most antagonistic to *R. solani.* Lenka *et al.,* (2012) evaluated several isolates of *Trichoderma* against *R. solani* and stated *T. Viride* (Bangalore isolate) was highly inhibited growth of mycelia and sclerotium formation. Next best were Bangalore isolate *T. harzianum* and Bhubaneswar isolate *T. viride.* Naeimi *et al.,*

(2010) have isolated more than 200 Trichoderma isolates, screened contrary to *R. solani.* Noted isolates *T. harzianum*, *T. virens* and *T. atroviride* showed better antagonism. Khan and Sinha (2007) have revealed that, *Trichoderma harzianum* was showed best growth inhibition of *R. solani* and next to it was *T. Viride.*

Screening of rhizobacteria for their antagonistic activity against *R. solani* by dual culture method

Four different rhizobacteria were isolated based on variation in their colony morphology and they were evaluated for their antagonistic effect against rice pathogen *R. solani* by dual culture technique. Radial growth of the *R. solani* has been taken after 72, 96 and 120 hours of inoculation. Readings on the radial growth (G in mm) and per cent growth inhibition (I) have been provided in Table 2 and visualized in Figure IV.

Date presented on Table 2 revealed that rhizobacteria RB1 (*Pseudomonas fluorescence*) had maximum antagonistic effect on *R. solani* as 20.86% of growth inhibition after 120 hours of inoculation compared with other rhizobacteria it has shown least antagonistic effect on pathogen.

Ahmed *et al.,* (2019) have screened five isolates of *Pseudomonas fluorescens* contrary to *R. solani,* which were isolated from rhizosphere. The isolate Pf 2 has shown 40.66 per cent inhibition compared to control. Maximum percent inhibition of sclerotia (88.64 per cent) and their dry weight also reduced to 82.72 per cent was observed when isolates Pf 2 was allowed to grow with *R. solani.* Nagendran *et al.,* (2019) have experimented sixty rhizosphere bacterial strains against sheath blight pathogen in *in vitro* condition, among the bacterial strains, *Pseudomonas fluorescens* Tu(Pf)19 and

Kk(Pf)24 and *Bacillus subtilis* Tn(Bs)7 and Kk(Bs)19 were showed maximum growth inhibition. Suman *et al.,* (2017) have isolated thirty native fluorescent *Pseudomonas* sp. and have screened them contrary to *R. solani*.

Among all three strains DMP1 (53.43%), DBP (51.53%) and PVP2 (48.26%) have been shown best growth inhibition results. Abdelbary *et al.,* (2015) have separated 17 isolates of *P. fluorescence* from rhizosphere. Among these Pf14 has shown maximum growth inhibition of *Rhizoctonia solani* as 60.49%.

In conclusion the results it can be say that, a total of twelve native soil bio agents (seven were rhizospheric mycoflora and four were rhizobacteria) were isolated and screened against sheath blight pathogen by dual culture method. All rhizospheric myco-flora have been shown a countable amount of antagonistic effect against mycilial growth of *Rhizoctonia solani* Kuhn; among these RMF1 (*Trichoderma harzianum*) and RMF2 (*Trichoderma viride*) showed best antagonism against *Rhizoctonia solani* as 55.56% and 48.70% of growth inhibition respectively after 120 hours of inoculation compared to control and remaining mycoflora were produced growth inhibition bellow 25%. Among four rhizobacteria, two were showed 20.86% (RB1-*Pseudomonas fluorescence*) and 9.30% (RB3) of growth inhibition and other two have been produced zero growth inhibition against sheath bight pathogen.

Acknowledgement

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