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Antagonistic Effect of *Pseudomonas fluorescens* on the Mycelial Growth and the Viability of the Sclerotia of *Rhizoctonia solani in vitro* and in Soil

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Pseudomonas fluorescens isolates NLR-B1, NLR-B2, NLR-B3 were tested in dual culture against *R. solani* among which NLR-B3 isolate was found to be more antagonistic *in vitro* against *Rhizoctonia solani* than other two isolates. Sclerotial germination was tested after incubating in NLR-B3 bacterial suspension in the concentrations from 10^{-4} to 10^{-8} CFU/ml, there was no inhibition in sclerotial germination at 10 min incubation at all concentrations of bacterial suspension, whereas at 20 min incubation, inhibition of sclerotial germination was 73.33 per cent at 10^{-4} concentration, 43.33 per cent at 10^{-5} concentration, 30 per cent at 10^{-6} concentration and there was no inhibition of sclerotial germination at 10^{-7} and 10^{-8} concentrations. At the incubation period of 30 min. inhibition in sclerotial germination was 53.33 per cent at 10^{-4} concentration, 36.67 percent at 10^{-5} concentration, 30 per cent at 10^{-6} concentration. Similar inhibition of 13.33 percent was observed both at 10^{-7} and at 10^{-8} concentration. Per cent inhibition increased with increase in concentration of bacterial suspension at 20 and 30 min incubation periods.The bacterial suspensions at the earlier concentrations were added to the soil and incubated for 10 days after which the sclerotia were retrieved and tested for their viability, the inhibition of sclerotial germination was 56.67 per cent at 10^{-4} concentration, 36.66 per cent at 10^{-5} concentration and 16.66 per cent at 10^{-6} concentration. However at lower concentration of 10^{-7} and 10^{-8} there was no inhibition of sclerotial germination. There was a significant increase in per cent inhibition of sclerotial germination with increase in concentration of the bacterial suspension.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops grown all over the world with a production of 550 million tonnes. In India, rice is grown over an area of 43.95 million hectares with production of 106.54 million tonnes and 2424 kg per hectare productivity. Rice is affected by several fungal, bacterial and viral diseases. As many as thirty five fungal, eight bacterial and twenty viral and mycoplasmal diseases were reported on rice (Ou, 1985). Of these rice sheath blight is second only to, and often rivals rice blast in importance. Rice Sheath blight is caused by *Rhizoctonia solani* Kuhn. The symptoms of the disease, usually appear as spots on the sheaths near the water line. The spots are first ellipsoid or ovoid, somewhat irregular, greenish grey, varying from 1 to 3 cm long.

These spots or lesions coalesce and advance from the leaf sheaths to the leaf blades. The presence of several large lesions upon a leaf sheath usually causes the death of the whole leaf and all the leaves may be blighted in severe cases. The fungus produces brown sclerotia depending upon environmental conditions. (Ou, 1985). The sclerotia survive for long periods and tend to accumulate in the soil (Lee and Rush, 1983). Therefore, the sclerotia of *R. solani* play an important role in the pathogen survival in rice fields. It has been reported that sclerotial viability in paddy soil is affected by soil microorganisms, making biological control an attractive alternative strategy for controlling rice sheath blight (Vasantha Devi *et al.,* 1989).

Materials and Methods

Isolation of Pathogen

The causal organism *R. solani* Kuhn was isolated from rice plants showing typical sheath blight symptoms under field conditions. Leaf sheath showing typical symptoms was washed in tap water for few minutes and leaf bits of 3-8 mm size were surface sterilized with 1% sodium hypochloride for 1minute and then rinsed with sterile distilled water to remove the traces of sodium hypochloride. These leaf bits are transferred to potato dextrose agar medium in petriplates and kept for incubation at 28 ± 2°C. When the growth of the fungus from the leaf bits was seen on the PDA surface, the hyphal bits from the periphery of the culture growing in the petriplates was transferred to the PDA in culture tubes. The culture was purified by hyphal tip method and pure culture was maintained on PDA by regular sub culturing at frequent intervals. Pathogen was also isolated from sclerotial bodies by keeping on Petri plate containing sterilized PDA after sterilizing with 70% ethanol followed by three washing in sterile distilled water. Plates were incubated at 28 ± 2°C and observed periodically for growth of the fungus. The culture was purified by single hyphal tip method and maintained on PDA by periodical transfer throughout the present investigation.

Isolation of native antagonistic bacteria from rhizosphere

Antagonistic bacteria were isolated by following serial dilution technique (Johnson and Curl, 1977). Composite soil sample was collected from rhizosphere of healthy plants. The soil was dried under shade and then used for serial dilution. To get 10^{-1} dilution, ten gram of this soil was dissolved in 90 ml of sterile distilled water. From this one ml of soil suspension was taken and added to nine ml of sterile distilled water to get 10^{-2} dilution. This was repeated until a final dilution of 10^{-4} for isolation of fungi and 10^{-6} for bacteria. Antagonistic bacteria were isolated on King's B medium by using a dilution of 10^{-6}. One ml of final dilution of soil suspension was poured into sterilized Petri plates, and then the melted and cooled media was poured. Plates were rotated gently on the laminar air flow bench to get uniform distribution of soil suspension in the medium. Then the plates were incubated at 28 ± 2ºC and observed at frequent intervals for the development of colonies. Dual culture technique was used to identify the potential antagonistic isolate of *Pseudomonas fluorescens*. Isolates used in the present investigation are listed in Table 1.

Fungal-Bacterial Interaction in Dual Culture

Pathogen, *R. solani* was inoculated at the center of 9.0 cm diameter PDA plate. Test bacterial cultures were streaked individually on both the sides of the *R. solani* at 2.5 cm distance leaving 2.0cm from periphery, Plates inoculated with *R. solani* alone were utilized as checks. Inoculated plates were incubated at

28 ± 2°C observations were recorded as zone of inhibition up to four days (when *R. solani* completely occupied the plate in monoculture check) (Lahlali *et al.,* 2007 and Reddy *et al.,* 2010).

Observations were recorded on mycelial growth of *R. solani* and per cent inhibition in *R. solani* growth was calculated using the following formula (Vincent *et al.,* 1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent reduction in growth of test pathogen, C = Radial growth (cm) in control, T = Radial growth (cm) in treatments.

Antagonistic effect of *P. fluorescens* on the viability of the sclerotia of *R. solani in vitro*

The potential antagonistic *P. fluorescens* NB-3 isolate against *R. solani* was identified in the Experiment 3.9.2 and it was used for this experiment. The bacterial culture was grown on Kings B medium for 48 hr and suspension was prepared and made serial dilutions from 10^{-4} to 10^{-8} CFU/ml. Sclerotia were soaked in each dilution for different time periods of 10, 20 and 30 minutes before transferring on to PDA for testing their viability (Bashar *et al.,* 2010). Experimental design used was CRD and three replications were maintained per concentration of the isolate. Per cent inhibition of sclerotial germination was calculated.

Per cent inhibition

$$\frac{\text{Total number of sclerotia - number of germinated sclerotia}}{\text{Total number of sclerotia}} \times 100$$

Antagonistic effect of *P. fluorescens* on the viability of the sclerotia of *R. solani* in soil

Dried paddy soil was used in this experiment. 10 g of soil was taken into plastic cups and ten sclerotia of *R. solani* was mixed with the soil. This is a unit representing a replication of a concentration of bacterial suspension. The bacterial suspension of *P. fluorescens* from 10^{-4} to 10^{-8} CFU/ml were added to the plastic cup containing sclerotia and soil mixture upto saturation and incubate for 10 d. After 10 d the sclerotia were retrieved and placed on PDA medium for testing their viability. Experimental design used was CRD and three replications were maintained per concentration of the isolate. Per cent inhibition of sclerotial germination.

Results and Discussion

Isolation of *R. solani*

The culture obtained on PDA at 28±1°C was light brown colour occupying 9 cm diameter Petriplate in 3 days of incubation. The pathogen produced dark brown, irregular, loose type of sclerotial bodies on PDA.

Effect of *P. fluorescens* on the mycelial growth of *R. solani in vitro* and identification of potential antagonistic isolate against *R. solani*

Three isolates of *P. fluorescens* were assessed for their antagonistic potential against *R. solani*. Bacterial isolates were screened based on their antagonistic potential against *R. solani in vitro* in dual culture. In control plates, *i.e.,* in monoculture, *R. solani* grew to the extent of 4.5 cm radius covering the entire plate in two days. Variation existed in the growth of *R. solani* with different *Pseudomonas* isolates in dual culture (Table 2). The data was analysed using CRD and the results was presented here with.

Based on the mean inhibition % observed in the growth of *R. solani* due to the presence of *P. fluorescens* in dual culture plates, the results obtained indicated that maximum inhibition was due to NLR-B3 (66.76%)

followed by NLR-B1 (63.32%). Least mean inhibition was observed with NLR-B2 (57.30%) (Fig.1).

Effect of *P. fluorescens* on the sclerotial viability of *R. solani in vitro*

The potential antagonistic *P. fluorescens* NLR-B-3 isolate against *R. solani* was identified during screening and it was used for this experiment. The bacterial culture was grown on Kings B medium for 48 h and suspension was prepared and made serial dilutions from 10^{-4} to 10^{-8} CFU/ml.

Sclerotia were soaked in each dilution for different time periods of 10, 20 and 30 min, sclerotia were soaked in distilled water for control and sclerotia were transfered on to PDA for testing their viability. The results were presented in the Table 3.

Among all the treatments, control showed 100 % sclerotial germination. Similarly at all the concentrations from 10^{-4} to 10^{-8} CFU/ml of bacterial suspension, there was no inhibition in sclerotial germination at 10 min incubation (Plate 3.3), whereas at 20 min incubation germination of sclerotia was inhibited. At 10^{-4} concentration inhibition was 73.33 %, at 10^{-5} concentration inhibition was 43.33 %, at 10^{-6} concentration inhibition was 30 %, no inhibition of sclerotial germination was observed.at 10^{-7} and 10^{-8} concentrations. At 30 min. incubation, inhibition in sclerotial germination was 53.33 % at 10^{-4} concentration, 36.67 % at 10^{-5} concentration, 30 % at 10^{-6} concentration, 13.33 % at 10^{-7} concentration and at 10^{-8} concentration 13.33 % inhibition was observed. Per cent inhibition increased with increase in concentration of bacterial suspension at 20 and 30 min incubation periods.

Table.1 Different *P. fluorescens* isolates tested

Isolate	Isolated from	Designated as
P. fluorescens	Rice rhizosphere	NLR-B1
P. fluorescens	Rice rhizosphere	NLR-B2
P. fluorescens	Rice rhizosphere	NLR-B3

Table.2 *In vitro* efficacy of bacterial antagonists on the mycelial growth of *Rhizoctonia solani*

S. No.	Isolate	Designated as	Radial growth in cm	Per cent Inhibition
1	*P. fluorescens*	NLR- B1	1.65	63.32 (52.70)
2	*P. fluorescens*	NLR- B2	1.92	57.30 (49.17)
3	*P. fluorescens*	NLR- B3	1.50	66.76 (54.77)
4	Control		4.50	0.00 (0.00)
	CD (P=0.01)			0.35
	SEm±			0.11
	SEd±			0.16
	CV%			0.57

Table.3 *In vitro* efficacy of bacterial antagonists on the sclerotial viability of *Rhizoctonia solani*

S. No	Bacteria isolate NLR-B3, CFU/ml	Per cent germination of sclerotia			Per cent inhibition of Sclerotia		
		10 min	20 min	30 min	10 min	20 min	30 min
1	10^{-4}	100.00	26.67 (30.98)	46.67 (43.06)	0.00	73.33 (58.98)**	53.33 (46.90)
2	10^{-5}	100.00	56.67 (48.83)	63.33 (52.75)	0.00	43.33 (41.13)	36.66 (37.21)
3	10^{-6}	100.00	70.00 (56.77)	70.00 (56.77)	0.00	30.00 (33.19)	30.00 (33.19)
4	10^{-7}	100.00	93.33 (81.14)	86.67 (68.83)	0.00	6.66 (8.85)	13.33 (21.13)
5	10^{-8}	100.00	100.00 (90.00)	86.67 (68.83)	0.00	0.00 (0.00)	13.33 (21.33)
6	Control	100.00	100.00 (90.00)	100.00 (90.00)	0.00	0.00 (0.00)	0.00 (0.00)
	CD (P=0.01)		11.87	6.02		11.86	6.01
	SEm±		3.81	1.93		3.80	1.93
	SEd±		5.39	2.73		5.38	2.73

****** Figures in parentheses are angular transformed values.

Table.4 Evaluation of bacterial antagonists on the sclerotial viability of *Rhizoctonia solani* in soil

S. No.	Bacteria isolate NLR-B3, CFU/ml	Per cent germination of sclerotia	Per cent inhibition of Sclerotia
1	10^{-4}	43.33 (41.14)	56.66 (48.82)**
2	10^{-5}	63.33 (52.75)	36.66 (37.21)
3	10^{-6}	83.33 (66.12)	16.66 (23.84)
4	10^{-7}	100.00 (90.00)	0.00 (0.00)
5	10^{-8}	100.00 (90.00)	0.00 (0.00)
6	Control	100.00 (90.00)	0.00 (0.00)
	CD (P=0.01)	4.94	4.93
	SEm±	1.58	1.58
	SEd±	2.24	2.24
	CV%	3.83	14.0

****** Figures in parentheses are angular transformed values.

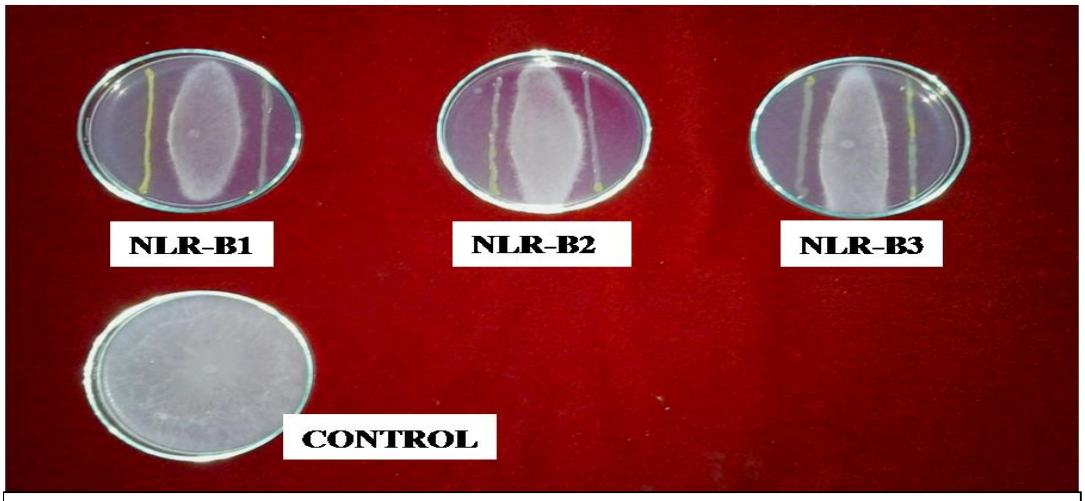

Fig.1 : *In vitro* efficacy of *P . fluorescens* isolates on mycelial growth of *R. solani* in dual culture

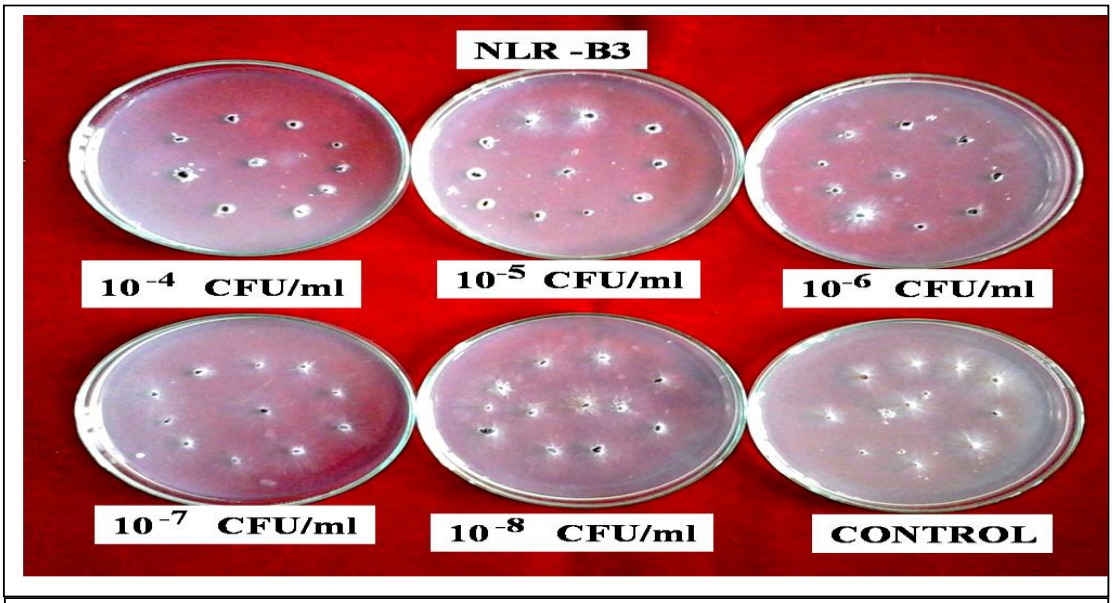

Fig.2: Efficacy of soil application of *P . fluorescens* isolates on sclerotiial viability of of *R. solani* at 10 d incubation

Per cent inhibition of sclerotial germination was recorded in the following order.

$10^{-4} > 10^{-5} > 10^{-6} > 10^{-7} > 10^{-8}$ CFU/ml.

Effect of *P. fluorescens* on the sclerotial viability of *R. solani* in soil

Dried paddy soil was used in this experiment. 10 g of soil was taken into plastic cups and ten sclerotia of *R. solani* was mixed with the soil. This is a unit representing a replication of a concentration of bacterial suspension.

The bacterial suspension of *P. fluorescens* from 10^{-4} to 10^{-8} CFU/ml were added to the plastic cups containing sclerotia and soil mixture upto saturation and incubated for 10

d. After 10 d the sclerotia were retrieved and placed on PDA medium for testing their viability. The results were presented in the Table 4.

Among all the treatments, the control showed no inhibition whereas the inhibition was 56.67 % at 10^{-4} concentration, 36.66 per cent at 10^{-5} concentration and 16.66 % at 10^{-6} concentration However at lower concentration 10^{-7} and 10^{-8} all sclerotia were germinated *i.e.*, no inhibition was observed. With increase in concentration of the bacterial suspension the per cent inhibition was also increased significantly in all the treatments (Fig. 2).

Vasantha Devi *et al.,* (1989) reported inhibition in sclerotial germination declined with prolonged incubation periods with bacteria.

In dual culture, among the three isolates of *Pseudomonas fluorescens,* NLR-B1, NLR-B2 and NLR-B3, the isolate NLR-B3 was found to be antagonistic to *R.* solani showing the 66.76% inhibition of mycelial growth of *R. solani.* There was no inhibition in sclerotial germination at 10 min incubation at all concentrations of bacterial suspension, whereas at 20 min incubation, inhibition of sclerotial germination was 73.33 per cent at 10^{-4} concentration, 43.33 per cent at 10^{-5} concentration, 30 per cent at 10^{-6} concentration and there was no inhibition of sclerotial germination at 10^{-7} and 10^{-8} concentrations. At the incubation period of 30 min. inhibition in sclerotial germination was 53.33 per cent at 10^{-4} concentration, 36.67 per cent at 10^{-5} conc., 30 per cent at 10^{-6} concentration. Similar inhibition of 13.33 per cent was observed both at 10^{-7} and at 10^{-8} concentration. When the bacterial suspensions at the earlier concentrations were added to the soil and incubated for 10 days, the inhibition of sclerotial germination was 56.67 per cent at 10^{-4} concentration, 36.66 per cent at 10^{-5} concentration and 16.66 per cent at 10^{-6} concentration. However at lower concentration of 10^{-7} and 10^{-8} there was no inhibition of sclerotial germination. There was a significant increase in per cent inhibition of sclerotial germination with increase in concentration of the bacterial suspension. Hence, the bacterial antagonist (*P. fluorescens*) found effective against *R. solani*.

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