



## ANTAGONISTIC EFFECT OF *Pseudomonas fluorescens* ON THE MYCELIAL GROWTH AND SCLEROTIAL VIABILITY OF *Rhizoctonia solani* *in vitro* AND IN SOIL

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ABSTRACT

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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 effective under *in vitro* against *Rhizoctonia solani* than other two isolates. Sclerotial germination was tested after incubating in NLR-B3 bacterial suspension at concentrations ranging 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, where as 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, 30.00 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  $10^{-5}$  concentration, 30.00 per cent at  $10^{-6}$  concentration. Similar inhibition of 13.33 per cent was observed both at  $10^{-7}$  and at  $10^{-8}$  concentration. The inhibition percentage increased with increase in concentration of bacterial suspension at 20 and 30 min incubation periods. The addition of higher concentrates bacterial suspensions to soil containing sclerotia with further incubation for 10 days and subsequent retrieval showed the bacterial germination inhibition of 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. The results indicated a significant increase in per cent inhibition of sclerotial germination with increase in concentration of the bacterial suspension.

**KEYWORDS:** *Pseudomonas fluorescens*, *Rhizoctonia solani*, Sclerotia, dual culture.

### 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)., 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 the rice sheath blight (Vasanth Devi *et al.*, 1989).

### MATERIAL AND METHODS

#### Isolation of Pathogen

The pathogen *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 1 minute 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  $28 \pm 2^\circ\text{C}$ . When the growth of the fungus from the leaf bits was seen on the PDA surface, the hyphal

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tips 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  $28 \pm 2^\circ\text{C}$  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. Composite soil sample was collected from rhizosphere of healthy plants. The soil was dried under shade and then used for serial dilution. 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  $28 \pm 2^\circ\text{C}$  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 below.

**Table 1. Different *P. fluorescens* isolates tested**

Isolate	Isolated region	Designated as
<i>P. fluorescens</i>	Rice rhizosphere	NLR-B1
<i>P. fluorescens</i>	Rice rhizosphere	NLR-B2
<i>P. fluorescens</i>	Rice rhizosphere	NLR-B3

### Pathogen –Antagonistic 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 2.0 cm from periphery, Plates inoculated with *R. solani* alone were utilized as checks. Inoculated plates were incubated at  $28 \pm 2^\circ\text{C}$  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 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* NLB-3 isolate against *R. solani* identified in the dual culture was used for this study. The bacterial culture was grown on Kings B medium for 48 hr and suspension was prepared and made serial dilutions from  $10^{-4}$  to  $10^{-8}$ . 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=

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

### Antagonistic 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* ranging from  $10^{-4}$  to  $10^{-8}$  dilutes were added to the plastic cup containing sclerotia and soil mixture upto saturation and incubated for 10 days. After 10 days the sclerotia were retrieved and placed on to the 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 was recorded.

## RESULTS AND DISCUSSION

### Isolation of *R. solani*

The culture obtained on PDA was light brown colour producing dark brown, irregular, loose type of sclerotial bodies on PDA (Fig. 1).



Fig. 1. Pure culture of *R. solani*

### 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, *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.

Based on the mean per cent inhibition in the growth of *R. solani* in dual culture against *P. fluorescens* in dual culture 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. 2).

### 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 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 varying from  $10^{-4}$  to  $10^{-8}$  dilutions. 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 transferred on to PDA for testing their viability. The results were presented in the Table 3.

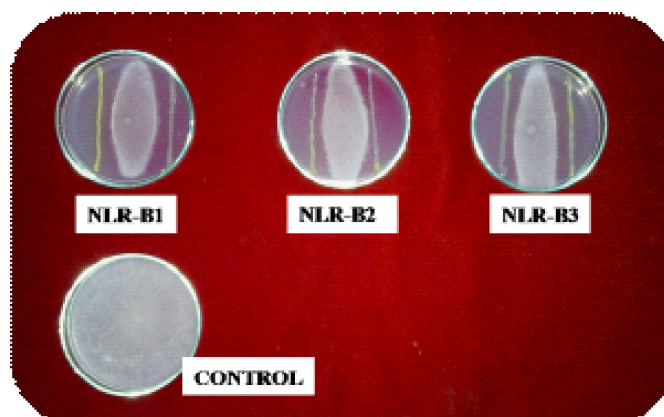


Fig. 2. *In vitro* efficacy of *P. fluorescens* isolates on mycelial growth of *R. solani* in dual culture

Among all the treatments, control showed 100 % sclerotial germination. Similarly at all the dilutions ranging from  $10^{-4}$  to  $10^{-8}$  of bacterial suspension, there was no inhibition in sclerotial germination at 10 min incubation (Plate 3.3). At 20 min incubation, inhibition of sclerotial germination was observed. A  $10^{-4}$  concentration inhibition per cent was 73.33 %, at  $10^{-5}$  concentration inhibition was 43.33 %, at  $10^{-6}$  concentration inhibition was 30.00 %, at  $10^{-7}$  concentration inhibition was 6.66 % and no inhibition was observed at  $10^{-8}$  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 (Fig. 3, 4, 5).

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

$$10^{-4} > 10^{-5} > 10^{-6} > 10^{-7} > 10^{-8} \text{ dilutions}$$

### 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* ranging from  $10^{-4}$  to  $10^{-8}$  dilutions were added to the plastic cups containing sclerotia and soil mixture upto saturation and incubated for 10 days. After 10 days the sclerotia were retrieved and placed on PDA medium for testing their viability. The results were presented in the Table 4.

Antagonistic effect of *Pseudomonas fluorescens*

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

S. No.	Isolate	Designated as	Radial growth (cm)	Per cent inhibition (%)
1	<i>P. fluorescens</i>	NLR- B1	1.65	63.32 (52.70)
2	<i>P. fluorescens</i>	NLR- B2	1.92	57.30 (49.17)
3	<i>P. fluorescens</i>	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.	Dilution factor of NLR-3 Bacteria isolate	Per cent germination of sclerotia			Per cent inhibition of sclerotia		
		10 min incubation	20 min incubation	30 min incubation	10 min incubation	20 min incubation	30 min incubation
1	10 <sup>-4</sup>	100.00	26.67 (30.98)	46.67 (43.06)	0.00	73.33 (58.98)**	53.33 (46.90)
2	10 <sup>-5</sup>	100.00	56.67 (48.83)	63.33 (52.75)	0.00	43.33 (41.13)	36.66 (37.21)
3	10 <sup>-6</sup>	100.00	70.00 (56.77)	70.00 (56.77)	0.00	30.00 (33.19)	30.00 (33.19)
4	10 <sup>-7</sup>	100.00	93.33 (81.14)	86.67 (68.83)	0.00	6.66 (8.85)	13.33 (21.13)
5	10 <sup>-8</sup>	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

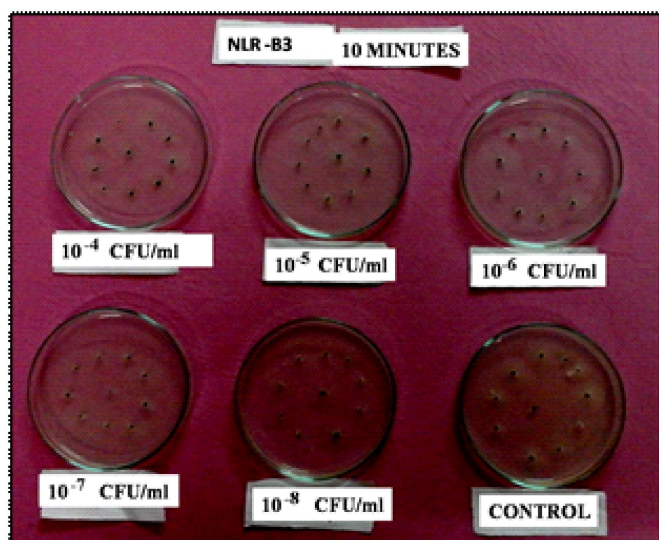


Fig. 3. *In vitro* efficacy of *P. fluorescens* isolates on sclerotial viability of *R. solani* at 10 min incubation

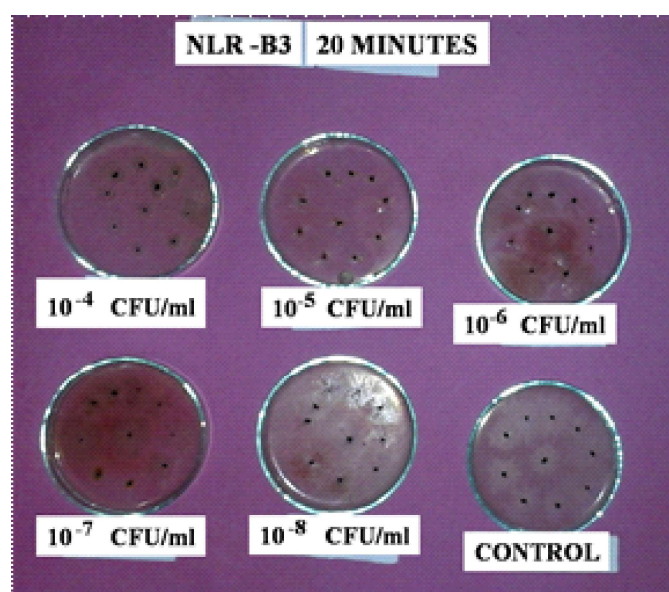


Fig. 4. *In vitro* efficacy of *P. fluorescens* isolates on sclerotial viability of *R. solani* at 20 min incubation

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. 6).

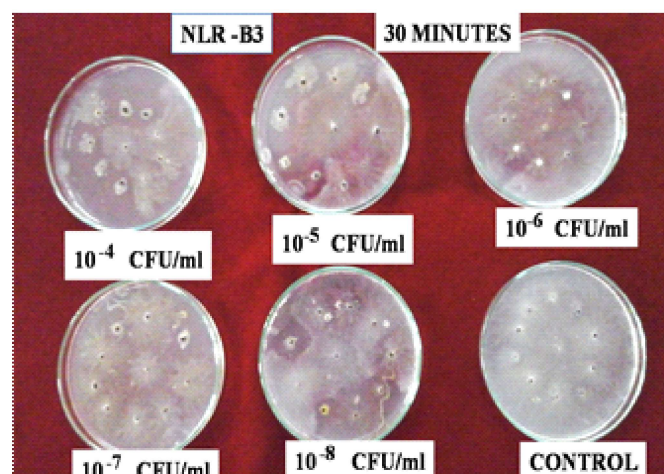


Fig. 5. *In vitro* efficacy of *P. fluorescens* isolates on sclerotial viability of *R. solani* at 30 min incubation



Fig. 6. Efficacy of soil application of *P. fluorescens* isolates on sclerotial viability of *R. solani* at 10 d incubation

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

## CONCLUSION

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 effective against *R. solani* showing maximum of mycelial growth of *R.*

**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 <sup>-4</sup>	43.33 (41.14)	56.66 (48.82)**
2	10 <sup>-5</sup>	63.33 (52.75)	36.66 (37.21)
3	10 <sup>-6</sup>	83.33 (66.12)	16.66 (23.84)
4	10 <sup>-7</sup>	100.00 (90.00)	0.00 (0.00)
5	10 <sup>-8</sup>	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

*solani*. There was no inhibition in sclerotial germination at 10 min incubation at all concentrations of bacterial suspension, where as at 20 min incubation, inhibition of sclerotial germination was 73.33 per cent at 10<sup>-4</sup> concentration, 43.33 per cent at 10<sup>-5</sup> concentration, 30 per cent at 10<sup>-6</sup> concentration and there was no inhibition of sclerotial germination at 10<sup>-7</sup> and 10<sup>-8</sup> concentrations. At the incubation period of 30 min. inhibition in sclerotial germination was 53.33 per cent at 10<sup>-4</sup> concentration, 36.67 per cent at 10<sup>-5</sup> conc., 30 per cent at 10<sup>-6</sup> concentration. Similar inhibition of 13.33 per cent was observed both at 10<sup>-7</sup> and at 10<sup>-8</sup> 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<sup>-4</sup> concentration, 36.66 per cent at 10<sup>-5</sup> concentration and 16.66 per cent at 10<sup>-6</sup> concentration. However at lower concentration of 10<sup>-7</sup> and 10<sup>-8</sup> there was no inhibition of sclerotial germination. Results indicated 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 biocontrol against *R. solani*.

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