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Molecular Characterization of *Pseudomonas* sp and *Bacillus* sp for Biological Management of Sheath Blight Pathogen in Rice

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Abstract

All the 20 isolates of *Psuedomonas fluorescens* showed positive result in all biochemical characteristics such as KOH test, HCN production, pigment production, gelatin liquefication, growth at 45 ºC and negative results were obtained in gram staining and growth at 4 ºC. Among the 20 isolates of *P. fluorescens*, *P.f.* I_{10} isolate was found to be effective by recording the mycelial growth reduction of 39.56 per cent followed by *P.f.* I_{20} isolate 30.67 per cent reduction when compared to check (TNAU *P.f.*1-37.33%). The result revealed that an amplicon of 560 bp. Metabolic compounds such as HCN, Sidereophore and Volatiles were analyzed to know their mode of action.

Keywords: Rice, sheath blight, molecular characterization, *Pseudomonas sp, Bacillus sp, and mycelial growth*

Introduction

Rice (*Oryza sativa* L.) is one of the widely cultivated important food crop in the world and over half of the world population relies on it for food. It is the chief food for more than 65% of the world's population and is India's pre-eminent crop. Rice provides 20 per cent of the world's dietary supply and is a good source of thiamine, riboflavin, niacin and dietary fiber. In Asia, where 95 per cent of the world's rice is produced and consumed; it contributes 40-80 per cent of the calories of Asian diet (Kanchana *et al.,* 2012). Globally, more than three million people have rice as staple food, and it accounts for 50 to 80 per cent of their daily calorie intake (Delseny *et al.,* 2001). Over the next 20 years, it is expected that demand for rice will grow by 2.5 per cent per year (Hobbs, 2001). In India rice is grown in 43.86 million ha, the production level is 104.80 million tones and the productivity is about 2390 kg/ha (Statistics, 2005). However, in Tamil Nadu rice crop was cultivated about 20 lakh hectare with an annual production of about 75.17 lakh tonnes with a productivity is 3758 kg/hectare during the year 2015-16 (INDIASTAT). Yadav *et al.,* 2020 found that sheath blight incited by *Rhioctonia solani* is one of the potentially devastating disease of rice in almost all over the rice growing countries of the world. In India, intensive and extensive cultivation of rice mostly under rice-wheat cropping system have resulted heavy losses to the crop ranging from 5.2 to 70 per cent in yield.

Diseases cause serious constraints to rice cultivation in India. More than 70 diseases caused by fungi, bacteria and viruses have been recorded on rice (Ou, 1985). Among them sheath blight caused by *Rhizoctonia solani* [Teleomorph: *Thanatephorus cucumeris* (Frank) Donk] anastomosis group 1 IA (AG-1 IA) is a serious disease worldwide (Rush and Lee, 1983). The sheath blight disease of rice is prevalent in almost all rice growing countries of the world. Sheath blight is an important soil borne fungal disease (Kuhn) causing upto 40 per cent of yield losses annually. The symptoms of the disease include greenish grey water-

soaked spots develops on the outer most sheath of rice. As the disease progresses spots enlarge, coalesce forming larger lesions with grayish white center surrounded by tan to brown irregular borders or outlines. Sclerotia surviving in the soil are the major source of infection (Damicone and Jackson, 1996). When sclerotia come into contact with a rice plant they germinate and the hyphae produce infection cushions on the exposed leaf sheath. Haustoria grow from the infection cushions, penetrate the host tissue, and typically green gray water-soaked lesions develop (Dodman and Flentje, 1970).

Among the cultivated rice varieties, the level of resistance to rice sheath blight is low. The conventional methods for the fungal diseases control were mainly by using synthetic fungicides. The chemical control using synthetic fungicides is less acceptable due to their increase incidence of development of resistance, lack of specificity towards the target pathogen, adverse effect on environment and beneficial microbes (Mcneil *et al.,* 2010). Fungicide application may not be economically feasible and the time of application appears to be important (Lee and Rush, 1983). Thus, there is a need for alternative disease management that provides effective control without side effects in the environment.

Biological control is proved to be a promising disease management technology against soil borne plant pathogens, when applied either alone or in combination with other management practices Papavizas (1965) directed towards the irrigation of cultural practices with chemical control (Damicone *et al.,* 1996). Cook and Baker (1983) defined biological control as "the reduction of inoculum or disease producing activity of the pathogen accomplished by use of beneficial bacteria as biological control agents to suppress plant disease and also eco-friendly alternative to the chemical fungicides. Biological control is the best solution for long term sustainability and effective management of soil borne diseases which can minimize the disease (Avila *et al.,* 2013).

A diverse group of biocontrol agents such as bacteria, fungi and viruses exist in nature. Among them bacterial antagonists are considered ideal candidates because of their rapid growth, ease of handling and aggressive colonizing character. Bacterial antagonists, *Pseudomonas* and *Bacillus* in particular, are good candidates for biological control.

Bacilli are gram positive endospore producing bacteria that are tolerant to heat and desiccation; a very good feature required for field application. They have an antagonistic character against many soil borne fungi such as *R. solani*. The fluorescent Pseudomonads are gram negative rods and have simple nutrition requirements; are excellent colonizers which widely prevalent in rice rhizosphere. They have been successfully used for biological control of several sclerotium producing fungi such as *Sclerotium rolfsii* (Laha *et al.,* 1996), *R. solani* (Rabindran and Vidhyasekaran, 1996) and *Macrophomina phaseolina* (Meena *et al.,* 2001). Bacterium applied (prior to pathogen inoculation) against several rice pathogens to the seed and rice plants can reduce disease severity by 20- 42% in a greenhouse and the field. Such seed bacterization of rice plants can enhance plant height, number of tillers and grain yield by 3 to 160 per cent. With this above said background, the following objectives were formulated to manage the sheath blight disease of rice effectively by biological method. 1. Isolation of the causal organism of rice sheath blight (*Rhizoctonia solani*) and proving the pathogenicity, 2. Isolation of bacterial antagonists from the rhizosphere region of rice plants and its characterization, 3. Testing the efficacy of bacterial antagonists against *R. solani* under *in vitro* conditions, 4. Testing the efficacy of bacterial antagonists against *R. solani* under field conditions. and 5. Studying the mode of action of antagonists against *R. solani*.

Materials and Methods

Molecular characterization of *Pseudomonas fluorescens*

Genomic DNA extraction

Two *Pseudomonas fluorescens* isolates were grown in Kings B broth for 72 h. The 1.5 ml of culture was centrifuged at 13000 rpm for 5 min. The pellet was suspended with 200µl of 0.1M HCl added with 200µl of lysis solution. Again the sample was added with 700µl of Phenol: Chlorophorm: Isoamyl alcohol (25 :24 :1) and centrifuged at 13000 rpm for 10 min. The aqueous layer was transferred to a new tube. DNA was precipitated by adding equal volume of ice cold ethanol and incubated at -20ºC at overnight. Then the pellet was air dried and added with 100µl of TE buffer (10 mM of Tris HCl with pH 8.0, one mM of EDTA with pH 8.0) and treated with in five µl of RNAse A (10mg/ml) at 37^{0}C for one hour. The isolated total DNA was tested by electrophoresis in 0.8 per cent agarose gel.

PCR amplification of 16S rRNA from *Pseudomonas fluorescens*

The 16s rRNA region of *Pseudomonas fluorescens* isolates was amplified using primers pairs ITS 1F- 5' CTTGGTCATTTAGAGGAAGTAA 3' and ITS 2R 2- 5' TGTGTTCTTCATCGATG -3' as per the protocol of Singh *et al.,* (2008) with the reaction conditions as follows. The PCR was carried out with a master cycler gradient (Eppendorf, Germany) programmed for pre-heating at 92ºC at 5min followed by denaturation at 92ºC at 4 min, annealing at 28ºC at 1 min and extension at 72ºC at 2 min for 25 cycles with the final extension at 72ºC at 10 min and final hold at 4ºC. The PCR products were separated by electrophoresis in the 1.2 per cent agarose gel stained with Ethidium bromide and visualized under UV light and documented using alpha imager (Alpha Innotech, California, USA). The 100 bp DNA ladder was used to measure the size of PCR products (Bangalore Genei, Pvt. Ltd.) was used.

Biochemical characterization of *Bacillus* sp.

The following biochemical tests were carried out for the characterization of *Bacillus* sp.

Staining reaction

Bacterial smear was prepared with 24 hrs old culture on microscopic slide, air dried and heat fixed. The slide was flooded with crystal violet for 60 seconds and then washed with tap water. Then it was flooded with Grams iodine mordant for 60 seconds and washed with tap water. Decolorized with 90% alcohol and washed again with tap water. Thus, the smear was counterstained with safranin for 45 seconds, washed with tap water, blot dried and observed under microscope (Gregersen, 1978).

Starch hydrolysis

Bacterial cultures were streaked on Petri dish containing Peptone sucrose agar incorporated with 0.2 per cent starch and incubated for 5 days. Lugol's iodine solution was flooded on the bacterial culture and observed for the development of clear zone around the bacterial growth which indicated the positive reaction (Dowson, 1957).

Catalase test

The bacterial cultures were grown on nutrient broth and incubated at 37ºC for 24 hrs. Then added with 1ml of 3 per cent hydrogen peroxide. Examined immediately and after 5 min for the evaluation of bubbles which indicates a positive test (Aneja, 1993).

Citrate utilization test

Bacterial cultures were steaked on the Petri dish containing Simmons citrate agar medium and incubated at 28±2ºC. The colour change in the medium around the bacterial cultures from green to blue colour indicates the utilization of citrate.

Molecular characterization of *Bacillus* sp.

Genomic DNA extraction

Two *Bacillus* sp. isolates were grown in the nutrient broth. About 25 ml of actively grown broth culture

was centrifuged at 6000 rpm for 5 min. The pellet obtained from centrifuged sample was suspended with 1ml of TE buffer and 0.5 ml of 1- Butanol. The emulsion was centrifuged at 6000 rpm for 5 min at 4°C. The pellet obtained was resuspended with 2 ml of TE buffer to remove the trace amount of ethanol and centrifuged at 6000 rpm at 4°C for 5 min. Again, the sample was resuspended in 1ml of TE buffer added with 100 µl (10 mg/ml) freshly prepared lysozyme and incubated at room temperature for 5 min. The sample was mixed with 200 µl of 5 mM NaCl; 150 µl of CTAB (10X stock) again incubated for 10 min. The cell wall lysate was deproteinised with 1ml of Phenol: Chloroform (1:1) and centrifuged at 6000 rpm for 4 °C. The aqueous layer was transferred to a new tube. DNA was precipitated by adding equal volume of ice cold isopropanol and incubated at -20 °C at overnight. DNA was collected by centrifugation at 10000 rpm for 15 min at 4 °C. The pellet was added with 300µl of ice cold ethanol and centrifuged at 15000 rpm at 4 °C. The supernatant was discarded and the pellet was dried at room temperature. The dried pellet was dissolved in 50 µl of TE buffer (10 mM of Tris HCl with pH 8.0, one mM of EDTA with pH 8.0) and treated in five µl of RNAse A (10 mg/ml) at 37 °C for one hour. The isolated total DNA was tested by electrophoresis in 0.8 per cent agarose gel.

PCR amplification of 16S rRNA from *Bacillus* sp.

The 16s rRNA region of *Bacillus* sp. isolates was amplified using primers pairs BCF1-5' CGGGAGGCAGCAGTAGGGAT-3' and BCR2-5' CTCCCCAGGCGGAGTGCTTAAT-3' as per the protocol of Singh *et al.,* (2008) with the reaction conditions as follows. The PCR was carried out with a master cycler gradient (Eppendorf, Germany) programmed for pre-heating at 95ºC at 5 min followed by denaturation at 94ºC at 1 min, annealing at 55ºC at 1 min and extension at 72ºC at 1 min for 25 cycles with the final extension at 72ºC at 5 min and final hold at 4ºC. The PCR products were separated by electrophoresis in the 1.2 per cent agarose gel stained with Ethidium bromide and visualized under UV light and documented using alpha imager (Alpha Innotech, California, USA). The 100 bp DNA ladder was used to measure the size of PCR products (Bangalore Genei, Pvt. Ltd., Bangalore) was used.

In vitro* screening of *Pseudomonas fluorescens* isolates against *R. solani

Twenty *Pseudomonas fluorescens* isolates were screened against the virulent *R. solani* isolate (Rs_2) under *in vitro* by adapting dual culture technique (Dennis and Webster, 1971). Each bacterial isolate was streaked at one side of Petri dish containing PDA at one cm away from the periphery. Nine mm mycelial disc from 5 days old culture of *R. solani* was placed at opposite side of Petri dish at one cm away from the periphery and perpendicular to the bacterial streak. Control plates were maintained without bacterial streak. Three replications were maintained and incubated at room temperature (28±2 °C) for 5 days. After ensuring complete growth of pathogen in the control, Per cent inhibition (PI) over control in each treatment was calculated separately using the following formula (Pandey *et al.,* 2000).

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

C = Average diameter of fungal growth (mm) in control

T = Average diameter of fungal growth (mm) in treatment

In vitro* screening of *Bacillus* sp. isolates against *R. solani

Twenty *Bacillus* sp. isolates were screened against the virulent *R. solani* isolate (Rs_2) under *in vitro* by adapting dual culture technique Dennis *et al.,* 1971.

Study on mode of action of *Pseudomonas fluorescens* isolates under *in vitro*

Hydrogen cyanide (HCN) production

HCN production of *Pseudomonas fluorescens* isolates was tested qualitatively by following the method of (Schippers *et al.,* 1987). Each bacterial isolate was

streaked separately on Kings' B medium amended with glycine at 4.4g/l. Sterile filter paper saturated with picric acid solution (2.5 g of picric acid, 12.5 g of Na_2CO_3, 1000 ml of distilled water) was placed in the upper lid of the Petri plate. The dishes were sealed with parafilm and incubated at 28ºC for 48 h. Change filter paper colour from yellow to light brown, brown or reddish- brown was recorded as weak (+) moderate (++) or strong (+++) reaction HCN production, respectively.

Siderophore production

The 60.5 mg of Chrome Azurol s (CAS) was dissolved in 5 ml of distilled water and mixed with 10 ml of iron solution (1 mM $FeCl_3$ 6 H_2O in 10 mM HCL). Then added to 72.9 mg of Hexadecyltrimethyl ammonium bromide (HDTMA) in 40 ml of distilled water. The dark blue coloured CAS reagent was then autoclaved for 15 minutes. This reagent was added to Nutrient agar before pouring the plates. The plates were inoculated with active culture of *Pseudomonas fluorescens* and plates are incubated at 48 h. Appearance of an orange zone around the bacterial culture against dark blue background suggest siderophore production (Alla, 1999).

Volatile production of *Pseudomonas fluorescens*

Pseudomonas fluorescens isolates were tested for their volatile production as described by Kazempour, (2004). Nine mm mycelial disc of five days old culture of *R. solani* was placed at the center of Petri plate containing PDA. Each *Pseudomonas fluorescens* isolate was streaked separately in the center of Petri plate containing KB medium. Plate streaked with bacteria was matched face to face over the Petri plate inoculated with fungal disc without any physical contact between them. The plates were sealed to isolate the inside atmosphere and to prevent the loss of volatile formed. Plates were incubated at room temperature for 4 days. Control plate was maintained without bacterial streak. After ensuring complete growth of pathogen in the control, Per cent inhibition (PI) over control by each isolate was calculated using the following formula (Pandey *et al.,* 2000).

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

C = Average diameter of fungal growth (mm) in control

T = Average diameter of fungal growth (mm) in treatment

Volatile production of *Bacillus* sp.

Bacillus sp. isolates were tested for their volatile production as described by Kazempour (2004). Nine mm mycelial disc of five days old culture of *R. solani* was placed at the center of Petri plate containing PDA. Each *Bacillus* sp. isolate was streaked separately in the center of Petri plate containing NA medium.

Results and Discussion

Survey on rice ShB

The maximum severity index of 64 per cent was recorded at TRRI, Aduthurai in Thanjavur district of Tamil Nadu followed by Tiruvayur district with 56% PDI. This might to be due to the high population and virulence of the pathogens, sclerotia present in the soil, continuous cropping of rice, intensive cultivation and the susceptibility of host to pathogen (Sathya *et al.,* 2020).

Biochemical characterization of *Bacillus* sp

The bacterial antagonists were biochemically characterized based on the following diagnostic tests detailed below (**Table 1**).

Table 1: Biochemical characteristics of *Bacillus* sp.

S. No	Biochemical test	*Bacillus* sp.	
		B.s.I_6	TNAU *B.s.*1
1.	Gram's staining	+	+
2.	Growth at 4 °C	-	-
3.	Growth in NaCl	+	+
4.	Growth at 45 °C	+	+
5.	Catalase test	+	+
6.	Utilization of citrate	+	+
7.	Starch hydrolysis	+	+

+ : Positive, - : Negative

Antifungal activity *Pseudomonas fluorescens*

The antifungal activity of *Pseudomonas fluorescens* isolates against the mycelial growth of *Rhizoctonia solani* (Rs2) under *invitro* conditions are **(Table 2)**.

Table 2: Antifungal activity of *Pseudomonas fluorescens* isolates against the mycelial growth of *Rhizoctonia solani* (Rs_2) under *in vitro* conditions

Sl. No	Treatments	Mycelial growth (cm)*	Per cent reduction over control (%)
1.	*P.f.* I_1	6.40	28.88
2.	*P.f.* I_2	7.09	21.22
3.	*P.f.* I_3	6.30	30.00
4.	*P.f.* I_4	7.30	18.88
5.	*P.f.* I_5	8.83	1.89
6.	*P.f.* I_6	6.42	28.67
7.	*P.f.* I_7	6.65	26.11
8.	*P.f.* I_8	6.50	27.78
9.	*P.f.* I_9	6.63	26.33
10.	*P.f.* I_{10}	5.44	39.56
11.	*P.f.* I_{11}	7.27	19.22
12	*P.f.* I_{12}	8.93	0.78
13	*P.f.* I_{13}	6.54	27.33
14	*P.f.* I_{14}	6.42	28.67
15	*P.f.* I_{15}	7.23	19.67
16	*P.f.* I_{16}	6.93	23
17	*P.f.* I_{17}	8.59	4.56
18	*P.f.* I_{18}	8.51	5.44
19	*P.f.* I_{19}	6.43	28.56
20	*P.f.* I_{20}	6.24	30.67
21	TNAU *P.f.* 1	5.64	37.33
22	Control	9	-
SEd		**0.35**	-
CD (P=0.05)		**0.70**	-

*Mean of three replications

Effect of *Bacillus* sp. on isolate (Rs_2) under *in vitro* conditions

In the present investigation, isolate *B.s.* I_{10} was found to reduce the mycelial growth of *R. solani* by 35.89 per cent which was found to be effective when compared to all other isolates over control. Among them, seven strains showed strong mycelial inhibition against the mycelial growth of *R. solani.* Shrestha *et al.,* (2016) screened 29 rice associated bacteria against the growth of *R. solani* under *in vitro* conditions. Among them, 26 rice associated bacteria showed antagonistic activity on the mycelial growth of *R. solani* **(Table 3)**.

Table 3: Antifungal activity of *Bacillus* sp. isolates against the mycelial growth of *Rhizoctonia solani* (Rs_2) under *in vitro* conditions

Sl. No	Isolates	Mycelial growth (cm)*	Per cent reduction over control (%)
1.	*B.s.*I_1	6.40	28.88
2.	*B.s.*I_2	7.17	20.33
3.	*B.s.*I_3	7.00	22.22
4.	*B.s.*I_4	8.60	4.44
5.	*B.s.*I_5	6.50	27.78
6.	*B.s.*I_6	5.77	35.89
7.	*B.s.*I_7	6.67	25.89
8.	*B.s.*I_8	8.90	1.11
9.	*B.s.*I_9	6.76	24.89
10.	*B.s.*I_{10}	8.40	6.66
11.	*B.s.*I_{11}	8.80	2.22
12	*B.s.*I_{12}	8.83	1.88
13	*B.s.*I_{13}	8.17	9.22
14	*B.s.*I_{14}	8.90	1.11
15	*B.s.*I_{15}	6.97	22.55
16	*B.s.*I_{16}	8.84	1.77
17	*B.s.*I_{17}	8.20	8.88
18	*B.s.*I_{18}	6.20	31.11
19	*B.s.*I_{19}	6.90	23.33
20	*B.s.*I_{20}	8.57	4.77
21	TNAU *B.s.* 1	5.53	38.55
22	Control	9	-
SEd		**0.20**	-
CD (P=0.05)		**0.42**	-

*Mean of three replications

Mode of action of bacterial antagonists

HCN production

In the present study, all isolates of *Pseudomonas fuorescens* and *Bacillus* sp. were tested for HCN production. All *Pseudomonas fluorescens* isolates showed positive result for HCN production. Among them *P.f.* I_{10} showed strong HCN production (colour of the filter paper turns yellow to reddish brown). Suresh *et al.,* (2010) reported that the ten strains of fluorescent Pseudomonads produced HCN **(Table 4)**.

Table 4: Siderophore, HCN and volatile compounds production by bacterial antagonists

S. No	*Pseudomonas* sp. Isolates	Test			*Bacillus* sp. Isolates	Test		
		Siderophore	HCN	Volatile		Siderophore	HCN	Volatile
1	*P.f.* I_1	+	+	+	*B.s.*I_1	-	-	+
2	*P.f.* I_2	+	+	+	*B.s.*I_2	-	-	+
3	*P.f.* I_3	+	+	+	*B.s.*I_3	-	-	+
4	*P.f.* I_4	+	+	+	*B.s.*I_4	-	-	+
5	*P.f.* I_5	+	+	+	*B.s.*I_5	-	-	+
6	*P.f.* I_6	+	+	+	*B.s.*I_6	-	-	+
7	*P.f.* I_7	+	+	+	*B.s.*I_7	-	-	+
8	*P.f.* I_8	+	+	+	*B.s.*I_8	-	-	+
9	*P.f.* I_9	+	+	+	*B.s.*I_9	-	-	+
10	*P.f.* I_{10}	+	+	+	*B.s.*I_{10}	-	-	+
11	*P.f.* I_{11}	+	+	+	*B.s.*I_{11}	-	-	+
12	*P.f.* I_{12}	+	+	+	*B.s.*I_{12}	-	-	+
13	*P.f.* I_{13}	+	+	+	*B.s.*I_{13}	-	-	+
14	*P.f.* I_{14}	+	+	+	*B.s.*I_{14}	-	-	+
15	*P.f.* I_{15}	+	+	+	*B.s.*I_{15}	-	-	+
16	*P.f.* I_{16}	+	+	+	*B.s.*I_{16}	-	-	+
17	*P.f.* I_{17}	+	+	+	*B.s.*I_{17}	-	-	+
18	*P.f.* I_{18}	+	+	+	*B.s.*I_{18}	-	-	+
19	*P.f.* I_{19}	+	+	+	*B.s.*I_{19}	-	-	+
20	*P.f.* I_{20}	+	+	+	*B.s.*I_{20}	-	-	+
21	Control	-	-	-	Control	-	-	+
22	TNAU *P.f.* 1	+	+	+	TNAU *B.s.*1	-	-	+

Siderophore production

In the present study, all the isolates of *Pseudomonas fluorescens* were tested for the production of siderophore. All the *Pseudomonas* isolates showed positive result for HCN production. Among them *P.f* I_{10} produced fluorescent yellow green pigment indicating the production of siderophore (pseudobactin). All the *Bacillus* isolates showed negative result for siderophore production. Meera and Balabaskar (2012) reported that Pf4, Pf6, Pf8, Pf11, Pf13, Pf20 isolates showed positive reaction on siderophore production. Saravanan *et al.,* (2013) reported that the fluorescent *Pseudomona*s produces siderophore under *in vitro* conditions. Showkat *et al.,* (2012) observed the siderophore production in the rhizosphere isolates of *Pseudomonas fluorescens.*

Effect of volatiles from bacterial antagonists against the growth of *R. solani*

Effect of volatiles from *Pseudomonas fluorescens* against the growth of *R.solani* under *in vitro* conditions

In the present investigation, among all the *Pseudomonas isolates* tested, *P.f.* I_{10} recorded maximum mycelial growth reduction of 38.88 per cent when compared to all other isolates of *Pseudomonas fluorescens.* Kazempour (2004) reported that the production of volatile from *Pseudomonas fluorescens* isolate B41 effectively reduced the mycelial growth (72.7 per cent) of *R. solani* under *in vitro* conditions and all the isolates showed inhibitory effect on mycelial growth **(Table 5)**.

Table 5: Activity of volatile from *Pseudomonas fluorescens* isolates against the mycelial growth of *Rhizoctonia solani* (Rs_2) under *in vitro* conditions

Sl. No	Isolates	Mycelial growth (cm)*	Per cent reduction over control (%)
1.	*P.f.* I_1	8.47	5.88
2.	*P.f.* I_2	8.42	6.44
3.	*P.f.* I_3	6.50	27.77
4.	*P.f.* I_4	8.64	4
5.	*P.f.* I_5	8.75	2.77
6.	*P.f.* I_6	5.93	34.11
7.	*P.f.* I_7	7.10	21.11
8.	*P.f.* I_8	7.27	19.22
9.	*P.f.* I_9	7.50	16.66
10.	*P.f.* I_{10}	5.50	38.88
11.	*P.f.* I_{11}	8.62	4.22
12	*P.f.* I_{12}	8.76	2.66
13	*P.f.* I_{13}	8.69	3.44
14	*P.f.* I_{14}	8.27	2.11
15	*P.f.* I_{15}	8.69	3.44
16	*P.f.* I_{16}	8.65	3.88
17	*P.f.* I_{17}	8.78	2.44
18	*P.f.* I_{18}	8.74	2.88
19	*P.f.* I_{19}	7.41	17.66
20	*P.f.* I_{20}	8.7	3.33
21	TNAU *P.f.* 1	7.49	16.77
22	Control	9	0
SEd		**0.22**	-
CD (P=0.05)		**0.44**	-

*Mean of three replication

Effect of volatiles from *Bacillus* sp. against the growth of *R. solani*

Though all the isolates of *B. subtilis* showed varying results in mycelial growth inhibiton of *R. solani*, *B.s.* I_6 isolate was highly effective in inhibiting the mycelial growth next to the *Bacillus* check isolate (TNAU *B.s.*1) from TNAU. Fiddaman and Rossall (1993) reported that the strain of *Bacillus* produces an antibiotic metabolite which is volatile in nature and antifungal to *R. solani* **(Table 6)**.

Table 6: Activity of volatile from *Bacillus* sp. isolates against the mycelial growth of *Rhizoctonia solani* (Rs_2) under *in vitro* conditions

Sl. No	Isolates	Mycelial growth (cm)*	Per cent reduction over control (%)
1.	*B.s.*I_1	7.5	16.66
2.	*B.s.*I_2	8.81	2.11
3.	*B.s.*I_3	8.60	4.44
4.	*B.s.*I_4	8.73	3
5.	*B.s.*I_5	7.06	21.55
6.	*B.s.*I_6	6.32	29.77
7.	*B.s.*I_7	8.54	5.11
8.	*B.s.*I_8	8.98	0.22
9.	*B.s.*I_9	8.89	1.22
10.	*B.s.*I_{10}	8.77	2.55
11.	*B.s.*I_{11}	8.51	5.44
12	*B.s.*I_{12}	8.60	4.44
13	*B.s.*I_{13}	6.68	25.77
14	*B.s.*I_{14}	7.87	12.55
15	*B.s.*I_{15}	8.79	2.33
16	*B.s.*I_{16}	8.81	2.11
17	*B.s.*I_{17}	8.69	3.44
18	*B.s.*I_{18}	8.81	2.11
19	*B.s.*I_{19}	8.83	1.88
20	*B.s.*I_{20}	7.41	17.66
21	TNAU *B.s.* 1	6.18	31.33
22	Control	9	0
SEd		**0.13**	-
CD (P = 0.05)		**0.27**	-

*Mean of three replications

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