Screening of rhizosphere and phyllo plane bacterial antagonist against *Sclerotium rolfsii (Sacc.)* in Tropical sugar beet ecosystems

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Abstract : Forty four isolates of Pseudomonas and six isolates of Bacillus tested their antagonistic activity against *Sclerotium rolfsii* . Out of the 44 pseudomonad isolates, *P. fluorescens* (SBHRPF 2) was found to be best against *Sclerotium rolfsii*, since its unique capability to suppress the growth of mycelium as well as the sclerotia formation and sclerotium germinations *S. rolfsii* in vitro conditions. In filed condition BA of neem cake @ 150 kg ha^{-1}+ *P.fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) @ 2.5 kg/ha reduction of *S. rolfsii* and enhancement of plant growth. In this study, PGPR isolates SBHRPF2 may be exploited to be used as potential biocontrol agents against *S. rolfsii* in agriculture system.

IndexTerms - Rhizosphere, Pseudomonas, *Sclerotium rolfsii*, Biocontrol, Management.

I. Introduction

The pathogen *Sclerotium rolfsii* Sacc. is distributed in tropical and subtropical region of the world where high temperature prevails, and it is common in Southern united states, Central America, Africa, Australia and India. It has a very extensive host range atleast 500 species belonging to about 100 plant families and it was named differently by various authors in different states, it was called as blight in Florida, seedling blight Utter Pradesh in India and also reported that *Sclerotium rolfsii* affected sugar beet varieties up to 15-59 per cent loss and reducing root yield in different cultivar, (1) . *S. rolfsii* affected sugar beet reduced root yield by 10 – 25 percent (2) Management of *S. rolfsii*, a major soilborne plant pathogen, through the application of fungicides but has environment problem is more to alternate control for soil borne pathogens using rhizosphere. *P. fluorescens* was most effective in inhibiting the growth of S. *rolfsii* (3) Fluorescent Pseudomonas PS I and PS II gave maximum growth inhibition of (73% and 70 %) against *Sclerotium rolfsii* rhizosphere bacteria are ultimate for use as biocontrol agents as they can provide the immediate defence for plant roots against the attack by various soilborne plant pathogens [4]. Among the rhizobacteria, *Pseudomonas* spp. Are emerged as the largest and most promising group of biocontrol agents outstanding to their potential of rapid and aggressive colonization, rhizosphere abundance, catabolic versatility, and their capacity to produce a diverse group of antifungal compounds [5]. Sequences application of *P. fluorescens* at different intervals regarding maximum disease reduction, increase plant growth and yield rice against sheath blight of rice, (6). Therefore, biocontrol agents have emerged to grasp promise in disease management.

2. Materials and Methods

2.1.Isolation of the pathogen.

The pathogen was isolated from the affected portion of the diseased plants collected from different districts separately by tissue segment method on sterile Potato Dextrose Agar (PDA) medium. The infected plants were pulled out with intact root showing the presence of white mycelial mat with small round brown sclerotia near the collar region are collected and gently tapped to remove the soil and dirt particle. The Infected crowns and tuber portions of diseased plants collected from different area were cut into small pieces of 1 to 1.5cm separately using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one min. and then washed in sterile distilled water thrice and then placed in a Petriplate at equidistance onto previously poured and solidified in Petriplate containing potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 2˚C) for five days and observed for the growth of the fungus. The hyphal tips of fungi growing from the pieces were transferred aseptically to PDA slants for maintenance of the culture.

2.2.Isolation of rhizosphere micro organism from tropical sugarbeet ecosystem

Antagonistic fungi and bacteria were isolated from the rhizosphere soil, collected from different tropical sugarbeet growing areas of Tamil Nadu. The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently. Ten gram of rhizosphere soil was transferred to 100ml of sterile water taken in 250 ml Erlenmeyer flask. After

thorough shaking, the antagonist in the suspension was isolated by dilution plate method. From the final dilution of 10^3 and10^6, one ml of aliquot was pipetted out, poured in sterilized Petri dish contain PDA medium. king's B medium and nutrient agar medium gently, rotate clockwise and anti clockwise for uniform distribution and incubated at room temperature (28±2ºC) for 24 hrs colonies were viewed under UV light at 366nm. Colonies with characteristics of *Bacillus* sp. and *Pseudomonas* sp. were isolated individually and purified by streaking them on Nutrient agar medium and King's B medium. *Pseudomonas chlororaphis* (PA23) was obtained from the University of Manitoba, Canada.

Pseudomonas sp. = SBHRPF1 to SBHRPF 21, SBHPPF22 to SBHRPF30, SBDPPF31 to SBDPPF36 and SBDRPF 37 to 44 *Bacillus* sp = SBHRBS 1, SBHPBS 2, SBDRBS3, SBHPBS 4, SBHRBS 5 and BHRBS 6.

2.3.Screening of the antagonism of *Pseudomonas* sp. and *Bacillus* sp. *in vitro* against *Sclerotium rolfsii*

Effect on linear growth of *Sclerotium rolfsii*

forty five isolates of *Pseudomonas* sp. and Six isolates of *B. subtilis* were streaked in a four cm line (1 cm away from the edge of the plate) on each PDA medium. A nine mm mycelial disc of *S.rolfsii* was placed to the most distal point of the Petri dish perpendicular to the bacterial streak . The plates were incubated at room temperature for four days and mycelial growths of the pathogen and inhibition zone (cm) were measured. The Petri plate inoculated with pathogen alone in the absence of antagonist served as control and the experiment was done in triplicates. The radial growth of fungal mycelium on each plate was measured and the per cent inhibition of growth over control (absence of antagonists) was determined using the formula:

$$I = 100 (C - T) / C$$

where, I = inhibition of mycelial growth, C = growth of the pathogen in the control plate and T = growth of the pathogen in dual cultures.

2.4.INTEGRATED MANAGEMENT OF ROOT ROT OF TROPICAL SUGARBEET

The experiments were conducted in randomized black design with sufficient replication each treatment. Individual application of organic biocides and its combination with organic amendments were applied in furrow system. The details of the treatments are given below. Susceptible variety Indus, 2 seeds were sown at 60 x 20 cm spacing. All normal agronomic practices were followed with regular intervals. The antagonists' population was assessed in 30 days intervals up to 150 days after sowing as already described. In addition growth parameters like height, number of crown, girth of tuber, tuber weight, and top yield from each treatment were recorded. Each treatment was replicated three and the treatment details includes,

3. Results and discussion

Attempts were made to isolate pathogen which is responsible for tropical sugar beet root rot. The pathogens Viz., *Sclerotium rolfsii* sacc. was isolated from the diseased tubers and roots collected from different parts of Tamil Nadu, root rot pathogen *Sclerotium rolfsii* Sacc was isolated and the same was purified and the axenic culture of these isolates were maintained in PDA slants for further studies.

3.1 *In vitro* antagonistic activity of *Pseudomonas* sp. and *Bacillus* sp. against *S. rolfsii.*

3.1.1.Effect on mycelial growth of *S. rolfsii*

Among the six isolates of *Pseudomonas* sp. tested for their antagonistic activity against *S. rolfsii* by dual culture, two isolates namely *P. fluorescens* (SBHRPF2) and *P. chlororaphis* (PA 23) were found to be effective (Table 1). *P. fluorescens* (SBHRP2) significantly exerted highest per cent mycelial growth inhibition of 66.36 followed by *P. fluorescens* (SBHRPF4) and *P. chlororaphis* (PA 23) recorded 65.27 and 64.77 per cent mycelial growth inhibition over the control.

Among the isolates of *B. subtilis,* screened for antifungal activity against *S. rolfsii,* SBHRBS 1 and SBHRBS 3 recorded 50.45 and 49.09 per cent reduction of mycelial growth over control (Table1). *P. fluorescens* inhibited the maximum mycelial growth (67.22%) followed by *B. subtilis* (55.56%) and *T. viride* (45.56%) (17)

3.1.2Sclerotial number

The sclerotial production of *S. rolfsii was* also assessed in dual culture technique with nine bacterial antagonists were tested. Among the isolates screened *P. fluorescens* (SBHRPF2) allowed minimum of sclerotial production (22.69/plate) followed by SBHRPF 4 and SBHRPF 18 which recorded 34.53 sclerotia / plate as against the control (148.01/palte) Among the Bacterial antagonist *B. subtilis* (SBHRBS1) allowed maximum sclerotia production with less inhibition 63.33 per cent when compare to other *P. fluorescens* isolates. (Table 1) Degradation of sclerotium rolfsii producing oxalic acid reduced by *P.fluorescens* (7)

3.1.3.Sclerotial germination

The sclerotial germination while assessing it was less in antagonists of *P. fluorescens* (SBHRPF1) which recorded 34.65 per cent germination compare to control (100%), followed by *B. subtilis*, *P. fluorescens* isolate SBHRPF8, SBHPPF38, and SBHRPF 4 recorded 40.59, 41.58, 42.57 and 43.56 per cent germination respectively as compared 100 per cent in the control. The maximum sclerotial germination of 69.30 per cent was recorded in SBHPBS2 which accounted by only 30.70 per cent reduction (Table 1). The best bacterial antagonists *P. fluorescens* isolate (SBHRPF2) was used throughout the study as it performed better than all other strains. eleven rhizobacteria antagonistic to *S.rolfsii* including *P. fluorescens, P. putida, Streptomyces* spp. and *Bacillus* spp. which completely inhibited *S.rolfsii* under dual culture by the production of antibiotics or lytic enzymes.

Table 1. Effect of bacterial antagonists' against *S. rolfsii in vitro*

S. No	Treatments	Mycelial growth (cm)		Number of sclerotia		Sclerotial germination (%)	
		***Mycelial growth of *S. rolfsii* (cm)**	**Per cent reduction over control**	**No. of sclerotia/ plate**	**Per cent reduction over control**	**Sclerotial germination (%)**	**Per cent reduction over control**
1	*P. fluorescens* (SBHRPF2)	2.96	66.36	22.69	84.66	34.65	65.35
2	*P. fluorescens* (SBHRPF4)	3.06	65.23	34.53	76.67	43.56	56.44
3	*P. fluorescens* (SBHRPF8)	3.50	60.22	39.46	73.33	41.58	58.42
4	*P. fluorescens* (SBHRPF14)	3.50	60.22	45.38	69.33	59.4	40.60
5	*P. fluorescens* (SBHRPF18)	4.50	48.86	34.53	76.33	57.42	42.58
6	*P. fluorescens* (SBHRPF38)	3.10	64.77	38.48	74.00	42.57	57.43
7	*P. chlororaphis* (PA 23)	3.50	60.22	42.42	71.33	51.48	48.52
8	*B. subtilis* (SBHRBS1)	4.36	50.45	54.27	63.33	40.59	59.41
9	*B. subtilis* (SBHRBS2)	4.48	49.09	52.29	64.67	69.3	30.70
10	Control	8.80	-	148.01	-	100	-
	CD (P=0.05)	**0.07**		**0.92**		**1.29**	

*** mean of three replications**

3.2.Integrated disease management

3.2.1. Root rot incidence

The effect of organic biocides (Rhizosphere and organic amendments) basal application individually and in combination were assessed against root rot of tropical sugarbeet. Delivering organic biocides through basal application reduced the root rot incidence under field conditions. Seed germination in all the treatments ranged from 68.00 to 77.50 per cent. Among the treatments BA of neem cake @ 150 kg ha^{-1}+ *P.fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) @ 2.5 kg/ha recorded least root rot disease incidence of 19.71 per cent which accounted for 71.52 per cent disease reduction over control Compared by carbendazim (0.1%) recorded 72.38 and followed by , *P.fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) 70.82 per cent reduction of disease incidence respectively over control and these treatments were on par with each other (Table 2). 186 bacterial strains of different morphological types were screened for their bio control activity against *S. rolfsii* causing collar rot in betelvine (8).*P. fluorescens* and *B. subtilis* were effective against sclerotial wilt of jasmine caused by *S. rolfsii.* The sclerotial germination was effectively inhibited by the antagonists under *in vitro* condition. Soil application of talc based commercial formulation of *P. fluorescens* @ 20 g/pot and *B. subtilis* @ 25 g/pot effectively reduced wilt incidence of jasmine in pot culture experiment (9).

3.2.2.Growth parameter

In this field experiment plant growth parameter viz., Number of leaves per plant, plant height, top yield, root yield Brix per cent and cost benefit ratio were studied and the results were furnished in Table 2.

3.2.3.Number of leaves per plant

The maximum number of leaves (18.37 leaves/plant) was recorded due to basal application of neem cake @ 150 kg ha^{-1}+ *P. fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) followed by *P. fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) which recorded 17.39 leaves /plant as against control, which recorded 11.20 leaves per plant(Table 2)

3.2.4.Plant height

Regarding, plant height basal application of neem cake @ 150 kg ha^{-1}+ *P. fluorescens* (SBHRPF2) @ 2.5 kg ha^{-1} recorded maximum plant height which recorded 49.0 cm per plant followed by BA of *P. fluorescens* (SBHRPF2) @ 2.5 kg ha^{-1} and Carbendazim (0.1%)which were recorded 48.59 and 48.53 cm of plant height respectively and these were on par with each other as against control (35.21 cm /plant) (Table 2)

3.2.5. Root yield

In case of root yield basal application of neem cake @ 150 kg ha^{-1}+ *P. fluorescens* (SBHRPF2) @ 2.5 kg ha^{-1} recorded maximum root yield 58.93 t ha^{-1} followed by individual application of *P. fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) recorded 56.66 t ha^{-1} as against control (25.50 t ha^{-1}) (Table 2)

3.2.6.Top yield

During harvest the top yield was observed and the results revealed that maximum top yield (7.56 t ha^{-1}) was observed in basal application of neem cake @ 150 kg ha^{-1}+ *P. fluorescens* (SBHRPF2) @ 2.5 kg ha^{-1} followed by BA *P. fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) applied individually recorded 7.37 t ha^{-1} as against control (3.43 t ha^{-1}) (Table 2)

3.2.7.Brix meter readings

After harvest to assess the sugar content present in tuber brix meter readings was taken and the results indicated that the mean brix per cent of sugarbeet juice was influenced by various levels of treatments it ranged from 10.00 to 20.00 per cent. The highest value of brix per cent was recorded in basal application neem cake

@ 150 kg ha^{-1}+ *P. fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) @ 2.5 kg ha^{-1} which recorded 19.0 and BA *P. fluorescens* (SBHRPF2) (2.5 kg ha^{-1}) 19.0 per cent on par with each other where as lowest value (10.00 %) was recorded in control (Table 2)

Table 2. Effect of basal application of organic biocides on growth parameter of tropical sugarbeet

S. No	Treatments	Germinations	Disease incidence	Per cent reduction over control	(Biocontrol agents @ 2.5 kg/ha Organic amendments @ 150 kg/ha)							
					Number of leaves /plant	Plant height (cm)	Root weight (kg/root)	Root yield	Top yield (t/ha)	Root yield increase over control (%)	BC ratio	Brix (%)
T 1	BA of *Pseudomonas fluorescens* (SBHRPF 2)	77.50	19.71	71.52	18.37	49.00	0.98	55.52	7.37	117.72	1:3.7	19.00
T 2	BA of *Bacillus subtilis* (SBHRPF2)	70.16	23.52	66.02	15.15	41.26	0.69	39.10	6.18	53.33	1: 2.6	15.00
T 3	BA of Neem cake + *P.fluorescens* (SBHRPF 2)	76.32	20.20	70.82	17.39	48.59	1.04	58.93	7.56	131.09	1: 3.6	19.00
T 4	BA of Neem cake+ *B. subtilis* (SBHRBS1)	71.20	21.10	69.52	17.10	43.36	1.00	56.66	7.00	122.19	1: 3.5	16.00
T 5	BA of Mahua cake + P. *fluorescens* (SBHRPF 2)	75.50	21.36	68.75	17.30	47.05	0.99	56.10	7.12	120.00	1: 3.4	18.00
T 6	BA of Mahua cake + *B subtilis* (SHRBS1) @2.5 kg/ha	68.20	22.63	67.31	16.30	39.51	0.72	40.80	6.31	60.00	1: 2.5	16.00
T 7	Soil drenching of Carbendazim (0.1%)	74.50	19.12	72.38	17.50	48.53	1.00	56.66	7.50	122.19	1: 3.6	17.00
T 8	Control	68.50	69.23	-	11.20	35.21	0.45	25.50	3.43	0.00	1: 1.7	10.00
	CD (P=0.05)	0.30	2.36	-	0.32	0.73	0.03	-	-	-	-	-

Conclusion

The plant growth promoting rhizobacteria *P.fluorescens* (SBHRPF 2) is effective against *Sclerotium rolfsii* out of 44 isolates in tropical sugar beet ecosystems and also potential biocontrol agents against *S. rolfsii* which may be exploited to be used as a potential biocontrol agent against *S. rolfsii* in agriculture system. its potential to suppress *S. rolfsii* and suggest the usefulness of this super bioinoculant as a component of IDM of *S. rolfsii*. Although the growth promoting traits in field condition promote plant growth in nature, tuber yield, Sugar under natural environmental conditions. If the potential of this isolate is confirmed, it could in future be used as a component of IDM, which will help in developing cost-effective integrated biological control methods in agriculture to combat the pathogen *S. rolfsii.*

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