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Evaluation of antagonists against jasmine wilt caused by *Sclerotium rolfsii* Sacc.

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ABSTRACT: The effectiveness of four species of *Trichoderma* namely *T. viride* Pres. ex. S. F. Gray, *T. longibrachiatum* Rifai, *T. reesei* Simmons, and *T. harzianum* Rifai, *Gliocladium virens* J. H. Miller, Giddens and Foster, two bacterial antagonists namely *Pseudomonas fluorescens* Migula and *Bacillus subtilis* (Chun) Praznowski and *Saccharomyces cerevisiae* Hansen was tested against *Sclerotium rolfsii* Sacc. causing jasmine (*Jasminum sambac* L.) wilt. *P. fluorescens, B. subtilis* and *T. viride* were found effective against sclerotial wilt. The volatile metabolites produced by these antagonists were also effective against *S. rolfsii in vitro*. The sclerotial germination of *S. rolfsii* was effectively inhibited by *P. fluorescens, B. subtilis* and *T. viride*. All the antagonists tested, except *S. cerevisiae* were effective in inhibiting the production of oxalic acid. Soil application of talc based commercial formulation of *P. fluorescens* @ 20 g/pot, *B. subtilis* and *T. viride* @ 25g/pot effectively reduced the wilt disease incidence in the pot culture experiment.

KEY WORDS: *Bacillus subtilis*, jasmine wilt, oxalic acid, *Pseudomonas fluorescens, Sclerotium rolfsii*, talc based commertial formulations, *Trichoderma viride*, volatile metabolites

Jasmine (*Jasminum sambac* L.) grown commercially in various parts of the country is affected by a serious sclerotial wilt disease caused by *Sclerotium rolfsii* Sacc. It has caused complete destruction of the jasmine nurseries in the Thangachimatam, Ramanathapuram district and also affected jasmine mainfield in Sathyamangalam, Erode district and Madurai district. Chemical control of the disease is costlier, has problem of toxic residues and can completely pollute the environment. Continuous usage of a single chemical induces resistance development in the target fungus. Biological control approach is ecofriendly. Hence, various antagonists were evaluated for the management of jasmine wilt.

MATERIALS AND METHODS

Efficacy of antagonists against *S. rolfsii in vitro*

The antagonists, *viz.*, *Trichoderma viride, T. reesei, T. longibrachiatum, T. harzianum, Gliocladium virens, Pseudomonas fluorescens, Bacillus subtilis* and *Saccharomyces cerevisiae* were tested against S. *rolfsii* for their antagonistic activity in dual culture technique (Dennis and Webster, 1971a) *in vitro*. Three replications were maintained per treatment. The radial growth of the pathogen was measured and the results were expressed as percent growth reduction over control.

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Effect of volatile metabolites of antagonists on *S. rolfsii- in vitro*

The method adopted by Dennis and Webster (1971b) was followed. PDA culture discs of 9mm, diameter of the fungal antagonists were placed at the centre of the Petri-plates containing sterilized PDA medium. In case of bacterial antagonists and yeast, 48 hour old culture of *P. fluorescens, B. subtilis* and *S. cerevisiae* were streaked at the center of the Petri-plate containing King's B, Nutrient and Potato Dextrose Agar Medium, respectively and were incubated at room temperature (28±2°C). After incubation period, the lid of each Petri-plate was replaced by another bottom plate containing PDA medium inoculated with 9mm actively growing culture disc of *S. rolfsii*. The two dishes were sealed together with an adhesive tape. The lids of control plates, which had not been inoculated with antagonists, were also replaced in the same way. Three replications were maintained for each treatment. The colony diameter of the pathogen was measured on the second and fifth day after inoculation. Data were expressed in percent reduction over control.

Effect of culture filtrates of antagonists on sclerotial germination of *S. rolfsii* - *in vitro*

The filtrates of the antagonists (15 day old fungal culture filtrate and 72 hour old bacterial and yeast culture filtrate) were obtained by filtering the broth cultures through Whatman No.1 filter paper. These filtrates were again passed through Seitz filter under vacuum to prevent contamination. The method adopted by Prasad *et al.* (1999) was followed. The antibiotic potential of the antagonists were tested by placing sclerotia on sterile filter paper impregnated with culture filtrates of antagonists and that impregnated with sterile distilled water served as control. Each treatment was replicated thrice. After incubation period of 5 days, sclerotial germination was recorded and expressed in percent reduction over control.

Effect of antagonists on the production of oxalic acid by *S. rolfsii* in *vitro*

To 100ml of previously sterilized PD broth in a conical flask, 9mm PDA culture disc of actively growing virulent isolate of *S. rolfsii* was inoculated on one side (2 cm away from the edge of the flask) and on the other side 9mm PDA culture disc of the fungal antagonists, *viz., T. viride, T. reesei, T. longibrachiatum, T. harziamum* and *Gliocladium virens* were inoculated. In the case of bacterial antagonists, the cell suspensions of *P. fluorescens, B. subtilis* and yeast (*S. cerevisiae*) were prepared from actively growing 48 hour old bacterial cells and yeast cells, respectively and inoculated within the flasks containing pathogen (*S. rolfsii*) on one side. Flasks inoculated with *S. rolfsii* alone served as control. Three replications were maintained and incubated for 15 days.

After incubation the mycelial mats were harvested in all the cases, the filtrate was centrifuged at 2,100 rpm for 30 minutes and the solution was assayed for oxalic acid. Oxalic acid production was estimated as per the method suggested by Mahadevan and Sridhar (1986).

Mass multiplication of *T. viride* - talc based commercial formulation

The *T. viride* was cultured onto 250ml conical flask containing 70ml of molasses - yeast broth (molasses 30g+yeast 5g+distilled water one litre). After 15 days of incubation, the mycelial mat of the fungus was homogenized and mixed with sterilized talc powder @ 1: 2 (v/w) and shade dried for 2 days. For each kg of talc powder, 5g of carboxy methylcellulose was added as adhesive.

Mass multiplication of bacterial antagonists – talc based commercial formulation

P. fluorescens and *B. subtilis* were multiplied in King's B and NA broth, respectively for 48 hours. The pH of the talc powder (substrate) was adjusted to 7 by adding calcium carbonate @ 150g/kg. Four hundred ml of *P. fluorescens* and *B. subtilis* suspensions were added to one kg of sterilized talc powder containing 5g of carboxy methyl cellulose and mixed well.

Effect of talc based commercial formulation of antagonists on *S. rolfsii* - Pot culture experiment

The antagonists; *viz.*, *T. viride*, *P. fluorescens* and *B. subtilis* commercial formulations were applied to each pot of size 25cm diameter and 23cm height containing 7kg of soil at different dosage levels, *viz.*, 5, 10, 15, 20, 25 and 30g, 48 hours before inoculation of the pathogen. Ten gram of the inoculum of *S. rolfsii* multiplied in sorghum grains was applied to the soil containing jasmine plants with different levels of talc based commercial formulation of antagonists. The plants inoculated with pathogen alone served as control. Three replications were maintained for each treatment.

RESULTS AND DISCUSSION

Among the antagonists tested, *in vitro*, *P. fluorescens* inhibited maximum mycelial growth (67.22 %) followed by *B. subtilis* (55.56 %) and *T. viride* (45.56 %). Sclerotial production was minimum (13.67) in *T. viride* followed by *P. fluorescens* (28) and *B. subtilis* (56). But the size of the sclerotia was maximum in *T. viride* when compared to *P. fluorescens* and *B. subtilis* (Table 1). Alippi and Manoco (1994) reported that *B. subtilis*, *B. pumilus* and *B. licheniformis* inhibited the growth of *S. rolfsii* and *Rhizoctonia solani*, which were responsible for the damping off of tomato *in vitro*. Alice *et al.* (1998) suggested that *T. harzianum* followed by *T. viride* were the best antagonists in inhibiting the mycelial growth of *S. rolfsii* by 70 and 60 percent, respectively in dual plate technique. Patil *et al.* (1998) also reported that the strains of *P. fluorescens* were antagonistic to collar rot pathogen of groundnut (*S. rolfsii*) and also inhibited sclerotial germination *in vitro*.

The *in vitro* experimental results (Table 1) revealed that volatile metabolites produced by all the antagonists except yeast inhibited the mycelial growth of the pathogen. The percent inhibition varied among the antagonists. *P. fluorescens* inhibited maximum mycelial growth (66.78%) of *S. rolfsii* followed by *T. viride* (61.11%) whereas *B. subtilis* caused 47.22 percent inhibition of growth. Similar results were established by Laha *et al.* (1996) as they reported that volatile cyanogenic metabolites produced by the isolates of *P. fluorescens* suppressed the growth of *S. rolfsii* causing cotton wilt *in vitro*. Alice *et al.* (1998) reported that volatile compounds produced by *T. harzianum* were inhibitory to the growth of *S. rolfsii* causing jasmine wilt. Zeppa *et al.* (1990) identified the volatile metabolites produced by *T. viride* as lactones, alcohols and terpene derivates. Mannina *et al.* (1997) isolated new tetracyclic diterpene (C_2OH_2 $8O_2$) from culture filtrates of a strain of *T. viride* which exhibited antifungal activity against *S. rolfsii*.

The results (Table 2) revealed that the culture filtrate of *P. fluorescens* completely inhibited the sclerotial germination, whereas minimum sclerotial germination of 11.11 and 13.33 percent were observed in *B. subtilis* and *T. viride*. Elad *et al.* (1983) suggested that, b-1-3 glucanase and chitinase in culture filtrates of *Trichoderma* spp. affected germination of sclerotia of *S. rolfsii*. Laha *et al.* (1996) also reported that, sclerotial viability of *S. rolfsii* causing cotton wilt was reduced when immersed in *P. fluorescens* cell suspension or in a cell free culture filtrate.

All the antagonists tested were effective in inhibiting the oxalic acid production except yeast. *P. fluorescens* had minimum production of oxalic acid followed by *T. viride* and *B. subtilis*. Dickman and Mitra (1992) and Dickman and Chet (1998) have identified that some pseudomonads were capable of degrading the toxin oxalic acid, produced by *S. rolfsii*.

The populations of antagonists, *viz.*, *T. viride*, *P. fluorescens* and *B. subtilis* were $92x10^6$ cfu/g, $78x10^7$cfu/g and $64x10^7$cfu/g, respectively of talc at the time of application in the pot culture experiment.

The talc based commercial formulation of antagonists were applied at 5, 10, 15, 20, 25 and 30 g/pot at the time of application to pot culture experiment. The results (Table 3) revealed that, as the dosage level of the antagonists increased, the percent disease incidence decreased. At 20g/pot of *P. fluorescens* the lowest disease incidence of 25 percent was recorded and this was followed by *T.*

Table 1. Effect of different antagonists and volatile metabolites against *S. rolfsii in vitro*

Antagonist	Dual culture (5th day)				Volatile metabolites			
					2nd Day		5th Day	
	Colony diam (cm)*	Growth inhibition (%)	Sclerotial production (no.)	Size of the sclerotia (mm)	Colony diam (cm)	Growth inhibition (%)	Colony diam (cm)	Growth inhibition (%)
Trichoderma viride	4.90	45.56	13.67	1950.00	2.20	56.86	3.50	61.11
T. longibrachiatum	5.85	35.00	78.00	1269.50	2.70	47.06	4.73	47.44
T. reesei	5.75	36.11	70.00	759.00	2.60	49.02	4.85	46.11
T. harzianum	6.15	31.67	89.67	940.00	3.20	37.25	5.50	38.89
Gliocladium virens	6.72	25.33	85.33	1133.50	3.10	39.22	5.40	40.00
Bacillus subtilis	4.00	55.56	56.00	1105.30	2.57	49.61	4.75	47.22
Pseudomonas fluorescens	2.95	67.22	28.00	1210.70	1.63	68.04	2.90	66.78
Saccharomyces cerevisiae	7.32	18.67	152.67	1265.70	4.83	5.29	9.00	0.00
Control	9.00		201.00	1275.00	5.10	0.00	9.00	0.00
CD (P=0.05)	0.48		7.68	6.85	0.17		0.16	

* Mean of three replications

Table 2. Effect of antagonists on sclerotial germination and production of oxalic acid by *S. rolfsii in vitro*

Antagonist	Germination of sclerotia in culture filtrate * (%)	Inhibition of sclerotial germination (%)	Amount of oxalic acid produced (mg/ml)
Trichoderma viride	13.33 (21.41)**	86.67	0.945
T. longibrachiatum	40.00(39.23)	60.00	1.147
T. reesei	48.89(44.35)	51.11	1.181
T. harzianum	51.11(45.64)	48.89	1.282
Gliocladium virens	53.33 (46.91)	46.67	1.358
Pseudomonas fluorescens	0.00 (0.74)	100.00	0.903
Bacillus subtilis	11.11 (19.27)	88.89	1.021
Saccharomyces cerevisiae	93.33 (75.04)	6.67	1.940
Control	100.00 (86.29)	0.00	1.991
CD (P = 0.05)	3.54		0.032

* Mean of three replications

** Data in the parentheses are arcsine transformed value.

Table 3. Incidence of jasmine wilt in potted plants treated with talc based commercial formulation of antagonists

Dose of antagonist (g/pot)	Wilt incidence (%)*		
	T. viride	*P. fluorescens*	*B. subtilis*
5	66.67 (54.74)**	58.33 (49.80)	66.67 (54.74)
10	50.00 (45.00)	41.67 (40.21)	58.33 (49.80)
15	47.22 (43.40)	36.11 (36.91)	40.00 (45.00)
20	41.67 (40.21)	25.00 (30.00)	44.45 (41.80)
25	36.11 (36.91)	25.00 (30.00)	38.89 (38.56)
30	33.33 (35.26)	22.22 (28.03)	33.33 (35.26)
Control	94.45 (76.73)	94.45 (76.73)	94.45 (76.73)
CD (P=0.05)	3.70	3.93	3.70

* Mean of three replications

** Data in the parentheses are arcsine transformed values.

viride and *B. subtilis* at 25g/pot with 36.11 and 38.89 percent disease incidence, respectively as against the control, which recorded 94.45 percent disease incidence. Ganesan and Gnanamanickam (1987) reported that, in greenhouse tests, 99 percent of groundnut plants were protected from *S. rolfsii* infection, when inoculated along with *P. fluorescens*. Yang *et al.* (1990) showed that *P. fluorescens* gave 52.2 percent disease control against *S. rolfsii*. Suriyachandraselvan (1997) also reported that soil application of 2.5kg/ha of *T. viride* and *P. fluorescens* in talc based formulations to sunflower recorded 33.33 and 36.67 percent disease incidence, respectively compared to 66.67 percent charcoal rot in control. The talc based formulation of *P. fluorescens* proved to be the best. *T. viride* and *B. subtilis* can also be successfully used for the management of jasmine wilt caused by *S. rolfsii*

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