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ANTAGONISTIC ACTIVITY OF FUNGAL AND BACTERIAL BIO-INOCULANTS AGAINST *SCLEROTIUM ROLFSII*

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ABSTRACT

The present studies were undertaken to investigate the effect of fungal and bacterial biocontrol agents against stem rot of groundnut. The result of the dual culture technique indicated that *Trichoderma* isolates inhibited the growth of *S. rolfsii*. Among the isolate *T. viride* (Tv_1) produced maximum reduction of mycelial growth. This was followed by the isolates *T. harziaum* and *T. virens* which restricted the mycelial growth when compared to control. Among the isolates *T. viride* (Tv_1) at a conc. of 10, 20, 30 and 40 per cent conc. showed an increase in the inhibition of the mycelia growth recording 22.15, 15.27, 8.75 and 0.00 mm respectively. The next best in antagonist was *T. harzianum.* Among the *Pseudomonas fluorescens* isolates, PfI_3 produced maximum reduction of mycelial growth accounting for 74.97 per cent reduction over control. Also, a general increase in the conc. of the Antagonistic culture filtrate showed an increase in the inhibition of the mycelial growth of the test pathogen.

Keywords: Groundnut, Stem rot, Antagonistic agent, *Trichoderma*, *Pseudomonas fluorescens*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as peanut, earth nut, wonder nut, monkey nut, goobers, is an annual leguminous plant. It is called as king of oil seed. Today groundnut is widely distributed and is cultivated in more than eighty countries in tropical and sub tropical regions of the world (Madhusudhana, 2013). The groundnut seed is a good source of edible oil and proteins in the form of oil cake, which can also be used as an animal fodder and fertilizer. It also has some industrial uses like in paint, varnish, lubricating oil, soap, furniture polish *etc.*. (Pratibha *et al.,* 2012). Groundnut seeds are valued for oil content (40-48%) and also contain protein (22-26%), carbohydrate (26%), fat (3%), high calcium, thiamine and niacin contents, which make a substantial contribution for human and animal nutrition (Rangarani, 2017).

Groundnut crop is affected by several fungal, bacterial and viral diseases. In India among the soil-borne fungal diseases stem rot caused by *Sclerotium rolfsii* Sacc. is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. The loss of yield caused by stem rot of groundnut incited by *S. rolfsii* and reported the diverse level of yield loss ranged from 45-80 per cent (Rangarani, 2017). In Tamil Nadu, the annual yield loss of groundnut due to this disease was estimated as 10 to 50 per cent (Rakholia *et al.*, 2012). Recently, the role of fungal biocontrol agents and Plant Growth Promoting Rhizobacteria (PGPR) *viz*., *Trichoderma* spp., *Chaetomium* spp*., Pseudomonas fluorescen*s and *Bacillus subtilis* in biocontrol approaches for managing the pathogen in crop plants are well reported. Plants have latent defense mechanism against pathogens, which can be systemically activated upon exposure of plants to stress or infection by pathogens (Baker *et al*., 1997). This phenomenon is called induced systemic resistance (Tuzun and Kuc, 1991). The present studies were undertaken to investigate the effect of fungal and bacterial biocontrol agents against stem rot of groundnut.

MATERIALS AND METHODS

Screening of potential bioagents by dual culture technique

The antagonistic activity of three *Trichoderma* spp. against *S. rolfsii* was determined by dual culture technique (Johnson *et al*., 1959). Mycelial discs measuring 5 mm diameter from four days old cultures of both fungal antagonists and the test pathogen were placed at equidistant on sterile petriplate containing PDA medium. The petriplates were then incubated at 28 ± 2°C. Three replications were maintained in each treatment. Suitable controls were kept without antagonist. Growth of antagonists, pathogen and zone of inhibition were measured after recording full growth in control plate and percentage inhibition of mycelial growth of test pathogen was calculated. Antagonistic potential was determined by using parameters *viz.,* degree of inhibition or intermingled zone between both the colonies.

The percentage inhibition of radial growth was calculated by using equation

Percentage of Inhibition I = C-T/C × 100

Where C= growth of pathogen in control

T = growth of pathogen in treatment

Poisoned food technique (Solid media)

The culture filtrates of *T. viride, T. harzianum, and T. virens* isolates was incorporated into sterilized PDA medium at 10, 20, 30 and 40 per cent conc. by adding the calculated quantity of culture filtrate to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The flasks were inoculated with 9 mm mycelial disc of *S. rolfsii* collected from the periphery of seven days old culture and incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *S. rolfsii* was measured when the mycelial growth fully covered the control plates.

Poisoned food technique (Liquid media)

A similar experiment as described above was conducted with incorporation of the culture filtrates in the liquid media. The flasks were incubated for 10 to 11 days room temperature (28 ± 2°C) and filtered through whatman No.41 filter paper under vacuum. Then the bio mass as dried until attaining constant weight in an oven and the mycelial dry weight was recorded in mm.

Isolation of *Chaetomium globosum*

Chaetomium species were originally isolated by soil plate technique and baiting technique according to the method described by Soytong (1989). Soil plate technique, soil samples were dried and ground to fine particles, 0.005-0015 g of each soil sample were placed to sterilized petri dishes and then overlaid with glucose-ammonium nitrate agar (GANA) medium. After 2-7 days incubation at room temperature in the dark, *Chaetomium* spp. were observed under microscope and isolated into pure culture by single spore isolation.

***In vitro* testing of fungal antagonists**

The antagonistic activity of bio control agents against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile petri dish containing 15 ml of sterilized and solidified PDA medium a 6 mm mycelial disc obtained from one five days old culture of *Chaetomium* spp. was placed under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the *Chaetomium* culture disc, a six mm mycelial disc obtained from seven days old culture of *S. rolfsii* was placed and incubated. A control was maintained by inoculating *S. rolfsii* alone at one end of the petri dish. The plates were incubated at room temperature (28± 2°C) for seven days. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927).

Per cent inhibition (I) =C-T/C × 100

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual plate

I -Inhibition per cent

Bioassay of culture filtrates of *C. globosum*

Poisoned food technique (Solid media)

The culture filtrates of *C. globosum* isolates was incorporated into sterilized PDA medium at 10, 20, 30 and 40 per cent conc. by adding the calculated quantity of culture filtrate to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The flasks were inoculated with 9 mm mycelial disc of *S. rolfsii* collected from the periphery of seven days old culture and incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *S. rolfsii* was measured when the mycelial growth fully covered the control plates.

Poisoned food technique (Liquid media)

A similar experiment as described above was conducted with incorporation of the culture filtrates in the liquid media. The flasks were incubated for 10 to 11 days room temperature (28 ± 2°C) and filtered through whatman No.41 filter paper under vacuum. Then the bio mass as dried until attaining constant weight in an oven and the mycelial dry weight was recorded in mm.

***In vitro* testing of bacterial antagonists**

The antagonistic activity of bio control agents against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile petri dish containing 15 ml of sterilized and solidified PDA medium a 6 mm mycelial disc obtained from seven days old culture of *S. rolfsii* was placed at 1.5 cm away from the margin of the petri dish. Similarly one cm long streak was gently made onto the medium using 48 h. old bacterial isolates just opposite to pathogenic culture at equidistance

under aseptic conditions. A control was maintained by inoculating *S. rolfsii* alone at one end of the petri dish. The plates were incubated at room temperature (28± 2°C) for seven days. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927).

Per cent inhibition (I) = C-T/C × 100

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual plate

I -Inhibition per cent

Table 1. Antagonistic activity of different fungal bio-inoculants against *S. rolfsii* (Dual culture technique)

S. No.	Antago-nists	Mycelial growth (mm)	Per cent inhibition over control
1	*T. viride*	18.83 [a]	79.07
2	*T. harzia-num*	22.77 [b]	74.70
3	*T. virens*	27.84 [c]	69.06
4	*C. globo-sum*	36.16 [d]	59.82
5	Control	90.00 [e]	0.00

Bioassay of culture filtrates of the bacterial isolates on the mycelial the growth of *S. rolfsii*

were filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Table 3. Antagonistic activity of P. fluorescens isolates against *S. rolfsii* (Dual culture technique)

S. No	Native Isolates	Mycelial growth (mm)	Per cent inhibition over control
1	PfI_1	29.17 [c]	67.58
2	PfI_2	32.75 [d]	63.61
3	PfI_3	22.52 [a]	74.97
4	PfI_4	32.36 [d]	64.04
5	PfI_5	41.92 [e]	53.42
6	PfI_6	24.86 [b]	72.37
7	Control	90.00 [f]	0.00

*Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Effect of culture filtrates on the mycelial growth of *S. rolfsii*

The culture filtrates of the antagonists were separately incorporated into sterile PDA medium at 10, 20, 30 and 40 per cent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile petri dishes separately @ 15 ml and allowed to solidify. Each plate was inoculated at the centre within seven days old (6 mm) PDA culture disc of *S. rolfsii*. Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *S. rolfsii* was measured when the mycelial growth fully covered the control plates.

Table 2. Effect of culture filtrate of fungal bio-inoculant at different conc. on the mycelial growth of *S. rolfsii* (Poisoned food technique)

S.No	Antagonists	Mycelial growth (mm)				Per cent inhibition over control			
		Conc. of culture filtrate (%)				Conc. of culture filtrate (%)			
		10	20	30	40	10	20	30	40
1	*T. viride*	22.15[a]	15.27[a]	8.75[a]	0.00[a]	75.38	83.03	90.27	100
2	*T. harzianum*	27.66[b]	22.45[b]	17.15[b]	6.78[b]	69.26	75.05	80.94	92.46
3	*T. virens*	33.85[c]	25.54[c]	21.32[c]	14.46[c]	62.38	71.62	76.31	83.93
4.	*Chaetomium globosum*	55.75[d]	43.67[d]	35.71[d]	26.49[d]	38.05	51.47	60.32	72.78
5	Control	90.00[e]	90.00[e]	90.00[e]	90.00[e]	0.00	0.00	0.00	0.00

* Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Preparation of the culture filtrate of *P. fluorescens*

The effective *P. fluorescens* isolates were inoculated into Erlenmeyer flasks containing 50 ml of sterile King's B broth and Nutrient agar medium, respectively and kept on a rotary shaker at 100 rpm for 48 h. Then the cultures

RESULTS AND DISCUSSION

Antagonistic activity of different fungal bio-inoculants against *S. rolfsii* (Dual culture technique)

The result of the dual culture technique indicated that *Trichoderma* isolates inhibited the growth of test fungus significantly table 1. Among the isolate *T. viride* (Tv_1) produced maximum reduction of mycelial growth (18.83 mm) accounting for 79.07 per cent reduction over control. This was followed by the isolates *T. harziaum* and *T. virens* which restricted the mycelial growth to (22.77 mm & 27.84 mm) when compared to control (90 mm). The least growth inhibition of pathogen 59.82 per cent was exhibited by the antagonist *C. globosum*. Fouzia and seleem, (2005) reported that *T. viride* was found to inhibit *in vitro* growth *S. rolfsii* by coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae. *T. viride* may also affect the growth of pathogen either through antibiosis (or) mycoparasitism. Besides they may also produce antifungal phenolic compounds (viridian, gliotoxin and Trichodermins) (Rahel Ratnakumari *et al*., 2011) which might be responsible for the inhibition of pathogen.

Effect of culture filtrates of fungal bio-inoculant different conc. on the mycelial growth of *S. rolfsii* (Poisoned food technique)

The results presented in table 2 revealed that all the *Trichoderma* isolates significantly inhibited the growth of *S. rolfsii.* In general, increase in the conc. of the culture filtrate showed an increase in the inhibition of the mycelia growth of the test pathogen. Among the isolates *T. viride* (Tv_1) at a conc. of 10, 20, 30 and 40 per cent conc. showed an increase in the inhibition of the mycelia growth recording 22.15, 15.27, 8.75 and 0.00 mm respectively. The next best in antagonist was *T. harzianum*. Among the isolates the culture filtrate of *T. harzianum* at 40 per cent conc. was found to be effective in reducing the mycelia growth to the minimum (6.78 mm) accounting for the per cent inhibition of (92.46 %) of the pathogen over control. Reports on inhibitory effect of *Trichoderma* culture filtrate and non-volatiles on the growth of *S. rolfsii* were reported earlier by Rudresh *et al.,* (2005). Antibiotics secreted by antagonistic agents may be volatile or non-volatile and *Trichoderma* produces both the types. They exert strong inhibitory action with Isopropyl alcohol, caryophyllene, pentadecane, demadin tricodedrmin, viridepyronone. The results are in agreement which early workers (Pan *et al*., 2013; Swathi *et al*., 2015; Dwivedi and Ganesh Prasad, 2016**;** Hirpara *et al*., 2017).

Antagonistic activity of *P. fluorescens* (PfI_3) isolates against *S. rolfsii* (Dual culture technique)

The result of the dual culture technique indicated that all the isolates inhibited the growth of test fungus significantly table 3. Among the isolates, PfI_3 produced maximum reduction of mycelial growth (22.52 mm) accounting for 74.97 per cent reduction over control. This was followed by the isolate PfI_6, PfI_1, PfI_4, PfI_2 and PfI_5 recording 72.37, 67.58, 64.04, 63.61 and 53.42 per cent respectively. The isolates PfI_5 was the least effective recording 53.42 per cent inhibition over control.

Effect of culture filtrates of *P. fluorences* (PfI_3) isolates at different conc. on the mycelial growth of *S. rolfsii* (Poisoned food technique)

The result presented in table 4 revealed that all the isolates showed reduction on mycelial growth of *S. rolfsii*. Among the isolates, PfI_3 at conc. of 10, 20, 30 and 40 per cent showed an increase in the inhibition of the mycelial growth recording 17.07, 10.87, 5.84 and 2.38 mm respectively. Also, a general increase in the conc. of the culture filtrate showed an increase in the inhibition of the mycelial growth of the test pathogen. Among the isolates the culture filtrate of PfI_3 at 40 per cent conc. was found to inhibit the mycelial growth to the minimum (2.38 mm) accounting for the highest per cent inhibition (97.35%) of the pathogen over control. This was followed by PfI_6 @ 40 per cent conc. recording (95.42 % inhibition) and PfI_1 (93.41 % inhibition). The least inhibition percentage (86.28%) was found with isolate PfI_5.

The mycoparasitic potential of *Pseudomonas* spp. is

Table 4. Effect of culture filtrates of P. fluorences isolates at different concentration on the mycelial growth of *S. rolfsii* (Poisoned food technique)

S.No	Native isolates	Mycelial growth (mm)				Per cent inhibition over control			
		Conc. of culture filtrate (%)				Conc. of culture filtrate (%)			
		10	20	30	40	10	20	30	40
1	PfI_1	20.61^c	13.25^c	8.82^c	5.93^c	77.10	85.27	90.20	93.41
2	PfI_2	25.75^e	18.76^e	13.63^e	10.24^e	71.38	79.15	84.85	88.62
3	PfI_3	17.07^a	10.87^a	5.84^a	2.38^a	81.03	87.92	93.51	97.35
4.	PfI_4	23.82^d	16.87^d	11.45^d	8.17^d	73.53	82.02	87.27	90.92
5	PfI_5	27.47^f	20.76^f	15.17^f	12.34^f	69.47	76.93	83.14	86.28
6	PfI_6	19.29^b	12.81^b	7.78^b	4.12^b	78.56	85.76	91.35	95.42
7	Control	90.00^g	90.00^g	90.00^g	90.00^g	0.00	0.00	0.00	0.00

* Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

well documented (Whipps, 1997) and this phenomenon has often been used as means for *in vitro* screening of biocontrol agents (Elad *et al*., 1980). Similarly, antifungal compounds such as pseudobactin, HCN, salicylic acid and 2-hydroxy phenazine produced by fluorescent pseudomonas suppressed plant pathogenic fungi (Pandey *et al*., 2006; Reddy *et al*., 2008). The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the growth of the pathogen and *P. fluorescens* were known to produce an array of low-molecular weight metabolites some of which were potential antifungal agents (O' Dowling and O' Gara, 1994). Pastor *et al*., (2010) reported that *Pseudomonas* f.sp. *monteilii* showed highest antagonistic active against *S. rolfsii.* Similarly, Chanutsa *et al.,* (2014) reported that the culture filtrate of three bacteria completely inhibited the growth of *S. rolfsii.* Earlier workers reported that *P. fluorescens* effectively reduced mycelial growth of other pathogens (Sundaramoorthy, *et al*., 2014; Chacko and Gokulapalan, 2014; Manoj Kumar *et al*., 2014; Thaveedu, 2017).

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