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EVALUATION OF FUNGAL AND BACTERIAL ANTAGONIST AGAINST *SCLEROTIUM ROLFSII* IN GROUNDNUT

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Abstract

Groundnut or peanut (*Arachis hypogaea* L.) is one of the important economic oilseed crops of the world. Among various soil borne pathogens, *Sclerotium rolfsii* causing stem rot or southern blight is the most important pathogen causing huge losses in groundnut. Management of soil borne pathogens with chemicals was found difficult, uneconomical and harmful for the environment. Biological control offers an interesting alternative to fungicides for sustainable management of soil borne diseases. Beneficial microorganisms have gained considerable attention as an eco-friendly and cost-effective measures for the stimulation of disease resistance through induced systemic resistance (ISR) and for the promotion of growth in plants for sustainable crop production. Amongst the growth-promoting fungi, *Trichoderma* and *Pseudomonas* species are potentially the most commonly used organisms for agricultural crop improvement. The application of these species in the form of seed, root or soil treatments improves the uptake of soil nutrients by crop plants, increases soil fertility and enhances the production of growth-promoting substances and bioactive metabolites that act as biopesticides. In the present investigation, efficiency of fungal and bacterial antagonist against the mycelial growth of *S. rolfsii*. The fungal antagonistic *T. asperellum* (Ta_2) and and the bacterial antagonistic *P. flourescens* (Pf_7) recorded the maximum inhibition zone of 78.92% and 77.57% respectively.

Key words: groundnut, *Sclerotium rolfsii, Trichoderma* and *Pseudomonas.*

Introduction

Groundnut (*Arachis hypogea* L.) is commonly called peanut, goober pea goober, manila nut, pygmy nut, pignut and monkey nut (Rathnakumar, 2013), grown extensively in various parts of the country in both Kharif and Rabi/ Summer seasons. Groundnut kernel is rich source of energy (567 calories per 100g), because of its oil content (44-48%), protein content (25-36%) and contain health benefiting nutrients, minerals, antioxidants and vitamins that are essential for growth and development (Rashmi *et al*., 2017). Several soil-borne fungal diseases affecting yield of groundnut, stem rot however, is most serious and wide spread under warm temperate and humid climate (Kumar *et al*., 2013). Stem rot caused by *Sclerotium rolfsii* is a major problem in most of the states accounting for 10-11 percent yield loss (Santha Lakshmi Prasad *et al.,* 2012). The pathogen survives in the soil as resistant structures called sclerotia that are found associated with plant debris or near the soil surface remaining viable for a long period in the absence of a susceptible host, serving as primary inoculum for disease. Management of soil borne diseases by chemical means is difficult and not economical and has already proved to be harmful to the environment. Notable success on disease control through the antagonistic microorganisms has been experimented during recent years (Vineela, 2017). Different *Trichoderma* species have been recorded to produce a variety of antibiotics such as trichodermin, trichodermol, harzianum A and harzianolide which may help in decreasing ill effects of dangerous pathogens (Mishra, 2010). *Trichoderma* isolates exhibited inhibition to the mycelial growth of all pathogens. This could be due to the production of diffusible components, such as lytic enzymes or water-soluble metabolites (Anees *et al*., 2010). Ability of pseudomonads to suppress soil-borne fungal pathogens depends on their ability to produce antibiotic metabolites, such as pyoluteorin, pyrrolnitrin, phenazine-1- carboxylic acid, 2, 4-diacetylphloroglucinol (DAPG) (Troppens *et al.* 2013).

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Materials and Methods

Isolation and identification of pathogen

The pathogen was isolated from the groundnut plants

Table 1: Isolation and identification of *Sclerotium rolfsii* from major groundnut growing area of Cuddalore district.

S. No	Isol-ates	Locality	Mycelial characters		Sclerotial character		
			Colony characters	Mycelial growth (mm) (5DAI)	No. of Sclerotia (15DAI)	Colour of sclerotia	Shape of Sclerotia
1	Sr_1	Poovanur	Fluffy white mycelium	88	251	Chocolate brown	Round
2	Sr_2	Sivapuri	Cottony profused mycelium	85	204	Dark brown	Spherical
3	Sr_3	Killai	Light cottony white mycelium	80	124	Brown	Round
4	Sr_4	Palakollai	Fluffy white mycelium	83	175	Light brown	Oval
5	Sr_5	Abathanapuram	Dense cottony white mycelium	90	325	Dark brown	Round
6	Sr_6	Vadalur	Profused cottony white mycelium	89	287	Chocolate brown	Round
7	Sr_7	T.S.Pillai	Light cottony white mycelium	79	116	Brown	Oval
8	Sr_8	Kurinjipadi	Light cottony white mycelium	81	138	Dark brown	Spherical
9	Sr_9	Nadiyappattu	Dull white profused mycelium	82	149	Light brown	Round
10	Sr_{10}	Kammapuram	Profused cottony white mycelium	87	243	Dark brown	Oval

showing typical symptoms of stem rot disease by tissue segment method (Rangaswami, 1958). The infected portion of the stem was cut into small bits, surface sterilized in 0.1 percent NaOH solution for 30 sec., washed in repeated changes of sterile distilled water and plated onto PDA medium in sterilized petridishes. The plates were incubated for room temperature 28±2°C for five days and were observed the fungal growth. The fungal isolates were purified by single hyphal tip method. The purified isolates were identified as *Sclerotium rolfsii* based on morphological and colony characteristics.

Isolation of fungal antagonist

The rhizosphere soil samples were collected from various groundnut growing locations used for the isolation of *Trichoderma* species by serial dilution method using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). The culture isolate were purified by single hyphal tip method and the culture were stored in test tube slants at 4°C for further studies. Identification of antagonists strains were confined by morphological and cultural characteristics of the colonies, measurement of hyphal diameter, conidiophores and conidia dimensions (Rifaee, 1969).

Isolation of native antagonistic bacteria

For isolation of Rhizobacteria, the method followed by (Manasa *et al*., 2017) was followed. In this procedure 10g of soil from each soil sample was taken in a conical flask of 90ml water. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. 1 ml of respective dilutions were spread on sterilized petri plates containing *Pseudomonas* agar. The petri plates were incubated at room temperatures (28±2°C) for 24-72h. Two replicates were maintained for each dilution. The plates were examined daily up to 3 days for bacterial colonies.

Dual culture technique (Dennis and Webster, 1971)

To test the efficacy of antagonistic fungus, fifteen ml of sterilized melted PDA was plated in petri plates (90mm) and allowed to solidify. Mycelial discs measuring six mm diameter from five day old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile Petri plate containing PDA medium. The petri plates with pathogen inoculated at one end alone, served as control. The petri plates were then incubated at 28±2°C. Three replications were maintained in each treatment. Growth of antagonists and pathogen were measured after recording full growth of the pathogen in control plate. Percent inhibition of mycelia growth of test pathogen was calculated by the formula suggested by Pandey *et al.,* (2000).

Percent inhibition (I) = C-T/C × 100

Where,

I = Per cent inhibition in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

Results and Discussion

Isolation and identification of *Sclerotium rolfsii* from major groundnut growing areas of Cuddalore district in Tamilnadu

All the ten isolates of *Sclerotium rolfsii* grown on potato dextrose agar medium showed variation in their colony characteristics as fluffy white mycelium, profuse cottony white mycelium and dull white profuse mycelium (Table 1). The isolate Sr_5 showed dense cottony white mycelium, whereas light cottony white mycelium was observed in the isolates Sr_3, Sr_7 and Sr_8 respectively.

Karthik Pandi *et al*., (2017) have collected eight isolates of *S. rolfsii*, among these three isolates namely

Table 2: Efficacy of *Trichoderma asperellum* isolate against *Sclerotium rolfsii* (Dual Culture).

S. No	Isolate	Mycelial growth (mm) (5DAI)	Percent reduction over control (%)
1	Ta_1	31.27[f]	65.26
2	Ta_2	24.32[a]	72.98
3	Ta_3	42.79[h]	52.46
4	Ta_4	30.02[e]	66.64
5	Ta_5	26.35[b]	70.72
6	Ta_6	38.72[g]	56.98
7	Ta_7	49.03[i]	45.52
8	Ta_8	28.15[d]	68.72
9	Ta_9	57.53[j]	36.08
10	Ta_{10}	27.69[c]	69.23
11	Control	90.00	-

*Mean of three replication; *Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

SFSR2, SFSR3 and SFSR6 have produced fluffy, dull white colonies, while other isolates produced compact and dull white colonies. All isolates of *S. rolfsii* differed in their mycelia dispersion and appearance in Petri plates and showed dispersed growth all over the plate to aggregated fashion and their appearance was loose to dense cottony with sparse or fluffy mycelium (Asish Mahato and Mohan Kumar Biswas, 2017). The variation in the morphological characters observed in this study might be due to different strains of *S. rolfsii.*

Efficacy of *Trichoderma asperellum* against the *Sclerotium rolfsii* Sr_5

Trichoderma asperellum collected from different locations of groundnut growing areas of cuddalore district were tested for their antagonistic activity against *Sclerotium rolfsii* by dual culture technique (Table 2). Among the tested isolates the *T. asperellum* (Ta_2) recorded the maximum inhibition zone (72.98) followed by *T. asperellum* (Ta_5) which recorded 70.72 per cent inhibition on the mycelial growth and the isolate *T. asperellum* (Ta_9) recorded the minimum inhibition zone (36.08).

Sai *et al.,* (2010) reported *Trichoderma* isolates TG2 was significantly inhibiting the mycelial growth of *S. rolfsii* to the extent of 67.83% due to the production of antifungal phenolic compounds *viz*., viridian, gliotoxin and trichodermin. Antagonistic activity of seven different species of *Trichoderma* was exhibited with measuring the pathogen growth by dual culture technique. The radial growth of *S. rolfsii* restricted by antagonistic strains of *Trichoderma* spp. and showed significant inhibition was observed that *T. viride* showed excellent antagonistic activity against pathogen with 68% of inhibition (Amna Ali and Arshad Javaid *et al*., 2015). Seven isolates of

Table 3: Efficacy of *Trichoderma asperellum* isolate against *Sclerotium rolfsii* (Dual Culture).

S. No	Isolate	Mycelial growth (mm) (5DAI)	Percent reduction over control (%)
1	Pf_1	31.27[h]	65.26
2	Pf_2	23.48[b]	73.91
3	Pf_3	26.00[e]	71.11
4	Pf_4	34.53[j]	61.63
5	Pf_5	25.34[d]	71.84
6	Pf_6	27.76[f]	69.16
7	Pf_7	20.19[a]	77.57
8	Pf_8	33.02[i]	63.31
9	Pf_9	24.91[c]	72.32
10	Pf_{10}	29.65[g]	67.05
11	Control	90	-

*Mean of three replication; *Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

Trichoderma spp. were tested against *S. rolfsii*, the maximum percentage inhibition (30 percent) was recorded the isolate ThrG1 (Rashmi *et al*., 2017). The inhibition of mycelial growth of *S. rolfsii* might be due to the production of antifungal phenolic compounds by the *Trichoderma* isolates.

Efficacy of *Pseudomonas fluorescens* against the *Sclerotium rolfsii* Sr_5

Pseudomonas fluorescens collected from different locations of groundnut growing areas of cuddalore district were tested for their antagonistic activity against *Sclerotium rolfsii* by dual culture technique (Table 3). Among the tested isolates *P. fluorescens* (Pf_7) recorded the maximum inhibition zone (77.57) followed by *P. fluorescens* (Pf_2) which recorded 73.91 percent inhibition on the mycelial growth and the isolate *P. fluorescens* (Pf_4) recorded the minimum inhibition zone (61.63).

Chanutsa *et al.,* (2014) observed the most effective isolate of *Pseudomonas* spp. (KK11EBa-3), which can inhibited mycelial growth of *S. rolfsii* by 60% due to the production of a wide range of antifungal compounds *i.e.*, fluorescent pigments siderophores, volatile compounds and antibiotics substances. *Pseudomonas* produce chitinase and lytic enzymes which can break down polymeric compounds *viz*., chitin, proteins, cellulose and hemicelluloses to degrade the cell wall of the pathogen (Janahiraman *et al*., 2016). 134 *Pseudomonas* isolates were tested against *S. rolfsii,* four isolates *viz*., AUDP 48, AUDP 276, AUDP 15 and AUDP 237 were most effective in inhibiting *S. rolfsii* showed highest inhibition of 100 % as compared to all rhizobacteria tested (Roopa and Krishnaraj, 2017). The results revealed in this table are line with the above observations.

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