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In vitro Influence of bio-controlling agents against Sclerotium rolfsii causing stem rot sickness of groundnut (*Arachis hypogaea* L.)

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Abstract
Groundnut stem rot caused by Sclerotium rolfsii Sacc causing stem rot on more than 500 plant species of agricultural and horticultural crops throughout the world. Most of the first symptom associated with S. rolfsii are usually yellowing and wilting of leaves following stem rot infections. Biological management of the disease through antagonists is an eco-friendly approach apart from better alternative to the use of chemicals. In the present study, the nine antagonistic microorganisms were evaluated by dual culture technique for their antagonistic effect against S. rolfsii under in-vitro conditions. Maximum inhibition of mycelial growth (71.67%) was noticed in Trichoderma harzianum (Gunthakal field isolate) which was followed by T. Viride (Kadiri field) (63.33%). Least inhibition was observed in T. harzianum RDGFI isolate (31.67%). The results indicated that the application of these micro-organisms successfully decreases the stem rot incidence and also increases the growth of the groundnut plants.

Keywords: Groundnut, stem rot, sclerotium rolfsii, biological control, trichoderma

Introduction
Peanut crop is prone to many diseases viz., tikka or leaf spot disease by Cercospora, collar rot by Aspergillus, seed rot and stem rot by Sclerotium rolfsii etc. Among these, stem rot caused by Sclerotium rolfsii which is gaining importance. S. rolfsii is an economically important pathogen on numerous crops worldwide [1]. Moist weather is conductive to sclerotial germination and mycelial growth. Consequently the diseases caused by the fungus are more serious in tropical and subtropical regions than in temperate regions. The huge number of sclerotia produced by S. rolfsii and their ability to persist in the soil for several years, as well as the profuse growth rate of the fungus make it well suited facultative parasite and a pathogen of major importance throughout the world [1]. The first confirmed report of losses due to the pathogen in USA was made by Rolfs in 1892 on tomato (*Lycopersicon esculentum* Miller) in Florida [2].

The fungus survives in soil mainly as sclerotia which represent the main source of inoculums and it remains viable in soil for several months [3]. Most of the first symptom associated with S. rolfsii are usually yellowing and wilting of leaves following stem rot infections. Natural management of the disease through antagonists is an eco-friendly approach apart from better alternative to the use of chemicals. Trichoderma sp. has been reported to be potential antagonists and these gained considerable success for the control of plant diseases [4, 5, 6].

The thought of a sustainable agricultural practice and environmental defense is enhancing the need of bio-control as an alternative technique to keep away from chemical hazards on both human beings and beneficial soil microorganisms [7]. The application of bio-control agents is the key elements for sustainable agriculture. Therefore, the adoption of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining worldwide recognition. In view of the above findings the present study was carried out by some of the beneficial bio agents collected from different farmer's fields and tested against the S. rolfsii to determine their antagonistic potential *in vitro*.

Material and Methods
Isolation and identification of the pathogen
The infected specimens was cut into small bits and washed in running water. These bits was surface sterilized with 1 per cent mercuric chloride solution and then aseptically transferred to

Petri plates containing sterilized PDA medium. The plates were incubated at 27±1°C for three days. The fungal growth on fourth day, which arise through the infected tissue was taken by inoculation loop and transferred aseptically to the PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture method under aseptic conditions [8].

Proving the pathogenicity

Sterilized soil was taken in earthen pots of size 45 x 30 cm. Thirty days old culture grown on sorghum grains was mixed thoroughly with soil to get sick soil. Then apparently healthy groundnut seeds (K-6 variety) were planted in pots filled with sick soil groundnut seeds sown in pots without inoculums served as control. Soil moisture was maintained at 25 per cent moisture holding capacity of soil by adding water on weight basis throughout the period. Re-isolation was made from such affected portion of the plant tissue and compared with that of original culture.

Evaluation of bio-agents

In vitro evaluation was carried out with Ten bio-agents (Table A) were collected from different farmer fields against GNATP Sr. (groundnut Ananthapuramu (dy)/dx S. rolfsii) isolate of S. rolfsii through dual culture technique.

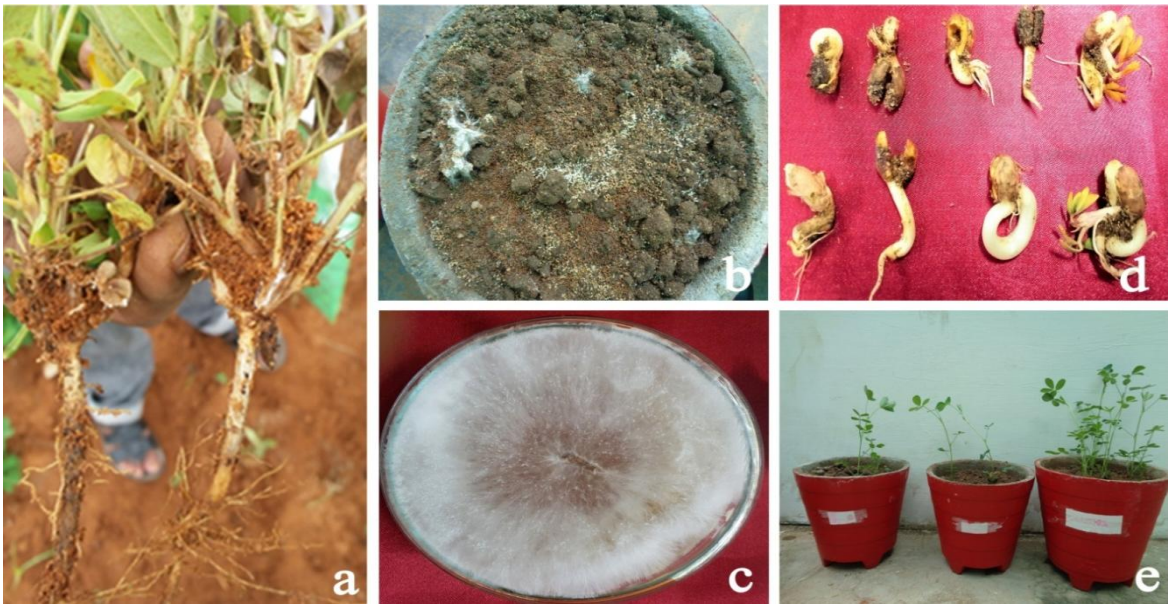
Dual culture technique

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus were inoculated at one end of the petriplate and antagonistic fungus was placed opposite to it on the other end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent [9].

I= (C-T)/CX100
Where,
I = per cent inhibition
C = growth in control
T = growth in treatment

Results and discussion

The pathogenicity test of S. rolfsii to groundnut was proved by soil inoculation method, carried out under glasshouse conditions and for this mass multiplication of the pathogen was done (Plate 1a to e) as per the procedure described in 'Material and Methods'. Control was maintained with sterilized soil. The pathogen infected first at stem region. Leaves of such infected plants became pale green followed by yellowing. During advanced stage of infection the white mycelium grew around the stem region and completely covered it. The plant gradually dried and toppled. The sclerotial bodies were formed on infected parts. The fungus was re-isolated from affected plant tissue and compared with the original culture (Plate c).



a) Infected Groundnut plant with Sclerotium rolfsii b) Sclerotium inoculated soil
c) Infected seedlings d) Pure culture of S. rolfsii e) Pot culture studies

Plate 1: Symptoms of stem rot of Groundnut

The nine antagonistic micro-organisms were evaluated by dual culture technique for their antagonistic effect against S. rolfsii under in-vitro conditions. Inhibition zone in mm was recorded and the per cent inhibition was calculated.

At 7 days after inoculation maximum inhibition of mycelial growth 71.67 per cent was observed in T. harzianum (GTLF field isolate), which was followed by T. viride (Kadiri field) 63.33 per cent and T. harzianum ATPFI 60.00 per cent. Least inhibition was recorded in T. harzianum RDGF isolate 31.67 per cent respectively (Table 1, Plate 2).

Some soil fungi isolated from the agricultural field soil were found to grow fast in dual culture with the pathogen i.e., S. rolfsii. In the present study the slow growth rate of the pathogen suggested a more rapid utilization of nutrients by the antagonists when grown together. Nutrient depletion, space and production of toxic substances (antibiotic and antibiotic like substances) by the fungi are known to play a dominant role in antagonism and these factors are usually

governed by the physico- chemical nature of the environment [10]. The present *in vitro* study results showing the positive antagonistic effect of the soil fungi which have restricted the growth of the pathogen under *in vitro* condition (i.e., Trichoderma sp.).

Number of Trichoderma species are known to be active hyper parasites of several soil fungi and hence they are used as a bio-control agent [11] (Ekefan *et al*., 2009). Control of plant diseases by the use of antagonistic microorganisms can be an effective means [12]. Various plant diseases have been successfully controlled through bacterial and fungal antagonists [13] (Cook and Baker, 1983; Campbell, 1989). Antagonistic microorganisms reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion. Antagonistic micro-organisms, such as Trichoderma, reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion [14].

Biological control is an effective, ecofriendly and alternative approach for management of any disease. Similar trend was observed when the test pathogen was placed at periphery where, maximum per cent inhibition of mycelial growth in T. harzianum (ATPFI isolate) (59.81%), followed by T. harzianum of GTLFI (57.97%) and least inhibition of mycelial growth was observed in Bacillus subtilis (10.74%). Similarly [15, 16] reported least inhibition by B. Subtilis and P. fluorescens as against higher inhibition by Trichoderma spp [17]. Screened the six Trichoderma strains among them T. viride (NBAII Tv 23) inhibited 61 per cent growth of S. rolfsii followed by T. harzianum (NBAII Th 1) 55 per cent, respectively.

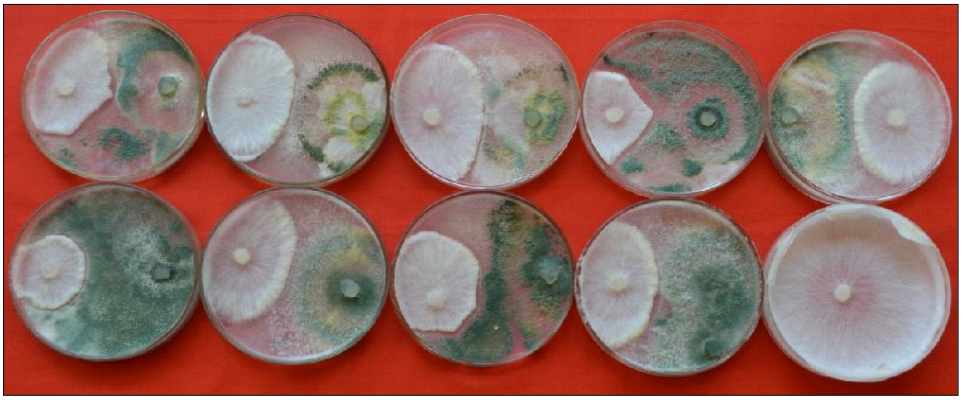


Plate 2: Mycelial inhibition by different bioagents in dual culture

1. Trichoderma harzianum (GTLFI), 2. Trichoderma viride (GTLFI), 3. Trichoderma harzianum (RDGFI), 4. Trichoderma viride (GTLFI), 5. Trichoderma asperullum (GTLFI), 6. Trichoderma harzianum (GTLFI), 7. Trichoderma harzianum (GTLTh-1), 8. Trichoderma harzianum-(GTLTh-6), 10. Trichoderma harzianum (GTLTh-16), 11. Control

Table 1: List of bio-agents used for *in vitro* evaluation a *Trichoderma harzianum*-(GTLTh-6), Against *Sclerotium rolfsii* causing stem rot of Groundnut

S. No.	Bio agents	Per cent inhibition of mycelial growth
1.	*Trichoderma harzianum (ATPFI)*	60.00
2.	. *Trichoderma viride (ATPFI)*	53.33
3.	*Trichoderma harzianum (RDGFI)*	31.67
4.	*Trichoderma viride (KDRFI)*	63.33
5.	*Trichoderma asperullum (GTLFI*	56.00
6.	*Trichoderma harzianum* (GTLTh-1)	71.67
7.	. *Trichoderma harzianum* (GTLTh-3)	50.00
8.	*Trichoderma harzianum* (GTLTh-6)	53.33
9.	*Trichoderma harzianum* (GTLTh-16)	48.00
10.	Control	0.00
	S.E.± C.D(P=0.01)	1.22 4.91

Table 2: List of bio-agents used for *in vitro* evaluation against *Sclerotium rolfsii* causing stem rot of groundnut

S. No.	Bio agents	Source / Isolate
1.	Trichoderma harzianum–(GTLFI)	GTL- Field Isolate
2.	Trichoderma viride-(KDRFI)	KDR- Field Isolat
3.	. Trichoderma harzianum	RDG- Field Isolate
4.	Trichoderma viride	GTL- Field Isolate
5.	. Trichoderma asperullum	GTL- Field Isolate
6.	. *Trichoderma harzianum-(Th-1)*	GTL- Field Isolate
7.	*Trichoderma harzianum-(Th 3)*	GTL- Field Isolate
8.	*Trichoderma harzianum-(Th-6)*	GTL- Field Isolate
9.	*Trichoderma harzianum-(Th-9)*	GTL- Field Isolate
10.	*Trichoderma harzianum*–(Th-16)	GTL- Field Isolate

Conclusion

From the *in vitro* findings, it can be recommended that the antagonists Trichoderma species. Can be used as a bio-control agent against S. rolfsii under field condition. It is also exposed that the microorganisms that naturally remain in the soil are having more or less similar potential antagonistic effect on the various crop disease caused by various pathogens. And some of them can be used as a potential bio- control agent under field condition to decrease the disease incidence and to increase crop productivity. Therefore, further work should be taken up to explore the possibility of the use of the antagonists study under field conditions.

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