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Isolation and Efficacy Evaluation of Forest Soil Microflora (FSM) Against *Sclerotium rolfsii* (Sacc.) causing Stem Rot of Groundnut under *in vitro* Conditions

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ABSTRACT: Major threat in chemical control of plant diseases is the fungicide resistance, environmental pollution and health hazards. Therefore, an ecofriendly approach such as biological control is necessary. Biological control play a powerful role for the treatment of bacterial and fungal plant diseases. Bacteria with a variety of mechanisms involved in limiting the spread of plant diseases are some of the most extensively studied biological control agents. A total of 42 bacteria and 36 fungi were isolated from the various regions of Katkur Reserve Forest and Tirumala kunta Reserve Forest under Bhadradri Kothagudem district following dilution method. The conventional dual culture technique was used to further confirm the antagonistic activity of these forest soil isolates. On the basis of the outcomes, the potential isolates were chosen. The potential isolates FSF10, FSB2, FSB4, FSB16 and FSF30 inhibited fungal growth by 76.44%, 61.12%, 63.78%, 60.76 % and 64.79% respectively, in a dual culture test. The study suggests that the FSF10 (*Trichoderma erinaceum*) has highest potential to be used as biocontrol agent against *Sclerotium rolfsii* causing groundnut stem rot.

Keywords: Antagonistics, Forest soil microbes, Groundnut stem rot, *Trichoderma erinaceum, Bacillus* spp.

INTRODUCTION

Groundnut [*Arachis hypogaea* L.], the king of oilseeds, is a member of sub- family *Papilionaceae* of the family *Fabaceae* is an edible oilseed crop that is extensively used for oil extraction, cooking, and domestic purposes. Also known as wonder nut and poor men's cashew nut, commercially it is used mainly for oil production but apart from oil, the by-products of groundnut contain many other functional compounds like proteins, fibers, polyphenols, antioxidants, vitamins, and minerals (Arya *et al*., 2016). The production and productivity of groundnut is affected by various fungal diseases, among them stem rot caused by *Sclerotium rolfsii* (Sacc.) is a major disease and causes 100% yield losses under favourable conditions (Joshi *et al*., 2020). Groundnut pod yield losses vary from 10 per cent to 25 per cent and in extensively infected crops of stem rot, they can exceed up to 80 per cent (Mehan *et al*., 1995). In groundnut, the yield losses caused by stem rot were reported upto 11% at normal condition and 10% to 25% in heavily infected fields and the losses can increase up to 80% under severe conditions (Ayyandurai *et al*., 2023).

Sclerotium rolfsii (Sacc.) is a necrotrophic, soil-borne fungal pathogen, with a wide host range, causing root rot and stem rot in tropics and subtropics regions of the world. The fungus develops white fluffy, branched, septate mycelium, with clamp connections only on the central hyphae, spreading like a fan. Sclerotia may be spherical or irregular in shape and at maturity resemble the mustard seed (Barnett and Hunter 1972). The sclerotia are initially white in color and turns light brown on maturity (Subramanian, 1964). The primary symptoms of stem rot in groundnut are browning and wilting of leaves and branches while still being attached to the plant. The fungus infects the stem preferentially by forming a whitish mycelial mat, but it can also infect any part of the plant including leaf, pod, and root. Infected plant portions develop sclerotia and yellow leaves as the infection gets worse, leading to withering and eventual death (Akgul *et al*., 2011). The quality of the pods and fodder is decreased by *S. rolfsii* infection, which also indirectly affects the dry weight and oil content of groundnut seeds (Bera *et al*., 2016a). *S. rolfsii* initiates the infection at the base of the stem and gradually turns yellow followed by the development of white mycelial threads which causes stem to rot (Pelealu *et al*., 2023). Stem rot symptoms were prevalent in areas of high moisture and high temperature. The complete wilting of plants was observed within 8-15 days of infection (Vamshi *et al*., 2023).

Chemical treatment is one of the widely practised methods for controlling the diseases. It has a number of disadvantages, including the emergence of pathogens resistant to fungicides, alteration of local ecologies, and health hazards for people. So, it's crucial to develop disease control systems that are effective and ecologically responsible.

MATERIALS AND METHODS

Isolation of Forest soil Microflora Isolates: Forest soil samples were collected from various forest locations in the Bhadradri Kothagudem District's of

Katkur Reserve Forest and Tirumala kunta Reserve Forest. Sampling was done by scraping the top 2-3 cm soil with the hand tiller (kurpie), at a depth of 15-25 cm, soil was collected randomly. A total of 23 samples were collected from different regions and the collected samples were brought to the laboratory for the isolation of soil microbes. For the isolation of fungi, dilutions of 10^{-3} and 10^{-4} were used, whereas dilutions of 10^{-5}, 10^{-6}, and 10^{-7} were utilised for the isolation of bacteria. 100µl of respective dilutions were spread onto potato dextrose agar medium (PDA) and Nutrient Agar (NA) for isolation of fungus and bacteria respectively. These media plates were incubated at 25 ± 2°C for 3–4 days, 28 ± 2°C for 24-48 hours for isolation of fungus and bacteria respectively.

Screening of forest soil isolates for their antagonistic activity against *Sclertium rolfsii* in dual plate assay: Screening of forest soil isolates was conducted to determine if they had an inhibitory impact on *S. rolfsii* growth. Using the dual culture method (Dennis and Webster, 1971), fungal and bacterial isolates were examined for their antagonistic nature towards the pathogen. A 5 mm mycelial disc of a 5 days old culture of *S. rolfsii* was placed at one end. And a loopful of bacterial pure cultures of the forest soil bacterial isolate that were 24 hours old, streaked 1 cm from the edge of PDA plates, and incubated at 25 ± 2°C. For screening fungal isolates a 5mm disc of 5 days old culture of forest fungal isolate is placed to the other end. Additionally, a control plate containing solely *S. rolfsii* was kept. Per cent inhibition of the mycelial growth of the pathogen by different test isolates was calculated using the formula given by Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100$$

I = Per cent inhibition of mycelial growth
C = Radial growth of the pathogen in control (mm)
T = Radial growth of the pathogen in treatment (mm)

RESULTS

Testing efficacy of Forest soil Bacterial isolates: Antagonistic activity of 42 isolates of forest soil bacteria against *Sclerotium rolfsii* was studied by adopting a dual culture technique. The study showed that the highest inhibition of radial growth over control was recorded with FSB2 (61.12 %), FSB4 (63.78 %), FSB16 (60.76 %) and FSB30(64.79 %) (Table 1, Fig. 2) followed by 18 isolates showing more than 40 percent of inhibition was recorded with FSB6 (42.34 %), FSB8 (45.67 %), FSB11 (51.89 %), FSB13(41.78 %), FSB18 (41.12 %), FSB24 (52.64 %), FSB26(41.67 %), FSB27 (40.56 %), FSB28 (46.67 %), FSB32 (42.23 %), FSB33 (47 %), FSB35 (41.78 %), FSB36 (41 %), FSB37 (48.89 %), FSB38(41.12 %), FSB39(40.12 %), FSB41(46.67 %) (Table 1).

Testing efficacy of Forest soil fungal isolates: Efficacy of 36 isolates of forest soil fungi tested against *Sclerotium rolfsii* results showed that the highest inhibition of radial growth over control was recorded with FSF10 (76.44 %) and 13 isolates were showing more than 40 percent of inhibition. These isolates recorded inhibition percent of FSF5 (47.12 %), FSF6 (48.34 %), FSF11 (42.67%), FSF12 (48.45%), FSF13 (60.67%), FSF14 (41.89%), FSF15 (45.02%), FSF16 (60.23%), FSF21 (59.56%), FSF22 (56.56%), FSF24 (59.00%), FSF31 (57.23%). Along with these isolates the efficacy of the available *Trichoderma viridae* is tested against *S. rolfsii* recorded with 66.5% of inhibition (Table 2, Fig. 1).

DISCUSSION

Forest soil bacterial and fungal isolates were isolated from the forest areas of bhadradri kothagudum district. Morphological and Molecular characterization study of FSF10 isolate showing highest inhibition percentage revealed that the isolate belongs to *Tricoderma erinaceum.* This isolate along with 4 potential isolates of bacteria FSB2, FSB4, FSB16, and FSB30 were used for their biological activity against *Sclerotium rolfsii* of groundnut.

Upadhyay and Mukhopadhyay (1986) discovered an isolate of *Trichoderma harzianum* directly attacked and dissolved the mycelium and sclerotia of *Sclerotium rolfsii*. Karthikeyan *et al*. (2006) examined three *Trichoderma viride* isolates; one isolate from each of *T. harzianum* and *Pseudomonas fluorescens* inhibited the growth of *Sclerotium rolfsii* (Sacc.), the cause of groundnut stem rot.

A total of 20 *T. harzianum* isolates collected from rhizosphere and rhizoplane of different crops was screened against *S. rolfsii* following dual plate culture technique. The screened isolates of *Trichoderma* showed significantly variable antagonism ranging from 65.01 to 83.06 percent reduction of radial growth of *S. rolfsii*. Among the screened antagonists, the isolate TH-18 of *T. harzianum* showed the highest 83.06 percent inhibition of radial growth of *S. rolfsii* (Bhuiyan *et al*., 2012).

Kumari *et al.* (2021) reported five rhizospheric *Bacillus* species *viz*., *B. subtilis* subsp. *subtilis* str.168, *B. siamensis* strain PDA 10, *B. amyloliquefaciens* strain 1034, *B. velezensis* strain FZB42165 and *B.atrophaeus* strain NBRC 15539 were assessed for their antagonistic potential against *S. rolfsii.* Among them, the maximum growth inhibition was (58%) exhibited by the strain NBRC 15539 of *B. atrophaeus*.

Suebrasri *et al*. (2020) reported the results of dual culture assay of four fungal isolates, Diaporthe *phaseolorum* BUP3/, *Macrophomina phaseolina* BUP2, *Daldinia eschscholtzii* 2NTYL11 and *Trichoderma erinaceum* ST-KKU2 isolated from stemona root and ginger could effectively inhibit the mycelial growth of *S. rolfsii* at 76.00, 41.20, 66.67 and 63.63%, respectively.

Karnewar *et al*. (2022) isolated the potential bioagents from the rhizospheric soil of banyan tree and tested their antagonistic potential against *S. rolfsii*. Among all the isolates *P. striata* found to be most effective showing highest inhibition zone of 74.44 per cent.

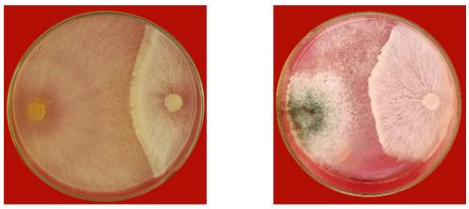

Fig. 1. Dual plate assay indicating the antagonistic activity of fungi isolates A: FSF10 (*Trichoderma erinaceum*) B: *Trichoderma viridae* (right side of the plate is *Sclerotium rolfsii*).

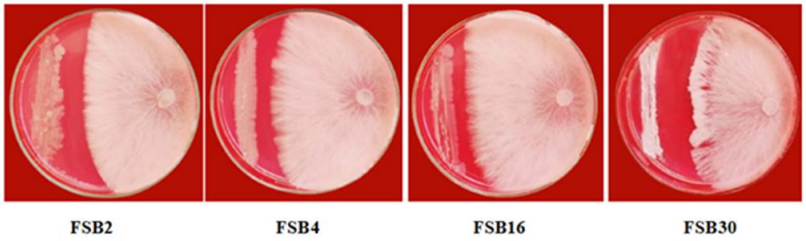

Fig. 2. Dual plate assay indicating the antagonistic activity of potential bacterial isolates.

Table 1: Antagonistic activity of Forest Soil Bacterial (FSB) isolates against *Sclerotium rolfsii* by dual culture technique.

Isolate	Radial growth of pathogen (cm)	Percent inhibition of radial growth over control
FSB1	5.53	38.56 (38.37)
FSB2	3.5	61.12(51.40)
FSB3	5.83	35.23 (36.41)
FSB4	3.26	63.78(53.00)
FSB5	6.17	31.45 (34.14)
FSB6	5.19	42.34 (40.62)
FSB7	5.87	34.78 (36.14)
FSB8	4.89	45.67(42.52)
FSB9	5.49	39(38.63)
FSB10	6.06	32.67(34.84)
FSB11	4.33	51.89(46.09)
FSB12	5.33	40.78(39.66)
FSB13	5.24	41.78(40.29)
FSB14	6.84	24(29.32)
FSB15	6.23	30.78(33.70)
FSB16	3.53	60.76(51.23)
FSB17	5.8	35.56(36.63)
FSB18	5.3	41.12(39.86)
FSB19	5.73	36.34(37.07)
FSB20	5.44	39.56(37.00)
FSB21	5.6	37.78(37.95)
FSB22	5.88	34.67(36.08)
FSB23	6.81	24.34(29.58)
FSB24	4.26	52.64(46.51)
FSB25	5.58	38(38.05)
FSB26	5.25	41.67(40.23)
FSB27	5.35	40.56(39.53)
FSB28	4.8	46.67(43.07)
FSB29	6.93	23(28.67)
FSB30	3.17	64.79(53.57)
FSB31	5.5	38.89(38.58)
FSB32	5.2	42.23(40.53)
FSB33	4.77	47(43.30)
FSB34	5.8	35.56(36.62)
FSB35	5.24	41.78(40.25)
FSB36	5.31	41(39.80)
FSB37	4.6	48.89(44.34)
FSB38	5.3	41.12(39.90)
FSB39	5.39	40.12(39.32)
FSB40	5.65	37.23(37.58)
FSB41	4.8	46.67(43.07)
FSB42	5.7	36.67(37.25)
C.D	0.360	
SE(m)	0.128	
SE(d)	0.181	
C.V	1.669	

Table 2: Antagonistic activity of Forest Soil Fungal (FSF) against *Sclerotium rolfsii.*

Isolate	Radial growth of pathogen (cm)	Percent inhibition of radial growth over control
FSF1	5.79	35.67 (36.68)
FSF2	6.64	26.23 (30.79)
FSF3	6.92	23.12 (28.75)
FSF4	5.82	35.34 (36.45)
FSF5	4.76	47.12 (43.32)
FSF6	4.65	48.34 (44.02)
FSF7	7.67	14.78 (22.59)
FSF8	7.41	17.67 (24.84)
FSF9	5.43	39.67 (39.04)
FSF10	2.12	76.44 (68.60)
FSF11	5.16	42.67 (40.81)
FSF12	4.64	48.45 (44.09)
FSF13	3.54	60.67 (51.18)
FSF14	5.23	41.89 (40.34)
FSF15	4.5	50 (45.02)
FSF16	2.68	60.23 (56.95)
FSF17	6.75	25 (30.01)
FSF18	8.1	10 (18.45)
FSF19	7.85	12.78 (21.00)
FSF20	6.61	26.56 (31.00)
FSF21	3.64	59.56 (50.49)
FSF22	3.91	56.56 (48.77)
FSF23	6.86	23.78 (29.17)
FSF24	3.69	59.00 (50.20)
FSF25	6.64	26.23 (30.81)
FSF26	6.77	24.78 (29.94)
FSF27	6.87	23.67 (29.10)
FSF28	6.55	27.23 (31.43)
FSF29	5.9	34.45 (35.95)
FSF30	5.91	34.34 (35.87)
FSF31	3.85	57.23 (49.17)
FSF32	6.85	23.89 (29.24)
FSF33	6.72	25.34 (30.23)
FSF34	5.97	33.67 (35.40)
FSF35	6.22	30.89 (33.79)
FSF36	5.92	34.23 (35.79)
Trichoderma viridae	3.43	66.5 (54.46)
C.D.	0.208	
SE(m)	0.074	
SE(d)	0.104	
C.V.	2.248	

CONCLUSIONS

In this experiment, 42 bacterial and 36 fungal cultures were isolated and screened against the groundnut stem rot causing pathogen *Sclerotium rolfsii*. through their biocontrol mechanisms. Among them 18 forest soil bacterial isolates and 13 fungal isolates were shown more than 40 % of inhibition of radial growth of pathogen. The isolates FSF10, FSB2, FSB4, FSB16, FSB30 were the potential isolates showing highest percent of inhibition with FSF10 showing maximum percent inhibition. Based on the morphological and molecular characterization the FSF10 isolate is identified as *Trichoderma erinaceaum.* So these isolates can be further screened at field level and can be used as biocontrol agents against groundnut stem rot disease as an ecofriendly control measure of disease.

FUTURE SCOPE

The isolated forest soil isolates identified in this study can be tested for plant growth promoting traits, yield parameters for commercial production.

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