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# Streptomyces is a Potential biocontrol agent for the management of Groundnut stem rot Pathogen *Sclerotiumrolfsii.*

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## ABSTRACT

*Native isolates of groundnut stem rot causal organism, SclerotiumrolfsiiSacc. were collected from major groundnut growing areas of Tamil Nadu. Selected isolates were screened, characterized and identified the virulent isolates. Several native bacterial and fungal antagonists were isolated against SclerotiumrolfsiiSacc.Two antagonistic actinomycetes isolates were found to have antagonistic effects the groundnut stem rot pathogen. Morphology and spore structure of isolated antagonists were studied under light microscopy, Biochemical test, Thin layer chromatography. and Biolog analysis. It confirmed the group of microorganisms as Streptomyces. The genus and species level of the antagonists were identified by Fatty Acids Methyl Esters (FAME) Analysis. The antagonistic activities of S. violaceusniger were found to be effective in reducing the mycelial growth, sclerotial formation, and sclerotial germination. When antagonists were combined together in various combinations with each other in pot experiments the treatment containing, seed treatment of P. fluorescens@ 5g kg$^{-1}$ +S. violaceusniger @ 5 g/kg was found to be effective in reducing the disease by 81.84 per cent over control followed by the seed treatment of S. violaceusniger @10 g/kg (75.06 per cent).This study provides a theoretical and practical explanation of an antagonist explored for control of stem rot caused by S. rolfsii.*

***Keywords:*** *Sclerotium rolfsii , Streptomyces, Groundnut stem rot, Biological control.*

## INTRODUCTION

Groundnut (*Arachishypogaea* L.) is an important oilseed crop in India and it is called as the 'king' of oilseeds. Groundnut is cultivated in about 40.12 lakh ha in 2021-22and production is 37.70 lakh tonnes and with an average yield of 931 kg/ha respectively. In spite of their important positions the national agricultural economy and the multiplicity of crops and crop-growing situations, the countries out of oilseeds are lagging far behind the requirement. Groundnut production is constrained by various factors and the major constraints include as frequent drought stress, low input use and socio economic infrastructure and higher incidence of disease and pest attacks. Though the groundnut is attacked by a number of diseases, the soil borne fungal disease, *Aspergillusniger* Van Tieghem, *Sclerotiumrolfsii*Sacc. and *Rhizoctoniabataticola*Taub have been reported to cause severe seedling mortality resulting patchy crop and reduced yield ranging from 25–40 per cent. Among soil borne pathogens*Sclerotiumrolfsii* has a wide host range, profile growth and ability to produce persistent sclerotia contributing the large economic losses. The excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution and the development of pathogen resistance to fungicides.

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Microbial antagonists are widely used for the biocontrol of fungal plant diseases due to lack of induction of pathogen resistance and reduction of chemical fungicide residues in the environment. Understanding the pathogen, developing and relay on a single antagonist become challenging and give way to explore and identifying the suitable alternate antagonist against the disease. *Streptomyces* are common inhabitants of the rhizosphere and act as beneficial microorganisms for plant growth and development. *Streptomyces* sp. RP1A-12 in managing groundnut stem rot disease caused by *S. rolfsii*under the greenhouse conditions [1]

## MATERIALS AND METHODS

Effect of biocontrol agents, organic amendments and biofertilizers on stem rot incidence on pot culture study. (Trial I)
A pot culture experiment was conducted with groundnut cv. VRI2 at Regional Research Station,TNAU, Vridhachalam. Groundnut seeds were treated with each antagonist separately and sown in the pathogen-inoculated (5 % w/w) soil in an earthen pot (15cm). Organic amendments were mixed as per the standard dosage with the soil in the pots of 15 cm diaseparately one week prior to the inoculation of the pathogen. Pathogen-alone inoculated soil served as a control. The details of the treatments are given below . Three replications were maintained for each treatment and different growth parameters *viz.* shoot length, root length, number of branches and number of nodules per plant were recorded 90 days after sowing. The population of each antagonist in the rhizosphere soil was also estimated separately at different intervals *viz*., 0,40,80 and 110 Days after sowing.

***Treatment details***

| | |
|---|---|
| 1 | ST with*P.fluroscent* (10g $kg^{-1}$) |
| 2 | ST with *T. viride* (3g $kg^{-1}$) |
| 3 | STwith *S. violaceusniger* (10g $kg^{-1}$) |
| 4 | STwith *S. exfoliatus* (10g $kg^{-1}$) |
| 5 | STwith *P.fluorescens* + *T. viride* |
| 6 | STwith *P.fluorescens* +*S.violaceusniger* |
| 7 | ST with *P.fluorescens* +*S. exfoliates* |
| 8 | ST with *S.violaceusniger* +*S. exfoliates* |
| 9 | ST with Carbendazim (0.1%) |
| 10 | Control I (pathogen uninoculated) |
| 11 | Control II (pathogen inoculated) |

## RESULTS

**Effect of antagonists and its combined application against stem rot of groundnut in pot culture**

In our studies, we identified two Streptomyces sp. *Streptomycesviolaceusniger* and *Streptomycesexfoliatus* with significant activity against *S. rolfsii* in various *in vitro* dual culture and in pot culture studies, the talc formulations of the organism and the water formulation of the crude extract were effective individually in reducing disease severity. The bio control agents *S. violaceusniger*, *S. exfoliatus*, *P.fluorescens*, and *T. viride* were taken with different combinations to find out the suitable combination to reduce the disease incidence. The pot culture experiment was carried out and the results revealed that all the treatments significantly reduced the intensity of stem rot disease caused by *S. rolfsii* (Table. 1). Treatment containing combined seed treatment of *P. fluorescens* +*S. Violaceusniger* ($T_6$) was found to be effective in reducing the disease by 87.82 per cent, followed by ($T_7$) combined seed treatment of *P. fluorescens* + *S. Exfoliates* (87.06 per cent)

The consistent reduction in disease incidence was observed from sowing to 90 days after sowing with a mean of 6.63 per-cent in the treatment ($T_6$)containing *P .fluorescens* + *S. violaceusniger* than the treatment ($T_7$) containing both *S. violaceusniger* + *S. exfoliatus* (11.09 per cent).

The efficacy of antagonists and their combined application on growth parameters was studied. Results revealed that some treatments had influenced the growth parameters of groundnut plants. The treatment ($T_6$) consisted of seed treatment of *P. fluorescens*5g $kg^{-1}$ + *S.violaceusniger* 5 g $kg^{-1}$ was found to be effective in increasing root length (19.10cm), number of nodules per plant (182.6) and number of branches per plant (9.33) (Table 2)The results of treatment $T_7$ and $T_8$ were statistically on par with $T_6$.

**Rhizosphere population of antagonists**

The amount of fungal and bacterial antagonists present in the groundnut rhizosphere was determined and presented in (Table 3) and the results revealed that the amount of *Pseudomonas* antagonist present in the groundnut rhizosphere was high $23.91x10^6$cfu/gm in the pot-treated with *P.fluorescens*@ 10g $kg^{-1}$ followed by the pot treated with *P.fluorescens*@ 5g $kg^{-1}$ + *S.violaceusniger* @ 5g $kg^{-1}$ ($22.09x10^6$cfu/gm). The high fungal antagonist population ($24.33 \times 10^3$cfu/gm) was observed in the pot treated ($T_2$) with *T. viride*(GNTV 1) @ 3g/kg. The high *Streptomyces* population ($22.49x10^5$cfu/gm)was observed in the pot treated ($T_3$) with *S.violaceusniger* @ 10g $kg^{-1}$.

## DISCUSSION

The groundnut production is constrained by various factors and the major constraints include as frequent drought stress, low input use and socio economic infrastructure and higher incidence of disease and pest attack. Though the groundnut is attacked by number of diseases, the soil borne fungal disease, stem rot caused by *Sclerotium rolfsii* is a potential threat to groundnut production and it causes yield losses over 25 % [2]. The wide host range, profile growth and ability to produce persistant sclerotia contribute the large economic losses associated with the pathogen.

Several strategies have been developed based on genetic, chemical, biological and cultural methods and are also combined in Integrated Disease Management framework. Among the various approaches, chemical fungicides are widely used to control the plant diseases in agricultural and horticultural crops. Chemical fungicides are known to leave harmful residues in the agricultural produce cause environmental pollution and other deleterious effects in the ecosystem and led to the development of resistant types in the pathogen. Indiscriminate and continuous use of chemicals also leads to biological imbalance in the agro- eco system. These inherent ill effects associated with the use of chemicals in plant disease management forced the plant pathologists all over the world to search for safer alternatives with a little or no negative impact on the environment, leads to the development of biocontrol formulations for environmentally friendly plant disease management [3]. Although the potential benefits of a single biocontrol agent application has been demonstrated in many studies, it also partially account for the inconsistent performance because, a single biocontrol agent is not likely to be active in all kinds of soil environments and all agricultural ecosystems [4]. Several approaches have been used to overcome these problems including combined application of two or more biocontrol strains to enhance the level and consistency in disease control [5]

Present study was planned to formulate a suitable groundnut stem rot management technology through isolation of location specific biocontrol agents and to investigate unexplored microorganisms for stem rot management as an alternate for existing biocontrol agents. The results of this study are discussed hereunder

Increase in the root length and shoot length of tomato, cucumber, lettuce and potato as a result of bacterization with *Pseudomonas* strain. The increase in plant growth might be associated with the secretion of auxins, gibberellins and cytokinins [6]**.** *Streptomyces* sp. produced different categories of growth-promoting compounds such as auxins, gibberellins, and cytokinins that directly influence plant growth [7]. Higher rate of germination, more vigorous growth and chlorophyll concentration in groundnut by application of growth promoting Consortium rhizobacteria [8].In microbial consortium application of RDF + seed treatment of Rhizobium + soil application of AMF + foliar spraying of PPFM and Bacillus altitudinis (FD 48) recorded the maximum plant height [9].

Similar results were also obtained by earlier workers, the combination of *P. fluorescens* and *Stenotrophomonasmaltophila* improved protection of sugar beet against *Pythium* damping off [10]. The individual PGPR strain IN937a and two PGPR strain mixtures significantly enhanced the length of the main runner compared with the non-bacterized control.

The above-said treated pots contained more rhizosphere antagonists population ($22.09 \times 10^6$cfu /g of *Pseudomonas* and $15.33 \times 10^3$cfu / g of *Trichoderma*) than the untreated control. When look at the growth parameter and rhizosphere population of antagonists, the above said treatment was given the results of

high shoot length of 38.53cm with more nodules 193.00 numbers than others [11]. The *Pseudomonas, Streptomyces* and *Trichoderma* antagonist populations were $10.08x10^6$, $17.74 x10^5$ and $10.93X10^3$cfu/g of soil respectively. first report of the additive contribution of several biocontrol mechanisms to total disease suppression is given by Guetsky [12].

The promising screened-out antagonist were combined together to have integrated disease control on *S.rolfsii*. The pot culture and field trials were conducted, and basal application of FYM @ 6.25t / ha + neem cake @ 150 kg / ha was applied commonly to all the treatments except control. Among the 13 treatments, treatment containing seed treatment of *P.fluorescens* @ 5g / kg plus *S. violaceusniger*@ 5g / kg was found to be effective in reducing the disease by 89.69 percent in the pot culture. It was observed increase in the *Streptomyces* population in soil after enrichment with organic matter [13&14].

The growth factors like root length (19.53cm), nodule numbers (182), and the number of branches (9) were high in the above said treatment in both pot culture experiments. The organic amendments acted through more than one mechanism either simultaneously or sequentially in the suppression of the disease [15]. These were also known to influence soil physical characteristics such as pore size, aeration, temperature, water retention capacity essential rapid extension of the root systems, better uptake of nutrients, retention of added nitrogen for a longer period and finally for better plant vigour for resisting the pathogen attack.

***Table 1. Effect of antagonists and their combination on stem rot incidence of groundnut plants in Pot culture (Trail 1)***

| S.No. | Treatments | Germination % | *Disease incidence (%) | | | Mean Disease incidence | Disease reductions over control (%) |
|---|---|---|---|---|---|---|---|
| | | | 30 DAS | 60 DAS | 90 DAS | | |
| 1 | ST with*P.fluorescens* (10g $kg^{-1}$) | 88.83 | 6.60 (14.88) | 13.30 (21.38) | 18.88 (25.75) | 12.92 (21.06) | 85.81 |
| 2 | ST with *T. viride* (3g $kg^{-1}$) | 94.33 | 5.50 (13.56) | 24.44 (29.62) | 13.30 (21.38) | 16.65 (24.08) | 81.72 |
| 3 | STwith *S. violaceusniger* (10g $kg^{-1}$) | 83.33 | 6.60 (14.88) | 13.30 (21.38) | 20.00 (26.56) | 13.30 (21.38) | 85.40 |
| 4 | STwith *S. exfoliatus* (10g $kg^{-1}$) | 88.83 | 6.60 (14.88) | 13.30 (21.38) | 20.00 (26.56) | 13.30 (21.38) | 85.40 |
| 5 | STwith*P.fluorescens* + *T. viride* | 88.83 | 5.50 (13.56) | 12.22 (20.44) | 18.88 (25.69) | 12.20 (20.44) | 86.60 |
| 6 | STwith*P.fluorescens* +*S.violaceusniger* | 88.83 | 0.00 (0.86) | 6.60 (14.88) | 13.30 (21.38) | 6.63 (14.92) | 87.82 |
| 7 | ST with*P.fluorescens* +*S. exfoliatus* | 77.66 | 12.22 (20.46) | 11.11 (19.47) | 16.66 (24.04) | 11.09 (19.45) | 87.06 |
| 8 | ST with *S.violaceusniger* +*S. exfoliatus* | 88.83 | 13.30 (21.36) | 13.30 ()21.38 | 36.60 (37.22) | 21.06 (27.31) | 76.88 |
| 9 | ST with Carbendazim (0.1%) | 83.33 | 12.22 (20.46) | 18.88 (25.75) | 36.60 (36.87) | 22.56 (28.35) | 75.23 |
| 10 | Control I (pathogen uninoculated) | 100.00 | 0.0 (0.86) | 0.0 (0.86) | 0.0 (0.86) | 0.0 (0.86) | 100.00 |
| 11 | Control II (pathogen inoculated) | 72.16 | 73.30 (58.88) | 100.00 (89.13) | 100.00 (89.13) | 91.1 (72.64) | |
| CD (P=0.05) | | 1.67 | 1.43 | | | - | - |

ST -Seed treatment; DAS-Days after sowing

*Mean of three replications; Figure in the parantheses are arc sine transformed values

***Table 2. Effects of antagonists and their combination on growth parameter of groundnut plant in pot culture (Trial I)***

| S.No | Treatments | *Shoot length (cm) | *Number of branches/ plant | *Root length (cm) | *Number of nodules /plant |
|---|---|---|---|---|---|
| 1 | ST with*P.fluorescens* 10g $kg^{-1}$ | 24.43 | 4.33 | 18.70 | 181.3 |
| 2 | ST with *T. viride*3g $kg^{-1}$ | 34.33 | 4.33 | 17.93 | 172.0 |
| 3 | STwith *S. violaceusniger*(10g $kg^{-1}$) | 17.33 | 7.33 | 19.90 | 164.0 |
| 4 | STwith *S. exfoliatus*(10g $kg^{-1}$) | 33.66 | 5.00 | 18.80 | 162.3 |
| 5 | STwith*P.fluorescens* + *T. viride* | 37.17 | 5.67 | 18.70 | 161.3 |
| 6 | STwith*P.fluorescens* +*S.violaceusniger* | 31.80 | 9.33 | 19.10 | 182.6 |
| 7 | ST with*P.fluorescens* +*S. exfoliatus* | 24.13 | 5.33 | 17.50 | 180.3 |
| 8 | ST with *S.violaceusniger* +*S. exfoliatus* | 26.20 | 5.67 | 21.43 | 180.0 |
| 9 | ST with Carbendazim (0.1%) | 37.80 | 5.00 | 20.60 | 165.3 |
| 10 | Control I (pathogen uninoculated) | 26.00 | 6.66 | 16.37 | 109.3 |
| 11 | Control II (pathogen inoculated) | 26.83 | 4.33 | 14.53 | 82.00 |
| CD (P=0.05) | | 1.35 | 2.09 | 1.54 | 1.57 |

ST -Seed treatment; BA- Basal application

*Mean of three replications

***Table 3. Effect of antagonists and their combined application on groundnut rhizosphere population of antagonists in pot culture (Trail I)***

| S.No. | Treatments | *Trichoderma* spp. 10 $^{3}$cfu/ gm (Days after sowing)* | | | | | *Pseudomonas* spp. 10 $^{6}$cfu/ gm (Days after sowing)* | | | | | *Sreptomyces* spp. 10 $^{5}$cfu/ gm (Days after sowing)* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 40 | 80 | 110 | Mean | 0 | 40 | 80 | 110 | Mean | 0 | 40 | 80 | 110 | Mean |
| 1 | ST with*P.fluorescens* (10g kg$^{-1}$) | 6.33 | 21.33 | 18.66 | 8.33 | 13.66 | 6.33 | 21.33 | 34.33 | 33.66 | 23.91 | 6.66 | 19.00 | 20.66 | 20.00 | 16.58 |
| 2 | ST with *T. viride* (3g kg$^{-1}$) | 5.33 | 29.33 | 34.00 | 28.66 | 24.33 | 5.66 | 15.00 | 11.00 | 06.33 | 09.49 | 7.00 | 16.33 | 18.00 | 16.66 | 14.49 |
| 3 | STwith *S. Violaceusniger* (10g kg$^{-1}$) | 6.33 | 21.33 | 13.33 | 09.30 | 12.57 | 5.33 | 15.66 | 14.00 | 12.00 | 11.74 | 8.00 | 20.33 | 31.66 | 30.00 | 22.49 |
| 4 | STwith *S. exfoliatus*(10g kg$^{-1}$) | 5.66 | 19.66 | 13.00 | 08.66 | 11.74 | 5.66 | 13.66 | 13.33 | 27.33 | 14.99 | 7.33 | 16.33 | 28.00 | 36.00 | 21.91 |
| 5 | STwith*P.fluorescens* + *T. viride* | 6.33 | 21.66 | 24.00 | 26.66 | 19.66 | 5.33 | 21.33 | 29.33 | 30.66 | 21.66 | 7.66 | 20.00 | 21.00 | 20.66 | 17.33 |
| 6 | STwith*P.fluorescens* + *S.violaceusniger* | 7.00 | 17.66 | 16.00 | 11.66 | 13.08 | 5.66 | 22.00 | 29.33 | 31.40 | 22.09 | 8.66 | 23.66 | 24.00 | 32.66 | 22.24 |
| 7 | ST with*P.fluorescens* + *S. exfoliatus* | 5.66 | 18.33 | 14.66 | 11.00 | 12.41 | 5.33 | 22.00 | 28.66 | 32.00 | 21.99 | 7.33 | 16.00 | 27.66 | 31.66 | 20.66 |
| 8 | ST with *S.violaceusniger* + *S. exfoliatus* | 6.00 | 18.66 | 10.66 | 11.00 | 11.58 | 5.33 | 13.66 | 10.33 | 07.33 | 09.16 | 7.33 | 16.00 | 27.66 | 25.33 | 19.08 |
| 9 | ST with Carbendazim (0.1%) | 5.66 | 18.33 | 14.66 | 10.00 | 12.16 | 6.33 | 16.33 | 09.66 | 23.00 | 13.83 | 7.66 | 10.00 | 10.66 | 09.00 | 09.33 |
| 10 | Control I (pathogen uninoculated) | 4.33 | 09.33 | 10.00 | 07.00 | 07.66 | 5.66 | 13.00 | 09.00 | 06.66 | 08.58 | 7.33 | 09.33 | 10.33 | 09.66 | 09.16 |
| 11 | Control II (pathogen inoculated) | 4.00 | 08.66 | 09.33 | 06.66 | 07.16 | 5.33 | 16.33 | 08.66 | 06.32 | 09.16 | 7.33 | 11.33 | 11.66 | 10.33 | 10.16 |
| | **CD (P=0.05)** | Treatment 1.16<br>DAYS 0.78<br>TX D 2.60 | | | | | Treatment 1.80<br>DAYS 1.21<br>TX D 4.03 | | | | | Treatment 1.72<br>DAYS 0.91<br>TX D 3.09 | | | | |

*Means of three replications

**Future Scope:** In this study we have identified a suitable groundnut stem rot management technology through isolation of location specific biocontrol agents for stem rot management as an alternate for existing biocontrol agents.

**Conflict of interest:** Authors have no conflict of interest

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