Original Research Article

Bio efficacy of bioagents against *Sclerotium rolfsii* – An incitant of stem rot disease in groundnut.

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ABSTRACT

Aims: An experiment was carried out to know the bio efficacy of ten bio agents against *Sclerotium rolfsii viz*, Bacillus subtilis, Pseudomonas fluorescens, Bacillus megaterium, Bacillus velenzensis,Trichoderma longibrachiatum, Trichoderma hamatum, Trichoderma harzianum, Trichoderma afroharzianum,Trichoderma asperelloides, Trichoderma asperellum
Study design: The experiment was carried out in Completely Randomised Design with three replications.
Place and Duration of Study: During 2023-2024 at Department of Plant Pathology, College of Agriculture, Shivamogga
Methodology: The research utilized 10 different bioagents sourced from the Department of Plant Pathology at the College of Agriculture in Shimoga.
Results: Statistically difference observed among the bio-agents evaluated with respect to per cent inhibition of mycelia growth of *S. rolfsii*. Among the fungal bioagents, the highest per cent inhibition of mycelial growth was observed in *T. longibrachiatum* (55.43%). Among bacterial bio-agents, *Bacillus subtilis* showed maximum inhibition (42.35 %).
Conclusion: This study indicates that fungal bioagents are more effective than bacterial bioagents in controlling the growth of pathogen.

Keywords: Groundnut, Stem rot, Sclerotium rolfsii, Bioagents, Trichoderma

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1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a versatile and economically significant legume crop cultivated globally in tropical, sub-tropical and warm temperate climates. Commonly known as peanuts, groundnuts are highly valued for their nutritional richness serving as a source of protein, healthy fats and various essential nutrients [1]

This resilient crop adapts to diverse climates making it staple in many agricultural systems. With a wide range of applications from culinary uses to industrial products like oils, groundnut cultivation plays a crucial role in ensuring global food security and economic sustainability. Groundnut kernels contain 48-50 per cent of edible oil and 26–28 per cent protein and are rich in dietary fibre, minerals and vitamins [2].

Among various diseases of groundnut, stem rot disease caused by *Sclerotium rolfsii* Sacc, is a serious and takes heavy toll of the economic produce. The disease is commonly referred by other names as southern blight, *Sclerotium* blight, white mould, *Sclerotium* wilt, *Sclerotium* rot, root rot and foot rot. The fungus is distributed throughout the world and is particularly prevalent in warm climates.

A more environmentally friendly method than using chemicals is to manage the disease naturally with antagonists. According to reports, Trichoderma sp. may be antagonistic, and this has proven to be rather effective in controlling plant diseases [4,5].The idea of environmentally friendly farming methods and sustainable defense against chemical risks is making bio-control an alternative method that protects both humans and beneficial soil microorganisms. Usage of bio-control agents is one of the main components of sustainable agriculture [6-8]. As a result, adopting ecologically friendly, sustainable farming practices that rely less on chemical fungicides is becoming more and more well-known on a global scale. Given the aforementioned results, the current investigation employed a few beneficial bio agents that were gathered from various farmer's fields and evaluated for their ability to impede *S. rolfsii in vitro.*

2. MATERIAL AND METHODS

2.1 Isolation and identification of the pathogen

The infected samples were cut into small bits of 4-5 mm and washed in running water. These bits were surface sterilized with 1 per cent sodium hypochlorite solution for one minute and then washed thoroughly with sterile distilled water for three times to remove the traces of sodium hypochlorite if any and then aseptically transferred to Petri plates containing the sterilized PDA medium. The plates were incubated at 27 ± 1 ºC for three days. The fungal growth on fourth day, which arose through the infected tissue was taken by inoculation loop and transferred aseptically to Petri plates containing the sterilized PDA medium. The pure culture of the fungus was maintained by further growing the culture and following hyphal tip culture under aseptic conditions.

2.2 Proving the pathogenicity

To confirm the pathogenicity test, pure culture of the *S. rolfsii* was used as an initial inoculxums and pathogenecity was proved by artificial inoculation of groundnut plant with the pathogen in pot culture experiment. Giant culture grown on sand sorghum meal medium was mixed with sterilized soil at twenty grams per pot. Plants in pots without inoculum served as control. Soil moisture was maintained by adding water throughout the period. After inoculation the plants showing the typical wilting symptoms were observed after 14 days of

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inoculation. Reisolation was made from such affected portion of the stem and compared with that of original culture for confirming the pathogenicity

2.3 Evaluation of bio-agents

In vitro evaluation was carried out with ten bioagents by dual culture technique

2.4 Dual culture technique

Bioagents were evaluated for their efficacy through dual culture technique. Twenty ml of sterilized potato dextrose agar was poured into sterile Petri plate and allowed to solidify. For evaluation of fungal bio control agents, mycelial disc of test fungus was inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at the one end of the Petri plates and mycelial disc of the test fungus was placed on another end of the plate. The plates were incubated at 27 ± 1 °C and zone of inhibition was recorded by measuring the radial growth of the test fungus and antagonistic organism. The colony diameter of the test fungus in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula given by Vincent (1947)[3]

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

Where, I = Per cent inhibition,

C = Radial growth of the pathogen in control plates

T = Radial growth of the pathogen in treated plates.

3. RESULTS AND DISCUSSION

The pathogenicity test of *S. rolfsii* on groundnut was proved by soil inoculation method with giant culture, carried out under glass house conditions as per the procedure described in 'Material and Methods'. Control was maintained without adding any inoculum. Pathogen infected first at stem region then leaves became pale green followed by yellowing. During advanced stage of infection, the white mycelium grew around the collar region of stem and completely covered it. The base of the stem was constricted and turned brown. The plant gradually dried and toppled. The sclerotial bodies were formed on infected stem. The fungus was reisolated from affected plant tissue and compared with the original culture, thus proving the pathogenicity following Koch's postulates (Plate 1).

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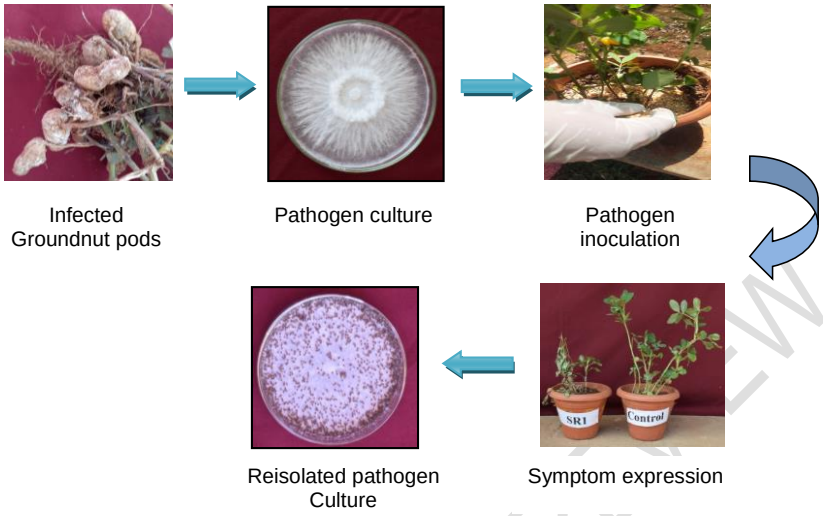

Plate 1: Proving Koch's postulates of *Sclerotium rolfsii*

The competitive ability of fungal bioagents *viz., T. harzianum, T. asperellum T. asperelloides, T. longibrachiatum T. hamatum* and *T. afroharzianum* and bacterial bioagents *viz*., *Pseudomonas fluorescens*, *Bacillus velenzensis, B. subtilis*, and *B. megaterium* were evaluated by dual culture technique against *S. rolfsii* under *in vitro* conditions as described in 'Material and Methods' (Plate 2).

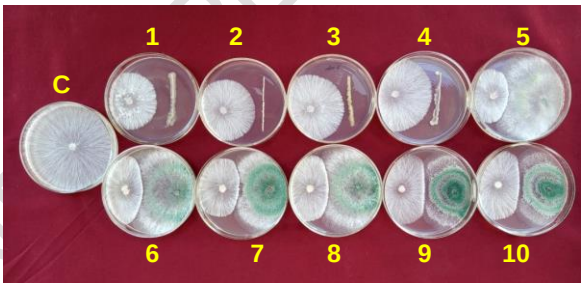

Plate 2: *In vitro* evaluation of bioagents against *Sclerotium rolfsii*

Statistically difference observed among the bio-agents evaluated with respect to per cent inhibition of mycelia growth of *S. rolfsii*. Among the fungal bioagents, the highest per cent inhibition of mycelial growth was observed in *T. longibrachiatum* (55.43%) and found to be statistically superior when compared to other bioagents, followed by *T. afroharzianum* (52.35 %), *T. hamatum* (41.85 %), *Trichoderma harzianum* (40.99 %), *Trichoderma asperelloides* (36.79 %) and *Trichoderma asperellum* (35.68 %) respectively.

Table 1: *In vitro* evaluation of bioagents against *S. rolfsii.*

Sl. No.	Bioagents	Per cent mycelial inhibition
1.	*Bacillus subtilis*	42.35[#] (40.58)[*]
2.	*Pseudomonas fluorescens*	40.74 (39.65)
3.	*Bacillus megaterium*	37.41 (37.68)
4.	*Bacillus velenzensis*	34.57 (35.99)
5.	*Trichoderma longibrachiatum*	55.43 (48.10)
6.	*Trichoderma hamatum*	41.85 (40.29)
7.	*Trichoderma harzianum*	40.99 (39.79)
8.	*Trichoderma afroharzianum*	52.35 (46.33)
9.	*Trichoderma asperelloides*	36.79 (37.33)
10.	*Trichoderma asperellum*	35.68 (36.65)
	S. Em±	0.70
	CD @ 1%	2.00

Mean of three replications
*Figures in parenthesis are arcsine transformed values

Among bacterial bio-agents, *Bacillus subtilis* showed maximum inhibition (42.35 %) which was followed by *Pseudomonas fluorescens* (40.74 %), *Bacillus megaterium* (37.41 %) and *Bacillus velenzensis* (34.57 %) respectively.

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4. CONCLUSION

Based on the *in vitro* results, it is suggested that Trichoderma species be used as antagonists. Under field conditions, it can be employed as a bio-control agent against S. rolfsii. Additionally, it has been shown that the microorganisms that live in the soil naturally have a somewhat comparable capacity to act as an antagonist against different crop diseases brought on by different pathogens. Additionally, some of them have the potential to be employed in the field as a biocontrol agent to lower the incidence of disease and boost agricultural output. Consequently, more research should be done to determine whether the antagonists study may be applied in real-world settings.

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REFERENCES

1.Chandrakala J, Pushpavathi, B., Sudini, H., Triveni, S. and Supriya, K., Changing scenario of groundnut stem and collar rot diseases in major groundnut growing areas of telangana. Int. J. Econ. Plants.2024;11: 166-179.

2.Leona G, Sudhakar R, Uma Devi G. and Uma Maheswari T, 2020. Management of stem rot of groundnut caused by *Sclerotium rolfsii* Sacc with actinomycetes. Int J Curr Microbiol Appl Sci. 2020; 9(12): 3587-3601.

3.Vincent JM, Distribution of fungal hyphae in the presence of certain inhibitors. Nature.1947;159: 850.

4.Pandav SM, Kadam JJ, Zagde PM, Joshi MS, Karande RA, Gurav NP. In vitro bio-efficacy of fungicides and bio-agents against Sclerotium rolfsii Sacc. causing collar rot disease in gerbera. Journal of Plant Disease Sciences. 2013;8(2):215-7.

5.Nagamma G, Nagaraja A, Konda S. In vitro Bio-Efficacy of Bio-Agents Against Sclerotium rolfsii Sacc. Causing Stem Rot Disease in Groundnut. Trends in Biosciences. 2015;8(10):2504-8.

6.Prasad G, Dwivedi SK. Efficacy of some bioagents against Sclerotium rolfsii Sacc. Causing root-rot in Zea mays L. Crop. European J. Biomedical & Pharmaceu. Sci. 2017;4(7):478-81.

7.Prasad G, Dwivedi SK. Efficacy of Bioagents, Plant extracts and chemical fungicides against root rot disease in Zea mays caused by Sclerotium rolfsii. Asia Pacific Journal of Multidisciplinary Research. 2020 Feb;8(1).

8. Parvin R, Aminuzzaman FM, Islam MS, Hasan N, Begum K, Roni MZ. In vitro efficacy of some fungicides, plant extracts and bio-agents against sclerotium rolfsii and control of foot and root rot disease of betel vine. Int. J. Bus. Soc. Sci. Res. 2016;4(3):211-20.

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