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Efficacy of Different Bioagents against Collar Rot Disease of Chickpea Incited by *Sclerotium rolfsii* under *In vitro* Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sclerotium rolfsii is a destructive soil-borne pathogen causes over 500 spp. in 100 families of agricultural and horticultural crops. Collar rot disease of chickpea caused by pathogen *Sclerotium rolfsii* Sacc. First symptom associated with *S. rolfsii* are usually girdling or rotting at basal or collar region of the stem resulting yellowing and wilting of entire plant. In the current study, the seven microorganisms were evaluated by dual culture technique for their antagonistic effect against *S.*

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rolfsii under *In-vitro* conditions. Maximum mycelial growth inhibition (80.97%) was noticed in *Trichoderma harzianum* which was followed by *Pseudomonas fluorescens* (74.04%). Least inhibition was observed in *T. virens* (45.53%). The results showed that the application of these bioagents were successfully decreases the disease incidence in chickpea.

Keywords: Chickpea; sclerotium rolfsii; collar rot; biological control; Trichoderma.

1. INTRODUCTION

Sclerotium rolfsii (Sacc.) is a polyphagous, omni pathogenic and facultative parasite [1]. This fungus mostly infects the lower stem part near the soil line but it also infects any part of a susceptible plant when favorable environmental conditions exist. The initial symptom is wilt. Wilted plants frequently weaken and die as a result of widespread lower stem rot [2]. This disease causes losses at any stage of crop growth. Yield loss is typically 25%, but it can reach 80-90% under wet weather conditions [3]. Whitish cottony mycelium is seen around the infected seedlings. When host plants are unavailable, the pathogen (*S. rolfsii*) can persist as a saprophyte on plant debris, including debris from non-host crops. It then creates the overwintering structures known as sclerotia; it serves as primary source of inoculum for disease development [4]. *Sclerotium rolfsii* is a Deuteromycete. It does not produce sexual spores and survived as sclerotia on plant debris and in soil [5]. In recent years, farmers applying efficient fungicides can effectively manage pathogens, but this approach cannot be regarded as a long-term solution due to concerns about exposure of carcinogenic risks, human health and environmental hazards, residue persistence, pathogen resistance emergence, contamination of food and ground water, and more production cost. Thus, the need for alternative approach to manage the pathogen has become vital. Therefore, a great deal of focus has been placed on the advantageous rhizosphere antagonistic microorganisms (fungal and bacterial biocontrol agents) in the management of soilborne diseases [6]. The biocontrol approaches include antibiosis, competition, mycoparasitism, the formation of volatile compounds and enzymes that break down cell walls and most is important induced resistance. Keeping these facts in mind, the main objectives of the present study was to suppress the growth of test fungus by using different bioagents under *in vitro* conditions and could be used for further exploitation under field condition for management of *S. rolfsii*.

2. MATERIALS AND METHODS

To evaluate the mycelial growth inhibition of test fungus and antagonistic properties of seven biocontrol agents (fungal and bacterial) under *in vitro* condition was carried out in the plant pathology laboratory of MPKV, Rahuri.

2.1 *In vitro* Evaluation of Biocontrol Agents Against Pathogen by Dual Culture Technique

The efficacy of seven antagonistic bio-agents *viz., Trichoderma virens*, *T. harzianum*, *T. Koningii*, *T. viride*, *Pseudomonas fluorescens*, *Bacillus cereus* and *Bacillus subtilis* were evaluated against *S. rolfsii* for mycelial growth inhibition on the PDA medium under *in vitro* condition. Dual culture technique was used. Single colony of the isolate was sub cultured in PDA medium and stored in refrigerator to maintain its genetic purity. For this study, fifteen to twenty ml of PDA medium was poured into a sterile Petri dish and allowed to solidify. Five mm diameter of disc from an actively growing colony of pathogen was cut with a sterile cork borer. Placed this disc near the periphery of the Petri plate containing PDA. Similarly, the bioagent was placed on the opposite side, i.e., at an angle of 180˚. Plates with no bioagents which served as control.

The plates were incubated at 28 ± 1°C for 7 days. Each treatment was replicated thrice.

The per cent inhibition of mycelial growth of pathogens was calculated using the formula given by Vincent [7].

$$I = C\text{-}T/C \times 100,$$

Where,

I = Per cent inhibition
C = Radial growth of test fungus in control Petri plate
T = Radial growth of test fungus in treated Petri plate

2.2 *In vitro* Efficacy of Fungal and Bacterial Antagonists Against *S. rolfsii*

In this experiment, the efficacy of seven antagonistic bio-agents (fungal and bacterial) were tested against *S. rolfsii* for radial growth inhibition on the PDA media using through dual culture technique under *in vitro* conditions and the results obtained are presented under the following heads with relevant discussion.

3. RESULTS AND DISCUSSION

In vitro studies on the antagonistic activities of seven bioagents against *S. rolfsii* indicated that there is a significant variations in the per cent inhibition of mycelial growth of *S. rolfsii* by all the bioagents tested. The effects of different *Trichoderma* spp., *P. fluorescens*, *B. subtilis* and *B. cereus* were tested against *S. rolfsii* by the dual culture technique.

The data from Table 1 and Plate 1 presented the mycelial inhibition (%) of test fungus by bioagents. Among the bioagents tested, *Trichoderma harzianum* was found to be significantly superior in inhibited the mycelial growth of pathogen which is 80.97% which was followed by *P. fluorescens* (74.04%), *B. subtilis* (64.85%), *B. cereus* (61.93%), *T. viride* (60.19%) and *T. koningii* (52.89%). Whereas, *T. virens* recorded the least inhibition which is 45.53%.

These results were conformity with the observations of Prasad *et al*. [8] tested the potentiality of twenty-four fungal and twelve bacterial bioagents against *S. rolfsii*. They found that the fungal antagonist *T. harzianum* and the bacterial bioagent *P. fluorescens* (Pf-3) considerably inhibited the mycelial growth of pathogen *S. rolfsii* under *in vitro* conditions. Similar results were reported by Nagamma and Nagaraja [9]- who found that *T. viride* (microbiology lab isolate) exhibited the highest inhibition of mycelial growth (63.33%), followed by *T. harzianum* (Bacteriology lab isolate) at 71.67%. The *T. harzianum* GKVK isolate showed the least inhibition (31.67%). Parmar *et al.* [10] evaluated six *Trichoderma* strains, *T. viride* (NBAIITv 23) suppressed the growth of *S. rolfsii* by 61%, followed by *T. harzianum* (NBAII Th 1) at 55%. They found that, the fungal antagonist *T. harzianum*-1 and bacterial bio-agents *P. fluorescens* (Pf-3) significantly inhibited the mycelial growth of *S. rolfsii* under *in vitro* conditions. These findings also support the results reported by Kumar *et al.* [11] observed that the effect of biocontrol agents on radial mycelial growth of collar rot of in chickpea caused by *S. rolfsii*. they showed that *T. harzianum* exhibited 85.55% inhibition followed by *P. fluorescens* which is 72.22% in collar rot of chickpea. Nandeesha and Ravindra [12] tested the four antagonistic biocontrol agents for their inhibitory effect on mycelial growth of *S. rolfsii* causing wilt disease. Among the biocontrol agents, *T. harzianum* showed maximum antagonistic effect and found to be significantly superior in inhibited the mycelial growth of *S. rolfsii* (62.64%). Nathawat and Mahendra [13] reported results where *Trichoderma harzianum* (Navsari) showed maximum growth inhibition (81.40 %) followed by *T. viride* (S. K. Nagar) as 77.40 % of collar rot pathogen.

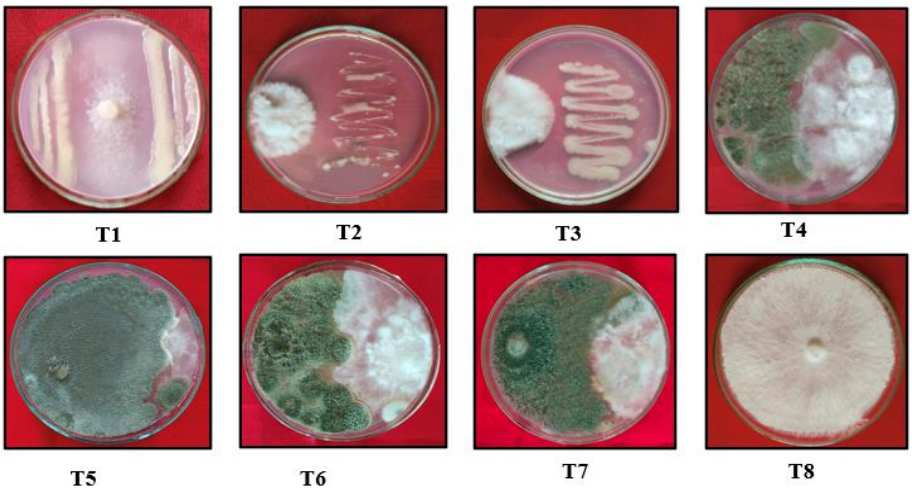

Plate 1. *In vitro* efficacy of bio agents against *S. rolfsii*

Table 1. *In vitro* efficacy of bioagents against *S. rolfsii* by dual culture technique

Tr. No.	Treatment	Growth inhibition (%)
T_1	*Pseudomonas fluorescens*	74.04 (59.37) *
T_2	*Bacillus subtilis*	64.85 (53.64)
T_3	*Bacillus cereus*	61.93 (51.90)
T_4	*Trichoderma virens*	45.53 (42.44)
T_5	*Trichoderma harzianum*	80.97 (64.14)
T_6	*Trichoderma koningii*	52.89 (46.67)
T_7	*Trichoderma viride*	60.19 (50.88)
T_8	Control	-
	S.E (m±)	0.62
	C.D at 1 %	1.86

**Figures in parentheses are angular transformed values*

4. CONCLUSION

Biological control is alternative to chemical management of plant disease. Farmers consider the bioagents to be more palatable and safer for the environment. Among the different bioagents tested, *T. harzianum* recorded the highest mycelial growth inhibition of the test pathogen. From this it can be concluded that *Trichoderma* is potential antagonist for the bio-control management of the disease if effective isolates could be obtained as it has shown both the inhibitory effect to the pathogen in *in vivo* and *in vitro* conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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