Efficacy of Ddifferent Bbio aAgents aAgainst Collar rRot dDisease of Chickpea iIncited Bby *Sclerotium rolfsii* Uunder *iIn Vvitro* cConditions

Abstract

Sclerotium rolfsii is a devastating soil borne pathogen causing more than 500 spp. in 100 families of agricultural and horticultural crops. Collar rotCollar rots disease of chickpea caused by *Sclerotium rolfsii* Sacc. Most of the 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 present study, the seven antagonistic microorganisms were evaluated by dual culture technique for their antagonistic effect against *S. rolfsii* under *in-vitro* conditions. Maximum inhibition of mycelial growth (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 indicated that the application of these bioagents were successfully decreases the collar rot incidence.

Keywords: Chickpea, Collar rRot, *Sclerotium rolfsii*, Bbiological cControl, *Trichoderma*

1. INTRODUCTION

Sclerotium rolfsii (Sacc.) is a polyphagous, ubiquitous, omni pathogenic and facultative parasite (Aycock,1966, 1966). This fungus mostly infects the lower stem part near the soil line but it also may infect any part of a susceptible plant as long as favorable environmental conditions exist. The first symptom is usually wiltwilted. Wilted plants decline and die rapidly as a result of rotting at basal part of stem. It causes severe damage to any growth stage of crop. Whitish cottony mycelium is seen around the infected seedlings.

The fungus can overwinter as mycelium in infected tissues or plant debris or as sclerotia near soil surface or buried in soil which serve as a major source of primary inoculum.

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 risks, health and environmental hazards, residue persistence, pathogen resistance emergence, contamination of food and ground water and development of carcinogenic risks and more so greater production cost. Thus, the need for alternative approach to manage the pathogen has become vital. Therefore, use of beneficial rhizosphere antagonistic microflora (fungal and bacterial biocontrol agents) in the management of soilborne diseases (Weller,1988, 1988). The mechanisms of biocontrol may antibiosis, competition,

mycoparasitism, production of cell wall degrading enzymes and volatile compounds and most important being 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 assess the growth inhibition of test fungus and antagonistic properties of seven biocontrol agents (fungal and bacterial) under *in vitro* experiment was carried out in the plant pathology laboratory of MPKV, Rahuri.

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 tested against *S. rolfsii* for radial growth inhibition on the PDA media by using dual culture technique under *in vitro* condition. Single colony of the isolate was sub- cultured in PDA and stored in refrigerator to maintain its genetic purity. For this study, twenty ml of PDA medium was poured into a sterile Petri plate and allowed to solidify. A 5 mm diameter of disc from an actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of the PDA plate. Similarly, the bioagent was placed on the other side, i.e., at an angle of 180˚. Plates with no antagonists served as control. The plates were incubated at 28 ± 1°C for 7 days. Each treatment was replicated three times. The per cent inhibition of mycelial growth of pathogens was calculated using the formula given by Vincent (1927).

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

Where,

I = Per cent inhibition

C = Radial growth of test fungus in control plate

T = Radial growth of test fungus in treated plate

3. RESULTS AND DISCUSSION

Efficacy of fungal and bacterial antagonists against *S. rolfsii* under *in vitro*

~~conditions~~Conditions:

In vitro studies on the antagonistic activities of seven bioagents against *S. rolfsii* revealed that there is a significant difference 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 evaluated 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 inhibiting the mycelial growth of pathogen 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%) . ~~W~~whereas, *T. virens* recorded the least inhibition which is 45.53%.

Table 1. *In vitro* Eefficacy 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	0.00 (0.00)
	SE m±	**0.62**
	C.D at 1 %	**1.86**

*Figures in parentheses are angular transformed values

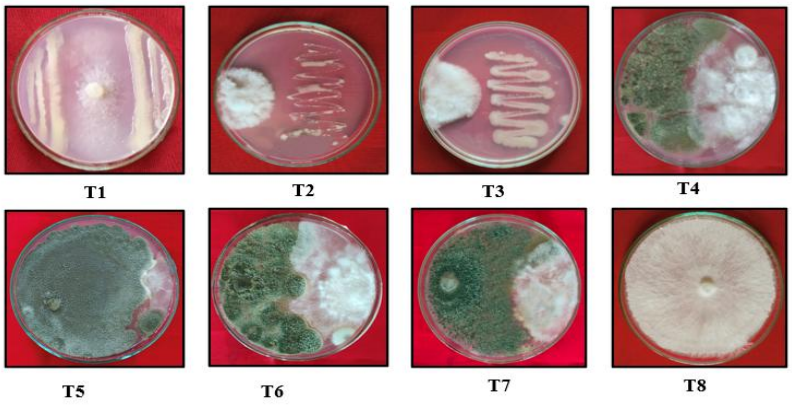

Plate 1 : *In vitro* evaluation of bio agents against *S. rolfsii*

These results were conformity with the observations of Prasad *et al*. (2017) evaluated 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) significantly inhibited the mycelial growth of *S. rolfsii* under *in vitro* conditions. Similar findings were reported by Nagamma and Nagaraja (2015) observed maximum inhibition of mycelial growth (71.67 %) in *T. harzianum* (Bacterial lab isolate), which was followed by *T. viride* (Microbial lab isolate) (63.33%). The least inhibition was observed in the *T. harzianum* GKVK isolate (31.67%). Parmar *et al.* (2015) screened the six *Trichoderma* spp., among ~~them,~~them; *T. viride* (NBAIITv 23) inhibited 61 per cent growth of *S. rolfsii*, followed by *T. harzianum* (NBAII Th 1), 55 per cent, respectively. Nathawat and Mahendra (2014) reported similar results where *Trichoderma harzianum* (Navsari) showed significantly maximum growth inhibition (81.40 %) followed by *T. viride* (S. K. Nagar) as 77.40 % growth inhibition of collar rot fungus.

4. CONCLUSION

Biological control is alternative to chemical management of plant disease. The bioagents are ecologically safe and culturally more acceptable among the farmers. 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.

5. REFERENCES

Aycock, R.1966. Stem rot and other diseases caused by *S. rolfsii*. Tech. Bull. No.174. *Agric. Expt*. Station, North Carolina State University, Raleigh~~:202~~: 202.

Nagamma, G. and Nagaraja, A. 2015. Efficacy of biocontrol agents against *Sclerotium rolfsii* causing collar rot disease of chickpea, under *in vitro* conditions. *Int. J. of Plant Prot.*, **8** (2):222-227.

Nathawat B.D.S. and Mahendra P. 2014 Evaluation of fungicides, botanical and *Trichoderma* spp. against collar ~~rot~~rots of Groundnut (*Arachis hypogaea* L.) ~~caused~~Caused by *Aspergillus niger* van Tiegham. *Annals of Pl. Prote.Sci*., 22(2):382-385.

Parmar, H. J., Mohamed M.H., Bodar, N.P., Umrania, V.V., Patel, S.V. and Lakhani, H. N. 2015. *In vitro* antagonism between phytopathogenic fungi *Sclerotium rolfsii* and *Trichoderma* strains. *Int. J. Appl. Sci. Biotechnol.*, **3** (1):16-19.

Prasad, M. R., Sagar, B. V., Devi, G. U. and Koteswarrao, S. R. 2017. *In vitro* evaluation of fungicides and biocontrol agents against damping off disease caused by *Sclerotium rolfsii* on tomato. *Int. J. Pure App. Biosci.*, **5** (4): 1247-1257.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159:850.

Weller, D.M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annual Rev. of Phytopath.*, **26**: 379-407.