*Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 25, No. (4) : 2023 : 648-650*

***ISSN-0972-3005***
**DOI No.: http://doi.org/10.53550/AJMBES.2023.v25i04.007**

# EFFICACY OF BIO-CONTROL AGENTS AGAINST *SCLEROTIUM ROLFSII* CAUSING COLLAR ROT DISEASE OF CHICKPEA, UNDER *IN VITRO* CONDITIONS

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(Received 16 May, 2023; Accepted 3 July, 2023)

***Key words:*** *Chickpea, Trichoderma, Pseudomonas, Inhibition and biological control.*

**Abstract–** Chickpea is the most important pulse crop widely grown in India, accounts for nearly 75 per cent of the total pulse production in the world. Chickpea crop is prone to many diseases. Among these, collar rot caused by *Sclerotium rolfsii* is one of the destructive soil-borne diseases of fungal origin and which is gaining importance elsewhere has been recently observed in and around different parts of country is a serious hazard. *S. rolfsii* survives in mycelium in the infected tissues and plant debris and as sclerotial structures in the soil or associated with plant debris and usually attacks the collar region of plants. Biological control of the disease through antagonists is an eco-friendly approach apart from superior alternative to the use of chemicals. In the present study, the four antagonistic micro-organisms were evaluated by dual culture and paper disc technique for their antagonistic effect against *S. rolfsii* under *in-vitro* conditions. Maximum inhibition of mycelial growth (78.61%) was noticed in *T. viride* which was followed by *T. harzianum* (75.28%). Least inhibition was observed in *P. fluorescence* (68.89%).

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop that belongs to the family *Leguminaceae* and is grown in tropical, subtropical, and temperate regions of the world. It occupies the third position among important grain legumes after the common bean and pea (Anwar *et al*., 2009). Chickpea is the most important pulse crop broadly grown in India and accounts for almost 75 percent of the total pulse production in the world (Keote *et al*., 2019). The chickpea crop is attacked by a total of 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes, and mycoplasma) from around the world (Nene *et al*., 1996).

Among them, only a few have the potential to destroy crops. Some of the severe diseases in order of importance are dry root rot (*Rhizoctonia bataticola*), wilt (*Fusarium oxysporum* f. sp. *ciceri*), collar rot (*Sclerotium rolfsii*), Ascochyta blight (*Ascochyta rabiei), and* wet root rot (*Rhizoctonia solani*). Among these, collar rot caused by *Sclerotium rolfsii* Sacc. is one of the devastating soil-borne diseases of fungal origin (Maurya *et al*., 2008), which is gaining importance elsewhere and has been recently observed in and around different parts of the country as a serious hazard. *S. rolfsii* is a soil-borne pathogen generally found in tropical and sub-tropical regions of the world (Sumi *et al*., 2018). *S. rolfsii* survives in mycelium in the infected tissues and plant debris, as sclerotial structures in the soil or associated with plant debris, and usually attacks the collar region of plants. Because of its high competitive saprophytic survival capacity, *S. rolfsii* has become more widespread in recent years in agricultural areas where sudden rainfall increases soil moisture for more prolonged periods combined with warm temperatures. With the availability of such an extensive range of natural hosts, *S. rolfsii* could even survive in dry climatic regions and persist in the soil for prolonged periods after several crop rotations. Lack of adequate information about the factors affecting collar rot development has made its control rather difficult (Tarafdar *et al*., 2018).

Biological management of the disease through antagonists is an eco-friendly approach that is a better alternative to the use of chemicals. Among the soil microorganisms, there are forms that inhibit the

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growth of other microbes; these are called antagonists (Campbel, 1989; Morang *et al.,* 2013). *Trichoderma* sp. has been reported to be a potential antagonist, and these have gained considerable success for the control of plant diseases (Denis and Webster, 1971; Duta, 1981; Upadhya and Mukhyopadhayay, 1986).

The application of biocontrol agents is one of the key elements of sustainable agriculture. Therefore, the adoption of sustainable agricultural practices using strategies that are environmentally friendly and less dependent on agricultural chemicals is gaining worldwide recognition. In view of the above findings, the present study was carried out using some of the beneficial bioagents collected from the institute and tested against *S. rolfsii* to determine their antagonistic potential *in vitro.*

## MATERIALS AND METHOD

### Collection, isolation and identification of the pathogen

Collection and isolation of pathogen Chickpea plants showing typical symptoms of collar rot were brought to the laboratory, and the pathogen was isolated by the tissue isolation method under aseptic conditions. The pure culture of the pathogen was subcultured by the hyphal tip method and maintained on Potato Dextrose Agar (PDA) medium. The pathogen was identified by its morphological characteristics. Various *Trichoderma* spp., *viz., Trichoderma harzianum*, *T. viride*, *T. asperellum,* and *P. fluorescens,* which were available in the Department of Plant Pathology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur, were screened to test their efficacy against *S. rolfsii*.

The colony and sclerotial morphology were the principal characters considered for the identification of pure culture isolates of *Sclerotium rolfsii*.

### Dual culture technique

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For the evaluation of fungal bio-control agents, mycelial discs of test fungus were inoculated at one end of the Petri plate and antagonistic fungus was placed opposite it on the other end. The plates were incubated at 25±1°C and the zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and the antagonistic organism. The colony diameter of pathogen in control plate was also recorded. *In-vitro* efficacy of different bio-control agents' *viz., T. viride, T. asperellum* and *T. harzianum* will be evaluated *in-vitro* using the "Dual culture" technique and *P. fluorescens* using the paper disc method. Percent inhibition of mycelial growth in treated plates will be calculated by applying the formula (Vincent, 1947).

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

Where, I = Per cent inhibition,
C = Control,
T = Treatment

## RESULTS

Three fungal and one bacterial antagonist were tested using dual culture and paper disc inoculation methods, respectively, on Potato Dextrose Agar medium. The study results depicted in Table 1 and Figure 1 indicated that all the four tested bioagents significantly inhibited the mycelial growth of *S. rolfsii*, and the per cent inhibition varied from 68.89

**Table 1.** *In vitro* mycelial growth inhibition of *S. rolfsii* by bio-control agents

| T. No. | Treatment name | Mycelial growth (mm) | Growth inhibition (%) |
|---|---|---|---|
| 1 | *Trichoderma viride* | 19.25(26.01) | 78.61 |
| 2 | *Trichoderma asperellum* | 25.25(30.15) | 71.94 |
| 3 | *Trichoderma harzianum* | 22.25(28.01) | 75.28 |
| 4 | *Pseudomonas fluorescence* | 28.00(31.93) | 68.89 |
| 5 | Control | 90.00(71.54) | 0.00 |
| | SE(m) | 0.66 | |
| | CD at 5% | 1.99 | |
| | CV (%) | 3.58 | |

* Figures in parentheses are angular transformed values

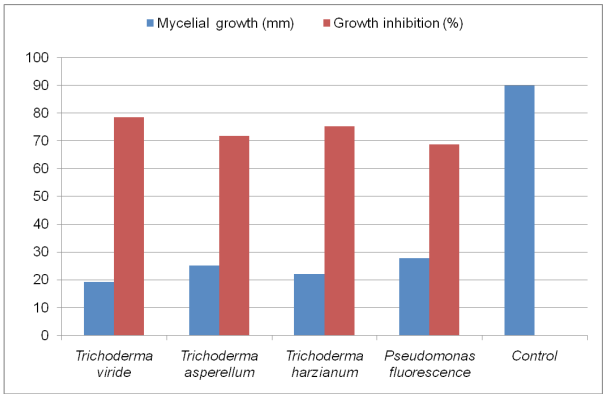


**Fig. 1.** *In vitro* mycelial growth inhibition of *S. rolfsii* by bio-control agents

to 78.61. Among fungal and bacterial antagonists, the maximum inhibition of mycelial growth was observed in *T. viride* isolate with 78.61 per cent, followed by *T. harzianum* with 75.28 per cent, which was statistically similar. Two other antagonists' *viz*., *T. asperellum* (71.94%) significantly inhibited mycelia growth, and the minimum inhibition was recorded in *P. fluorescens with* 68.89 per cent. Whereas minimum mycelium growth was found in *T. viride* 19.25 mm, followed by *T. harzianum* 22.25 mm, *T. asperellum* 25.25 mm, and maximum mycelium growth was found in *P. fluorescens* 28.00 mm.

## DISCUSSION

Similar observations on mycelial growth inhibition of *S. rolfsii* pathogenic to chickpea and certain other hosts using different antagonists, *viz*., *Trichoderma* spp. and *P. fluorescens,* were observed by several workers. Singh *et al*. (2022) found a similar result when, under *in-vitro* condition, four species of *Trichoderma* were tested against *S. rolfsii*. Minimum radial growth of the *S. rolfsii* was recorded in the *T. viride* (20.40 mm), 4 days after inoculation, with maximum growth inhibition of 54.67 per cent, superior among all *Trichoderma* spp. Tested, which was followed by *T. harzianum* with radial growth (22.20 mm) and inhibition 50.67 per cent. The same finding was made by Archana *et al*. (2018) for the treatment *Trichoderma viride* and *T. harzianum* which showed inhibition of 65.55 per cent and 64.44 per cent and colony diameters 2.2 cm and 2.3 cm, respectively. Ali *et al*. (2015) gave the same findings *T. viride* showed the best antagonistic performance approximately followed by *T. harzianum* causing 68 per cent and 57 per cent reduction in pathogen growth.

## CONCLUSION

*In vitro* efficacy of different bio-control agents was evaluated against *S. rolfsii*. *Trichoderma viride* was found to be the best among tested plant bio-control agents, respectively. From the *in vitro* findings, it can be suggested that the antagonists *Trichoderma species* can be used as a bio-control agent against *S. rolfsii* under field conditions.

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