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Research Paper

Efficacy of biocontrol-agents and plant extracts against *Sclerotinia sclerotiorum* causing stem rot of chickpea

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ABSTRACT

The present study was conducted to evaluate biocontrol-agents, including *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* and plant extracts, Ginger, Garlic, Tulsi, Lemon grass and Neem against *Sclerotinia sclerotiorum* (ON831560.1) under *in vitro* condition. All the biocontrol-agents suppressed the mycelial growth of *S. sclerotiorum.* Maximum suppression (69.29%) was observed with *T. harzianum*, followed by *T. viride*, which was on par with each other. The *Pseudomonas fluorescens* was observed to have minimum suppression (61.51%) but more than the control. Among the plant extracts maximum percent inhibition of the pathogenic fungus was observed in treatment with Neem leaf extract (42.58%), followed by garlic clove extract, ginger rhizome extract, and lemon grass leaf, while the minimum per cent inhibition was observed in Tulsi leaf extract (12.30%).

Key words: Bio-agent, Chickpea, Management, Plant extract, *Sclerotinia sclerotiorum*.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop grown all over the world (San *et al*. 2022). It is an important pulse crop grown and consumed all over the world, especially in Afro-Asian countries. Chickpea seed is a good source of carbohydrates (52.40 to 70.90%), protein (12.40 to 30.60%), and minerals like Ca, Fe, Mg, K, P, Zn, and Cu. It also has vitamin A and B-carotene (Khalifa *et al.* 2013). All of the important amino acids are present in significant quantities in chickpeas. India contributes more than 70 percent of the total world production of chickpea, and it is grown over 98.5 lakh hectares with a production of 119.9 lakh metric tons and a productivity of 1217 kg/hectare (Anonymous 2021), but the cultivation of chickpea in recent years has been greatly hampered due to biotic and abiotic stresses. The crop is suffering from various fungal, bacterial, and viral diseases throughout the growing season. Besides Ascochyta blight and Fusarium wilt, *Sclerotinia sclerotiorum* causes stem rot or white mold and has been found to cause considerable economic losses to the crop (Sheshma *et al.* 2022). As a legume, it plays a significant role in the nutrition of the rural and urban poor of the developing world by providing protein-rich supplements to cereal-based diets (Buhariwalla *et al*. 2005). The productivity of chickpeas is not at the level of satisfaction. The current work sought to explore alternative natural compounds for biocontrol assessment by analyzing the inhibitory efficacy of several prospective biofungicide sources, such as certain plant extracts and BCAs, against *Sclerotinia sclerotiorum*.

MATERIALS AND METHODS

The present investigation was conducted in the Laboratory of the Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya 224229 (UP). Different potential isolates of bio-agents namely, *Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* were collected from the Division of Crop Protection, ICAR-Indian Institute of Pulse Research, Kanpur, India, where it was maintained at controlled (–20°C) condition.

Efficacy of biocontrol agents against S. sclerotiorum

To test the efficacy of three bio-control agents, namely, *T. viride*, *T. harzianum,* and *P. florescence,* against *S. sclerotiorum* in terms of percent mycelium inhibition, we used the dual culture technique (Morton and Stroube 1955). A mycelial disc of 5 mm diameter was taken from the actively grown fungal mycelium of each bio-control agent using a sterile cork borer and placed at the periphery of the Petri plate containing PDA (2%). Similarly, a mycelium disc of *S. sclerotiorum* was also placed on the periphery of the medium, exactly opposite the bio-control agent, and incubated at 27±1°C. Petri plates were inoculated with only *S. sclerotiorum* as the control. The experiment was set up in a completely randomized design (CRD) with four replications for each treatment. The diameter of the colonies of respective bio-control agents and *S. sclerotiorum* was measured at 3 days after incubation. The percent inhibition of mycelial growth was calculated using the formula.

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

Where,

I= Percent growth inhibition,

C = Colony diameter of pathogen in control,

T = Colony diameter/radial growth of pathogen in treatment.

Efficacy of plant extracts against S. sclerotiorum

A hundred grams of each washed plant part were ground in mortar and pestle by adding an equal amount (100 ml) of sterilized distilled water (1:1 W/V) and boiled at 80°C for 10 min in a hot water bath. The ground material was filtered through muslin cloth followed by filtering through sterilized Whatman No. 1 filter paper and treated as standard 100 percent plant extract and required concentrations of five percent of each plant extract were prepared. The 90 ml sterilized Potato dextrose agar medium mixed with 10 ml plant extract (10%) and 95 ml sterilized Potato dextrose agar medium mixed with 5 ml (5%) of plant extract contained in 150 ml conical flask and sterilized in an autoclave at 1.1 kg pressure/sq. cm for 5 minutes (Nene and Thapliyal 1982). Then sterilized media was poured into three sterilized Petri plates and after solidification 5 ml disc of the 2-day-old culture of the pathogen was transferred into the centre of Petri dishes and incubated at 27±1°C temperature. PDA not amended with leaf extract served as control. The experiment was set up in a completely randomized design (CRD) with four replications for each treatment. Observation of radial growth of the test fungus was recorded after 3 days of incubation.

The radial growth of fungal mycelium was recorded 7 days after inoculation and percent growth was calculated by the following formula

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

Where,

C = Radial growth in controls

T = Radial growth in treatments.

The data recorded on radial growth were statistically analyzed using completely randomized block design (Web Agri Stat Package 1.0).

RESULTS AND DISCUSSION

Evaluation of biocontrol agents against S. sclerotiorum by dual culture assay

Bio-agents, including *T. viride*, *T. harzianum,* and *P. fluorescens,* were evaluated for their antagonistic potential against *S. sclerotiorum* (ON831560.1) *under in vitro* conditions by a dual culture assay. The results presented in Table 2 indicated that all the biocontrol-agents suppressed the colony growth of *S. sclerotiorum* (Figure 1).

Among the tested biocontrol-agents against *S. sclerotiorum*, the maximum suppression was observed with *T. harzianum* (69.29%), followed by *T. viride* (62.96%), which was on par with each other. The *P. fluorescens* was observed to have a minimum (61.51%) suppression, but more than the control (Table 2 and Plate 1). Similar findings were also reported by earlier workers. For example, Pandey *et al.* (2011) reported that bioagents, *viz., Trichoderma*

Table 1. List of botanicals used against *S. sclerotiorum* by poison food technique

Treatment	Botanicals	Scientific name	Family	Plant parts used	Dose (per cent)	
T_1	Ginger	*Zingiber officinalis*	Amaryllidaceae	Clove	5	10
T_2	Garlic	*Allium sativum*	Zingiberaceae	Rhizome	5	10
T_3	Tulsi	*Ocimum sanctum*	Lamiaceae	Leaf	5	10
T_4	Lemon grass	*Cymbopogan citrates*	Poaceae	Leaf	5	10
T_5	Neem	*Azadirachta indica*	Neliaceae	Leaf	5	10
T_6	Control (untreated)					

harzianum (62.70%) and *T. virens* (60.41%), inhibited the mycelial growth of *S. sclerotiorum in vitro*. Manhas *et al.* (2022) studied the antagonistic activity of fungal biocontrol agents and reported that *T. harzianum* yielded better results by inhibiting 63.41 percent growth of *Sclerotinia sclerotiorum*, followed by *T. viride* (60.41%) by dual culture. *Trichoderma* spp. antagonize the major pulse pathogens has been reported by Arif *et al.* (2024), Sharma and Kumari (2018).

Evaluation of plant extract against S. sclerotiorum by poison food technique

Table 2. Effect of biocontrol-agents on mycelial growth of *S. sclerotiorum* in dual culture

Treatment		7 DAI	
		Colony growth (mm)	Inhibition (%)
T_1	*T. viride*	33.33 (35.24)	62.96 (52.49)
T_2	*T. harzianum*	27.63 (31.69)	69.29 (56.32)
T_3	*P. fluorescens*	34.63 (36.03)	61.51 (51.63)
T_4	Control	90.00 (71.53)	0.00 (0.00)
SEm (±)		0.282	0.314
CD (p=0.01)		0.933	1.039

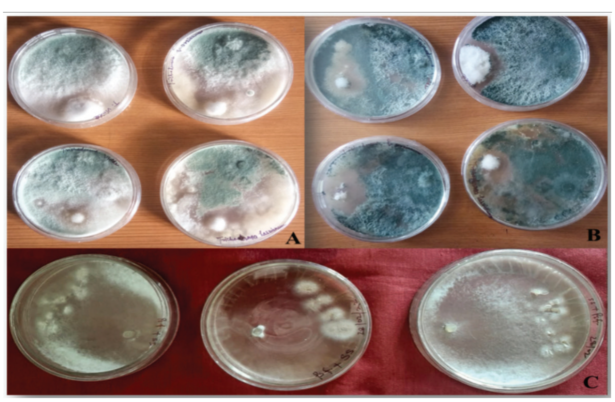

Plate 1. Antagonism of *T. harzianum* and *T. viridae*, (A), 7 days after inoculation (DAI) (B) and *P. fluorescens* (C) against *S. sclerotiorum*

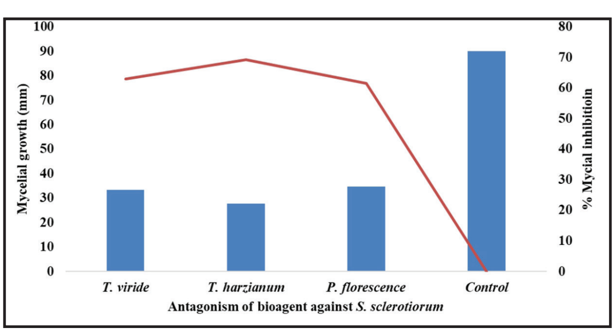

Figure 1. Efficacy of biocontrol-agents on *Sclerotinia sclerotiorum* (*in vitro*)

Five plant extracts were evaluated for their inhibitory action against the *S. sclerotiorum* (ON831560.1) in *in-vitro* conditions at 5 and 10 percent concentrations (Plate 2, Table 3, Figure 2).

Table 3. Effect of Plant extract on mycelial growth of *S. sclerotiorum* in poison food technique

Plant extract	7th DAI			
	5%		10%	
	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition
T_1 (Ginger rhizome extract)	81.46 (64.47)	9.48 (17.92)	66.66 (54.71)	25.77 (30.49)
T_2 (Garlic clove extract)	81.49 (64.49)	9.45 (17.89)	65.96 (54.28)	26.54 (31.00)
T_3 (Lemon grass leaf)	82.54 (65.28)	8.27 (16.71)	74.53 (59.66)	17.01 (24.34)
T_4 (Tulsi leaf extract)	86.26 (68.22)	4.09 (11.65)	78.75 (62.52)	12.30 (20.52)
T_5 (Neem leaf extract)	67.35 (55.13)	25.15 (30.09)	51.56 (45.87)	42.58 (40.72)
T_6 (Control)	90.00 (71.53)	0.00 (0.00)	90.00 (71.53)	0.00 (0.00)
SEm (±)	0.169	0.177	0.134	0.149
CD (p=0.01)	0.505	0.529	0.401	0.447

The maximum percent inhibition of the pathogenic fungus was observed at 5 percent concentration in treatment with Neem leaf extract (25.15%) followed by Garlic clove extract (9.48%), Ginger rhizome extract (9.45%), and Lemon grass leaf (8.27%), while the minimum percent inhibition was observed in Tulsi leaf extract (4.09%).

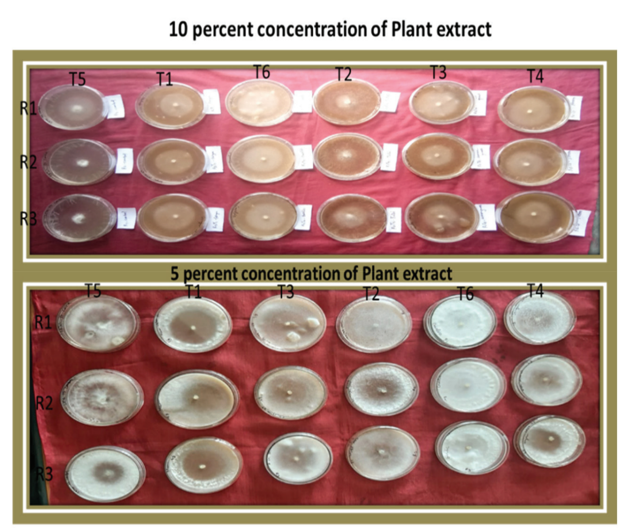

Plate 2. Effect of Plant extract on mycelial growth of *S. sclerotiorum* in poison food technique

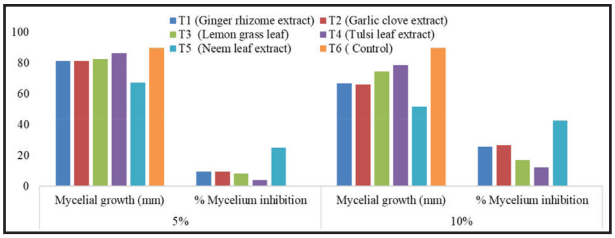

Fig. 2. Effect of Plant extract on mycelial growth of *S. sclerotiorum* in poison food technique.

The maximum percent inhibition was observed at a 10 percent concentration in treatment with Neem leaf extract (42.58%) followed by Garlic clove extract (26.54%), Ginger rhizome extract (25.77%) and Lemon grass leaf (17.01%), while the minimum inhibition percent is observed in Tulsi leaf extract (12.30%). Yadav *et al*. (2009) evaluated ten plant extracts for their antimicrobial capacity against five isolates of *S. sclerotiorum*. Among them, *Calotropis gigantean* and *Azadirachta indica* extracts were very effective in reducing the radial growth of *S. sclerotiorum*. Kewate *et al*. (2020) reported that leaf extracts of neem, Nilgiri, Tobacco, Oak and Beshram significantly inhibited the growth of *S. sclerotiorum*. Gupta *et al*. (2012) also reported similar findings.

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