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Integration of bioagents and chemical fungicides against Sclerotinia sclerotiorum causing stem rot in chickpea

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ABSTRACT

One of the most harmful infections to chickpea plants, Sclerotinia sclerotiorum, causes stem rot and causes financial losses all over the world. Beneath the soil's surface, as sclerotia, the pathogen can live for a very long time alongside the detritus. As a result, the condition is extremely difficult to manage, has nearly no known treatments, and only a few types have been shown to be somewhat successful against the infection. Thus, the primary goal of the research is to investigate the effects of a variety of treatments, including *Rhizobium leguminosarum* + *Pseudomonas fluorescens* combination, *Trichoderma viride*, Saaf (Carbendazim 12%+ Mancozeb 63% WP), Vitavax Power (Carboxin 37.5%+ Thiram 37.5%), Hexaconazole, and *Rhizobium leguminosarum* + *Pseudomonas fluorescens*, to investigate their impact on *S. sclerotiorum* suppression. While maintaining sustainability, bioagents improve the soil's properties and productivity. Fungicides have been found to be effective when bio-agents are not able to control a disease. Therefore, in this investigation, both methods of treatment were used. The results of an experiment conducted in both in vitro and in vivo conditions showed that chemical treatment with Saaf fungicide and seed treatment with *T. viride* were highly efficient against *S. sclerotiorum*.

Keywords: Bio-agents, seed treatment, *S. sclerotiorum*, sclerotia

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INTRODUCTION

One of the earliest crops in the Leguminaceae family to be domesticated was the chickpea (*Cicer arietinum* L.), a member of the Fabaceae family. Chickpeas may fix up to 140 kg N/ha of environmental nitrogen by symbiotic nitrogen fixation, which meets 80% of their nitrogen needs (Saraf *et al.*, 1998). Because chickpeas are high in protein, dietary fiber, and essential minerals, they are a crucial component in the fight against hunger and poverty in many poor nations (Jukanthi *et al.*, 2012). The three most prevalent diseases that cause serious losses in chickpea plants are stem rot, collar rot, and damping-off.

Sclerotiorum causes stem rot, also referred to as Sclerotinia wilt or white mold, which is a very harmful disease in chickpea (Sheshma *et al.*, 2022). According to Kukreja (2018), some of the fungi that seriously affect chickpea crops are *Alternaria* sp., *Ascochyta pisi*, *Uromyces* sp., Botrytis sp., *Fussarium* sp., *Sclerotinia* sp., and *Phytophthora medicaginis*. Chickpea is one of the several plant species that *S. scterotiorum* (Lib.) de Bary infects in subtropical and temperate climates. The structure of Sclerotinia that overwinters is called Sclerotia. Destructive and widely distributed plant diseases, Sclerotinia spp., induce stem and crown rot in a variety of horticultural and agronomic crops as well as wild species. Sclerotinia stem rot resulted in crop losses of 10 million harvests (270 million kg) between 1996 and 2009 (Koenning and Wrather, 2010). White

mold, another name for the fungus that causes stem rot, thrives in high humidity and temperatures between 18 and 23 °C. Long, wet sores that swiftly extend to the petiole and into the stem are the first indications on the stem. The infection may create shot hole symptoms on the leaves during ascospore emergence. This disease is present on a wide range of crops worldwide and has a broad host range (Boland, 1994). Saaf was discovered to be the most efficient fungicide, totally inhibiting the pathogen's radial growth (Goshwami *et al.,* 2020). The antagonistic activity of *Trichderma viride, T. harzianum, Pseudomonas fluorescence,* and *Bacillus subtilis* as fungal biocontrol agents in dual culture. It was discovered that *Trichoderma harzianum* yielded better outcomes than Trichoderma viride (Yuen *et al.,* 1991).The hyphae and sclerotial walls of S. sclerotiorum can be broken down and degraded by Trichoderma spp. that have cellulose-lying enzymes (p-l, 3-glucanase, and chitinase) (Jones and Watson, 1969). In light of the devastating nature of the disease, the significance of chickpeas as a grain for the country's economy, the scarcity of information regarding biocontrol and chemical treatments against the pathogen, and the existence of limited resistance sources, the current study aimed to examine various disease management approaches.

MATERIAL AND METHODS

Saaf (Carbendazim 12%+ Mancozeb 63% WP), *Rhizobium leguminosarum, Trichoderma viride, Pseudomonas fluorescens,* Vitavax Power (Carboxin 37.5% + Thiram 37.5%), and Hexaconazole 5% were used as treatments. The study was conducted during the Rabi season at the Agricultural research field of LPU, Punjab using a randomised block design (RBD) with three replications and sample was collected from the infected area of the chickpea plant using the single hyphal tip method (Pandey *et al*., 2011). Sclerotinia rot infected plants were collected at various stages for pathogen isolation and identification. GNG-469 variety was used to study the *in-vivo* effect of various treatments on *S. sclerotiorum* disease incidence.

Fungicides against *S. sclerotiorum*in *in-vitro* condition.

The efficacy of fungicides against *S. Sclerotiorum* was evaluated using the Poison Food Technique. A small bit of mycelial was cut using a cork borer of 1mm size from pure culture and transferred to fresh culture material using a sterile inoculating loop and was kept in an incubator at 25±1°C for 6 days. PDA (working Solution) was prepared along with Stock solutions (Fungicides) and the stock solutions were used to poison the working media. Mycelium with a diameter of 1 mm was cut from the fresh culture of *S. sclerotiorum* using a cork borer and put in the centre of a Petri plate containing poisoned media. Three Fungicides *viz.* Saaf (Carbendazim 12 % + Mancozeb 63 % WP), Vitavax power (Carboxin 37.5 % + Thiram 37.5 % WS), and Hexaconazole 5% SC measured at 120µL, 480µL, 600µL mg were studied at three concentrations of 50, 80, and 100 ppm, with untreated media serving as a control. Radial growth was checked on a frequent interval after incubation. Percent inhibition of the mycelial growth (Eksteen *et al*., 2001) was calculated by using following formula:

Percent of mycelial growth inhibition (I) = (C-T)/C*100

Where,

I = Percent inhibition

C = colony diameter.

T = treatment colony diameter

The following formula was used to calculate radial growth inhibition in comparison to control growth (Garrett, 1965):

Percentage Inhibition of radial mycelial growth (I) = [(C-T)／C] × 100

Where,

I represent Percent inhibition,

C = the pathogen radial growth in control plates and

T=the pathogen's mycelial growth in the presence of bioagents.

Bioagents against *S. sclerotiorum* in *in-vitro* condition

Three bioagents *viz., Trichoderma viride, Rhizobium leguminosarum,* and *Pseudomonas fluorescens* were screened for antagonistic potential against the pathogen by using Dual culture technique (Dennis and Webster, 1971) in which the mycelial piece of 5 mm diameter *Trichoderma* culture was cut with a cork borer and placed on potato dextrose agar, around 1 cm from the edge of each petri dish. A mycelial fragment of *Sclerotinia sclerotiorum*, taken from a 4-day-old culture, was placed to the same petri dish and positioned 5.5 cm apart from the *Trichoderma leguminosarum*. As controls, Petri plates inoculated with *Trichoderma* and *Sclerotiorum* isolates alone were employed. Plates were then incubated at 25±1°C for 4 days and analyzed for the establishment of inhibitory zones between *Trichoderma* and *Sclerotinia sclerotiorum* isolates after 6 and 14 days. Mycelial growth was assessed at the end of the incubation period. *Pseudomonas fluorescens* and *Rhizobium leguminosarum* cultures were prepared using the serial dilution method, then placed into Petri plates with PDA and evenly distributed. It was isolated from the culture into a new petri plate using the streak plate method along with *S. sclerotiorum* and kept for incubation at 25±1°C.

***Sclerotinia sclerotiorum* disease incidence under field condition**

The effect of different treatments i.e., Saaf (Carbendazim 12 % + Mancozeb 63 % WP), Vitavax power (Carboxin 37.5 % + Thiram 37.5 % WS), and Hexaconazole 5% SC and biocontrol agents i.e., *Rhizobium leguminosarum, Trichoderma viride, Pseudomonas fluorescens* on disease incidence was measured after 30, 60, and 120 days of sowing using the following formula:

$$\text{Disease incidence} = \frac{No.\ of\ diseased\ plants \times 100}{Total\ Plant\ examined}$$

RESULT AND DISCUSSION

Poison Food Technique under *in-vitro* condition

In the experiment, Mycelial linear growth was measured at 6 and 14 days and it was observed that as the chemical concentration increased, percent inhibition of radial growth also increased (Table 1). Hexaconazole @50 ppm gave 84.56 growth inhibition percentage followed by Vitavax power @50ppm gave 76.78 inhibition percentage. At all concentrations of 50, 80, and 100ppm, Saaf showed complete inhibition (Table 2). Goshwami *et al*. (2020) reported SAAF to be the best treatment that inhibits pathogen radial growth completely. Carbendazim fungicide from the benzimidazole group prevents *S. sclerotiorum* from producing energy and forming cell walls (Nene and Thapliyal, 1973). Chattopadhyay *et al*. (2002) also found that a higher dose of carbendazim (0.1 percent active ingredients) and mancozeb completely inhibited fungal growth (0.2 percent active ingredients). The data implies that @80 and 100ppm all fungicides gave complete mycelial growth inhibition of *S. sclerotiorum* and SAAF was found to be the most effective fungicide. Vitavax power and Hexaconazole was found to be least effective in comparison to SAAF.

Table 1. *In-vitro* evaluation of biocontrol agents against *S. sclerotiorum*

Treatments	Mycelial Growth of *Sclerotinia sclerotiorum* in the presence ofbioagents (mm)		Inhibition (%)
	6 days	14 days	
Trichoderma viride	42	15	83.33
Rhizobium leguminosarum	31	58	35.55
Pseudomonas fluorescens	35	62	31.11
Control	90	90	
SE (m)	0.333	0.257	0.349
CD @ 5%	1.344	1.036	1.408
CV	1.604	0.977	1.216

At 6 and 14 days, mycelial radial growth was recorded for both pathogen and bioagents to estimate the inhibition percentage.

The inhibition rate of biocontrol agents such as *T. viride*, *R. leguminosarum*, and *P. fluorescens* against *S. sclerotiorum* was assessed after 14 days, using the dual culture method which showed that among all the bioagents *Trichoderma viride* was found to be most effective with 83.3 inhibition percentage followed by *R. leguminosarum*, and *P. fluorescens*.

Table 2. Efficacy of different concentrations of fungicides on radial growth of *S. sclerotiorum*.

Treatments	Conc. 50ppm		Conc. 80 ppm		Conc. 100 ppm	
	14 Days		**14 Days**		**14 Days**	
	R.G (mm)	I (%)	RG (mm)	I (%)	RG (mm)	I (%)
Vitavax Power	20.9	76.7778	0	100	0	100
Hexaconazole	13.9	84.5556	0	100	0	100
Saaf	0	100	0	100	0	100
Control	90		90		90	
SE (m)	Factor(A) 0.807		Factor(B) 0.807		Factor (A X B) 1.398	
CD @ 5%	Factor(A) 2.440		Factor(B) 2.440		Factor (A X B) 4.227	

I-inhibition percentage, R.G- Radial Growth.

Jones and Watson (1969) reported that with cellulose-lying enzymes (p-l,3-glucanase, and chitinase), Trichoderma spp. can disintegrate and degrade the hyphae and sclerotial walls of *S.Sclerotiorum*. Sharma (1994) reported a similar result where *T. harzanium* showed maximum inhibition percentage followed by *Trchoderma viride* and showed to be highly effective in comparison to control against *S. sclerotiorum.* It was found that T1 (*Trichoderma viride)* was significantly different from T2 *(P. fluorescens)* and T3 *(R. leguminosarum),* whereas T2 (*Pseudomonas fluorescens)* and T3 *(Rhizobium leguminosarum)* was significantly similar to each other.

S. sclerotiorum showed white, thick mycelial development, with no conidial formation, and an abundance of black sclerotia of varied sizes and shapes arranged in a ring pattern, and sclerotia formation was observed on the edges of the ring. Sharma (1979), verified that sclerotia grown in culture were morphologically identical to those formed on the host. The pathogen's morphological characteristics matched the taxonomic keys provided by Willetts and Wong (1980), proving the pathogen as *S. sclerotiorum*. Hyphae are multinucleate, septate, branched, and hyaline. In culture, mycelium appears white to tan. There are no asexual conidia formed. Depending on the environment, sclerotia can germinate and produce mycelia or apothecia. The hyphae measured in breath from 2.0 to 9 µm and included thick granular protoplasm. Micro conidia were formed on vegetative mycelium conidiophores and measured 1 to 3.5 µm in culture. In culture, the sclerotia are sub-spherical to irregular in shape, sometimes flattened, with a diameter of 0.5-7 mm, dark to brownish. Sclerotia started off with white radial growth, but gradually became light brown and took on a bean-like look. Furthermore, the sclerotia on the affected area were irregular (superficial, round, or ellipsoidal) in shape and 0.5 mm - 5 mm in diameter

S. sclerotiorum disease incidence under field conditions

Disease incidence was measured after 30, 90 and 120 days after sowing for different replication and treatments to check the total infection caused by the pathogen (Table 3). The data given indicates that minimum disease incidence was observed in T2-*T. viride* followed by T4-Saaf and T6- *P. fluorescens* + *R. leguminosarum*. Srivastava (2010) during the research demonstrated that combining various antagonistic bacteria improves the plant's level of defence, and T1-*R. leguminosarum*, T7 Contaf Plus, T3-*P.*

Table 3. Disease Incidence of stem rot disease caused by *Sclerotium sclerotiorum* in chickpea plant under field condition during 2021-2022

Treatments	30 DAS	60 DAS	120 DAS	Mean
Rhizobium leguminosarum	6.66	11.66	15	11.11
Trichoderma viride	5	8.33	11.66	8.33
Pseudomonas fluorescens	8.33	13.33	18.33	13.33
Carbandazim 12% + Mancozeb 63 WP (Saaf)	4	9	13	8.66
Carboxin 37.5%+ Thiram 37.5% (Vitavax power)	10	15	20	15
Pseudomonas fluorescens + *Rhizobium leguminosarum*	5	10	13.33	9.44
Hexaconazole 5% SC (Contaf plus)	6.66	11.66	15	11.11
Control	18.33	23.33	28.33	23.33
SE (m)	0.43	0.607	0.751	0.571
CD @ 5%	1.33	1.89	2.30	1.75
CV	7.83	8.06	7.08	7.24

fluorescens, T5- Vitavax power, and maximum disease incidence was observed in control. T1, T4 were found to be significantly similar to each other but different to T8 (Control). It can be concluded that *T. viride* as the best treatment against the pathogen followed by SAAF.

From the data and observations, it can be concluded that the minimum disease incidence and radial growth of pathogen was found in *T. viride* and Carbendazim 12% + Mancozeb 63% WP (Saaf) against *S. sclerotiorum* under in vitro as well as in vivo condition. The soil-borne fungus not only cause losses but also impacts the environment due to unsustainable management practices. The combination of bio-control agents with chemical fungicides might be a better way for eco-friendly management of Sclerotinia rot. The present study will give an idea for the disease management in the field conditions with the combination of chemical as well as biological methods for getting the optimum yield with minimum losses.

Contribution of authors

Mini Chakma did the whole research during the post-graduate programme. Akash Jangid carried ou the experiments. Kalpna Gairola and Parul Chudhary data collection. Anju A Andrews analysed the data. Seweta Srivastava preparation of the manuscript. Meenakshi Rana supervised the research.

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