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Combined Application of Bio-agents and Novel Fungicides for Management of Collar Rot of Chickpea

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ABSTRACT

Background: The chickpea, scientifically known as *Cicer arietinum* L., is a significant legume crop that serves as a valuable source of vegetable protein. The chickpea crop is susceptible to various pests and illnesses. Collar rot, induced by the fungal pathogen *Sclerotium rolfsii*, is a highly significant and extremely damaging disease that affects chickpea crops. The disease causes seedling mortality ranging from 54.7 to 95 per cent and field conditions result in yield decrease ranging from 22 to 50 per cent. The present study aimed to investigate the effectiveness of using a combination of fungicides, bio-agents and organic amendments for the management of collar rot in chickpea.

Methods: The investigations were conducted in the Rabi seasons of 2019-20 and 2020-21. The experiments involved the integration of fungicides, fungal biocontrol agents (*Trichoderma* spp.), FYM and vermicompost to control Collar rot disease in Chickpea caused by *S. rolfsii*. Six indigenous fungal antagonists (*Trichoderma* spp.) were assessed in a laboratory setting against *S. rolfsii* using both dual culture and non-volatile (culture filtrate) methods. The efficacy of the fungicides was assessed using the poison food technique. Nine fungicides were assessed in a laboratory setting to determine their effectiveness against a pathogen and *Trichoderma harzianum*-2. The fungicides were tested at four different concentrations: 50, 100, 500 and 1000 ppm. The goal was to identify fungicides that are extremely toxic to *S. rolfsii* at lower concentrations, while being less harmful to the bioagent *Trichoderma* spp. Pot culture studies were conducted using a completely randomised design (CRD), while field experiments were conducted using a randomised block design (RBD).

Result: *Trichoderma harzianum*-2 (TH-2) was found to be highly efficient against the pathogen. It reduced the growth of the pathogen by 75.18% in the dual culture technique and by 61.85% in the culture filtrate approach. Among the nine fungicides tested, four of them, specifically propineb, mancozeb, captan 70% + hexaconazole 5% WP and penflufen 13.28% w/w + trifloxystrobin, showed lower inhibitory effects on *Trichoderma harzianum* at doses ranging from 50 to 1000 ppm. The treatment that resulted in the highest seed germination rate (100%) and the lowest occurrence of collar rot was the one where the seeds were treated with captan 70% + hexaconazole 5% WP and the soil was supplemented with *Trichoderma harzianum* through vermicompost application.

Key words: Bio control agents, Chickpea, Collar rot, Fungicides, Management.

INTRODUCTION

The chickpea, scientifically known as *Cicer arietinum* L., is the third most significant pulse crop globally, following beans and peas. India is responsible for nearly 75% of the world's chickpea production. The total land area occupied by this entity is 9.70 million hectares. Its production output is 11.08 million tonnes, with an average productivity of 1142 kg per hectare, according to the Directorate of Economics and Statistics in 2019-20. According to the Directorate of Economics and Statistics, 2019-20, Uttar Pradesh is the fourth highest producer of chickpea in India, with a production of 0.85 million tonnes. The states of Madhya Pradesh, Rajasthan and Maharashtra hold the first, second and third places, with the production of 2.73, 2.66 and 2.24 million tonnes, respectively. Bundelkhand, located in southern part of Uttar Pradesh, is the primary region for cultivating pulses in the state. It is renowned as the main hub for pulse production in Uttar Pradesh. The chickpea is the predominant pulse crop in the Bundelkhand region of Uttar Pradesh. Overall, the crop productivity in this region is significantly limited by various biotic and abiotic causes Overall, the crop productivity in this region is significantly limited by various biotic and abiotic restrictions (Narayan and Kumar, 2015; Jha *et al.,* 2018). Chickpea cultivation is significantly impacted by a variety of diseases and insect pests. One of the major diseases affecting chickpea is collar rot, caused by *Sclerotium rolfsii*. This disease has become more prevalent due to climate change. The study conducted by Gurha and

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Dubey in 1982 found that it results in a much greater rate of seedling death, ranging from 55% to 95%. Due to its soil-borne origin, polyphagous behaviour and extended persistence in soil, controlling the pathogen on its own appears to be ineffectual and economically unviable. Various fungicides can be used to effectively manage soil borne plant diseases, such as *Sclerotium rolfsii*. In addition to their detrimental effects on the biotic and abiotic environments, fungicides pose a significant hazard for human and farm animals. Controlling collar rot of chickpea (*Sclerotium rolfsii*) cannot be achieved by the use of a single approach of plant disease control. The application of bioagents (*Trichoderma harzianum*, *T. virens* and *Pseudo-monas fluorescens*) were successfully decreases the collar rot incidence in chickpea (Singh *et al.,* 2022; Bagul *et al.,* 2024). The management can be achieved by the integration of all existing control mechanisms. Consequently, considering the aforementioned facts, tests were carried out to investigate the integration of Trichoderma, FYM, vermicompost and fungicides for the purpose of managing Collar rot disease in chickpea.

MATERIALS AND METHODS

Isolation and purification of causal pathogen

The pathogenic fungus (*Sclerotium rolfsii)* was isolated and purified from infected plants exhibiting characteristic collar rot symptoms, which were taken from chickpea fields of Crop Research Farm of BUAT, Banda. The collar region, exhibiting characteristic signs of decomposition, was dissected into minute fragments. These fragments were washed in sterilized water. Then the parts underwent surface sterilization using the 1% sodium hypochlorite solution for a duration of 60 seconds. The pieces were rinsed extensively in sterile distilled water two to three times to eliminate any remaining sodium hypochlorite and then transferred to sterilized potato dextrose agar (PDA) plates under aseptic conditions. The plates were placed in an incubator set at a temperature of 27±1°C for a duration of four days. After this period, the plates were inspected to determine if there was any growth of the pathogen. After a duration of four days, the newly growing and expanding hyphal fragments were transferred to newly prepared PDA plates using the hyphal tip procedure as described by Karr and Albersheim (1970).

Collection and maintenance of cultures of bio-agents (*Trichoderma* spp.)

Trichoderma isolates *i.e.* one isolate of *Trichoderma viride*-1 (TV-1) and 5 isolates of *Trichoderma harzianum*-1 (TH-1), *Trichoderma harzianum*-2 (TH-2), *Trichoderma harzia-num*-3 (TH-3), *Trichoderma harzianum*-4 (TH-4) and *Tricho-derma harzianum*-5 (TH-5) were collected from Plant Pathology Laboratory, Department of Plant Pathology, B.U.A.T., Banda.

In vitro efficacy of fungal antagonists (*Trichoderma* spp.) against pathogen (*Sclerotium rolfsii*)

The effectiveness of fungal antagonists (*Trichoderma* spp.) against the pathogen (*Sclerotium rolfsii*) was evaluated using dual culture and culture filtrate procedures. The antagonistic potential of *Trichoderma harzianum* (TH-2) against *S. rolfsii* was tested using a dual culture approach developed by Morton and Stroube (1955). The antagonists and pathogens were grown on PDA medium. Aseptically, 20 ml of sterilized PDA media was poured into a sterilized Petri plate with a diameter of 90 mm. A 5 mm mycelial disc obtained from the five-day-old vigorously growing *Trichoderma* spp culture was moved to one side of a Petri dish. Similarly, the opposite side of the Petri plate was inoculated with five days-old culture of *S. rolfsii*. The test pathogen was inoculated at centre of the Petri plate for the purpose of comparison. The plates that were treated with an inoculum were placed in a BOD incubator at the temperature of 27±1°C. This process was repeated three times. The percentage of inhibition in the growth of test pathogens in the presence of *Trichoderma* spp was determined by using the formula provided by Bliss (1934) to compare it with the control.

Per cent inhibition

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

Where,

I = Per cent inhibition.

C = Growth of test pathogen in control.

T = Growth of test pathogen.

Efficacy of culture filtrates of the *Trichoderma* spp. against *S. rolfsii*

The *Trichoderma* spp. and S. *rolfsii* isolates were cultivated on PDA medium in Petri plates at a temperature of 27±s1°C. for a duration of 4 days. Two blocks of Trichoderma species, each measuring 5 mm in size, were taken from the actively growing edges of 4-day-old cultures. These blocks were then separately placed into 250 ml conical flasks, with each flask holding 100 ml of sterilised potato dextrose broth. This process was repeated three times to ensure triplicate samples. Following a 10-day period of incubation at a temperature of 27±1°C, the static cultures were passed through Whatman filter paper number 42 into sterilized flasks and subsequently through a cellulose Millipore membrane filter. To get the desired concentration of culture filtrate, 5 ml of the filtrate was added to 95 ml of melted PDA media before pouring. The modified medium was carefully poured into sterilized Petri plates and reproduced three times for each treatment. The solid plates were inoculated at the centre with a 5 mm diameter disc of mycelium from the pathogen S. *rolfsii*. The plates were then incubated at a temperature of 27±1°C for a period of 5-6 days. The PDA media without the addition of culture filtrate of *Trichoderma* and infected with the pathogen was used as the control. The expansion of mycelium in the test pathogens was quantified and the percentage of inhibition in mycelial growth was computed.

Determination of tolerance in pathogen (*Sclerotium rolfsii*) and fungal bio-agent agent (*Trichoderma harzianum*) to novel fungicides

Two set of experiments were conducted to evaluate novel fungicides against *S. rolfsii* and *T. harzianum*-2 (TH-2)

through Poisoned food technique (Schmitz 1930). Nine different fungicides *viz*; carbendazim 12% + mancozeb 63% WP, captan 70% + hexaconazole 5% WP, Propineb 70% WP, carbendazim 50% WP, mancozeb 75% WP, Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS, propiconazole 25% EC., hexaconazole 5% SC and tebuconazole 25.9% w/w EC were tested at four concentrations (50 ppm, 100 ppm, 500 ppm and 1000 ppm) *in vitro*. Hundred ml of PDA medium was sterilized in conical flask, requisite quantity of fungicide was incorporated in laminar air flow in molten media to make 50, 100, 500 and 1000 ppm concen-tration. Media was then poured aseptically in sterilized Petri plates. After solidification of media five mm discs of the *S. rolfsii* was cut with the help of sterilized cork borer from 5-6 days old culture and placed centrally in each Petri plates. Three replications of each treatment were maintained and control treatment maintained without the added of fungicide. The Inoculated plates were incubated at 27±1°C in BOD incubator. Observation of linear growth of fungus was recorded after 24 hours of incubation.

Another set of experiment was conducted for evaluation of fungicides against *T. harzianum* in which nine fungicides, *viz*., carbendazim 12% + mancozeb 63% WP), captan 70% + hexaconazole 5% WP, propineb 70% W, carbendazim 50% WP, mancozeb 75% WP, Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS, propiconazole 25% E.C., hexaconazole 5% SC and tebuconazole 25.9% w/w EC were tested at four concentrations *i.e.* 50 ppm, 100 ppm, 500 ppm and 1000 ppm, *in vitro* using poisoned food technique (Table 4). Three replications were maintained by inoculating 5 mm disc of 3 days old cultures of *T. harzianum* (TH-2) control plates without any fungicide were also inoculated simultaneously for comparison. Inoculated plates were incubated at 25±1°C for seven days. Colony diameter in each plate was measured to find the fungicidal and fungistatic behaviour.

Integrated management of collar rot of chickpea in pots and field condition

Pot culture experiment

The pot culture experiments were conducted during *Rabi* 2019-20 and 2020-21 for evaluating the effect of biocontrol agents, fungicides alone or various integrated treatments in complete randomized design (CRD). Plastic pots were filled with sterilized soil. The formulation of *T. harzianum*-3 (TH-3) was multiplied on FYM and vermicompost was mixed separately in pots @ 100 g/pot. After 5-7 days mass culture of *S. rolfsii* was mixed in pots @ 25 g/kg soil. Seeds of chickpea were surface sterilized with sodium hypochlorite solution for 3 minutes, rinsed thoroughly in sterilized distilled water. Seeds of collar rot susceptible chickpea cultivar L550 were sown with different treatments seed treatment. Data on seedling emergence and mortality per cent were recorded 10-15 days after sowing and final plant stand was counted after 60 days of sowing.

Field experiment

Field experiments were carried out during the Rabi season of 2019-20 and 2020-21 at the Crop Research Centre, BUAT, Banda. The trials followed a randomised block design (RBD) with ten treatments and three replications. The chickpea cultivar L550, which is prone to susceptibility to collar rot, was planted in a plot measuring 6.0 m^2. The spacing between each plant was 30 cm × 10 cm. This setup was replicated for each treatment. Chickpea seeds, which had been treated with bio-agents and fungicide according to the specified treatments, were planted in six rows in each plot, with a total of 180 seeds. The fungicide Captan 70% + Hexaconazole 5% EC and a mixture of Bavistin+Thiram (1:1) were applied at a rate of 2 grams per kilogram of seed, while a talc formulation of *T. harzianum* was applied at a rate of 5 grammes per kilograms of seed. *T. harzianum*, with a concentration of 2 × 10^8 colony forming units per gramme, was applied to the soil together with enriched farmyard manure at a rate of 5 kilograms of bioagent per tonne of manure. The application was done in furrows at a rate of 1 ton per hectare or 100 grams per square meter, according to the specified treatments. The occurrence of collar rot was monitored at 20-days intervals until the crop reached maturity and the total number of diseased plants was tallied. The grain yield observations were recorded post-harvest.

Treatment number	Treatments detail
T1	Seed treatment with Thiram + carbendazim (1:1) @ 2 g/ kg of seed.
T2	Seed treatment with Captan 70% + Hexaconazole 5%EC (Taquat) @ 2 g/kg of seed.
T3	Seed treatment with *Trichoderma harzianum* @ 5 g/kg seed.
T4	Soil application with *Trichoderma harzianum* enriched FYM@ 100 g/m^2.
T5	Soil application with *Trichoderma harzianum* enriched FYM vermicompost @ 100 g/m^2.
T6	Seed treatment with Captan 70% + Hexaconazole 5% EC (Taquat) @ 2 g/kg of seed + soil application *Trichoderma harzianum* enriched FYM @ 100 g/m^2.
T7	Seed treatment with Captan 70% + Hexaconazole 5%EC (Taquat) @ 2 g/kg of seed+ soil application *Trichoderma harzianum* enriched vermicompost@100 g/m^2.
T8	Seed treatment with *Trichoderma harzianum* @ 5 g/kg seed + soil application with *Trichoderma harzianum* enriched FYM @100 g/m^2.
T9	Seed treatment with *Trichoderma harzianum* @ 5 g/kg seed + soil application with *Trichoderma harzianum* enriched vermicompost @100 g/m^2.
T10	Control.

Per cent disease incidence

Per cent disease incidence =

$$\frac{\text{Number of plants infected in a microplot}}{\text{Total number of plants in a microplot}} \times 100$$

Per cent disease control

Per cent disease control =

$$\frac{\text{PDI in control - PDI in treated}}{\text{PDI in control}} \times 100$$

Per cent increase yield

Per cent yield Increase =

$$\frac{\text{Yield under protected - Yield under unprotected}}{\text{Yield under protected}} \times 100$$

RESULTS AND DISCUSSION

In vitro efficacy of antagonists (*Trichoderma* spp.) against *Sclerotium rolfsii*

The antagonistic actions of five indigenous isolates of *Trichoderma harzianum*, namely TH-1, TH-2, TH-3, TH-4 and TH-5, were demonstrated by assessing the growth of the pathogen using the dual culture technique. Additionally, one isolate of *Trichoderma viride* (TV-1) was also included in the study. The radial expansion of S. *rolfsii* was limited by the presence of antagonistic strains of *Trichoderma* spp., resulting in considerable suppression. Among the six *Trichoderma* isolates, *Trichoderma viride*-1 exhibited the highest level of growth inhibition against S. *rolfsii*, with a rate of 75.44%. *Trichoderma harzianum*-2 also showed a similar level of inhibition at 75.18%, making it comparable to *T. viride*-1. The subsequent potent antagonist was *T. harzianum*-4, which effectively suppressed 71.43% of the pathogen's mycelial growth. This was followed by *T. harzianum*-3 (70.70%) and T. *harzianum*-1 (68.81%), demonstrating their relative superiority. There was no substantial difference between them in terms of their ability to prevent the growth of the infection. Nevertheless, the antagonist *T. harzianum*-5 exhibited the lowest level of inhibition (64.25%) on the growth of the pathogen's mycelium, as seen in Table 1. Ali and Javaid (2016) and Darvin *et al.* (2013) have verified that *T. harzianum* and *T. viride* exhibit antagonistic effects in dual culture against *S. rolfsii.*

Effect of non-volatile (cultural filtrate) metabolites of potential *Trichoderma* spp on mycelial growth of *Sclerotium rolfsii*

The impact of *Trichoderma* species' non-volatile compounds was assessed using the procedures outlined by Dennis and Webster (1971) as described in the materials and methods section. The data in Table 2 shows that among the six isolates of *Trichoderma* spp, *T. harzianum*- 2 exhibited the highest inhibition of mycelial growth of *S. rolfsii* through the production of non-volatile compounds, with a rate of 61.85%. This was followed by *T. harzianum*-1 with a rate of 57.77% and *T. harzianum*-4 with a rate of 52.40%. These differences in inhibition rates between the isolates were statistically significant. *T. viride* -1, the subsequent potent antagonist, hindered 42.22% of the pathogen's mycelial growth by producing an inhibitory metabolite. Nevertheless, the lowest level of inhibition (22.95%) in the growth of *S. rolfsii* was seen when exposed to *T. harzianum*-5. A study conducted by several workers focused on the development of antibiotics, both volatile and non-volatile. The study found that *T. harzianum* and *T. viride* were particularly successful in inhibiting the radial growth of S. *rolfsii* through the production of these compounds. This conclusion was reached by Rao and Kulkarni (2003). *Trichoderma* spp. isolates have the ability to create both volatile and non-volatile compounds that exhibit activity against a broad spectrum of fungi (Dennis and Webster, 1971). In their study, Kucuk and Kivanc (2003) found that the culture filtrate of *T. harzianum* exhibited inhibitory properties against various plant pathogenic fungi.

In vitro evaluation of the efficacy novel fungicides against *Sclerotium rolfsii*

The data from Table 3 showed that out of the nine fungicides tested, namely captan 70% + hexaconazole 5% WP

Table 1: Evaluation of fungal antagonists (*Trichoderma* spp.) against *S. rolfsii* through dual culture.

Fungal antagonists	Mycelial growth of *Sclerotium rolfsii* (mm) after 96 hrs	Per cent inhibition of mycelial growth of *Sclerotium rolfsii* over control
Trichoderma viride (TV-1)	20.60	75.44 (61.39)
Trichoderma harzianum (TH-1)	28.06	68.81 (56.03)
Trichoderma harzianum (TH-2)	22.33	75.18 (60.10)
Trichoderma harzianum (TH-3)	26.36	70.7 (57.23)
Trichoderma harzianum (TH-4)	25.70	71.43 (57.67)
Trichoderma harzianum (TH-5)	32.17	64.25 (53.26)
Control	90.00	-
C.D.	2.59	2.00
C.V.	4.19	1.93

*Values given in parentheses are Arcsin $\sqrt{}$ transformaion.

(Taquat), penflufen 13.28% w/w + trifloxystrobin (Evergold), hexaconazole 5%SC (Contaf) and tebuconazole 25.9% w/w EC (Folicur), all of them were highly effective at all concentrations. They achieved a 100% inhibition of mycelial growth of *S. rolfsii*. Propiconazole 25% EC (Arihant) demonstrated a 70.92% inhibition of mycelia growth of the pathogen at a concentration of 50 ppm and an 82.96% inhibition at a concentration of 100 ppm. At higher concentrations of 500 and 1000 ppm, it completely inhibited the mycelial growth of the pathogen. Propineb 70 WP (Antracol) showed a lower inhibition rate of 8.51% and 61.66% at concentrations of 50 and 100 ppm respectively, but achieved 100% inhibition at concentrations of 500 and 1000 ppm in the mycelia growth of *S. rolfsii*. The effectiveness of Mancozeb 75% WP (Indofil M-45) in inhibiting the growth of the pathogen's mycelia was found to be the least. It demonstrated inhibition rates of 24.03%, 26.29%, 54.07% and 70.92% at doses of 50, 100, 500 and 1000 ppm, respectively. Nevertheless, the fungicide carbendazim 50% WP (Bavistin) proved to be ineffective in inhibiting the growth of the pathogen's mycelium at all dosages tested. These findings were corroborated by other prior researchers, specifically Arunasri *et al.* (2011), who documented that Triazoles (Hexaconazole, Propiconazole, Difenconazole) had significant efficacy in suppressing the growth of *S. rolfsii*. According to Manu *et al.* (2012), Hexaconazole, Tebuconazole and Propiconazole were found to strongly suppress the growth of *S.rolfsii* isolated from finger millet, even at lower concentrations. According to Das *et al.* (2014), Hexaconazole and Tebuconazole had high efficacy at all concentrations against *S. rolfsii*. Propiconazole showed moderate inhibition, while Thiophanate methyl and Bavistin exhibited the least inhibition.

Determination of tolerance of *Trichoderma harzianum* to novel fungicides

The nine previously mentioned fungicides were assessed at four different concentrations: 50 ppm, 100 ppm, 500 ppm and 1000 ppm (Table 4). The results demonstrated that lower concentrations of fungicides exhibited a reduced inhibitory impact in comparison to larger concentrations. The combination of Carbendazim 12%+ Mancozeb 63% (Saff), Carbendazim (Bavistin), Propiconazole (Arihant), Hexaconazole 5% SC (Contaf plus) and Tebuconazole 25.9% EC (Folicur) completely prevented the growth of *T. harzianum*-2 at all tested concentrations of 50 ppm, 100 ppm, 500 ppm and 1000 ppm. The next fungicide in terms of effectiveness was a combination of Captan 70% + hexaconazole 5% WP (Taquat). This combination inhibited 35.36% and 52.77% of TH-2 growth at concentrations of 50 ppm and 100 ppm, respectively. It completely inhibited the growth of the bio-agent at concentrations of 500 ppm and 1000 ppm. Following this, the fungicide Penflufen 13.28% w/w + Trifloxystrobin (Evergold) inhibited 38.7%, 41.11%, 43.33% and 46.29% of pathogen mycelial growth at concentrations of 50 ppm, 100 ppm, 500 ppm and 1000 ppm, respectively. Propineb (Antracol) was determined to have the lowest effectiveness but was very compatible with *Trichoderma harzianum* isolates. It inhibited the growth of *T. harzianum*-2 by 2.4%, 5.55%, 12.59% and 21.44% at concentrations of 50 ppm, 100 ppm, 500 ppm and 1000 ppm, respectively. According to Bagwan's research in 2010, it was found that thiram (0.2%), copper oxychloride (0.2%) and mancozeb (0.2%) are suitable to be used together with *Trichoderma harzianum*. Rai *et al.* (2016) reported that *Trichoderma harzianum* (Th-14) demonstrated compatibility with Mancozeb and Metalaxyl at low dosages. This study presents facts regarding the compatibility and incompatibility of *Trichoderma harzianum* with fungicides. Dubey *et al.* (2015) have also reported a similar study on the compatibility of *T. harzianum* with fungicides and other agrochemicals.

Evaluation of the effect of different integrated treatments for management of collar rot in pots under net house conditions

The findings from net-house studies (Table 5) carried out in pots with pathogen inoculation during the *Rabi* crop seasons of 2019-20 and 2020-21 shown that all the treatments effectively improved seed germination and decreased the occurrence of collar rot compared to the control group. The seeds treated with Captan 70% WP +

Table 2: Evaluation of fungal antagonists (*Trichoderma* spp.) against *S. rolfsii* through non- volatile technique.

Fungal antagonists	Mycelial growth of *Sclerotium rolfsii* (mm) after 96 hrs	Percent inhibition of mycelial growth of *Sclerotium rolfsii* over control
Trichoderma viride (TV-1)	52	42.22 (40.51)
Trichoderma harzianum (TH-1)	38	57.77 (49.452)
Trichoderma harzianum (TH-2)	34.33	61.85 (51.84)
Trichoderma harzianum (TH-3)	59.83	33.51 (35.36)
Trichoderma harzianum (TH-4)	42.83	52.4 (46.36)
Trichoderma harzianum (TH-5)	69.33	22.95 (28.62)
Control	90	0.00
C.D.	1.68	1.22
C.V.	1.72	1.62

*Values given in parentheses are Arcsine $\sqrt{}$ transformaion.

hexaconazole 5% EC at a rate of 2 g/kg of seed, along with soil application of *Trichoderma harzianum*-2 enriched vermi-compost at 100 g/pot, resulted in the highest germination rate of 100% and the lowest disease incidence of 18.05%. This was followed by seed treatment with Captan 70% WP + Hexaconazole 5% EC at a rate of 2 g/kg of seed, along with soil application of *T. harzianum*-2 enriched FYM at 100 g/pot, which provided a germination rate of 96.33% and an incidence of collar rot of 19.68%. This trend was consistent across both years of experimentation and in the combined data. Nevertheless, the lowest germination rate of 74.33% and the highest disease incidence of 39.06% were observed after applying *T. harzianum*-2 enriched FYM at a rate of 100 g per pot. Veena and Reddy (2016)

Table 3: Evaluation of the efficacy of novel fungicides against *S. rolfsii.*

Common and trade name of fungicides	Doses (ppm)	Radial growth (mm)	Per cent Inhibition over control
Carbendazim 12% + Mancozeb 63% WP (Saff)	50	82.50	8.33(16.69)
	100	67.50	24.98(29.98)
	500	41.50	53.87(47.22)
	1000	0.00	100(90.00)
Captan 70% + Hexaconazole 5% WP (Taquat)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
		1000	0.00
100(90.00)			
Propineb70% WP (Antracol)	50	82.33	8.51(16.93)
	100	34.50	61.66(51.74)
	500	0.00	88.51(78.02)
	1000	0.00	100(90.00)
Carbendazim50% WP (Bavistin)	50	90.00	0.00(0.00)
	100	90.00	0.00(0.00)
	500	90.00	0.00(0.00)
	1000	90.00	0.00(0.00)
Mancozeb75% W.P. (Indofil M-45)	50	70.16	24.03(29.26)
	100	66.33	26.29(30.77)
	500	41.33	54.07(47.33)
	1000	28.66	70.92(57.50)
Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS (Evergold)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Propiconazole25% E.C. (Arihant)	50	26.16	70.92(57.37)
	100	15.33	82.96(65.67)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Hexaconazole5% SC (Contaf plus)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Tebuconazole25.9% w/w EC (Folicur)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Control		90.00	-
CD for fungicide		0.91	3.08
CD for concentration		0.58	2.00
CD for interaction		1.82	6.03

*Values given in parentheses are Arcsin $\sqrt{}$ transformaion.

conducted a study to assess the impact of various organic amendments, such as FYM, vermicompost and neem cake, both individually and in combination with the fungal antagonist Trichoderma isolate, on the root rot of chickpea. They found that treating the seeds with the fungicide Copper oxychloride and applying the potential fungal antagonist *Trichoderma harzianum* to the soil, along with a bacterial biocontrol agent, resulted in the best outcomes. This treatment achieved a germination percentage of 100% and the lowest incidence of disease at 16%. A study conducted by Ahsan *et al.* (2020) found that the most effective method for controlling collar rot in chickpea was the combination of soil application of *Trichoderma harzianum* (10 g/pot) with Carboxin Vitavax seed treatment at a rate of 2 g/kg seed. Combining biocontrol agents (*Trichoderma* spp) with fungicides resulted in significantly improved disease control in various crops (Sugar beetroot, cauliflower and chickpea) compared to using either the biocontrol agent or

Table 4: Evaluation of novel fungicides against bioagent (*Trichoderma harzianum*-2).

Common name of fungicides	Doses (ppm)	Radial growth (mm)	Per cent inhibition over control
Carbendazim 12% + Mancozeb 63% WP (Saff)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Captan 70% + Hexaconazole 5% WP (Taquat)	50	58.16	35.36(36.48)
	100	42.50	52.77(46.58)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Propineb 70% WP (Antracol)	50	87.83	2.40(8.09)
	100	85.00	5.55(13.58)
	500	78.66	12.59(20.77)
	1000	69.5	21.44(27.56)
Carbendazim50% WP (Bavistin)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Mancozeb75% W.P (Indofil M-45)	50	83.00	7.77(16.15)
	100	78.00	18.88(25.74)
	500	73.00	13.33(21.40)
	1000	71.00	21.11(27.34)
Penflufen 13.28% w/w +	50	55.16	38.70(38.46)
Trifloxystrobin 13.28% w/w FS (Evergold)	100	53.00	41.11(39.87)
	500	51.00	43.33(41.16)
	1000	48.33	46.29(42.87)
Propiconazole25% E.C. (Arihant)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Hexaconazole5% SC (Contaf plus)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Tebuconazole25.9% w/w EC (Folicur)	50	0.00	100(90.00)
	100	0.00	100(90.00)
	500	0.00	100(90.00)
	1000	0.00	100(90.00)
Control		90.00	-
CD for fungicide		0.54	0.50
CD for concentration		0.34	0.33
CD for interaction		1.09	1.01

*Values given in parentheses are Arcsin $\sqrt{}$ transformaion.

fungicide alone (Upadhay and Mukhopadhyay, 1986 and Dubey *et al.,* 2015). The current findings are consistent with earlier findings.

Evaluation of the effect of various integrated treatments under field conditions on collar incidence and grain yield

Integrated disease management refers to a compre-hensive approach that incorporates many ways to promote the healthy growth of crop plants, resulting in good yields (Youdeowei, 2004). The utilization of fungicides, bioagents and organic amendments has the potential to decrease disease occurrence and enhance grain production. Utilizing a combination of seed treatment using fungicide and soil application with bioagent (*T. harzianum*-2), along with soil amendment, resulted in a decrease in disease occurrence and an increase in grain yield compared to the control group in both the consecutive years (2019-20 and 2020-21) as well as in the combined data. The most effective treatment among those tested was seed treatment with captan 70% WP + hexaconazole 5% EC (Taquat) combined with soil application of *T. harzianum*-2 enriched vermicompost. This treatment resulted in the lowest disease incidence (11.67%) and the highest grain yield (15.27 q/ha) compared to the control. The second most effective treatment was seed treatment with captan 70% WP + hexaconazole 5% EC combined with soil application of *T. harzianum*-2 enriched FYM, which had a disease incidence of 13.51% and a grain yield of 15.07 q/ha (Table 6). The results showed that the combined use of fungicides as seed treatment and bio-control agents as soil treatment was successful in reducing collar rot disease in chickpea and increasing grain production. This could be attributed to the immediate impact of fungicides and the long-term effectiveness of bio-control agents. In 2013-14 and 2014-15, Singh *et al.* (2017) conducted an experiment to investigate the effectiveness of integrating *Trichoderma, Pseudomonas* and fungicides in controlling collar rot disease in chickpea. The results showed that the treatment that had the greatest effectiveness was the application of *Trichoderma harzianum* enriched FYM at a rate of 8 q/ha^{-1} (Soil) combined with

Table 5: Evaluation of various integrated treatments against collar rot in pots under net house conditions.

Treatments	Seed germination (%)			Collar rot incidence (%)		
	2019-20	2020-21	Pooled	2019-20	2021	Pooled
T_1: Seed treatment with thiram + carbendazim (1:1) @ 2 g/kg seed	82.66 (65.40)	81.60 (64.63)	82.16 (65.01)	28.18 (32.04)	28.84 (32.45)	28.51 (32.25)
T_2: Seed treatment with captan 70% WP + hexaconazole 5% EC @ 2 g/kg seed	86.66 (68.66)	85.33 (67.61)	86 (68.13)	24.48 (29.83)	25.33 (30.14)	25.08 (29.98)
T_3: Seed treatment with *T. harzianum* @ 5 g/kg seed	78.33 (62.28)	80 (63.42)	79.16 (62.85)	29.09 (32.62)	29.42 (32.82)	29.25 (32.72)
T_4: Soil application with *T. harzianum* enriched FYM @ 100 g/pot enriched FYM @ 100 g/pot	73.33 (58.90)	75.33 (60.22)	74.33 (59.56)	38.78 (38.50)	39.33 (38.81)	39.06 (38.65)
T_5: Soil application with *Trichoderma harzianum* enriched vemicompost @ 100 g/pot	76.66 (61.11)	79.66 (63.19)	78.16 (62.14)	35.45 (36.49)	36.12 (36.90)	35.78 (36.70)
T_6: Seed treatment with captan 70% WP + hexaconazole 5% EC @ 2 g/kg seed + soil application with *T. harzianum* enriched FYM @ 100 g/pot	96.00 (78.68)	96.66 (79.56)	96.33 (79.10)	19.45 (26.15)	19.91 (26.48)	19.68 (26.31)
T_7: Seed treatment with captan 70% WP + hexaconazole 5% EC 2 g/kg seed + soil application with *T. harzianum* enriched vermicompost @ 100 g/pot	100.00 (90.00)	100 (90.00)	100 (90.00)	17.77 (24.91)	18.33 (25.34)	18.05 (25.12)
T_8: Seed treatment with *T. harzianum* seed @ 5 g/kg seed + soil application with *T. harzianum* enriched FYM @ 100 g/pot	83.66 (66.76)	84.66 (66.95)	84.16 (66.56)	24.84 (29.83)	25.5 (30.29)	25.17 (30.06)
T_9: Seed treatment with *T. harzianum* @ 5 g/kg seed + soil application with *T. harzianum* enriched vermicompost @ 100 g/pot	90.00 (71.66)	91 (72.64)	90.5 (72.14)	22.82 (28.47)	23.25 (28.77)	23.2 (28.74)
T_{10}: Control	56.66 (48.82)	55.33 (48.05)	56 (48.43)	77.85 (62.03)	78.66 (62.16)	77.92 (62.09)
C.D.	3.92	3.75	3.78	4.01	4.00	3.96
C.V.	3.40	3.23		6.87	6.79	6.74

*Values given in parentheses are Arcsin $\sqrt{}$ transformaion.

Table 6: Evaluation of various integrated treatments under field conditions on collar incidence and grain yield.

Treatments	Collar rot incidence (%)			Grain yield q/ha		
	(2019-20)	(2020-21)	Pooled	(2019-20)	(2020-21)	Pooled
T_1: Seed treatment with thiram + carbendazim (1:1) @ 2 g/kg seed	18.88 (25.72)	19.58 (26.25)	19.23 (25.99)	13.56	14.00	13.78
T_2: Seed treatment with captan 70% WP + hexaconazole 5% EC @ 2 g/kg seed	16.26 (23.76)	17.73 (24.88)	16.99 (24.33)	14.03	14.50	14.26
T_3: Seed treatment with *T. harzianum* @ 5 g/kg seed	23.60 (29.06)	25.15 (30.08)	24.37 (29.57)	11.7	12.00	11.85
T_4: Soil application with *T. harzianum* enriched FYM @100 g/m^2	28.55 (32.28)	30.21 (33.32)	29.38 (32.80)	10.16	10.55	10.35
T_5: Soil application with *Trichoderma harzianum* enriched vermicompost @100 g/m^2	28.33 (31.24)	27.61 (32.14)	26.90 (31.68)	11.15	11.13	11.14
T_6: Seed treatment with captan 70% WP + hexaconazole 5%EC @ 2 g/kg seed + soil application with *T. harzianum* enriched FYM @100 g/m^2	13.02 (21.11)	14.00 (21.96)	13.51 (21.55)	14.64	15.50	15.07
T_7: Seed treatment with captan 70% WP + hexaconazole 5% EC 2 g/kg seed + soil application with *T. harzianum* enriched Vermicompost @ 100 g/m^2	11.06 (19.36)	12.28 (20.48)	11.67 (19.96)	14.8	15.75	15.27
T_8: Seed treatment with *T. harzianum* seed @ 5 g/kg seed + soil application with *T. harzianum* enriched FYM @ 100 g/m^2	21.12 (27.35)	21.40 (27.54)	21.26 (27.44)	12.46	13.45	12.96
T_9: Seed treatment with *T. harzianum* @ 5 g/kg seed + soil application with *T. harzianum* enriched vermicompost @100 g/m^2	19.78 (26.39)	20.72 (27.05)	20.25 (26.72)	13.26	13.54	13.4
T_{10}: Control	40.58 (39.55)	41.33 (39.99)	40.96 (39.77)	8.86	9.44	9.12
C.D.	2.19	1.37	0.98	0.80	1.39	0.78
C.V.	4.59	2.81	0.32	3.72	5.83	3.50

*Values given in parentheses are Arcsin $\sqrt{}$ transformaion.

Hexaconazole at a rate of 3 ml/kg^{-1} seed. This treatment resulted in the lowest mortality rate (4.30% and 2.25%) and the biggest increase in grain production (44.85%). Pandey *et al.* (2017) found that applying *Trichoderma viride* or *T. harzianum* (2 ×10^8 cfu/g) enriched FYM (10 kg bioagent/ tonne FYM) to the soil in furrows at a rate of 1 tonne/ ha, followed by soaking chickpea seeds in a suspension of talc-based formulation 1% WP (2 ×10^8 cfu/g) of *T. viride* or *T. harzianum* for 10 hours at a rate of 50 g product/ 250 ml of water/kg seed and shade drying, was effective in managing wilt and root rot complex. The conclusions of prior researchers strongly corroborate our findings.

Conclusion

The present study's results indicated that it provided information on the effectiveness and compatibility of fungal antagonists (*Trichoderma* spp.) and new fungicides. The module comprises the formulation of a putative native bioagent, *Trichoderma harzianum*-2, along with organic amendments such as vermicompost, farmyard manure (FYM) and a fungicide. The formulation of Captan 70% WP + Hexaconazole 5% EC has been specifically designed to effectively control collar rot and maximize the grain yield of chickpea through integrated management techniques.

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Authors contribution

Arvind Kumar: Investigation, data curation, formal analysis and draft preparation. Vivek Singh: designed the research and experiments, supervision, original draft preparation, review and editing. Harshita: draft preparation, formal analysis, review and editing. Girijesh Jaisval: data curation, draft preparation, formal analysis. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of interest

On behalf of all the authors, I wish to confirm that there is no conflict of interest in the publication of this manuscript.

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