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Efficacy of bio-agents and fungicides against *Sclerotium rolfsii* Sacc. Causing collar rot in chickpea (*Cicer arietinum* L.) *In-vitro*

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Abstract
In the present investigation, nine commercially available fungicides were tested for their efficacy in inhibiting the growth of the pathogen *in vitro.* 100 per cent inhibition of mycelial growth of *Sclerotium rolfsii* was found in the treatment with Cymoxanil 8%+ Mancozeb 64%, Thifluzamide 24%SC, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 5% SC, Propiconazole 13.9% + Difenconazole13.9% EC at all three concentrations 50ppm, 100ppm and 150ppm followed by 50.00%, 77.77% and 100% in Famoxadone16.6% + Cymoxanil 22% SC. Minimum 33.33% inhibition of mycelial was found in Thiram at 150ppm concentration. Different potent bio- control agents were evaluated for their antagonistic effect against *Sclerotium rolfsii.* The maximum mycelial growth inhibition of *Sclerotium rolfsii* was recorded with *Chaetomium globosum* 82.6% after 96 hours, which is significantly superior from all the tested bio-agents followed by *Pseudomonas fluorescence* (76.3%), *Trichoderma viridae* (72.22%) and *Trichoderma harzianum* (71.48%). While least mycelial growth inhibition was recorded with *Bacillus subtilis* (71.11%).

Keywords: *Sclerotium rolfsii,* bio-agents and mycelial

Introduction
Chickpea (*Cicer arietinum* L.) is the premier pulse crop grown in more than 50 countries originated in south west Asia and cultivated from ancient times both in Asia and European countries. Chickpea is a major pulse crop of India accounting for more than 40% of the total pulses area and production. From a mere 3.86 million tonnes (mt) during 2000-01, chickpea production rose steadily to an all- time high of 11.23 mt during 2017–18 (Dixit *et al.* 2019) [4]. In India chickpea is grown on an area of 105.73 lakh hectares with a production of 111.18 lakh tons and productivity 1056 kg/ha (anonymous 2018) [1].

Chickpea is not only good for human health but also for soil health. It means 80% of its nitrogen (N) requirement are met from symbiotic rhizoibial interactions. It leaves substantial amount of residual nitrogen behind for subsequent crops and adds much needed organic matter to maintain and improve soil health. It has long-term fertility and sustainability of the ecosystems, boon to the resource-poor marginal farmers in the tropics (Saraf *et al.*, 1998) [14].

The chickpea crop is attacked by nearly 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 Nematodes and Mycoplasma) from all over the world (Nene *et al.* 1996) [12]. Among all, only a few of them have the potential to devastate the crops. Some of the serious diseases in order of their importance are wilt (*Fusarium oxysborum f. sp. ciceri),* wet root rot (*Rhizoctonia solani),* dry root rot (*Rhizoctonia bataticola),* Ascochyta blight (*Ascocthya rabiei)* and collar rot *(Sclerotium rolfsi).* Among these, Collar rot caused by *Sclerotium rolfsii* Sacc. is gaining importance. This disease is a serious threat, which under favorable conditions causes 55-95% mortality of the crop at seedling stage (Gurha and Dubey, 1982) [5].

Collar rot is a fast spreading and destructive disease of chickpea. It causes significant losses in yield where ever the crop is grown under environmental conditions favorable for its development. This soil-borne pathogen causes rot of collar region on a wide range of plant species belonging to families Compositae and Leguminosae where as members of Gramineae are less susceptible to this disease (Mahen *et al.*, 1995) [7]. The most common hosts are legumes, crucifers, vegetables and cucurbits. Seedling mortality from 54.7 to 95.0% in chickpea due to infection of *S. rolfsii* has been reported (Mathur & Sinha 1968, 1970 and Kotastthane *et al.*, 1976) [8, 9, 6]. Control of soil borne pathogens has become one of the major concerns in agriculture.

However effective and efficient management of crop diseases is generally achieved by the use of synthetic pesticides. These pesticides are known to pollute the environment, soil and water besides causing deleterious effects on human health and biosphere. In the present investigation is related to the study of the management of collar rot disease of chickpea by different fungicides, bioagents and comparing the efficacy of both.

Material and Methods

Isolation, purification and identification of antagonistic microorganisms

Isolation of bio-agents was done by serial dilutions technique. 10 gm of rhizospheric soil was dissolved in 100 ml of sterile distilled water to get 10^{-1} dilution. 1 ml of soil suspension was taken from stock and added to 9 ml of sterile distilled water to get 10^{-2} dilution. This is further repeated until a final dilution of 10^{-7} was obtained. 1 ml of each soil suspension was poured in sterilized petriplates containing nutrient medium and incubated at 25 ± 1°C and observed at frequent intervals for the development of colonies of bio-agents. Bio-agents were identified based on cultural and mycological characters described by Barnett and Hunter.

Isolation and purification of the pathogen

The diseased plant showing the symptoms were washed thoroughly with tap water, small pieces from infected parts 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with the help of sterilized blade. These pieces were surface sterilized with 1% sodium hypochlorite solution for 30 seconds and finally washed well in three changes of sterilized distilled water to remove trace of sodium hypochlorite. The pieces were then transferred aseptically to petri plates containing Potato Dextrose Agar. Inoculated petri plates were incubated at 26 ±1ºC for three to five days and examined at frequently intervals to see the growth of the fungus. The pure colonies were picked and inoculate in culture tube for further research work.

Dual culture technique

Antagonistic activities of bio control agent were tested against soil borne pathogen *Sclerotium rolfsii* by employing dual culture techniques of Morton and Stroube (1955) [10] on PDA. A mycelial disc (5 mm.), obtained from the peripheral region of 5-7 day old culture of pathogen on PDA, was placed on fresh PDA plate (3 cm from centre) then a 5 mm mycelial disc, obtained from the periphery of a 5-7 day old culture of fungal bio agents were placed 3cm away from the inoculum of the pathogen, for bacterial bio agents were streaked 3 cm away from the inoculum of the pathogen. Three replication of each treatment were maintained with one control set without inoculating the bio inoculants. Then the plates were incubated at 26+1 ^{0}C. At the end of incubation period, radial growth was measured. The inoculated plates with culture discs of pathogen without bioagents served as control. After 48, 96 and 144 hrs of incubation at 26+1 ^{0}C, radial growth of pathogen and percent inhibition was recorded.

Poisoned food technique

Bioassay test of fungicides were done by poisoned food technique (Schmitz, 1930) [15] in the laboratory to find out the toxicity fungi of different fungicides against the pathogen. The efficacy of nine systemic and non-systemic fungicides was assayed against pathogen at different concentrations. The required concentrations of chemicals were prepared and incorporated into sterilized, cooled Potato Dextrose Agar. 20 ml of medium was poured into 90 mm sterilized petri plates and all plates were inoculated with actively growing 5 mm mycelial disc of *Sclerotium rolfsii* three replications were maintained for each treatment. These plates were incubated at 26±1°C for five days in an incubator and colony diameter was recorded.

Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947) [47].

$$\% \text{ inhibition of mycelial growth} = \frac{C-T}{C} \times 100$$

Where,
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Result and Discussion

Effect of bio-control agents against *Sclerotium rolfsii*

Five bio-agents were evaluated for their efficacy against *Sclerotium rolfsii* through dual culture technique as explained in material and method. The result revealed that there was significant mycelial growth inhibition of *Sclerotium rolfsii* by all the tested bio agents. The maximum inhibition of *Sclerotium rolfsii* was recorded with *Chaetomium globosum* 82.6% after 96 hours, which is significantly superior from all the tested bio-agents followed by *Pseudomonas fluorescence* 76.3%, *Trichoderma viridae* 72.22% and *Trichoderma harzianum* 71.48%. While least mycelial growth inhibition was recorded with *Bacillus subtilis* 71.11%. Similarly, Uikey *et al.* (2019) [16] was also reported, maximum inhibition (46.66%) of *Sclerotium spp.* in treatment *Chaetomium*. Banakar *et al.* (2017) [2] was observed similar result efficacy of five bio-agents and observed that *Trichoderma viride* and *Trichoderma harzianum* recorded 61% and 44% inhibition respectively.

Table 1: Effect of Bio-agents on radial growth of *Sclerotium rolfsii in vitro.*

Name of Antagonist	48 hrs		72 hours		96 hours	
	Mycelial growth (mm)	% Inhibition	Mycelial growth (mm)	% Inhibition	Mycelial growth (mm)	% Inhibition
Trichoderma viride	23.00	66.00	28.00	68.88	25.00	72.22
Trichoderma harzianum	23.50	65.26	28.66	68.15	25.66	71.48
Chaetomium globosum	14.00	79.30	19.00	78.88	15.66	82.6
Bacillus subtilis	25.00	63.05	30.00	66.66	26.00	71.11
Pseudomonas florescence	21.00	68.96	25.00	72.22	21.33	76.3
Control	67.66	0.00	90.00	0.00	90.00	0.00
C.D.$_{(0.05)}$	2.213		2.360		1.272	
S.E.(m)	0.710		0.758		0.408	

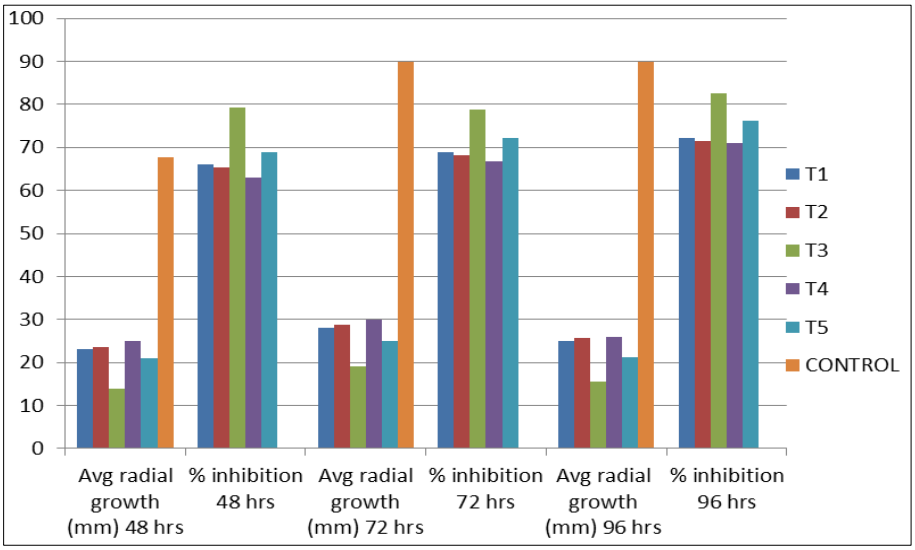

Fig 1: Effect of Bio-agents on radial growth of *Sclerotium rolsii.*

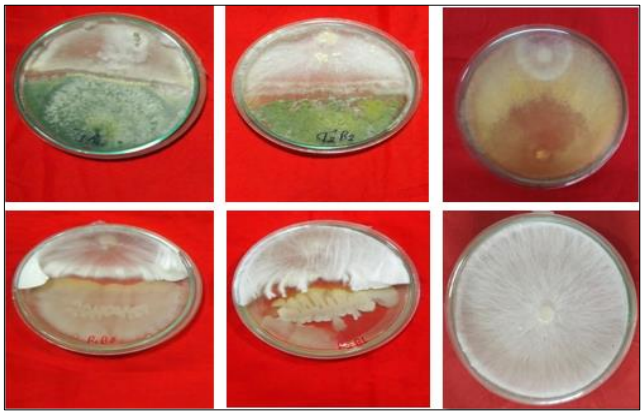

Plate 1: Efficacy of bio-agents on mycelial growth of *Sclerotium rolfsii*

Efficacy of fungicides on inhibiting mycelial growth of *Sclerotium rolfsii*

The results thus obtained have been presented in Table-2 and Fig-2. The results revealed that there is significant difference in per cent inhibition of mycelial growth of *Sclerotium rolfsii* with the fungicides which were tested. 100 per cent inhibition of mycelial growth of *Sclerotium rolfsii* was found in the treatment with Cymoxanil 8% + Mancozeb 64%, Thifluzamide 24% SC, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 5% SC, Propiconazole 13.9% + Difenconazole13.9% EC inhibition of mycelial growth of *Sclerotium rolfsii* was at all three concentrations (50ppm, 100ppm and 150ppm) followed by (50.00%, 77.77% and 100%) in Famoxadone16.6% + Cymoxanil 22% SC and (0.00%, 0.00% and 33.33%) in Thiram at all three concentrations (50ppm, 100ppm and 150ppm) respectively. The 0 per cent of mycelial growth was recorded in Chlorothalonil 75%WP followed by (0.00%, 0.00% and 24.44%) in Copper hydroxide 46.1% DF at all three concentrations (50ppm, 100ppm and 150ppm) after 144 hrs. incubation respectively. Similarly the result was more or less in agreement with Rajendra Prasad *et al.* (2017) [13] where Cymoxanil + Mancozeb and Tebuconazole + Trifloxysrobin recorded maximum inhibition (100%) of *S. rolfsii.* Begum *et al.* tested the efficacy of eight fungicides and found that Propiconazole, Hexaconazole and Carbendazim were effective in controlling the growth of *S. rolfsii* at higher concentrations. Fungicides that are toxic to fungi may cause mycelium to cease growing, change metabolic processes or be killed, spores may fail to germinate or be killed (Neely, 1969) [11].

Table 2: Effect of different fungicides on the mycelial growth of *Sclerotium rolfsii* after 144 hours at different concentrations. *In vitro*

Treatments	Concentration					
	50 ppm		100 ppm		150 ppm	
	Average Mycelial Growth of pathogen (mm)	% Inhibition over control	Avg Mycelial Growth of pathogen (mm)	% Inhibition over control	Avg Mycelial Growth of pathogen (mm)	% Inhibition Over control
Thiram (T1)	90.00	0.00	90.00	0.00	60.00	33.33
Famoxadone16.6% + Cymoxanil 22% SC (T2)	45.00	50	20.00	77.77	0.00	100
Cymoxanil 8% + Mancozeb 64% (T3)	0.00	100	0.00	100	0.00	100
Thifluzamide 24%SC (T4)	0.00	100	0.00	100	0.00	100
Tebuconazole 50% + Trifloxystrobin 255 WG (T5)	0.00	100	0.00	100	0.00	100
Hexaconazole (T6)	0.00	100	0.00	100	0.00	100
Propiconazole 13.9% + Difenconazole 13.9%EC (T7)	0.00	100	0.00	100	0.00	100
Chlorothalonil (T8)	90.00	0.00	90.00	0.00	90.00	0.00
Copperhydroxide(T9)	90.00	0.00	90.00	0.00	68.00	24.44
Control (T10)	90.00	0.00	90.00	0.00	90.00	0.00
SE(m)	0.183		0.365		1.033	
CD (5%)	0.542		1.085		3.068	

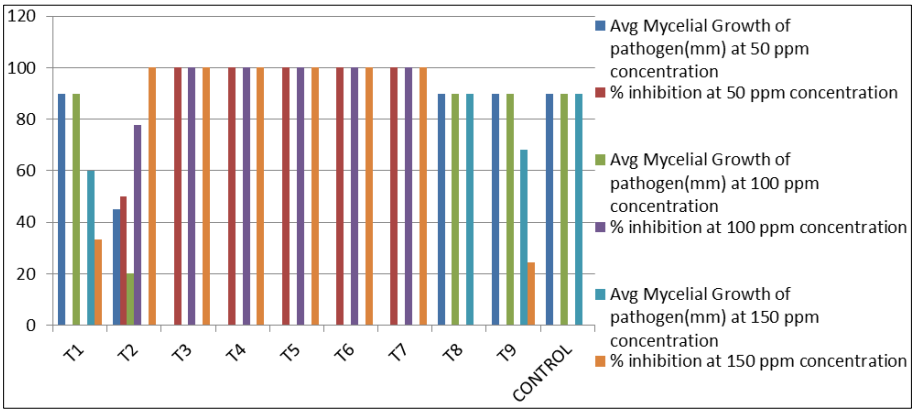

Fig 2: Effect of fungicides on mycelial growth of *Sclerotium rolfsii*

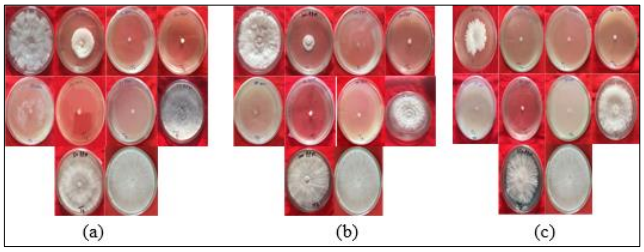

Plate 2: Efficacy of different fungicide (a) at 50 ppm concentration, (b) at 100 ppm concentration (c) at 150 ppm concentration, inhibition on mycelial growth of *Sclerotium rolfsii*.

Conclusion

Chaetomium globosum, *Pseudomonas fluorescence*, *Trichoderma viridae* and *Trichoderma harzianum* have capability to inhibit the mycelial growth of pathogen by competition, parasitism and antibiosis mechanisms.

In the fungicide, Cymoxanil 8% + Mancozeb 64%, Thifluzamide 24% SC, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 5% SC, Propiconazole 13.9% + Difenconazole 13.9% EC are most effective fungicide for inhibition of mycelial growth of pathogen. The results reported here suggest that above bio-agents and fungicide were more capable of influencing the growth of pathogens in dual culture under controlled condition, and may be used as broad spectrum under field condition.

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