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Bio-Rational Management of Collar Rot of Sunflower Incited by *Sclerotium rolfsii* Sacc.

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ABSTRACT

The present study was carried out under *in vitro* conditions in the Department of Plant Pathology, CCS Haryana Agricultural University, Hisar to test the efficacy of different non-conventional chemicals, botanicals and bio-agents against *Sclerotium rolfsii.* The non-conventional chemicals and botanicals were tested using the poison food technique and bioagents were tested using dual culture technique under *in vitro* conditions. Efficacy of non-conventional chemicals showed that salicylic acid, acetyl salicylic acid and indole butyric acid completely inhibited mycelial growth up to 100% at 200 ppm concentration. Indole acetic acid was found least effective among all the non-conventional chemicals as this chemical inhibited only 21.9% of mycelial growth even at 200 ppm concentration. Among four botanicals *Azadirachta indica* was found most effective with 59.5% mycelial growth inhibition at 20% followed by *Cannabis sativa* and *Pongamia pinnata*. *Eucalyptus* spp. found least effective in inhibition of mycelium growth (4.7%) even at 20% concentration. Botanical also effective in reducing the sclerotial formation of *S. rolfsii*. Minimum number of sclerotia 433.7 and 247.5 were also formed at 5% and 20% concentration with *Azadirachta indica* as compare to other treatments. Among bio-agents *Trichoderma harzianum* showed maximum antifungal activity with 85.6% inhibition of mycelial growth followed by *T. harzianum* (Darjeeling isolate) and *T. viride*. *Coniothyrium* sp. found least effective in inhibition mycelial growth. The finding suggested that bio-rational components help to reduce the growth of *S. rolfsii* significantly. These bio-rational components are environmentally safe and economically profitable for cultivation of sunflower and they can replace the use of fungicides for management of *S. rolfsii*.

Keywords *Sclerotium rolfsii*, Sunflower, Non-conventional chemicals, Botanicals, Bio-agents.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the major oilseed crops which ranked third after soyabean and groundnut in the world. India accounts for 10% of total world production with the production of 0.216 million tonnes and 0.262 million hectares area (Indiastat 2019). In spite of significant increase in the production and productivity, a huge gap exists between the potential yield and actual yield at the farmer's

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Plate 1. Pure culture of *S.rolfsii* in Petri plates.

field, which is mainly due to biotic and abiotic stresses. Biotic stresses mainly include the damage caused by the living organism like bacteria, fungi, viruses, phanerogamic parasites. Sunflower crop is attacked severely by various kind of fungi, bacteria, viruses and phytoplasma. Among them, it suffers extensive losses from the fungal pathogen *Sclerotium rolfsii* Sacc., which causes collar rot disease. *Sclerotium rolfsii* Sacc., is a very devastating soil borne pathogen which has wide host range (500 plant species) and is distributed world-wide. There are several reports on incidence of collar rot by *S. rolfsii* on many dicots and monocots plant species (Mordue 1974). This pathogen survives through sclerotia which are the extremely hard and dormant structures (Singh *et al.* 2003). Despite decades of research, management attempts have generally met with little success due to the pathogen's wide host range, extensive growth and production of enormous numbers of soil borne sclerotia that can remain in soil for several years. The bioagents like *Trichoderma hazianum*, *T. viride, Bacillus subtilis*, *Pseudomonas flourescens*, botanicals with neem, *Pongamia pinnata, Eukalyptus* spp. and many fungicides have been used extensively against *S. rolfsii* that gave the promising results (Punja 1985, Anahosur 1999, Sarma *et al.* 2002, Singh *et al*. 2003). Under field condition, the bioagents *T. viride*, *P. fluorescens, B. subtilis* and *S. cerevisiae* was found most effective in controlling the pre and post emergence damping off of bean seedling (Eid 2014). Natural occurring plants have antimicrobial properties and proved to be as good substitute for synthetic pesticides

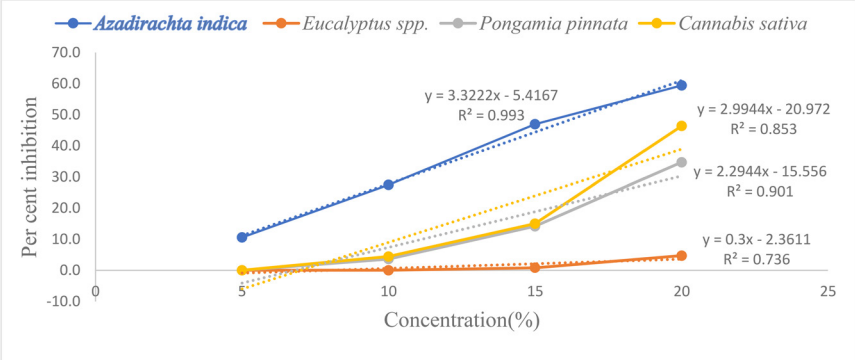

Fig. 1. Functional relationship between concentration of botanicals and per cent inhibition of mycelial growth.

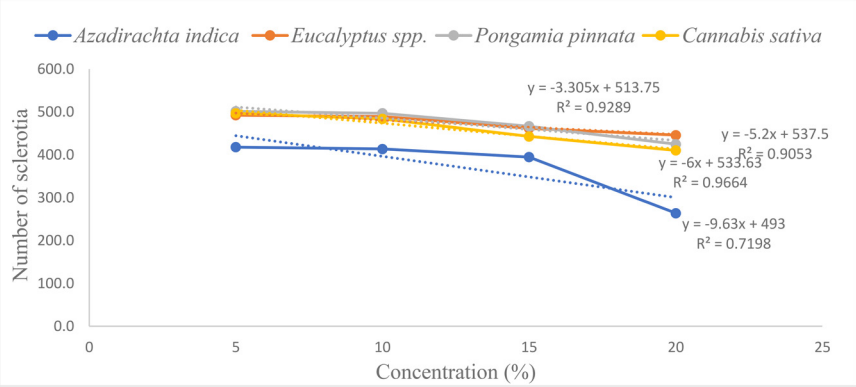

Fig. 2. Functional relationship between concentration of botanicals and average number of sclerotia.

(Sajeena 2019). Fungicides application demonstrated encouraging results in reducing the disease, but their widespread usage is prevented by fungicidal residue and phytotoxicity, as well as their environmental pollution and human health risks. As a result, for the environment safety and economically profitable agriculture the fungicides are replaced with bio-rational/ or products. Now a days new group of chemicals called non-conventional chemicals came in existence that increase the plant resistance to the pathogen by activating the systemic acquired resistance (SAR) in the plant. In this context, the present investigation was carried out to evaluate the bio-rational components and non-conventional chemicals for the management of collar rot of sunflower.

MATERIALS AND METHODS

Experimental Site

The crop was sown during the *rabi* season on 10th February 2020 and harvested on 11th May 2020 at the Research Field, Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (29º 10´N latitude, 75º 46´E longitudes). Disease samples were collected from infected sunflower plant and experiments was performed in the laboratory of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar.

Isolation of fungus

The sclerotia of *S. rolfsii* were collected from infect plants in the field. Sclerotia were washed with running tap water and sterilized with mercuric chloride (0.1%) solution for 30 seconds and washed thoroughly in sterile distilled water for three times and aseptically transferred to sterilized PDA media containing Petri plates in laminar air flow chamber and were incubated at 25 ± 1ºC for mycelial growth of fungus. The pure culture of the fungus thus obtained were used in the experiments by further subculturing (Plate 1).

Table 1. Effect of different botanicals on mycelial growth of *S. rolfsii.* Mean ± SEM of four replications. Duncan's multiple range test (DMRT) was followed, mean with the same letters are not significant.

Botanicals	Inhibition percentage at different concentration			
	5%	10%	15%	20%
Azadirachta indica	10.6 ± 1.15^{f}	27.5 ± 2.29^{d}	46.9 ± 1.24^{b}	59.4 ± .071^{a}
Eucalyptus spp.	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}	0.8 ± 0.53^{hi}	4.7 ± 0.96^{g}
Pongamia pinnata	0.0 ± 0.0^{i}	3.6 ± 0.85^{gh}	14.2 ± 1.40^{e}	34.7 ± 0.85^{c}
Cannabis sativa	0.0 ± 0.0^{i}	4.4 ± 1.03^{g}	15.0 ± .074^{e}	46.4 ± 0.96^{b}

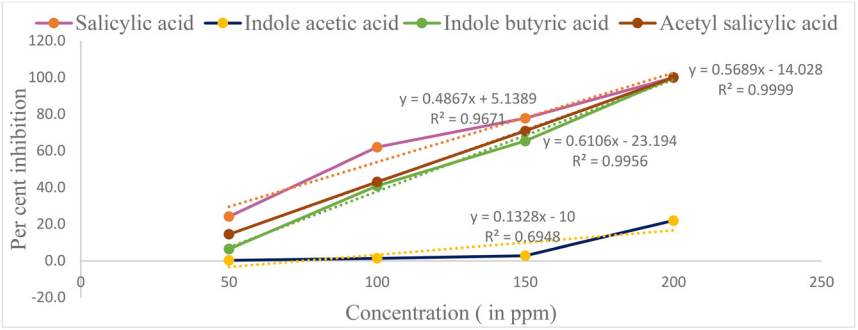

Fig. 3. Functional relationship between concentration of non-conventional chemicals and per cent inhibition of mycelial growth.

In vitro evaluation of non-conventional chemicals

Four non-conventional chemicals viz., salicylic acid, acetyl salicylic acid, indole acetic acid and indole butyric acid each with four different concentration levels (50, 100, 150, 200 ppm) were evaluated against *S. rolfsii* under *in vitro* using standard procedure of poisoned food technique as given by Mayer (1962). Stock solution of each non-conventional chemical was prepared in double concentration i.e., 100, 200, 300 and 400 ppm in a measured volume of sterilized distilled water. The double strength potato dextrose agar medium was also prepared at 121.6 temperature and 15 psi pressure for 20 minutes. An equal volume of non-conventional chemicals and PDA medium was mixed and poured in Petri dishes in laminar air flow chamber. Each Petri plate was centrally inoculated with 5 mm mycelial bit from 7 days old culture of *S. rolfsii* and incubated at 25 ± 1°C with suitable control. Each chemical was replicated four times with and CRD experimental design. Mycelial growth (radial) was measured after 7 days of inoculation.

Per cent growth inhibition was calculated by using the formula of Vincent (1947).

$$GI\ (\%) = \frac{(C-T)}{C} \times 100$$

Where,
GI= Per cent growth inhibition
C = Radial growth of *S. rolfsii* in control
T = Radial growth of *S. rolfsii* in treatment.

In vitro evaluation of botanicals

The effect of four plants extracts viz., *Eucalyptus* spp., *Azadirachta indica*, *Pongamia pinnata* and *Cannabis sativa* leaves were evaluated under *in vitro* by poisoned food technique against *S. rolfsii.* The

Plate 2. Growth inhibition by *Azadirachta indica*.

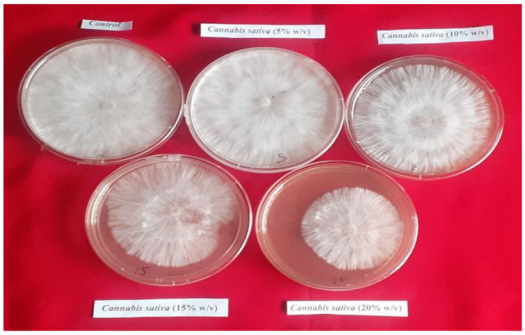

Plate 3. Growth inhibition by *Cannabis sativa*.

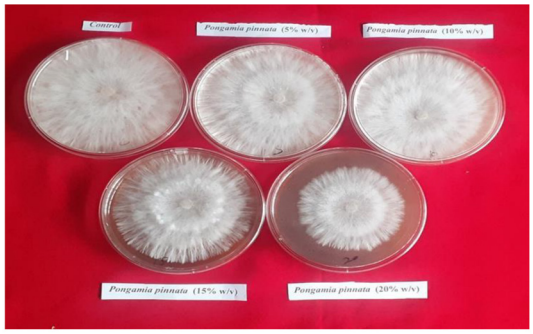

Plate 4. Growth inhibition by *Pongamia pinnata*.

Plate 6. Growth inhibition by salicylic acid.

fresh leaves of respective plants were collected and washed thoroughly with distilled water after that the leaves were macerated separately in blender with appropriate volume of water (1:1 w/v). The macerated material was passed through double fold muslin cloth and the filtrate was filtered through Whatman filter paper No.1. The aqueous extracts collected were subjected to filter sterilization. The filtrates were centrifuged at 4000-6000 rpm for 20 minutes and supernatant obtained were filtered through Millipore filter attached to a glass syringe (5 ml) and collected in sterile screw cap vials for further use. The crude plant extracts served as 100% concentration. Stock solution of each plant extract in double concentrations (10, 20, 30 and 40 %) was prepared by dissolving in a measured volume of sterilized distilled water. The double strength potato dextrose agar medium was also prepared and sterilized at 121.6 temperature and 15 psi pressure for 20 minutes. An equal volume of plant extract and medium was mixed and pour aseptically in the Petri dishes. After solidification of the medium each Petri dish was centrally inoculated with 5 mm disc of fungus from 7 days old pure culture of *S. rolfsii* with the help of sterilized cork borer and incubated at 25 ± 1°C. Suitable controls were also maintained. Each chemical was replicated four times with and CRD experimental design. Per cent growth inhibition was calculated by using the formula given by Vincent (1947). Sclerotial formation per plate in each treatment was also recorded.

In vitro evaluation of bio-agents

Efficacy of bio-agents viz., *Trichoderma harzianum, Trichoderma viride*, *Coniothyrium* sp., *Gliocladium virens* and *Pseudomonas fluorescens* were evaluated against *S. rolfsii* by dual culture technique under *in vitro* conditions. Mycelial discs (5 mm) of *S. rolfsii* and fungal bioagents from 3 to 4 days old pure culture were transferred simultaneously on the periphery,

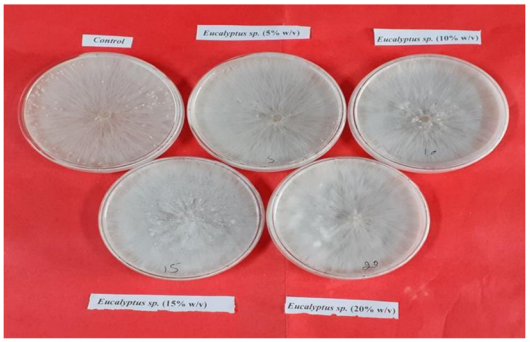

Plate 5. Growth inhibition by *Eucalyptus* spp.

Plate 7. Growth inhibition by acetyl salicylic acid.

Plate 8. Growth inhibition by Indole butyric acid.

Plate 10. Growth inhibition by *T. harzianum*.

about 1cm from the edges of the Petri plates (9 cm diameter) at opposite sides. The streaking (2-3) of *Pseudomonas fluorescens* was done with the help of inoculating loop on opposite side of the mycelium disc of *S. rolfsii* on media. The fungus was cultured on the PDA while the bacteria on NA (Nutrient agar) media while the antagonistic effects of the bio-agents were tested on PDA. Control plate was maintained by inoculating only with mycelial disc of *S. rolfsii.* Inoculated plates were incubated at 25 ± 1 in BOD incubator. Observations on growth of the pathogen were measured and inhibition percentage was calculated by comparing with control plate. Inhibition percentage was calculated by using the Vincent (1947) formula.

Statistical analysis

Data obtained on various Lab experiments were assessed using Duncan's multiple range test (DMRT) at $p < 0.5$.

RESULTS AND DISCUSSION

Effect of botanicals on mycelial growth and sclerotial production of the *S. rolfsii* at different concentration

In the present study, four botanicals were evaluated *in vitro* against *S. rolfsii* in order to observe the effectiveness against the pathogen. All the botanicals have significant effect against the *S. rolfsii* in term of mycelial as well as sclerotial inhibition. The inhibition of mycelial growth was significantly increase with increase in the concentration of different botanicals. Regression equations (Fig. 1) revealed that 99%, 85%, 90% and 73% of the variation in increased per cent inhibition of mycelial growth could be explained by

Plate 9. Growth inhibition by indole acetic acid.

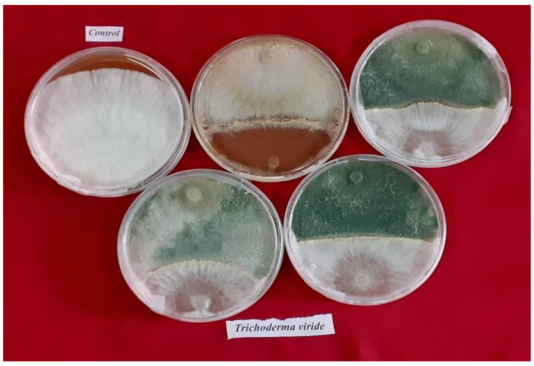

Plate 11. Growth inhibition by *T. viride.*

Plate 12. Growth inhibition by *P. fluorescens.*

Plate 14. Growth inhibition by *Coniothyrium sp.*

the increase in concentration of *Azadirachta indica*, *Cannabis sativa, Pongamia pinnata* and *Eucalyptus* spp., respectively. *Azadirachta indica* found most effective among all the botanicals (Plate 2). From the Table 1 maximum mycelial inhibition (59.4%) was observed in case of *Azadirachta indica* at 20% concentration followed by *Azadirachta indica* (46.9%) at 15% and *Cannabis sativa* (46.4%) (Plate 3) at 20% concentration. *Pongamia pinnata* inhibited 34.6% mycelial growth at 20% concentration (Plate 4). *Eucalyptus* spp. found least effective among all the botanicals even at the highest concentration (Plate 5).

In case of the sclerotial formation, average number of sclerotia decrease with increase in the botanical's concentration. Regression equations (Fig. 2) revealed that 71%, 96%, 90% and 92% of the variation in decreased average number of sclerotia could be explained by the increase in concentration of *Azadirachta indica*, *Cannabis sativa, Pongamia pinnata* and *Eucalyptus* spp., respectively. Minimum number of sclerotia (263.8) were produced at 20% concentration of the *Azadirachta indica* which were significantly different from the control (521) (Table 2). All other treatment of *Azadirachta indica*, *Cannabis sativa, Pongamia pinnata* and *Eucalyptus* spp., were found at par with control. Similar result was reported by Mundhe *et al* (2009), they observed that maximum per cent mycelial inhibition was due to Sarpagandha (87.7%) followed by Neem (85.5%). However, Glyricidia (25.22%), Tulsi (24.44%), Karanj (14.11%) and Bougainvillea (12.22%) were found less effective. Plant extract reduced the mycelium growth as well as the sclerotial production of *S. rolfsii* under *in vitro* conditions (Singh and Dwevedi 1989, Bhaskar and Ali 2005). Different plants can be used as botanicals in the form of extracts, gum, resins, essentials oils that have bioactivity against the various plant pathogens (Romanazzi *et al.* 2012). These plants have the ability to produce secondary metabolites like phenols, quinones, flavones, flavonoids, tannis and coumarins that shows the antimicrobial activities (Ngumah, 2012, Aidah *et al*. 2014). Neem contains approximately 18 bioactive compounds among them azadirachtin found most effective antimicrobial secondary metabolite (Silva-Aguayo 2013).

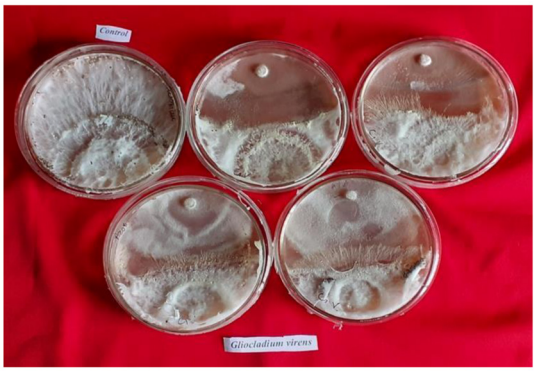

Plate 13. Growth inhibition by *Glocladium virens.*

Effect of non-conventional chemicals on mycelial growth of the *S. rolfsii* at different concentration

Four non-conventional chemicals have been evaluated against the *S. rolfsii* under in vitro condition. All the test non-conventional chemicals found effective against the *S. rolfsii*. Regression equation (Fig. 3)

Table 2. Effect of different botanicals on sclerotial formation of *S. rolfsii*. Mean ± SEM of four replications Duncan's multiple range test (DMRT) was followed, mean with the same letters are not significant.

Botanicals	Average number of sclerotia at different concentration 5%	10%	15%	20%	Control
Azadirachta indica	418 ± 91.6^{ab}	413.8 ± 79.8^{ab}	395 ± 62.7^{ab}	263.8 ± 21.9^{b}	
Eucalyptus spp.	492.3 ± 28.6^{a}	489.3 ± 11.8^{a}	462 ±10.6^{a}	446.3 ± 24.0^{a}	521 ± 5.7a
Pongamia pinnata	501.5 ± 23.6^{a}	497 ± 52.2^{a}	466.5 ± 39.3^{a}	425 ± 35.6^{a}	
Cannabis sativa	497 ± 58.5^{a}	483.8 ± 31.4^{a}	443.3 ± 36.0^{a}	410.5 ± 37.7^{ab}	

between concentration of the non-conventional and percent mycelial growth inhibition revealed that 96%, 99.9%, 99.5, 69.4% of the variation in increased per cent inhibition could be explain by increase in the concentration of salicylic acid, acetyl salicylic acid, indole butyric acid and indole acetic acid, respectively. The maximum per cent mycelial growth (100%) inhibition was observed @ 200 ppm concentration with the salicylic acid (Plate 6), acetyl salicylic acid (Plate 7) and indole butyric acid (Plate 8) followed by salicylic acid (77.8%) and acetyl salicylic acid (70.9%) at 150 ppm concentration (Table 3). Salicylic acid @ 100 ppm (61.9%) and Indole butyric acid @ 150 ppm (65.3%) found statistically similar with each other. The lowest per cent mycelial inhibition was observed by Indole acetic acid which showed only 21.9% inhibition even at 200 ppm concentration (Plate 9). Very few reports are available in literature that these chemicals reduced the *S. rolfsii* infection but the non-conventional chemical were tested against many other pathogens. Sarma *et al.* (2007) found that sodium selenite (10-4 mmol) proved to be highly antifungal against *S. sclerotium* under *in vitro* condition as it drastically inhibited mycelial growth of the pathogen. Oxalic acid also inhibited mycelial growth but only at higher concentrations (10^{-6} mmol). However, Zinc sulfate and sodium malonate had no effect on mycelial growth of the pathogen and a similar mycelial growth was recorded as in the control. Rohilla *et al.* (2001) while studying mustard *Albugo candida* system reported that salicylic acid showed curative effect. Kumar *et al.* (2015) also reported that K_2SO_4 found effective followed by $ZnSO_4$ at higher dose of 1000 ppm in inhibition of mycelial growth of *Alternaria brassicae* under *in vitro* conditions.

Effect of bioagents on mycelial growth of the *S. rolfsii*

In present investigation, six bio-agents viz., *Trichoderma hazianum* (Darjeeling), *Trichoderma hazianum, Glocladium virens, Trichoderma viride*, *Pseudomonas fluorescens* and *Coniothyrium* sp were evaluated against *S. rolfsii.* The highest antifungal activity was observed with *T. hazianum* (85.6%) (Plate 10) and *T. hazianum* (Darjeeling) (80%) followed by *T. viride* (62.2%) (Plate 11), *G. virens* (60%) (Plate 13) and *P. fluorescens* (53.1%) (Plate 12) (Table 4). The lowest inhibition of mycelial growth (17.2%) has been observed with *Coniothyrium* sp. (Plate 14).

Table 3. Effect of different non-conventional chemicals on mycelial growth of *S. rolfsii.* Mean ± SEM of four replications. Duncan's multiple range test (DMRT) was followed, mean with the same letters are not significant.

Non-conventional chemical	Inhibition percentage at different concentration 50 ppm	100 ppm	150 ppm	200 ppm
Salicylic acid	24.2± 1.32^{f}	61.9 ± 4.61^{d}	77.8 ± 0.90^{b}	100 ± 0.00^{a}
Indole acetic acid	0.3 ± 0.28^{h}	1.4 ± 0.83^{h}	2.8 ± 1.33^{h}	21.9 ± 0.94^{f}
Indole butyric acid	6.4 ± 2.41^{h}	40.8 ± 0.83^{e}	65.3 ± 2.66^{cd}	100 ± 0.00^{a}
Acetyl salicylic acid	14.5 ± 1.03^{g}	43.1 ± 4.17^{e}	70.9 ± 2.66^{c}	100 ± 0.00^{a}

Table 4. Efficacy of different bioagents against of *S. rolfsii*. Mean ± SEM of four replications. Duncan's multiple range test (DMRT) was followed, mean with the same letters are not significant.

Bio-agents	Inhibition percentage of mycelium
Trichoderma hazianum (Darjeeling)	80 ± 4.03[a]
Trichoderma hazianum	85.6 ± 1.20[a]
Glocladium.virens	60 ± 2.03[b]
Trichoderma viride	62.2 ± 1.51[b]
Pseudomonas fluorescens	53.1 ± 0.95[c]
Coniothyrium sp.	17.2 ± 1.16[d]

Results of the investigation are in agreement with the finding of Vinella *et al*. (2020), they reported that *Trichoderma harzianum* inhibited per cent mycelial growth up to 74.7% followed by *Trichoderma viride* (71.9%). Prasad *et al.* (1999) reported that *T. harzianum* isolates inhibited mycelia growth of *S. rolfsii* up to 61.40% in dual culture. The antagonists' action of the bioagents involves the direct or indirect mechanism which includes antibiosis, mycoparasitism, secretion of hydrolytic enzyme (Wilson *et al.* 1991).

CONCLUSION

To maintain the sustainability of crop production system, the bio-rational components involved in the management of the disease should be used in integrated way that help in stabilizing the crop production. Among different botanicals treatments evaluated for the efficacy in the mycelial growth and sclerotial inhibition *Azadirachta indica* was most effective followed by *Cannabis sativa* and *Pongamia pinnataa.* The inhibition of the mycelial growth increases with increase the concentration of the non-conventional chemicals. Salicylic acid was found best in inhibition of mycelial growth followed by acetyl salicylic acid and indole butyric acid. Among the bio-agents *T. hazianum* was found best in antifungal activity against *S. rolfsii*. Thus, from the experiments it can be concluded that bio-rational components helps to reduce the growth of *S. rolfsii* and they can be replaced with conventional chemicals for management of *S. rolfsii* in an ecofriendly way.

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