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Antifungal Activity of Botanicals and Biological Agents against *Sclerotium rolfsii* Causing Foot and Root Rot Disease of Lentil

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ABSTRACT

Background: Foot and root rot disease caused by *Sclerotium rolfsii* is a destructive soil borne disease of lentil (*Lens culinaris*). The present experiment was conducted to determine the inhibitory effect of thirteen plant extracts and two bio-control agents on *in vitro* mycelium growth of *S. rolfsii*.

Methods: The experiment was conducted in the Laboratory of Pulse Research Center, RARS, BARI, Ishurdi, Pabna, Bangladesh during *rabi* season in 2015. Seven isolates of *S. rolfsii* were collected from foot and root rot affected lentil plant. Antifungal activity of thirteen botanicals and two biocontrol agents (*T. harziznum* and *T. viride*) was tested against a fast growing isolate (Sc-38) of the pathogen following poisoned food technique.

Result: Out of thirteen botanicals, only Black cumin inhibited colony growth of *S. rolfsii*, where the colony diameter ranged 0.00-6.00 cm at different days after incubation. A moderate inhibition was achieved with Tulsi at 3, 6 and 9 DAI compared to control. Amendment of PDA with compost and liquid *T. harziznum* and *T. viride* remarkably inhibited the colony growth of *S. rolfsii* compared to control. Efficacy of four treatments of biocontrol agents was much better than botanical treatments. Two botanical extracts (Black cumin and Tulsi) and biocontrol agents (*T. harzianum* and *T. viride*) were found effective against *S. rolfsii*.

Key words: Antifungal, Biocontrol, Botanicals, Lentil, *Sclerotium rolfsii*.

INTRODUCTION

Lentil (*Lens culinaris*) is the second most important pulse crop in Bangladesh (Ali *et al*., 2003). Pulses are the cheapest source of proteins and Indians fulfill 20 to 30 per cent of their protein requirement from pulses (Grover and Singh, 2015).

Lentil suffers from the attack of a number of seed borne diseases such as vascular wilt, collar rot, root rot, stem rot, foot and root rot, rust, powdery mildew and downy mildew caused by *Fusarium oxysporum* f. sp. *lentis*, *Sclerotium rolfsii, Rhizoctonia solani*, *Uromyces fabae, Erysiphe polygoni* and *Peronos poralentis*, respectively (Khare *et al*., 1979). The foot and root rot of lentil caused by *S. rolfsii* is very destructive disease of the crop causes reduction in plant stand and ultimately seed yield. Due to attack of *S. rolfsii* develops necrotic lesions on the stem base of lentil plants at any growth stage. The lesion girdles stem causing upper plant parts to become chlorotic and wilted; plants become necrotic after they die; disease often causes a patchwork of symptomatic plants throughout a field with plants initially becoming chlorotic and finally dying; fungus causes characteristic white lesions on stems which may be covered in a fluffy white growth during periods of wet weather.

Management of fungal disease using synthetic fungicides is not so effective because fungus displays strong ability to survive in soil through the formation of spherical sclerotia that have strong resistance to chemicals. Synthetic fungicides have potential harmful effects on human health and the environment. Therefore, search for alternative disease control strategies like plant based formulations are considered as promising alternates to synthetic fungicides (Amin *et al*., 2013 and Darvin, 2013).

Biocontrol agents like *Trichoderma harzianum, T. viride* and *T. asperellum* may be effective to control *S. rolfsii* the causal fungus of foot and root rot of pulse crops. *Trichoderma* is the most widely known biocontrol agent in agriculture and several species have success-fully been used against important fungal plant pathogens. *Trichoderma* have gained considerable recognition as biological agent. Several strains of *Trichoderma* have been found to be effective as bio-control agents against various soil borne plant pathogenic fungi such as *Fusarium, Sclerotium* and *Rhizoctonia* (Chet and Invar, 1994).

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The present study was conducted to investigate the inhibitory effect of botanicals and biological agents on *in vitro* mycelial growth of *Sclerotium rolfsii*, the causal fungus of foot and root rot of lentil. The specific objectives of the investigation were as follows:

(a) To isolate, purify and identify *S. rolfsii* causing foot and root rot of lentil isolated from infected plants.

(b) To evaluate the efficacy of selected botanical extracts and compost and liquid formulations of *T. harzianum* and *T. viride* to inhibit mycelium growth of *S. rolfsii*.

MATERIALS AND METHODS

Isolation, purification and identification of *Sclerotium rolfsii*

Isolation of *S. rolfsii* was done following tissue planting method (Tuite, 1969). Lentil plant specimens having typical symptoms of foot and root rot disease were collected from the experimental fields of Regional Agricultural Research Station (RARS), Ishurdi, Pabna, Bangladesh during rabi season of 2015. The collected samples were put in polyethylene bags and brought to the laboratory of Pulse Research Center, RARS, Ishurdi, Pabna, Bangladesh. Later the specimens were washed with tap water to make free from soil particles. Diseased specimens were cut into small pieces (0.5 cm) along with a portion of healthy tissues and surface sterilized with chlorox (1.0% sodium hypochloride solution) for one minute. Surface sterilized plant pieces were rinsed with sterilized water thrice and placed on filter paper to remove excess water. Three pieces of diseased specimens were plated on solidified PDA in Petri dishes maintaining equal distances under sterile conditions. The plates were incubated at room temperature and observation was made regularly to record growth of *S. rolfsii* from the plant tissue. The fungus was transferred to fresh PDA plates. The fungus was purified following hyphal tip culture method and identified using appropriate Key Book (Booth, 1971). Pure culture of the fungus was multiplied on PDA in test tube slants and preserved at 5±1°C for future use.

Comparison of *in vitro* colony growth of isolates of *Sclerotium rolfsii*

Seven isolates of *S. rolfsii* were isolated from infected lentil plants. The isolates of *S. rolfsii* were designated as Sc-7, Sc-12, Sc-13, Sc-27, Sc-33, SC-35 and Sc-38 where Sc represents first two letters of the genera *Sclerotium* and number represents serial number of isolates. Colony growth of the isolates of *S. rolfsii* was compared on fresh PDA plates incubated at 25°C. The colony diameter was measured using a ruler two days after incubation. In this study, three replicates used for each isolate (Punja and Damiani, 1996). The fast growing isolate (Sc-38) was selected as a test fungus for *in vitro* test of plant extracts as well as *Trichoderma* spp.

Collection of botanicals and *Trichoderma* spp.

Leaves of Allamanda (*Allamanda carthertica*), Tulsi (*Ocimum basilicum*), Creat (*Andrographis paniculata*), Khokhssha (*Ficus glumerata*), Henna (*Lawsonia inermis*), Neem (*Azadirata indica*), Tobacco (*Nicotiana tabacum*) and Bashok (*Justicia adhatoda*), seeds of Black cumin (*Nigella sativa*), Jute (*Corchorus capsularis*) and Mahogoni (*Sweteria macrophylla*), rhizome of Zinger (*Zinger officinalis*) and cloves of Garlic (*Allium sativum*) were collected from Pabna and Rajshahi districts of Bangladesh. The plant materials was chopped into small pieces, 50 ml of sterilized water was added and ground in a wiring blender. The sap thus extracted was first passed through four layers of muslin cloth and then filtered through Whatman filter paper no. 2 into 250 conical flasks. The mouth of the flask was closed with aluminum foil until use. The extracts were used as crude aqueous extract for evaluating their antifungal activity.

Both compost and liquid formulations of *T. harzianum* and *T. viride* were collected from Natore Development Society, Natore, Bangladesh. To prepare 15% liquid formulation of *T. harzianum* and *T. viride*, 15 ml liquid was mixed with 85 ml distilled water in 100 ml sterilized conical flask and shaken well. To prepare 15% extract of compost, 15 g compost of *T. harzianum* and *T. viride* were mixed with 85 ml distilled water in 100 ml sterilized conical flask and shaken well. The mixtures were first passed through four layers of muslin cloth and then filtered through Whatman filter paper no.2 into 250 conical flasks. The mouth of the flask containing diluted liquid or extract of compost of *T. harzianum* and *T. viride* was closed with aluminum foil until use.

Amendment of PDA with botanicals and biocontrol agents

Potato Dextrose Agar (PDA) medium was prepared by mixing extract of 200 g peeled potato slices, 15 g dextrose, 20 g agar and distilled water to make the volume 1000 ml. The mixture was cooked on a hot plate for melting the agar properly. To prepare 15% (v/v) plant extracts medium, 150 ml liquid PDA was poured into 250 ml sterilized conical flask and 27 ml of plant extract was poured into each conical flask with the help of pipette. After thorough mixing with the extract, the medium was sterilized at 121°C under 1.1 kg/cm^2 pressure for 20 minutes in an autoclave. After sterilization, PDA amended with plant extracts was poured into 90 mm Petri dishes at approximately 20 ml/dish. The pH of the medium was adjusted to 6.5 using pH meter with the help of 1N HCl and 1N NaOH. The amended PDA was poured into Petri dishes (9 cm) at 20 ml per dish. The Petri dishes containing PDA were autoclaves. After solidification, 15% liquid or 15% compost extract of *T. harzianum* and *T. viride* were poured onto surface of dishes containing PDA for absorption. After full saturation of PDA with bio-control agents, the plates were used for the antifungal test. The plates having unamended PDA served as control.

Antifungal test of plant extract and biocontrol agents

After solidification of PDA, the plates were inoculated with 5 mm mycelial disc of *S. rolfsii*. One disc of test fungus (Sc-38) was placed upside down in agar media at the center of the petriplates and incubated at room temperature. The plates were arranged on the laboratory desks following completely randomized design (CRD) with three replications.

Mycelial growth of the fungus was measured by taking the diameter in two directions and the average was computed. The growth reading was recorded at 3, 6, 9, 12 and 15 days after incubation.

Statistical analysis

The data were analyzed statistically. Analysis of variance was computed following CRD according to Gomez and Gomez (1984). Analysis was performed using MSTAT-C computer package. Mean differences were compared following Duncan´s multiple range test.

RESULTS AND DISCUSSION

Comparative growth of the isolates of *Sclerotium rolfsii*

Comparative radial mycelium growth of seven isolates of *S. rolfsii* on fresh PDA is shown in (Table 1). The maximum colony diameter was found for Sc-38 at all stages of data collection. At 3 and 6 DAI, the colony diameter ranged 1.70-5.57 and 4.37-8.53 cm, respectively. Significantly the highest colony diameter was recorded from Sc-38 at these two stages compared to other isolates. At 9 DAI, the colony diameter of different isolates varied from 5.67 to 9.00 cm. The radial colony diameter of seven isolates ranged 6.83-9.00 and 8.00-9.00 cm at 12 and 15 DAI, respectively. The lowest growth was found in Sc-33 which was statistically similar to Sc-13 at 15 DAI. Banakar *et al*. (2017) observed the maximum colony diameter (9.0 cm) of *S. rolfsii* in potato dextrose agar (PDA) within 48 hours of incubation. Hussain *et al*. (2003) found the maximum radial growth (8.5 cm) of *S. rolfsii* within 7 days of inoculation in cornmeal agar media.

From the results of this experiment, it was evident that isolate Sc-38 of *S. rolfsii* was a fast growing fungus, which was selected as the test fungus to study efficacy of botanical extracts and biocontrol agents on radial colony growth of *S. rolfsii* (Table 1).

Efficacy of botanical and biocontrol agents to inhibit *in vitro* growth of *Sclerotium rolfsii*

In control (PDA without any amendment), the radial colony diameter of *S. rolfsii* was 3.83, 7.43, 9.00, 9.00 and 9.00 cm at 3, 6, 9, 12 and 15 DAI, respectively. The radial colony diameter on PDA amended with thirteen botanical extracts ranged 0.00-8.67, 0.83-9.00, 4.27-9.00, 5.50-9.00 and 6.00-9.00 cm at 3, 6, 9, 12 and 15 DAI, respectively. Among the botanicals only black cumin significantly inhibited colony growth of *S. rolfsii* at all stages and significant inhibition was also obtained with tulsi extract at 3, 6 and 9 DAI compared to control. Amendment of PDA with extracts of garlic, henna and zinger reduced the colony growth to 0.67, 1.47 and 2.82 cm at 3 DAI which were significant compared to respective control. On the contrary, the radial mycelial growth was increased to 5.22, 5.17, 67.67, 8.00 and 8.67 cm due to amendment of PDA with extracts of Allamanda, Creat, Jute seed, Mahogani and Basak compare to control. Diameter of *S. rolfsii* under control and other treatments was statistically similar. At 12 and 15 DAI, colony diameter under control and treatments with all botanical extracts was also statistically similar except only black cumin (Table 2). Amin *et al*. (2013) tested different plants *viz*., rhizome ginger, neem leaf, tobacco leaf and rhizome of turmeric. All plant extracts inhibited the growth of *S. rolfsii* at higher concentration, while rhizome of turmeric inhibited the growth at a low level. Bangladesh is enriched with high plant diversity so many desirable antifungal compounds are isolated from various parts like root, leaf and stem (Jahan and Rahman, 2022).

Amendment of PDA with compost and liquid formulations of *T. harziznum* and *T. viride* significantly reduced the colony diameter of *S. rolfsii* compared to control at all stages of measurement. The colony diameter ranged 1.50-2.97, 1.50-3.08, 1.33-2.95 1.33-2.92 and 1.33-2.92 cm under different amendment with two biocontrol agents at 3, 6, 9, 12 and 15 DAI, respectively. Efficacy of four treatments biocontrol agents was better than all treatments with botanicals (Table 2). Radawan *et al*. (2006) reported that *T. harzianum* and *T. hamatum* were most effective against *S. rolfsii*. Patro and Madhur, (2013) observed that *T. harzianum* inhibits *in vitro* mycelial growth of *S. rolfsii,*which cause foot rot in finger millet. Khalid, (2013) isolated four bioagents *viz*, *Bacillus subtilis, Pseudomonas,* yeast and *T. viride* which inhibited damping-off disease of bean caused by *S. rolfsii*. Hooda *et al*. (2008) and Darvin *et al*. (2013) selected three species of *Trichoderma* (*T. viride, T. harzianum, T. longibrachiatum*) for inhibition of radial growth of *S.rolfsii*. *Trichoderma viride* and *T. harzianum* have highest radial growth inhibition and *T. longibrachiatum* has lowest radial growth inhibition of

Table 1: Comparative growth study of the isolates of *Sclerotium rolfsii* at different intervals on PDA.

Isolates	Radial colony growth of *S. rolfsii* (cm) at different days after inoculation				
	3	6	9	12	15
Sc-7	3.10c	5.17c	*7.30abc	8.00a	8.17a
Sc-12	3.33bc	5.50c	6.90bc	*8.07a	*8.97a
Sc-13	1.93d	4.93c	6.27bc	7.27a	8.27a
Sc-27	2.83c	5.17c	6.70bc	7.77a	8.87a
Sc-33	3.97b	6.80b	*7.77ab	*8.50a	*8.97a
Sc-35	1.70d	4.37c	5.67c	6.83a	8.00a
Sc-38	5.57a	8.53a	*9.00a	*9.00a	*9.00a

Means within the same column with a common letter (s) do not differ significantly (P=0.01).

Table 2: Radial colony diameter of *Sclerotium rolfsii* at different days after incubation on PDA amended with different botanicals and biological agents.

Common and scientific names of botanicals and biocontrol agents	Radial mycelial growth of *Sclerotium rolfsii* (cm) at different days of intervals				
	3	6	9	12	15
Allamanda (*Allamanda carthertica*) leaf	5.22c	8.67ab	9.00a	9.00a	9.00a
Tulsi leaf (*Ocimum basilicum*)	1.50gh	5.10d	7.50b	8.83a	9.00a
Creat (*Andrographis paniculata*) leaf	5.17c	8.83ab	9.00a	9.00a	9.00a
Black cumin (*Nigella sativa*) seed	0.00i	0.83f	4.27c	5.50b	6.00b
Garlic (*Allium sativum*) clove	0.67hi	7.17c	9.00a	9.00a	9.00a
Khokhsha(*Ficus glumerata*) leaf	3.35de	6.97c	9.00a	9.00a	9.00a
Henna (*Lawsonia inermis*) leaf	1.47gh	6.87c	8.07ab	8.77a	9.00a
Neem (*Azadirata indica*)	3.53de	9.00a	9.00a	9.00a	9.00a
Tobacco (*Nicotiana tabacum*)	2.17fg	7.17c	9.00a	9.00a	9.00a
Jute (*Corchorus capsularis*) seed	7.67b	9.00a	9.00a	9.00a	9.00a
Zinger (*Zinger officinalis*) Rhizome	2.82ef	7.57abc	9.00a	9.00a	9.00a
Bashok (*Justicia adhatoda*) leaf	8.67a	9.00a	9.00a	9.00a	9.00a
Mahogoni (*Sweteria macrophylla*) seed	8.00ab	9.00a	9.00a	9.00a	9.00a
Trichoderma harzianum compost	1.65g	1.65f	1.67e	1.67de	1.55e
Trichoderma harzianum liquid	1.67g	1.83ef	1.87e	2.10d	2.10d
Trichoderma viride compost	2.97ef	3.08e	2.95d	2.92c	2.92c
Trichoderma viride liquid	1.50gh	1.50f	1.33e	1.33e	1.33e
Control (Unamended PDA)	3.83d	7.43bc	9.00a	9.00a	9.00a

Means within the same column with a common letter (s) do not differ significantly (P=0.01).

S. rolfsii using dual culture technique *in vitro*. Basumatary *et al.* (2015) and (Swathi *et al.*, 2015) reported that *T. harzianum* and *T. virens* were more active against *S. rolfsii* with 100% inhibition under *in vitro* condition.

CONCLUSION

Based on the results of present experiment, the botanical extracts of black cumin seed and Tulsi leaf were found effective against growth of *S. rolfsii*. Liquid and compost formulations of *T. harziznum* and *T. viride* were found prospective bio-control against *S. rolfsi*. The extracts of botanicals and bio-control agents may be effective to control foot and root rot disease of lentil.

Conflict of interest: None.

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