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ANTIFUNGAL ACTIVITY OF BOTANICALS AND BIOCONTROL AGENTS AGAINST *SCLEROTIUM ROLFSII* CAUSING FOOT AND ROOT ROT DISEASE OF LENTIL

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ABSTRACT

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.* The experiment was conducted in the Laboratory of Pulse Research Center, RARS, BARI, Ishwardi, Pabna. 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. Among the botanicals only Black cumin inhibited colony growth of *S. rolfsii* and the colony diameter ranged 0.00, 0.83, 4.27 and 6.00 cm at 3, 6, 9 & 15 days after incubation, respectively. Amendment of PDA with compost and culture filtrate *T. harziznum* and *T. viride* remarkably inhibited the colony growth of *S. rolfsii* compared to control. Efficacy of four treatments of biocontrol agents was better than botanical treatments. Remarkable inhibition was achieved with Tulsi at 3, 6 and 9 DAI compared to control. Botanical extracts (Black cumin and Tulsi) and biocontrol agents (*T. harzianum* and *T. viride*) are found effective against the fungus of *Sclerotium rolfsii*.

Keywords: Lentil, foot and root rot disease, *Sclerotium rolfsii,* botanicals, biocontrol agents

Introduction

Lentil *Lens culinaris* is the second most important pulse crop in Bangladesh (Ali *et al*. 2003). 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*, *Selerotion. rolfsii, Rhizextonic. solani*, *Uromyces fabae, Erysiphe polygoni* and *Peronos poralentis*, respectively (Shahiduzzaman 2015). 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*, necrotic lesions develop 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, and fungus causes characteristic of 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 (Jagtap and Kamble 2010, Osemwegie *et al.* 2010, Darvin 2013, Amin *et al.* 2013, Rivera *et al.* 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 successfully been used against important fungal plant pathogens (Verma *et al*. 2007). *Trichoderma* have gained considerable

recognition as biological agent (Papavizas 1985). 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).

The present study was conducted to investigate the inhibitory effect of botanicals and biological agents on in-vitro mycelial growth of *S. rolfsii*, the causal fungus of foot and root rot of lentil. The specific objectives of the investigation were to isolate, purify and identify *S. rolfsii* causing foot and root rot of lentil isolated from infected lentil plants, and to evaluate the efficacy of thirteen 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), Ishwardi, Pabna during rabi season of 2015. The collected samples were put in polyethylene bags and brought to the laboratory of Pulse Research Center, RARS, Ishwardi, Pabna. 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±1ºC for future use. The collected isolates of *Sclerotium rolfsii* was maintained each separately and designed as shown in Table 1.

Table 1. Sample number and isolated *Sclerotium rolfsii* from host lentil plant

Sample No. of isolates	Isolated *Sclerotium rolfsii*
LG-4	Sc-7
LPath-6	Sc-12
LPath-7	Sc-13
LPb2-14	Sc-27
LPb4-17	Sc-33
LPb4-18	Sc-35
LPb4-19	Sc-38

The isolates were designed by using the first letter of the pathogen and host plant were designed by using the first letter of host adding with the first letter of location, e.g., Sc-33, where Sc stands for *Sclerotium rolfsii,* 33 for the serial number of the isolates and host plant L stands for Lentil. Path stands for Pathology field, G stands for Genetics and Plant Breeding field, Pb2 stands for Pulse Research Centre field block 2 and Pb4 stands for Pulse Research Centre field block 4.

Comparison of in-vitro colony growth of isolates of *S. 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. The colony growth of the isolates was compared on fresh PDA plates and a 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 culture filtrate 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 culture filtrate 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 121ºC 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% culture filtrate 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 15days 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 radial mycelium growth of seven isolates of *S. rolfsii* on fresh PDA is presented in Table 2. The maximum colony diameter was found under 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.

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 *F. solani* (Table 2).

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 where the colony diameter ranged 0.00, 0.83, 4.27 and 6.00 cm at 3, 6, 9 & 15 days after incubation, respectively. A 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 (Plate 1 and Table 3).

Amendment of PDA with compost and culture filtrate 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 (Plate 2 and Table 3).

Results of the present experiment reveal that botanical extracts of Black cumin seed and Tulsi leaf have potentiality to inhibit in-vitro colony growth of *S. rolfsii*. On the other hand, all four treatments with compost and culture filtrate *T. harziznum* and *T. viride* were found as prospective bio-control agents against the fungal pathogen.

Sindhu and Dadarwal (2001) and Biswas and Sen (2000) reported that *T. harzianum* inhibit stem-rot of groundnut caused by *S. rolfsii*. Radawan *et al*. (2006) and NalisaI *et al*. (2005) reported that *T. harzianum* and *T. hamatum* were most effective against *S. rolfsii*. Anand and Reddy (2009) reported that *Trichoderma, Penicillium* and *Aspergillus* species significantly inhibited the growth of *S. rolfsii* under in vitro condition.

Chandra (2014), Elad (2000), Haran *et al*. (1996), Lewis and Papavizas (1991) and Patro and Madhur (2013) reported 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*. Valle *et al*. (2013) observed that *T. viride, T. harzianum, Pochoniach lamydosporia, Pseudomonas fluorescens* and *Bacillus subtilis* are effective as biocontrol agent against *S. rolfsii* responsible for causing southern blight disease in *Zea mays*.

Table 2. Comparative growth study of seven isolates of *Sclerotium rolfsii* at different intervals on PDA without amendment

Isolates	Radial growth of *S. rolfsii* (cm) at different days after inoculation (DAI)				
	3	6	9	12	15
Sc-7	3.10 c	5.17 c	7.30 abc	8.00 a	8.17 a
Sc-12	3.33 bc	5.50 c	6.90 bc	8.07 a	8.97 a
Sc-13	1.93 d	4.93 c	6.27 bc	7.27 a	8.27 a
Sc-27	2.83 c	5.17 c	6.70 bc	7.77 a	8.87 a
Sc-33	3.97 b	6.80 b	7.77 ab	8.50 a	8.97 a
Sc-35	1.70 d	4.37 c	5.67 c	6.83 a	8.00 a
Sc-38	5.57 a	8.53 a	9.00 a	9.00 a	9.00 a
Cv (%)	13.38	12.69	13.56	10.90	9.07
Lsd	0.7512	1.284	1.682	-	
F-test	*	*	*	NS	NS

Means in a column, similar letter (s) do not differ significantly at 1 % (**) and 5% (*) level of probability. NS= non-significant


Allamada leaf and control


Tulsi leaf and control


Creat leaf and control


Black cumin seeds and control


Garlic clove and control


Khoksha leaf and control


Henna leaf and control


Neem leaf and control


Tobacco leaf and control


Jute seeds and control


Zinger seeds and control


Bashok leaf and control


Mahogoni seeds and control

Plate 1. Effect of botanicals on radial mycelial growth of *Sclerotium rolfsii* at 15 DAI.



T. harzianum compost and control



T. viride compost and control



T. harzianum filtrate and control



T. viride filtrate and control

Plate 2. Effect of biological control agents on radial mycelial growth of *Sclerotium rolfsii* at 15 DAI.

Table 3. Radial mycelial growth of *S. rolfsii* (cm) at different days of intervals

Common and scientific names of botanicals and Biocontrol agents	Days				
	3	6	9	12	15
Allamanda *Allamanda carthertica* leaf	5.22 c	8.67 ab	9.00 a	9.00 a	9.00 a
Tulsi leaf *Ocimum basilicum*	1.50 gh	5.10 d	7.50 b	8.83 a	9.00 a
Creat *Andrographis paniculata* leaf	5.17 c	8.83 ab	9.00 a	9.00 a	9.00 a
Black cumin*(Nigella sativa* seed	0.00 i	0.83 f	4.27 c	5.50 b	6.00 b
Garlic *Allium sativum* clove	0.67 hi	7.17 c	9.00 a	9.00 a	9.00 a
Khokhsha *Ficus glumerata* leaf	3.35 de	6.97 c	9.00 a	9.00 a	9.00 a
Henna *Lawsonia inermis* leaf	1.47 gh	6.87 c	8.07 ab	8.77 a	9.00 a
Neem *Azadirata indica*	3.53 de	9.00 a	9.00 a	9.00 a	9.00 a
Tobacco *Nicotiana tabacum*	2.17 fg	7.17 c	9.00 a	9.00 a	9.00 a
Jute *Corchorus capsularis* seed	7.67 b	9.00 a	9.00 a	9.00 a	9.00 a
Zinger *Zinger officinalis* rhizome	2.82 ef	7.57 abc	9.00 a	9.00 a	9.00 a
Bashok *Justicia adhatoda* leaf	8.67 a	9.00 a	9.00 a	9.00 a	9.00 a
Mahogoni *Sweteria macrophylla* seed	8.00 ab	9.00 a	9.00 a	9.00 a	9.00 a
Trichoderma harzianum compost	1.65 g	1.65f	1.67 e	1.67 de	1.55 e
Trichoderma harzianum culture filtrate	1.67 g	1.83 ef	1.87 e	2.10 d	2.10 d
Trichoderma viride compost	2.97 ef	3.08 e	2.95 d	2.92 c	2.92 c
Trichoderma viride culture filtrate	1.50 gh	1.50 f	1.33 e	1.33 e	1.33 e
Control (Unamended PDA)	3.83 d	7.43 bc	9.00 a	9.00 a	9.00 a
Cv (%)	13.73	12.95	6.91	5.67	4.27
Lsd	0.7802	1.319	0.8044	0.6787	0.5131
F-test	*	*	*	*	*

Means in a column, similar letter (s) do not differ significantly at 1 % (**) and 5% (*) level of probability. DAI= Days After Inoculation

Hooda *et al*. (2008), Mohiddin and Khan (2013) and Darvin *et al.* (2013) selected three species of *Trichoderma* (*T. viride, T. harzianum, T. longibrachiatum*) for inhibition of radial growth of *S. rolfsii*. *T. viride* and *T. harzianum* have highest radial growth inhibition and *T. longibrachiatum* has lowest radial growth inhibition of *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.

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.

Conclusions

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

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