African Journal of Biotechnology Vol. 11(82), pp. 14822-14827, 11 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1463
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

In vitro screening of fungicides and antagonists against *Sclerotium rolfsii*

Bhuiyan, M. A. H. B.*, Rahman, M. T. and Bhuiyan, K. A.

Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1706, Bangladesh.

Accepted 29 June, 2012

A study was conducted in the microbiology laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, during 2010 to 2011 to control *Sclerotium rolfsii* with fungicides and *Trichoderma harzianum*. Six fungicides namely Provax-200, Bavistin, Ridomil, Dithane M-45, Rovral 50 WP and Tilt were screened at 100, 200 and 400 ppm concentration for their efficacy against the radial colony growth of *S. rolfsii*. The complete inhibition was obtained with Provax-200 at all the selected concentrations. Complete inhibition also obtained at the highest concentration of Tilt. The highest concentration of Rovral 50WP inhibits 93.88% radial growth and significantly superior to Dithane M-45 at the highest concentration. Bavistin and Ridomil were found to be significantly lower when used against the test pathogen. A total of 20 *T. harzianum* isolates collected from rhizosphere and rhizoplane of different crops were screened against *S. rolfsii* following dual plate culture technique. The screened isolates of *Trichoderma* showed significantly variable antagonism ranging from 65.01 to 83.06% reduction of radial growth of *S. rolfsii*. Among the screened antagonists, the isolate TH-18 of *T. harzianum* showed the highest (83.06%) inhibition of radial growth of *S. rolfsii*.

Key words: *Sclerotium rolfsii, Trichoderma harzianum*, fungicides, antagonist.

INTRODUCTION

Sclerotium rolfsii, an omnivorous ad soil-borne fungal pathogen causes disease in a wide range of agricultural and horticultural crops. *S. rolfsii* has at least 500 species hosts in 100 families (Ferreira and Boley, 1992). The most common hosts are the legumes, crucifers and cucurbits. The diseases caused by the fungus are more serious in tropical and sub-tropical regions. The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years, as well as the profuse growth rate of the fungus make it well suited facultative parasite and a pathogen of major importance throughout the world (Punja, 1985). The first confirmed report of losses due to the pathogen in USA was made by Rolfs in 1892 on tomato (*Lycopersicon esculentum* Miller) in Florida (Aycock, 1966). Of the various methods used to control plant diseases, the use of chemical Fungicides is very common. However, in view of the complexities arising from the use of chemical pesticides, such as harmful effect on environment and non-target organisms including man, domestic animals, beneficial insects, wild life, the use of microorganisms as biocontrol agents has provided a very promising alternative and less hazardous method for plant disease control. Antagonists may act against pathogens in one or more of the following mechanisms: competition, antibiosis, parasitism, predation or induce resistance in plant; hydrolytic enzymes excreted by antagonists is a well-known feature of mycoparasitism (Henis and Chet, 1975). Though, fungicides have enormous killing capacity but, indiscriminate use of fungicides is not only hazardous to living being but disrupt the natural ecological balance by killing the beneficial soil microbe (Ansari, 1995). However, biological control of plant pathogens is a potential non-chemical means for plant disease control and can serve as an alternative for costly chemical treatment (Omorusi, et al., 2007). *T. harzianum* was found to be an effective

*Corresponding author. E-mail: baki_136@yahoo.com.

Table 1. *Sclerotium* isolates collected from different crop fields.

Isolate no.	Source of isolate
Sr-1	Soybean
Sr-2	Potato
Sr-3	Chick Pea
Sr-4	Bush Bean
Sr-5	Strawberry
Sr-6	Brinjal
Sr-7	Radish
Sr-8	Lentil
Sr-9	Cotton
Sr-10	Chilli

biological control agent for protecting a number of crop plants from damage induced by *S. rolfsii* under both greenhouse and field conditions (Elad et al., 1980). *T. harzianum* is a common soil species and is used in biological control of a variety of plant-pathogenic fungi. The application of fungi as biological control agents, especially *T. harzianum*, parasitized *S. rolfsii* (Desai and Schlosser, 1999). Considering the above facts, the present study has therefore been undertaken with the following objectives: 1) to isolate and identify *S. rolfsii* from infected crop fields and select the most virulent isolate; 2) to select the most effective fungicide with its suitable concentration in controlling *S. rolfsii in vitro* and 3) to isolate and identify *T. harzianum* and to select the most promising antagonistic isolate of *T. harzianum* in controlling *S. rolfsii in vitro*.

MATERIALS AND METHODS

Sample collection, isolation and identification

S. rolfsii was collected from three thanas of three districts of Bangladesh. The crop seasons were kharif -1, kharif -2 and Rabi/2010. The pathogens associated with diseased specimens were collected from rhizosphere and rhizoplane of plants (Mian, 1995). The infected tissues with healthy tissues were cut into small pieces of 0.5 to 1.0 cm long bits. The bits were surface sterilized by dipping in 1% sodium hypochloride (NaOCl) solution for 3 min. The treated plant tissues were rinsed three times in sterilized water. The excess water on the surface of the pieces was removed by blotting on sterile blotting paper.

The sterilized pieces were placed on water agar medium containing 2% agar, 200 ppm streptomycin. The plates were incubated at 25 ± 1°C and examined daily for the growth of mycelium of *Sclerotium* species. Hyphal tip transfer was made aseptically to potato dextrose agar (PDA) plates and if necessary, to PDA amended with 200 ppm streptomycin. After subsequent growth, to ensure it is not contaminated with bacteria, the isolates were transferred to PDA slants in test tubes and were periodically transferred to new slants and stored at 10°C. The pathogen was identified following a standard key (Bernett, 1980). A total of 10 *Sclerotium* spp. isolates were collected from different crop fields (Table 1).

Isolation and identification of *Trichoderma* isolates

A total of 100 soil samples were collected from rhizosphere and rhizoplane of brinjal (*Solanum melongena*), tomato (*Solanum Lycopersicum*), bean (*Phaseolus vulgaris*), cauliflower (*Brassica oleracea cv. botrytis*), cabbage (*Brassica oleracea cv. capitata*), Lentil (*Lens culinaris*), potato (*Solanum tuberosum*), carrot (*Daucus carota*), indian spinach (*Spinacia oleracea*), red amaranth (*Amaranthus cruentus*) vegetable garden, bare land and kitchen garden from different locations of BSMRAU, Comilla, Bogra and farmers field of Gazipur districts by soil dilution plate method as described by Dhingra and Sinclair (1985). The soil samples of each crop, collected from different locations were mixed together and seven composite samples were made. Then, 10 g of composite soil sample of each crop was taken in a 250 ml Erlenmeyer flask. Sterilized water was added to flasks at 100 ml/flasks. The flasks were agitated on a vortex for 2 min for thorough mixing and 1 ml sub sample was transferred from each flask to another flask containing 9 ml sterile water and finally, five folds serial dilution of the soil suspension was prepared. From this fivefold serial dilution, 0.1 ml each was incorporated into melted PDA in Petri dish (9 cm diameter). The suspension was thoroughly mixed with the medium using a turntable. The Petri dishes were incubated for five days at room temperature (25 ± 3°C). Colonies of *Trichoderma* were identified following a standard key (Bernett, 1980). A total of 20 fungal isolates (Table 2) were identified as *Trichoderma* spp. out of 50 isolates of different fungi. The other isolated fungi except, *Trichoderma* spp. were discarded. The isolates of *Trichoderma* were purified on PDA plates following hyphal tip technique (Tuite, 1969). After purification, all of them were preserved in PDA slants at 10°C as stock culture for further use.

Preparation of inocula of test pathogens

Wheat grain was used for preparation of inocula. Wheat grains were soaked in water for 24 h. After soaking, excess water was drained off and water soaked grains were poured into 500 ml Erlenmeyer flask, sealed with cotton plug. Then, the flasks were autoclaved for 50 min at 121°C temperature under 15 pound pressures on two consecutive days. Inocula of each isolate were prepared in separate flask. Five millimeter diameter mycelial disc was taken from the edge of the three day old colony of each isolate grown on PDA. 10 mycelial discs were transferred into each flask and mixed with the autoclaved wheat grains on a clean bench. Flasks were incubated at 25 ± 2°C for 15 days. They were shaken by hand after two to three days interval for even growth. Inocula of all isolates were prepared separately. The colonized wheat grain was air dried on brown paper for two days and stored at 4°C for future use.

Pathogenicity tests for selection of *Sclerotium rolfsii* isolate as test pathogen

10 isolates of *S. rolfsii* were tested for their ability to cause foot and root rot disease of soybean by soil infestation method in pot culture experiment under shade condition in front of the plant pathology laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University. Each earthen pot was filled with 1.0 kg sterilized soil. *S. rolfsii* was thoroughly mixed with sterilized soil at the rate of 20 g/kg soil. Controls were prepared using sterilized soil only. Seven pieces of seeds (soybean) were sown in each pot. Disease development was observed regularly and recorded at seven to 30 days after sowing to estimate the effect of pathogens in causing pre-emergence and post-emergence seedling mortality. The causal agent of pre-emergent seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds, infected root and stems.

Table 2. *Trichoderma* isolates collected from different crop fields.

Isolate	Source/crop
TH-1	Tomato
TH -2	Cauliflower
TH -3	Lentil
TH -4	Tomato
TH -6	Cabbage
TH -8	Red amaranth
TH -10	Bean
TH -12	Lentil
TH -12 C	Brinjal
TH -16	Indian spinach
TH -17	Carrot
TH -18	Tomato
TH -19	Lentil
TH -20	Potato
TH -16	Carrot
TH -23	Potato
TH -52	Veg. garden
TH-75	Country bean
TH -76 I	Bare land
TH -77 C	kitchen garden

Screening of *Trichoderma* isolates against *S. rolfsii*

In vitro tests were conducted to evaluate the antagonistic effect of 20 isolates of *T. harzianum* against *S. rolsii* on PDA medium by dual culture technique (Dhingra and Sinclair, 1985). The medium was prepared by mixing infusion of 200 g pealed potato, 20 g dextrose and 20 g agar per liter of medium. One mycelial disc (5 mm) of individual isolate of *T. harzianum* and one disc (5 mm) of test pathogen was placed simultaneously on the edge of each PDA Petri dish plate at opposite direction. Three plates (replication) were used for each isolate of *T. harzianum* and a test pathogen. The plates were arranged in the laboratory desks following completely randomized design. The plates that received only mycelial disc of *S. rolfsii* served as control. The plates were incubated in the laboratory having ambient temperature of 25 ± 2°C. Thereafter, inhibition percentage of *S. rolfsii* was calculated on the growth of pathogen on PDA plate after three days of incubation following the formula as suggested by Sundar et al. (1995).

$$\text{Inhibition of growth (\%)} = \frac{X - Y}{X} \times 100$$

Where, X = mycelial growth of pathogen in the absence of *T. harzianum* (control), Y = mycelial growth of pathogen in the presence of *T. harzianum.*

Effect of fungicides on radial growth of *S. rolfsii*

Six fungicides namely Bavistin, Ridomil, Rovral, Dithane M45, Cupravit and Provax 200 at three different concentrations viz. 100, 200 and 400 ppm were tested *in vitro* to evaluate their effect on radial colony growth using poison food technique (Dingra and Sinclair, 1985). Requisite quantity of individual fungicides was added to melted PDA to have concentration of 100, 200 and 400 ppm. After thorough mixing, amended medium was autoclaved at 121°C under 1 kg/cm^2 for 20 min. Approximately 20 ml of melted PDA mixed with fungicides was poured into each 90 mm diameter Petri dish. After solidification, the inocula (5 mm disc) from three days old culture of test organism *S. rolfsii* were placed at the center of the test plate. There are three replications for each treatment. The plates were incubated in the laboratory having ambient temperature of 25 ± 2°C. Data on radial colony diameter was recorded after three days of incubation when the whole plate under control was covered with the growth of test organism. Inhibition of radial growth based on colony diameter was calculated following the formula as suggested by Sundar et al. (1995).

Experimental design and data analysis

The experiments were conducted following completely random design (CRD) with three replications. Data were analyzed by using MSTAT-C program. The significant difference, if any, among the means were compared by Duncan's multiple range test (DMRT). Whenever necessary, the data were transformed before statistical analysis following appropriate method.

RESULTS

Pathogenicity test of *S. rolfsii* against soybean plants

10 isolates of *S. rolfsii*: Sr-1, Sr-2, Sr-3, Sr-4, Sr-5, Sr-6, Sr-7, Sr-8, Sr-9 and Sr-10 were collected from soybean, potato, chickpea, bush bean, strawberry, brinjal, radish, lentil, cotton and chilli, respectively. All (10) isolates of *S. rolfsii* were tested to evaluate their ability to cause disease in soybean seedlings in the pot culture experiment. All the tested isolates of the pathogens were found to be pathogenic against soybean seedlings causing 63.25 to 98.20% seedling mortality in soybean variety 'Shohag'. The results of the pathogenicity test of *S. rolfsii* against soybean seedlings are presented in the Table 3. Isolates collected from the infected soybean crop might be the most virulent isolate (Sr-1) causing 98.20% seedling mortality.

Preliminary laboratory evaluation of fungicides on radial growth of *S. rolfsii* isolate Sr-1

The results of the preliminary evaluation of fungicides on the radial growth of *S. rolfsii* isolate Sr-1 are presented in Table 4. The results of the experiment show that all the Ridomil showed the lowest inhibition (0%) of mycelial growth at 100 ppm concentration. At 200 and 400 ppm concentrations, 3.33 and 52.97% reduction of mycelial growth were achieved. Rovral 50WP and Tilt appeared to be the most effective in inhibiting the radial growth of *S. rolfsii.* At 100, 200 and 400 ppm concentrations, Rovral inhibited 57.4, 74.81 and 93.88% radial growth, respectively. Tilt showed 84.81, 90.99 and 100% inhibition of radial growth at 100, 200 and 400 ppm concentration,

Table 3. Pathogenicity test of 10 isolates of *S. rolfsii* against soybean seedlings variety 'Shohag'.

S. rolfsii isolate	Mortality		
	Pre-emergence mortality (%)	Post-emergence mortality (%)	Total mortality (%)
Sr1	77.70	20.50	98.20
Sr2	66.50	14.50	81.00
Sr3	70.00	10.50	80.50
Sr4	60.00	13.00	73.00
Sr5	73.50	11.25	84.75
Sr6	61.75	22.75	84.50
Sr7	78.80	11.50	90.30
Sr8	70.00	10.50	80.50
Sr9	40.50	22.75	63.25
Sr10	30.00	34.50	64.50
Control	0.00	0.00	0.00

Table 4. Preliminary laboratory evaluation of fungicides on radial growth of isolate Sr-1 of *S. rolfsii*.

Fungicide	Concentrations (ppm)	Inhibition of radial growth (%)
Provax-200	100	100.0^{a}** $(84.76)^{\phi}$
	200	100.0^{a} (84.76)
	400	100.0^{a} (84.76)
Bavistin	100	5.75^{l} (13.07)
	200	6.07^{l} (14.26)
	400	6.64^{k} (14.93)
Ridomil	100	0.0^{n} (1.654)
	200	3.33^{m} (10.51)
	400	52.97^{l} (46.70)
Dithane M 45	100	25.14^{j} (30.09)
	200	63.10^{g} (52.59)
	400	80.63^{e} (64.03)
Rovral 50WP	100	57.40^{h} (49.26)
	200	74.81^{f} (59.88)
	400	93.88^{b} (75.68)
Tilt	100	84.81^{d} (67.06)
	200	90.99^{c} (72.55)
	400	100^{a} (84.76)
Control	90.00 mm	

$^{\phi}$Value within a column with same latter do not differ significantly (P=0.05), Figure within the parenthesis are arcsin transformation ($Y = \sin^{-1}\sqrt{X}$) value.

respectively. Dithane M-45 inhibited 25.14, 63.10 and 80.63% radial growth of *S. rolfsii* at 100, 200 and 400 ppm concentration, respectively. Radial growth of *S. rolfsii* was least inhibited by Bavistin by only 5.75, 6.07

Table 5. Screening of 20 isolates of *T. harzianum* against *S. rolfsii* (Sr-1) in dual culture technique.

Isolates of *T. harzianum*	Inhibition (%)
TH-1	66.22^{ef}*
TH -2	74.19^{b}
TH -3	70.24^{bcde}
TH -4	66.44^{ef}
TH -6	68.09^{def}
TH -8	72.27^{bc}
TH -10	71.54^{bcd}
TH -12	72.02^{bcd}
TH -12 C	65.01^{f}
TH -16	65.81^{f}
TH -17	68.50^{cdef}
TH -18	83.06^{a}
TH -19	68.71^{cdef}
TH -20	71.79^{bcd}
TH -16	68.10^{def}
TH -23	67.04^{ef}
TH -52	71.99^{bcd}
TH-70	66.84^{ef}
TH -76 I	71.49^{bcd}
TH -77 C	69.98^{cde}

*Value within a column with same latter do not differ significantly (P = 0.05).

Figure 1. Dual culture of *S. rolfsii* (isolate Sr-1) and *T. harzianum* isolate TH-18 on PDA plate (A, inhibition of colony growth of *S. rolfsii* in the presence of isolate TH-18; B, colony growth of *S. rolfsii* in the absence of isolate TH-18.).

and 6.64% at 100, 200 and 400 ppm concentration, respectively. Among the tested fungicides, Provax-200 appeared to be the best in inhibiting the hyphal growth of the pathogen *S. rolfsii* in all the concentration. Rovral and Tilt were also effective in controlling radial mycelium growth of the pathogen but Provax-200 gave complete inhibition of radial mycelial growth of *S. rolfsii* even at the lowest concentration. These findings for Provax-200 in this study were supported by the findings of Rubayet (2011) who found that complete inhibition of sclerotia formation of the tested pathogen was achieved at the highest 250 and 500 ppm concentration of Provax-200 but sclerotia formation of *S. rolfsii* was completely inhibited at the 100, 250 and 500 ppm concentration.

Screening of 20 isolates of *T. harzianum* against *S. rolfsii* (Sr-1) in dual culture technique

20 isolates of *T. harzianum* were tested against S*. rolfsii* on PDA and the results are presented in Table 5. All the tested isolates of *Trichoderma* showed more than 60% inhibition of the radial growth of the test pathogens *S. rolfsii* over control. Among the tested isolates, TH-18 showed the highest (83.06%) reduction of the radial growth (Figure 1) followed by TH-2 (74.19%). Among the tested isolates, TH-2, TH-3, TH-8, TH-10, TH-20 and TH 76 I showed significantly similar results in inhibiting *S. rolfsii*. The lowest radial growth inhibition of *S. rolfsii* was observed in isolate TH-12 C (65.01%) and TH-16 (65.81%), respectively.

Results of the experiment showed that all the tested isolates were effective in reducing mycelial growth of *S. rolfsii* on culture media. Significant reduction of mycelial growth of *S. rolfsii* in the presence of *Trichoderma* spp. were also reported by many other workers (Mondal, 1999; Akhter, 1999; Khan, 2003) and the results of the present investigations are in agreement with that of the above mentioned investigators.

REFERENCES

Akhter N (1999). Biological control of seedling mortality of different crops caused by *Sclerotium rolfsii* using antagonistic fungi. MS thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh p. 76.

Ansari MM (1995). Control of sheath blight of rice by Plant extracts. Indian. Phytopathology 48(3):268-270.

Aycock R (1966). Stem rot and other diseases caused by *Sclerotium rolfsii*. NC State Univ. Tech. Bull. 174:202.

Bernett HL (1980). Compendium of Soil Fungi. Buress Pub. Co. Minneapolis, USA p.223.

Desai S, Schlosser E (1999). Parasitism of *Sclerotium rolfsii* by *Trichoderma*. Indian Phytopathol. 52(1):47-50.

Dhingra OD, Sinclair JB (1985). Basics plant pathology methods. CRC Press. Inc. Boca Raton Florida. p. 13-44.

Elad Y, Chet I, Katan J (1980). *T. harzianum*: A biocontrol agent effective against *S. rolfsii* and *Rhizoctonia solani.* Phytopathology 70(2):119-121.

Ferreira SA, Boley AR (1992). *Sclerotium rolfsii*. Extension Plant Pathologist. Department of Plant Pathology, CTAHR. University of Hawaii at Manoa.

Henis Y, Chet I (1975). Microbiological control of Plant Pathogens. Adv. Appl. Microbiol. 19:85-111.

Khan MAA (2003). Integrated management of seedling mortality of chickpea caused by *Sclerotium rolfsii*. MSc thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh p.48.

Mian IH (1995). Methods in plant pathology. IPSA-JICA Project Publication 24:100.

Mondal PK (1999). Biological control of seedling mortality of chickpea caused by *Sclerotium rolfsii* using antagonistic fungi. MS thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh p.96.

Omorusi VI, Evueh GA, Ogbebor NO (2007). *In vitro* assessment of biological control of white root rot of rubber (*Hevea brasiliensis*) by antagonistic fungi. In: Proc. 5th Int. Conf. Nig. Soc. Exp. Biol. Anyigba 5:5-6.

Punja ZK (1985). The biology, ecology and control of *Sclerotium rolfsii*. Annual Rev. Phytopathol. 23:97-127.

Rubayet MT (2011). Management of stem rot of Potato caused by *Sclerotium rolfsii.* MS thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh p.76.

Sundar AR, Das ND, Krishnaveni D (1995). *In-vitro* Antagonism of *Trichoderma* spp. against two fungal pathogens of Castor. Indian J. Plant Prot. 23(2):152-155.

Tuite J (1969). Plant Pathological Methods: Fungi and Bacteria Burgess Pub. Co., Minneapolis, Minn. USA p.293.