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Isolation of Stem rot Disease Causing Organism of Brinjal and their *in-vitro* Inhibition with Fungicides and Bio-control Agents

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Abstract

Different strains of *Sclerotinia sclerotiorum* were isolated from the diseased samples collected from different hosts and locations. Among the 14 isolates, 12 isolates colonies covered the entire Petri plates within 96 hours but, two isolates from fababean and yellow mustard showed slow colony growth within 96 hours. All isolates produced sclerotia which were varied in number, but the fenugreek isolate produced maximum (43) number of sclerotia and lambs quarter isolate produced minimum number of sclerotia (12) on PDA medium. To examine inhibitory effect of fungicide on the mycelial growth of the pathogen, 9 fungicides were tested *in vitro* against *Sclerotinia sclerotiorum,* of those carbendazim, carboxin, topsin-M and carbendazim+ mancozeb (SAAF) were found most effective and inhibited the mycelial growth of pathogen up to 100 per cent at 0.05%, 0.1%, and 0.2% concentration. The effect of different bioagents viz., *Trichoderma harzianum, T. viride, T. koningii, T. atroviride, T. longibraciatum, Aspergillus niger, Chaetomium globosome* and *Penicillium notatum* in inhibiting the growth of *Sclerotinia sclerotiorum* was studied through "Dual Culture Technique". The data showed that among the eight biocontrol agent six were fond effective. The maximum inhibition was found by *T. harzianum* causing 70.82% inhibition of mycelial growth of the pathogen *S. sclerotiorum*.

Keywords: *Sclerotinia sclerotiorum;* fungicides and biocontrol agents.

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a soil-borne plant pathogen that infects over 400 plant species at all stages of growth, development and harvested products. The diseases it causes are commonly known as white mold, sclerotinia wilt or stalk rot [1]. In addition, as most inoculum is ascosporic, a few germinating sclerotia can lead to significant infection levels in the field [2]. Also, ascospores could travel long distance from neighboring fields and infect petals [3]. During the growing season, depending on various environmental factors, the sclerotia germinate and form

either mycelium, which can infect plants or produce ascospores by developing an apothecium [4]. Ascospores are the primary inoculum for epidemics in many crops [1, 5]. *S. sclerotiorum* is capable of infecting flowers, leaves, fruits or stems. Management of disease with crop rotations is unrealistic due to the persistence of survival structures (sclerotia) in the soil for long periods and due to its wide host range [6]. These factors necessitate the use of fungicides, which have been known to have adverse effects on non-target organisms [7]. Biological control as a disease management strategy in protected agricultural areas would not only be economical but also durable by sustaining the reduction of inoculum potential and amount of disease produced by *S. sclerotiorum*. A number of biocontrol agents have been characterized for the control of *S. sclerotiorum* [8]. Antagonists such as *Ulocladium atrum* is an effective biocontrol agent as a mycoparasitic fungus on *S. sclerotiorum* [6] and *Pseudomonas sp*. inhibit the germination of ascospores of *S. sclerotiorum* by antibiosis [9,10]. The suppression of *S. sclerotiorum* by *Trichoderma harzianum* (Th38) and *Epicoccum purpurescence* was due to colonization on petals of brinjal [9]. Although, most of the species of *Trichoderma* and *Bacillus* are effective BCAs and very few species have been tested on *S. sclerotiorum* [9, 10]. Therefore, there is a need for research into biocontrol of *S. sclerotiorum* on brinjal crop. In this paper we studies the morphological variations among the different isolates from different host of *S. sclerotiorum*, check the efficacy of different fungicides against the pathogen *S. sclerotiorum in vitro* and evaluate different bio-control agents against the pathogen *S. sclerotiorum in vitro*.

Material and method

1. Collection of diseased samples from different hosts and locations

The samples (plant/plant parts infected with *Sclerotinia sclerotiorum*) were collected from different hosts and locations under natural condition to isolate the strains of *S. sclerotiorum*. The plant/plant parts showing prominent symptoms of the disease were examined for the presence of the causal organism.

In vitro studies were done in Centre of Excellence for Sanitary and Phytosanitary (SPS), Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut during 2012-2014.

2. Isolation and purification of the pathogen

The diseased part i.e. fruits and stem of brinjal showing typical symptoms along with sclerotia were collected for isolation of the causal organism. The selected plant parts and sclerotia were thoroughly washed with sterilized water for removing the dust and other surface contaminants. The diseased plant parts were initially cut into small pieces with the help of sterilized knife. These pieces were first washed in sterilized water and then dipped in 0.1 per cent mercuric chloride ($Hgcl_2$) solution for 30 seconds and were thoroughly washed in sterilize water three times to remove the traces of mercuric chloride. Excess moisture of these pieces was removed by putting them in two folds of sterilized blotting paper under aseptic condition and then three to five pieces of infected parts and sclerotia were transferred into sterilized petri dishes containing potato dextrose agar (PDA) medium and incubated at room temperature. After about 24-48 hours of incubation, the whitish mycelia growth appeared out from the pieces and sclerotia. The hyphal tips of mycelium were transferred aseptically to PDA medium plates. The culture obtained from different diseased pieces and sclerotia were subjected to preliminary microscopic examination, which revealed the presence of the pathogen responsible for disease.

3. Host range of the pathogen

To ascertain host range of the fungus *Sclerotinia sclerotiorum,* the selected host plants of different species of different families were artificially inoculated with mycelial discs, mycelial suspension and sclerotia. The inoculated plants were covered with polythene bags for 7-10 days, irrigated and maintained throughout the experiment. After 10 days of inoculation the polythene bags were removed from the inoculated plants. The critical observations were recorded for the appearance of diseased symptoms on artificially inoculated plants.

4. Morphological variation among different isolates of *Sclerotinia sclerotiorum* by observing its mycelial and sclerotial characters

The mycelia and colony characters of different 14 isolates of *S. sclerotiorum* were studied on PDA medium. The Petri dishes containing the medium were inoculated with 5.0 mm disc of each isolates which were taken from 3 days old actively growing culture. The Petri dishes were incubated

at 25 ± 1° C in BOD incubator. The mycelial colony characters were examined after 7 days incubation. On these plates after production of sclerotia by different *S. sclerotiorum* isolates were studied and observed its characters.

5. Efficacy of fungicides against the pathogen

Nine fungicides belonging to the different group were tested against the pathogen *Sclerotinia sclerotiorum in vitro* to select the best effective fungicide, which could inhibit the growth of the pathogen in culture to maximum extent. This could be achieved by "poisoned food technique" [11]. The requisite quantity of each fungicide was incorporated in PDA medium, thoroughly mixed by shaking prior to pouring in sterilized petri plates and were allowed to solidify. These petri plates were inoculated with 5 mm discs of three day old culture of the pathogen with three replications. The petri plates were incubated at 25 ± 1° C with one set of control. The data on radial growth of fungal colony were measured in millimeter (mm) after every 24 hours till the control petri plates were not filled up. The percent inhibition over control was calculated by the following formula suggested by Bliss (1934) [12].

Per cent growth inhibition over control= dc – dt /dc × 100

where,

dc = colony diameter in control

dt = colony diameter in treatment

6. Evaluation of bioagents against the pathogen

Eight isolates of different bio-control agents were collected from different location of North India viz. *Trichoderma harzianum* and *T. viride* collected from Bikaner (RAU), *Trichoderma koningii* from IARI (4302), *Trichoderma atroviride, T. longibrachiatum, Chaetomium globosum,* and *Aspergillus niger* from Kanpur (CSA), and *Penicillium notatum* from Meerut (SVP), were evaluated by dual culture technique [13] (Morton, D.J. and Strouvle, W.H. 1955). Discs of 5 mm diameter were taken from the actively growing colonies of the test pathogen (*S. sclerotiorum*) and antagonists with the help of sterilized cork borer. The disc of the pathogen were placed on one side of poured PDA plates aseptically while, the discs of antagonists were placed on opposite side of pathogen in same petri plates and the control were also maintain. The experiments were carried out in three replications. These petri plates were incubated at 25 ± 1°C. After 7 days of incubation, the mechanism of interaction was observed and the data were recorded as per cent inhibition by following formula [12].

Per cent growth inhibition over control= dc – dt /dc × 100

where,

dc = colony diameter in control

dt = colony diameter in treatment

Results

1. Isolation and purification of the pathogen

The isolation of the fungus *Sclerotinia sclerotiorum* from diseased sample collected from different hosts and locations/places were done from the affected plant parts showing characteristic symptoms of the disease on PDA medium. After isolating the fungus *Sclerotinia sclerotiorum* was purified by subsequent transferring of fungal mycelium into culture tubes (PDA poured) and then these purified culture tubes were kept in refrigerator at 5°C temperatures for further research purpose.

2. Host range of the pathogen

The host plants have an important role in occurrence and spread of the disease in crop season by maintaining, survival and multiplication of the inoculum during off season. Therefore, the knowledge of host range of the pathogen is very useful in controlling the disease. The 14 isolates of *Sclerotinia sclerotiorum* were inoculated on 25 plant species including crop plants, medicinal and weed plants belonging to different families with mycelial disc and sclerotia of the fungus.

The results presented in table-1 revealed that out of 25 plants, 16 plant species belonging to seven different families were infected by the pathogen. The mycelial mat and black sclerotia were also noticed on highly susceptible plants. It is clear that the pathogen had a wide host range in different plant families and these species might help pathogen in its survival during the off season and spread the inoculums early in the crop season.

Table 1: Host range of *S. sclerotiorum* under artificial inoculation condition

S. N.	Name of host plant inoculated with *S. sclerotiorum*		Family	Pathogenic reaction
	Common Name	**Botanical Name**		
1	Aloe vera	*Aloe barbadensis*	*Liliaceae*	-
2	Ashwagandha	*Withania somnifera*	*Solanaceae*	-
3	Fababean	*Vicia faba*	*Leguminoseae*	+
4	Lambs quarters	*Chaenopodium album*	*Chenopodiaceae*	+
5	Indian Hemp	*Canabis sativa*	Geraniaceae	-
6	Brinjal	*Solanum melongena*	*Solanaceae*	+
7	Brocolii	*Brassica oleracea*	*Cruciferae*	+
8	Cabbage	*Brassica oleracea*	*Cruciferae*	+
9	Chickpea	*Cicer arietinum*	*Leguminoseae*	+
10	Coriander	*Coriander sativum*	*Umbellifereae*	-
11	Fenugreek	*Trigonella foenugracum*	*Leguminoseae*	+
12	Field bind weed	*Convolvulus arvensis*	*Convolvulaceae*	+
13	Lentil	*Lens esculenta*	*Leguminoseae*	-
14	Indian sweet clover	*Melilotus indica*	*Fabaceae*	-
15	Mustard	*Brassica juncea*	*Cruciferae*	+
16	Parthenium	*Parthenium heyterophorus*	*Asteraceae*	+
17	Pea	*Pisum sativum*	*Leguminoseae*	+
18	Potato	*Solanum tuberosum*	*Solanaceae*	+
19	Fennel	*Foeniculum vulgare*	*Umbellifereae*	-
20	Sowthistale	*Sonchus arvensis*	*Asteraceae*	+
21	Canarygrass	*Phalaris minor*	*Gramineae*	-
22	Wheat	*Triticum aestivum*	*Gramineae*	-
23	Pippapra	*Fumaria parviflora*	*Fumariaceae*	+
24	Sour dock	*Rumex dentatus*	*Polypogonaceae*	-
25	Yellow mustard	*B. brassica*	*Cruciferae*	+

Here (+) infection and (-) no infection.

3. Morphological variation among different isolates of *Sclerotinia sclerotiorum* by observing its mycelial and sclerotial characters

The colony color of yellow mustard isolate was found blackish and mustard isolate colony color was yellow, Field bind weed isolate showed brown color and remaining other isolates did not have much differences. The colonies of other 11 isolates were found white, creamy, compact, uniform, and circular with irregular margin. Among the 14 isolates, 12 isolates colonies covered the entire petri plates within 96 hrs. But, in the colony of two isolates i.e. fababean and yellow mustard, colony growth did not cover the entire petri plates within stated period (Figure-1, plates-1 to 14). The initial sclerotial formation started after 120 hours of inoculation. The colony color of all isolates turned to blackish, creamy, and brown and the hyphal arrangement were closely septate, laterally branched; variable and different.

The sclerotia produced by different 14 isolates were found varied in color from black, grey to brown and differed in shape (irregular to branch). The fenugreek isolate produced maximum (43) number of sclerotia and Lambs quarters produced minimum number of sclerotia (12) on PDA plates after 10 days. These sclerotia were formed terminally, arranged in 1-2 or more concentric rings and sometimes sclerotia were also formed scattered on old PDA plates (Table 2).

Figure-1 *S. sclerotiorum* isolates from different plant species

Plate-1 brinjal

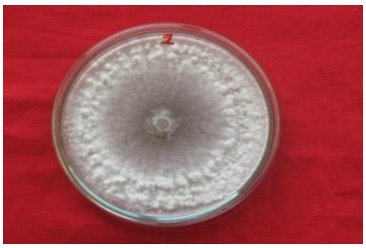

Plate-2 mustard

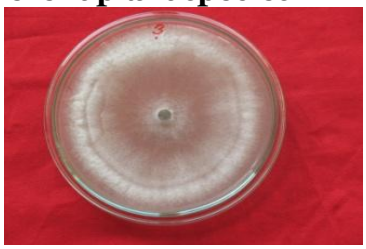

Plate-3 Parthenium

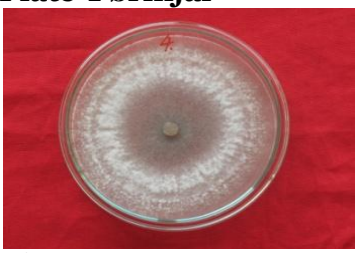

Plate-4 Pea

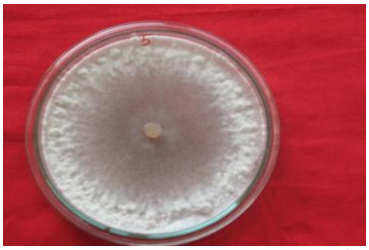

Plate-5 Potato

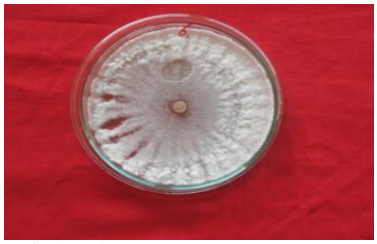

Plate-6 faba bea

Plate-7 sowthistle

Plate-8 wild fumeria

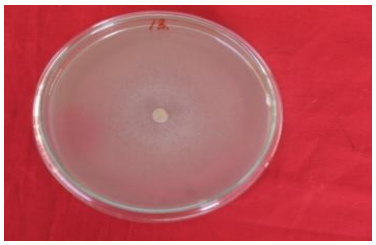

Plate-9 fenugreek

Plate-10 chickpea

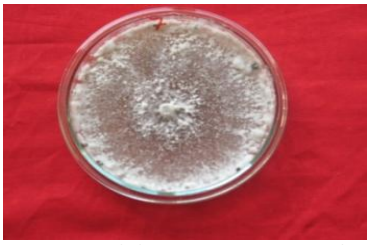

Plate-11 cabbage

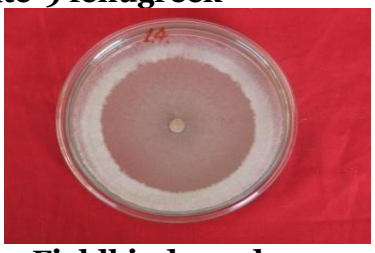

Plate-12 Fieldbind weed

Plate-13 Lambs quarter

Plate-14 yellow mustard

Table 2: Colony and Sclerotial character of *S. sclerotiorum*

S.N.	Isolates from different host	Characteristic features of *S. sclerotiorum* on PDA					
		Colony characters		Sclerotial characters			
		Color	Morpho-logy	Color	Shape	Formation (hrs.)	No. of sclerotia
1	Brinjal	Beige	Compact	Grey	Round	120-144	32
2	Mustard	yellow	Compact	Black	Semispherical	96-120	19
3	Pea	Brown	Compact	Black	Round	120	18
4	Parthenium	White	Fluffy	Grey	Semispherical	144	17
5	Sowthistale	White	Fluffy	Black	Round	120	29

6	Potato	White	Compact	Black	Round	120	15
7	Cabbage	White	Compact	Black	Branched	144	31
8	Fababean	White	Fluffy	Black	Round	156	16
9	Yellow mustard	Blackish	Fluffy	Brown	Irregular	120	14
10	Lambs quarters	Creamy	Compact	Black	Semispherical	144	12
11	Chickpea	Beige	Fluffy	Black	Semispherical	120	34
12	Wild fumeria	Creamy	Compact	Black	Round	120	19
13	Fenugreek	Brown	fluffy	Black	Round	144	43
14	Field bind weed	brown	fluffy	Black	Round	120	24

4. Efficacy of fungicides against the pathogen

A preliminary screening of nine fungicides viz. carbendazim, thiram, tricyclazole, carboxin, copper oxy-chloride, captan, mancozeb, topsin-M, carbendazim + mancozeb (SAAF) belonging to different groups were used at different concentration viz. 0.05%, 0.1% and 0.2% against pathogen, the results were presented in table 3.

The fungicide carbendazim, carboxin, topsin-M, and SAAF (carbendazim + mancozeb) were found most effective at 0.05%, 0.1% and 0.2%, but at 0.1% the fungicide thiram and at 0.2% tricyclazole also completely inhibited the growth of the pathogen. The other fungicides (captan, mancozeb) were found partially effective against the pathogen. The least effective fungicide was copper oxy-chloride.

Table 3: *In-vitro* inhibitory effect of fungicides at different concentration and time on growth of pathogen

S.N.	Fungicides	% Inhibition over control								
		at 0.05%			at 0.1%			at 0.2%		
		24 hr.	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
1	Carbendazim	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2	Thiram	100.0	100.0	97.1	100.0	100.0	100.0	100.0	100.0	100.0
3	Trycyclazole	100.0	95.2	91.4	100.0	96.7	92.2	100.0	100.0	100.0
4	Carboxin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	94.8
5	Copper oxy chloride	27.3	12.7	14.3	16.7	30.0	25.2	39.9	42.4	10.4
6	Captan	100.0	95.2	94.3	100.0	100.0	100.0	100.0	100.0	95.6
7	Mancozeb	63.8	25.4	41.9	58.3	33.4	48.5	100.0	62.7	43.7
8	Topsin-M	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
9	SAAF (C+M)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

5. Evaluation of different bio-control agents against the pathogen

The antagonist effect of *Trichoderma harzianum, T. viride, T. koningii, T. atroviride, T. longibrachiatum, Aspergillus niger, Chaetomium globosome* and *Penicillium notatum* against *Sclerotinia sclerotiorum* (*in vitro*) were evaluated on the basis of the percentage inhibition of pathogenic strains, the results were presented in table 4.

Six bio-control agents suppressed the mycelial growth of the pathogen (*Sclerotinia sclerotiorum)*. The data showed that the bioagent *Trichoderma harzianum* was found most effective and caused 70.82 per cent inhibition of mycelial growth of *S. sclerotiorum* followed by *Trichoderma koningii* (22.96 %). *Aspergillus niger* and *Penicillium notatum* did not inhibited the mycelia growth of the pathogen.

Table 4: *In-vitro* inhibitory effect of different bio-control agents on growth of pathogen

S.N.	Bio-control agents	% inhibition over control		
		4 days incubation	8 days incubation	12 days incubation
1	*Trichoderma harzianum*	24.92	54.67	70.82
2	*Trichoderma viride*	-	4.23	4.44
3	*Trichoderma koningii*	7.67	16. 25	22.96
4	*Trichoderma atroviride*	5.44	9.33	11.84
5	*Trichoderma longibrachiatum*	-	4.75	5.93
6	*Chaetomium globosum*	2.66	5. 44	6.67
7	*Aspergillus niger*	-3.93	-12.67	-15.55
8	*Penicillium notatum*	-2.23	-6.92	-8.88
9	Control	0.00	0.00	0.00

Discussion

Host range of the pathogen revealed that the fungus *S. sclerotiorum* was capable of infecting 16 plants of 9 different families. Most of the infected plants belonging to plant families viz., *Asteraceae, Brassicaceae, Chaenopodaceae, Convalvulaceae, Cruciferae, Fumeraceae, Solanaceae, Leguminocae,* may help in inoculum buildup, before and after sowing of brinjal plants and help in secondary infection or spread of the disease. Roy (1973) reported 27 different hosts of *S. sclerotiorum*, of which 13 host were records from India [14], Liu XueMin *et al.* (2002) reported 9 families, 15 genera and 15 species [15], which showed the more or less similarity with present investigation. After isolating the 14 strain of *S. sclerotiorum* from the collected diseased samples from different hosts and locations and their morphological characters were studied on PDA medium. The isolated strains produced mycelium fluffy and compact and also found variable in color (Beige, yellow, Brown, White, brown, Creamy to Blackish). The colony color of yellow mustard isolate was found to be blackish and mustard isolate colony color was yellow, Field bind weed isolate showed brown in color and remaining other isolates have not much more differences. The colonies of other 11 isolates were found white, creamy, compact, and uniform to circular with irregular margin. All the 14 isolates produced sclerotia which were varied in number of sclerotia, but the fenugreek isolate produced maximum (43) number of sclerotia and lambs quarter isolate produced minimum number of sclerotia (12) on PDA medium. Willet and Wong (1980) reported morphological character of *S. sclerotiorum* sclerotia were black, round or semispherical in shape measuring 3-10 mm in size [16]. Kolte (1985) reported the mycelium of *S. sclerotiorum* which was varies from 9 to 18 micrometer in diameter with numerous lateral branches of smaller diameter than the main hyphae [17]. Garg *et al.*, (2010) found no correlation between pigmentation and colony diameter of different isolates of *S. sclerotiorum* [17].

To examine inhibitory effect of fungicide on the mycelial growth of the pathogen, 9 fungicides were tested *in vitro* against *Sclerotinia sclerotiorum,* of those carbendazim, carboxin, topsin-M and SAAF were found most effective and inhibited the mycelial growth of pathogen up to 100 per cent at 0.05%, 0.1%, and 0.2% concentration, and at 0.1% conc. the fungicide thiram and at 0.2% tricyclazole were completely inhibit the growth of the pathogen in culture plates and were found significantly superior to remaining fungicides. However, in the present study most of the fungicides were found effective *in vitro* against *Sclerotinia sclerotiorum*. Mueller *et al.* (2002) reported thiophanate methyl and vinclozolin inhibited mycelial growth up to 18 to 93% and 93 to 99% [18]. Iqbal *et al.* (2003) reported Benlate, Ridomil gold, Tecto-60 and Topsin-M at 50 and 100 ppm concentrations found effective [19]. Singh *et al.* (2008) found that the Bavistin (0.1%), Vitavax (0.1%), and Topsin-M (0.15%) to be the most effective in inhibiting the growth of pathogen *in vitro* [20]. Prajapati and Narain (2008) reported Vitavax, Companion, Bavistin, Score, Mancozeb and Thiram to be the most effective as they inhibited the growth of fungus completely (100%) [21].

With more awareness of people and environmental scientists concerned about biological control is a supplement to the chemical control. The effect of different bio-control agents was studied and the data showed that among the eight biocontrol agent six were found effective. The

maximum inhibition was found with *T. harzianum* which showed 70.82% inhibition while *A. niger* and *P. nitatum* were found ineffective. However, the present finding are in consonance with the observation of Cundom *et al.* (2000) they reported *Trichoderma* species significantly inhibited the mycelial growth of *S. sclerotiorum* in dual culture [22]. Das *et al.* (2002) found *Trichoderma harzianum, Gliocladium virens* and *Aspergillus flavus* effective against *S. sclerotiorum in vitro* [23]. Singh *et al.,* (2008) found *T. viride* exerted the maximum activity against *Sclerotinia sclerotiorum* (33.4 %) [20].

Conclusion

The pathogen present in different weed host plant may cause disease in crop plants in same and or other plant families. It is clear from above study that the pathogen had a wide host range in different plant families and these species might help pathogens survival during the off season and spread the inoculums in the next crop season. The fungicide carbendazim, carboxin, Topsin-M, and SAAF (Carbendazim + Mancozeb) were found most effective at 0.05% concentration were provide 100 % inhibition, so the above fungicides should be recommended at 0.05 % concentration to make best cost: benefit ratio and for biological control of the pathogen *Trichoderma harzianum* was found most effective about 70.82% inhibition of mycelial growth of *S. sclerotiorum.*

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