P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(1): 2968-2973
© 2020 IJCS
Received: 19-11-2019
Accepted: 23-12-2019

Shashank Mishra
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Prashant Mishra
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Ravikant Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Jaskaran Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Anjali Arya
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Ankit Kumar
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Corresponding Author:
Shashank Mishra
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Screening of fungicides and bio-control agents against *Sclerotium rolfsii* (Sacc.) causing collar rot in Lentil

Shashank Mishra, Prashant Mishra, Ravikant Singh, Jaskaran Singh, Anjali Arya, and Ankit Kumar

DOI: https://doi.org/10.22271/chemi.2020.v8.i1as.8721

Abstract
Lentil is an important pulse crop, contributes about 8-9% of the total pulse production in India and is also a major source of protein, minerals and vitamins. Collar rot of lentil is an important seedling disease caused by *Sclerotium rolfsii* Sacc. In this study, twenty isolates (Th1-Th20) of *Trichoderma harzianum*, one isolate of *Pseudomonas fluorescence* and nine commercially available fungicides were tested *in vitro* for their efficacy in inhibiting the growth of the pathogen. *Trichoderma harzianum* isolate Th_{16} and Th_{14} were found to inhibit maximum mycelial growth of the pathogen. The complete inhibition of mycelial growth of *Sclerotium rolfsii* was found with Propiconazole, Tabuconazole, Hexaconazole, Ridomil and Carbendazim+ Mancozeb at all (0.03%, 0.05% and 0.1%) concentrations. Other fungicides (Captan, Mancozeb and Carbendazim) also showed significant inhibition of the mycelial growth of the pathogen. No inhibition was recorded in the treatment with copper oxychloride even at 0.2% concentration.

Keywords: Lentil, *Sclerotium rolfsii, Trichoderma harzianum, Pseudomonas flourescens* and fungicide

Introduction
Lentil, (*Lens culineris*) is a bushy, annual shrub plant that is popular for its lens shaped seeds and nutritious value of the seeds that is rich in protein, fibers having second highest levels of proteins and fiber after soybeans. The tap root system of the plant usually grows to a depth of around 15 cm that makes it a moderately drought resistant crop. India is second top producer of lentil in the world after Canada. In India, total area covered by lentil producers is about ~1.3 million hectares (APEDA, 2015) [1]. World lentil production has been increasing in recent years with most of the production coming from North American and Asian countries. Productivity of lentil in India varies from region to region due to variation in environment and biotic factors.
Lentil is attacked by fungal, viral and bacterial pathogens. *Sclerotium rolfsii* is one of the important soil borne fungal pathogens having a wide host range and world-wide distribution (Punja, 1988) [8], causing collar rot, root rot, stem rot and wilt on more than 500 plant species including almost all the agricultural and horticultural crops. The fungus is a soil borne pathogen of very aggressive nature and causes considerable damage to young seedlings causing collar rot resulting in substantial yield losses.
S. rolfsii produces sclerotia which is the principal structure to survive under adverse conditions. Control of soil borne pathogens has become one of the major concerns in agriculture. However effective and efficient management of crop diseases is generally achieved by the use of synthetic pesticides. These pesticides are known to pollute the environment, soil and water, besides causing deleterious effects on human health and biosphere. The present investigation is related to the study of the management of collar rot disease of Lentil by different fungicides and bioagents and comparing the efficacy of both.

Material and Methods
Experimental site, Location and Collection of diseased specimens-
The experiments were conducted at Department of Plant Pathology, Sardar Vallabhbhai Patel University of agriculture and Technology, Meerut (Uttar Pradesh).

Isolation and identification of pathogen- Infected lentil plants showing the typical symptoms of collar rot collected from Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut and from farmers' field in the vicinity of Meerut. All specimens were collected and placed in a moist chamber at 25ºC. Besides this the collar portion of the infected plants were cut in 3-5 mm thick tissue sections and sterilized with 0.1% sodium hypochlorite solution for 30 seconds, rinsed thrice in sterilized distilled water (SDW) and dried on sterilized filter paper at room temperature. The tissue sections were then placed on potato dextrose agar (PDA) amended with 100 µg/ml streptomycin sulphate and incubated at 25ºC for fifteen days. The pure culture of the pathogen was isolated and subsequently maintained on PDA.

Isolation of biocontrol agent- Soil samples were collected from the rhizospheric region of healthy plants from different locations of Meerut district for isolation of biocontrol agents. By using dilution plate techniques, Bio control agents were isolated, purified and identified on the basis of their morphological characters (Nashwa *et al*, 2008; Rifai, 1969) [6, 9] The obtained Bioagents (*Fungi- Trichoderma Spp.*) were maintained in PDA and (Bacterial- *P. fluorescens*) maintained in King's B agar slants.

In vitro* evaluation of bio-agents against *S. rolfsii

The tested isolates of *Trichoderma* spp. were grown on PDA medium at 20°C, for 6-days and used as inocula. Culture discs from each isolate of *Trichoderma* spp. and the pathogen (*S. rolfsii*) were inoculated opposite to each other at 4cm apart in the petriplate containing 20 ml PDA and the antagonistic properties of the test bioagents were exhibited (dual culture technique). Five replicates were used for each isolate of *Trichoderma* spp.. The inoculated plates with culture discs of pathogen without bioagents served as control. After 24, 48, 72 and 96 hrs of incubation at 20°C, radial growth of pathogen and percent inhibition was recorded. Inhibition percent of pathogen growth was calculated using the following formula:

Vincent (1927).

$$I = \frac{C - T}{C} X100$$

Where,
I = Per cent inhibition
C = Growth in control
T= Treatment

Table 1: List of Isolates of Bio-agents

S. N.	Bioagent	Isolated from crop	Location
`1.	T1	Wheat	Budaun
2.	T2	Wheat	Budaun
3.	T3	Wheat	Budaun
4.	T4	Wheat	Meerut
5.	T5	Berseem	Budaun
6.	T6	Berseem	Budaun
7.	T7	Berseem	Meerut
8.	T8	Berseem	Meerut
9.	T9	Chickpea	Budaun
10.	T10	Chickpea	Budaun
11.	T11	Chickpea	Meerut
12.	T12	Chickpea	Meerut
13.	T13	Lentil	Budaun
14.	T14	Lentil	Budaun
15.	T15	Lentil	Budaun
16.	T16	Lentil	Meerut
17.	T17	Lentil	Meerut
18.	T18	Sugarcane	Budaun
19.	T19	Sugarcane	Budaun
20.	T20	Sugarcane	Meerut

In vitro* evaluation of fungicides against *S. rolfsii

Effect of eight fungicides belonging to different groups (listed in Table-2) with different concentrations (viz., 0.03, 0.05 and 0.1 per cent for systematic fungicide and 0.05, 0.1 and 0.2 per cent for non-systemic fungicides) were tested *in vitro* for evaluating their efficacy to inhibit the growth of the pathogen. Effect on the growth of *S. rolfsii* was studied using poisoned food technique (Nene and Thapliyal, 1982) [7]. 20 ml of poisoned medium was poured into each of the 90 mm sterilized petriplates. Each plate was inoculated with five mm disc of mycelium at the center and incubated at 27± 1^0C. Three replications were maintained for each treatment. Potato dextrose agar medium without any fungicide served as control. All plates were incubated at 27± 1^0C till the growth completed in control plate. The per cent inhibition of the growth over control was calculated by following the equation given by Vincent (1927).

$$I = \frac{C-T}{C} X100$$

Where,
I = Per cent inhibition
C = Growth in control
T = Growth in treatment

Table 2: List of fungicides used

S. No.	Common Name	Chemical name	Concentrations
T_1	Carbendazim (Bavistin)	$C_9H_9N_3O_2$	0.03,0.05 and 0.1 %
T_2	Propiconazole (Tilt)	$C_{15}H_{17}Cl_2N_3O_2$	0.03,0.05 and 0.1 %
T_3	Tebuconazole(Torque)	$C_{16}H_{22}ClN_3O$	0.03,0.05 and 0.1 %
T_4	Hexaconazole(Contaf)	$C_{14}H_{17}Cl_2N_3O$	0.03,0.05 and 0.1 %
T_5	Ridomil(Metalaxyl)	$C_{15}H_{21}NO_4$	0.03,0.05 and 0.1 %
T_6	Mancozeb(Dithane M-45)	$C_4H_8MnN_2S_4Zn$	0.05, 0.1 and 0.2 %
T_7	Captan	$C_9H_8Cl_3NO_2S$	0.05, 0.1 and 0.2 %
T_8	Copper Oxy Chloride (Blitox50)	$CuCl_2.3CuH_2O_2$	0.05, 0.1 and 0.2 %
T_9	Carbendazim + Mancozeb (Saaf)	--------------------------	0.05, 0.1 and 0.2 %

Results

Isolation and identification of pathogen: On PDA plates, the fungus produced silky, white mycelium, which gradually losses its luster and became somewhat dull in appearance. Aerial hyphae grow fast, were not uniformly distributed. The hyphae were septet, hyaline, clamp connections occurred in the broader threads and branching was at right angles behind the cross wall. The individual hyphal cells were 60-350 µm in length and 2-8 µm in diameter.

After the mycelial mat develops, the sclerotial initials formed from hyphal strands lying parallel. A spherical shape was assumed by the loose mass of hyphae. The fully matured sclerotia were spherical, light to dark brown in colour, embedded in fuzzy mycelial mat and measured 1.5 to 4.2 mm in diameter. Initiation of sclerotial bodies was observed from fifth day onwards after inoculation. Sclerotia may be seen in the mycelium, on diseased tissues above or below ground, on soil surfaces, or in soil crevices also (West, 1961) [11].

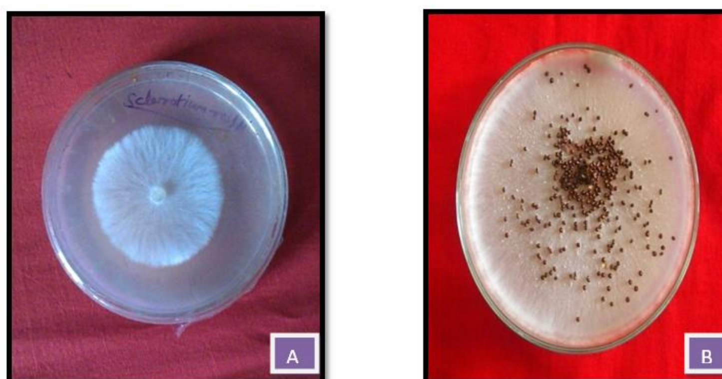

Fig 1: Mycelial Growth (A) and Sclerotia formation in *Sclerotium rolfsii* (B)

Fig 2: Mass culture of *Sclerotium rolfsii* (C) Pure culture (D)

In vitro* evaluation of bio-agents against *S. rolfsii:
Antagonistic activities of twenty isolates of *Trichoderma* sp. and one strain of *Pseudomonas* sp. were evaluated against *Sclerotium rolfsii* in *vitro* as indicated in Table: 4.2, Fig-1, Plate-4. The results from the table revealed that, significant difference in per cent inhibition of mycelial growth of *Sclerotium rolfsii* by all the tested bioagents. Among them maximum inhibition per cent (70.7%) of *Sclerotium rolfsii* were recorded in *Trichoderma* isolate T16.which is significantly superior from all the tested isolates and it is followed by T14 (70.0%), T2 (69.3%), T11 (68.5%), T8 (68.1%), and other isolates T1, T4, T7, T3, T5, were found 67.7% mycelial inhibition. whereas T10. T18, T15, T20, T17, T12, T16, T19 and T13 inhibited the mycelial growth of the pathogen between the range of 61.1% to 66.6% and the growth was significant as compared to control. However, the other bioagent *Pseudomonas fluorescence* inhibits 52.5 % mycelial growth of the pathogen. The minimum inhibition per cent of mycelial growth was recorded in *Trichoderma* isolates T19 (47.0%). All the tested bioagents were statistically significant but among the isolates T1, T4, T7, T3, T5 and T6, T12, T17, T20, T15 were non-significant among each other, but significant over control.

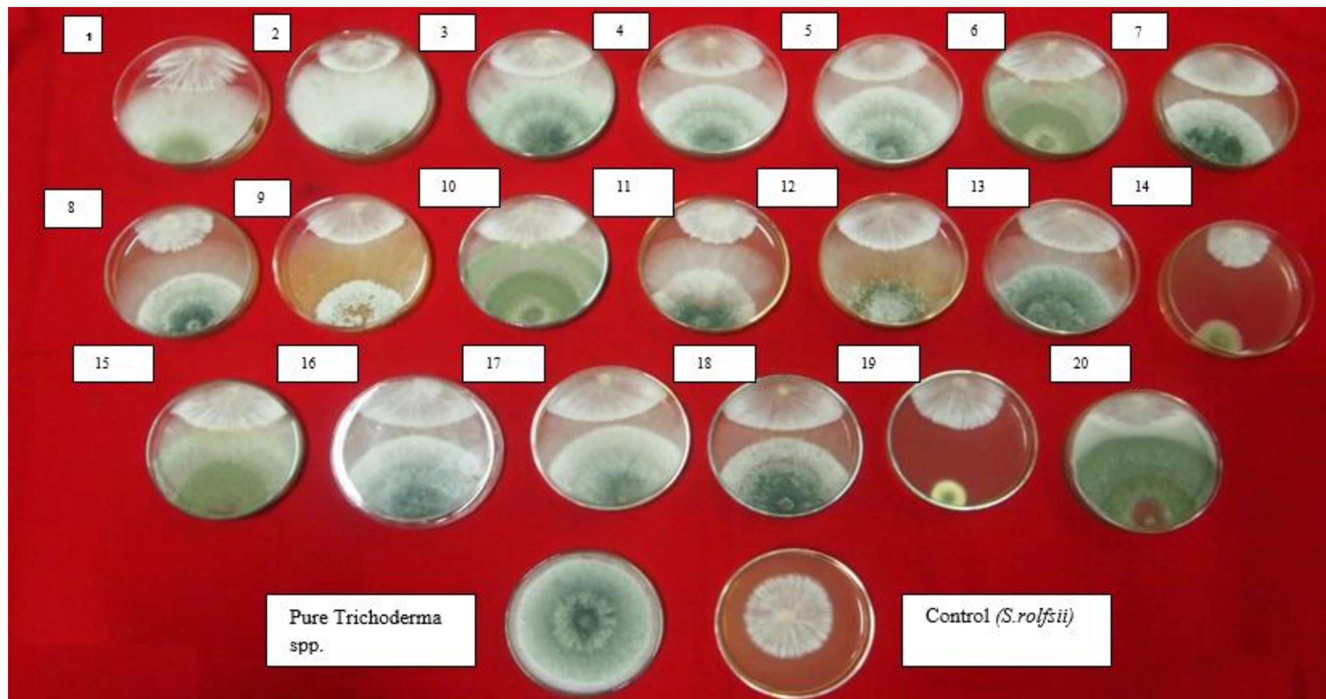

Fig 2: *in-vitro* evaluation of bio agents (left to right 1st three rows as T1 – T20, 4th row pure bioagent and control)

Table: 3 Effect of different Bioagents on radial growth of *sclerotium rolfsii in vitro.*

Treatments	Inhibition after 24 hr.		Inhibition after48 hr.		Inhibition after 72hr.		Inhibition after 96 hr.	
	colony diameter (mm)	Per cent inhibition	colony diameter (mm)	Per cent inhibition	colony diameter (mm)	Per cent inhibition	colony diameter (mm)	Per cent inhibition
T1	8.0	38.4	15.0	55.8	22.0	64.5	29.0	67.7
T2	9.0	30.7	15.3	55.0	25.0	59.6	27.6	69.3
T3	9.0	30.7	18.7	45.0	28.0	54.8	29.3	67.4
T4	8.0	38.4	19.0	44.1	25.7	58.5	29.0	67.7
T5	8.0	38.4	17.0	50.0	25.3	59.1	29.3	67.4
T6	10.0	23.1	18.7	45.0	31.3	49.5	33.0	63.3
T7	7.7	40.7	15.0	55.8	21.0	66.1	29.0	67.7
T8	10.0	23.1	13.7	59.7	22.0	64.5	28.7	68.1
T9	9.0	30.7	16.0	52.9	27.0	56.4	31.0	65.5
T10	9.7	25.3	18.7	45.0	30.3	51.1	35.0	61.1
T11	8.3	36.1	11.7	65.5	20.0	67.7	28.3	68.5
T12	7.7	40.7	16.0	52.9	25.0	59.6	33.0	63.3
T13	9.0	30.7	17.3	49.1	25.3	19.1	30.0	66.6
T14	8.0	38.4	12.0	64.7	19.0	69.3	27.0	70.0
T15	11.0	15.3	21.0	38.2	32.3	47.9	34.3	61.8
T16	9.0	30.7	13.0	61.7	24.0	61.2	26.3	70.7
T17	8.0	38.4	20.0	41.1	29.3	52.7	33.0	63.3
T18	9.0	30.7	20.0	41.1	30.0	51.6	35.0	61.1
T19	9.0	30.7	18.0	47.0	30.7	50.4	47.7	47.0
T20	10.0	23.1	19.3	43.2	30.3	51.1	33.3	63.0
Tp	8.7	33.1	19.0	44.1	29.3	52.7	42.7	52.5
Control	13.0		34.0		62.0		90.0	
CD at 5% level	Interval (A) 0.3397 Treatments (B) 0.7966 Factor (A X B) 1.593							
SE(m)	Interval (A) 0.1217 Treatments (B) 0.2854 Factor (A X B) 0.5708							

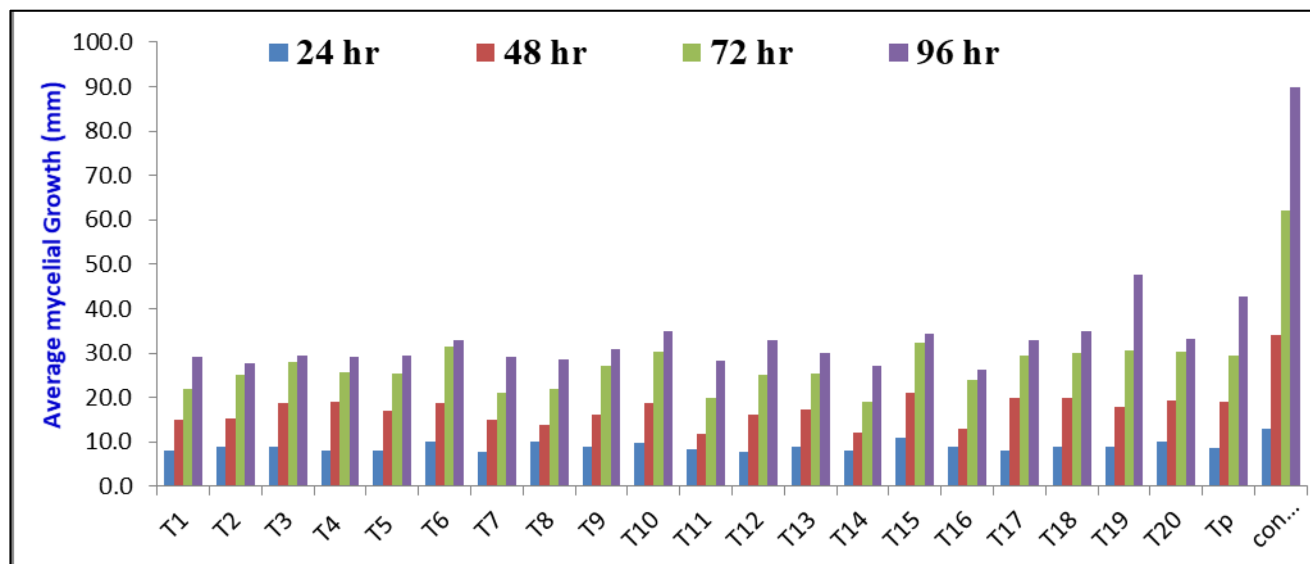

Fig 1: Effect of different Bioagents on radial growth of *S. rolfsii*

In vitro evaluation of systemic and non-systemic fungicides

Efficacy of five systemic and four non systemic fungicides were tested at different concentrations by poisoned food technique. The results thus obtained have been presented in Table-4.6, Fig-5 and Plate-6. The results from table – revealed that there is significant difference in per cent inhibition of mycelial growth of *Sclerotium rolfsii* with the fungicides which were tested. The per cent inhibition of mycelial growth of *Sclerotium rolfsii* was found highest (100%) in the treatment with Propiconazole, Tabuconazole, Hexaconazole and Ridomil at all three concentrations (0.03%, 0.05% and0.1%) and Carbendazim+ Mancozeb at all three concentrations (0.05%, 0.1%, and 0.2%). Other fungicides also inhibited the growth of the pathogen sigficantly. Captan (0.2%) and Mancozeb (0.2%) showed 88.5 per cent and 48.9 per cent inhibitions respectively and were found significantly superior over remaining treatment tested. No inhibition was recorded in the treatment with copper oxy chloride at all concentrations.

Table: 4 Effect of different Fungicides on the growth of *Sclerotium rolfsii in vitro.*

Treatment		Inhibition after 24 hrs.		Inhibition after 48 hrs.		Inhibition after 72 hrs.	
		Colony diameter (mm)	% inhibition	Colony diameter (mm)	% inhibition	Colony diameter (mm)	% Inhibition
Carbendazym	(0.03%)	8.0	61.9	24.0	54.9	65.3	27.4
	(0.05%)	7.0	75.0	19.7	63.0	57.0	36.7
	(0.1%)	6.0	71.4	16.0	70.0	49.0	45.5
Propiconazole	(0.03%)	0.0	100	0.0	100	0.0	100
	(0.05%)	0.0	100	0.0	100	0.0	100
	(0.1%)	0.0	100	0.0	100	0.0	100
Tabuconazole	(0.03%)	0.0	100	0.0	100	0.0	100
	(0.05%)	0.0	100	0.0	100	0.0	100
	(0.1%)	0.0	100	0.0	100	0.0	100
Hexaconazole	(0.03%)	0.0	100	0.0	100	0.0	100
	(0.05%)	0.0	100	0.0	100	0.0	100
	(0.1%)	0.0	100	0.0	100	0.0	100
Ridomil	(0.03%)	0.0	100	0.0	100	0.0	100
	(0.05%)	0.0	100	0.0	100	0.0	100
	(0.1%)	0.0	100	0.0	100	0.0	100
Mancozeb	(0.05%)	8.0	61.9	20.7	61.1	69.3	23.0
	(0.1%)	5.7	72.8	17.0	68.1	59.0	34.5
	(0.2%)	0.0	100	13.7	74.3	46.0	48.9
Captan	(0.05%)	0.0	100	8.0	75.7	30.3	66.3
	(0.1%)	0.0	100	5.7	82.7	26.3	70.8
	(0.2%)	0.0	100	0.0	100	10.3	88.5
Blitox	(0.05%)	15.0	28.6	57.0	-6	90.0	0
	(0.1%)	13.3	36.7	45.0	15.6	90.0	0
	(0.2%)	10.0	52.4	34.0	36.2	90.0	0
Carbendazym+ Mancozeb	(0.05%)	0.0	100	0.0	100	0.0	100
	(0.1%)	0.0	100	0.0	100	0.0	100
	(0.2%)	0.0	100	0.0	100	0.0	
Control		21.0		53.3		90.0	

CD at 5% level	Interval (A) 0.2173 Treatments (B) 0.5143 Factor (A X B) 1.150
SE(m)	Interval (A) 0.0781 Treatments (B) 0.1847 Factor (A X B) 0.4131

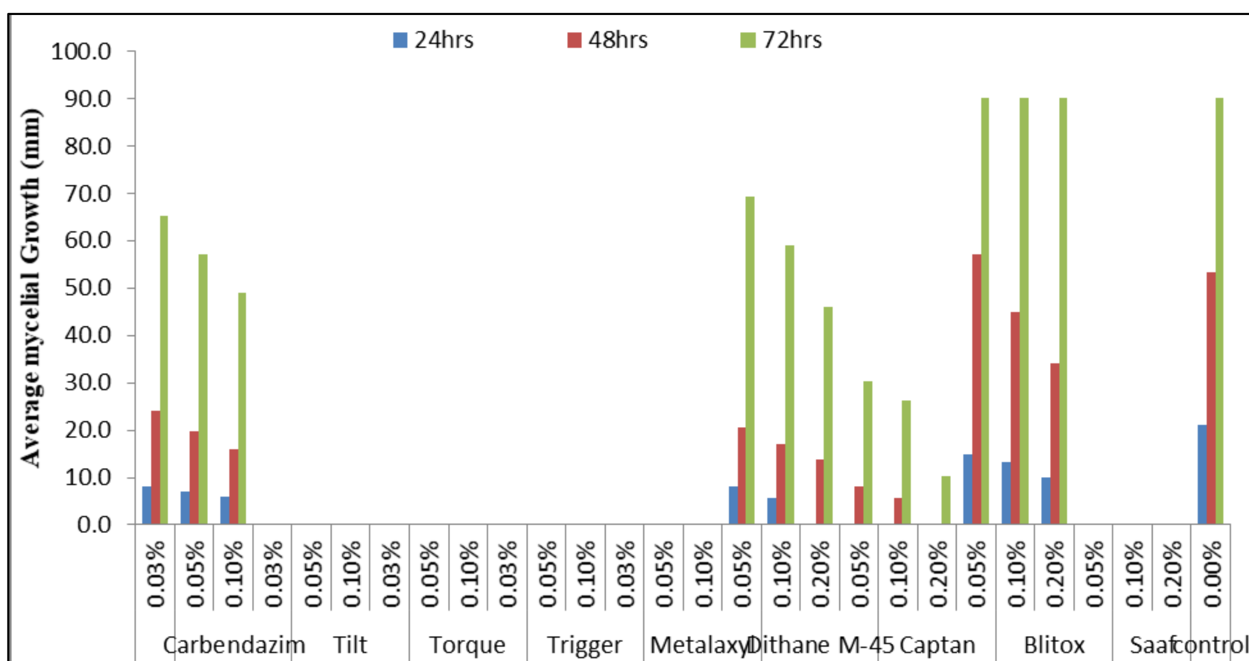

Fig 2: Effect of different Fungicides on *Sclerotium rolfsii*

Discussion

Bhuiyan *et al*, 2012 [2] studied the effect of six fungicides namely Provax-200, Bavistin, Ridomil, Dithane M-45, Rovral 50 WP and Tilt (at 100, 200 and 400 ppm concentration) for their efficacy against the radial colony growth of *S. rolfsii* and found that, complete inhibition was obtained with Provax-200 and Tilt at all concentrations.

These findings for Provax-200 in this study were supported by the findings of Rubayet (2011) who concluded that complete inhibition of sclerotia formation of the tested pathogen was achieved highest at 250 ppm and 500 ppm concentration of Provax-200 but sclerotia formation of *S. rolfsii* was completely inhibited at 100 ppm, 250 ppm and 500 ppm concentration.

For management of plant disease, Biological control is an efficient, environment friendly and alternative approach. Many microbial species have been reported that act efficiently against different pathogens. *Trichoderma spp., Bacillus spp*. and other microbial spp. have showed efficient inhibition of pathogens *in-vitro* and *in-vivo*. Kumar *et al*., 2012 studied the antagonistic properties of *Trichoderma* isolates and found that *Trichoderma* have higher potential antagonism against S. rolfsii with percent inhibition of 44.67 and 47.88.

Similarly, Kulkarni (2007) [4], found maximum inhibition of mycelial growth in *T. harzianum* (59.81%), followed by T. harzianum (57.97%) and least inhibition of mycelial growth was observed in Bacillus subtilis (10.74%). Bisht *et al*., 2013 tested different strains of Trichoderma spp. against *Curvularia* leaf spot of maize and found that *Trichoderma harzianum*, Th-13 showed maximum mycelial growth inhibition (83.82 %) followed by Th-9 (80.29 %) and Th-3 (79.12 %).

References

1. APEDA. Agricultural and Processed Food Products Export Development Authority, 2015. http://agriexchange.apeda.gov.in/IntTrade/IntTrade.aspx
2. Bhuiyan MA, Rahman HB, MT Bhuiyan KA. In vitro screening of fungicides and antagonists against *Sclerotium rolfsii*. African Journal of Biotechnology. 2012; 11(82):14822-14827.
3. Bisht SC, Mishra PK, Joshi JK. Genetic and functional diversity among root-associated psychrotrophic Pseudomonad's isolated from the Himalayan plants. Archives of Microbiology. 2013; 45:1237-1245.
4. Kulkarni M, Chaudhari R, Chaudhari A. Novel tensio-active microbial compounds for bio-control applications. In: General concepts in integrated pest and disease management (eds) Ciancio A and Mukerji KG, Springer, 2007, 295-304
5. Kumar A, Saini S, Wray V, Nimtz M, Prakash A, Johri BN. Characterization of an antifungal compound produced by *Bacillus* sp. Strain A3F that inhibits *S. sclerotiorum.* Journal of Basic Microbiology. 2012; 52:670-678.
6. Nashwa Sallam MA, Abo-Elyousr KAM, Hassan MA. Evaluation of *Trichoderma* species as biocontrol agent for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. Egypt Journal of Phytopathology. 2008; 36:81-93.
7. Nene YL, Thapliyal PN. Fungicides in Plant Disease Control, Oxford abd IBH Publishing House, New Delhi, 1982, 163.
8. Punja ZK. *Sclerotium (Athelia) rolfsii*, A pathogen of many plant species. In: Sidhu GS, ed. Genetics of plant pathogenic fungi. Vol. 6. London: Academic Press, 1988, 523-534
9. Rifai MA. A revision of the genus *Trichoderma*. Mycology Papers. 1969; 116:1-116.
10. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 1947; 150:850.
11. West E. *Sclerotium rolfsii*, history, taxonomy, host range, and distribution. Phytopathology. 1961; 51:108-109.