Potential of some fungicides on the growth and development of *Sclerotium rolfsii Sacc. in vitro*

N. C. Das[1], B. K. Dutta[1], D.C.Ray[2]

[1]Microbial and Agricultural Ecology & Biodiversity Conservation laboratory, Department of Ecology & Environmental Science, Assam University, silchar-788011
[2]Integrated pest management Laboratory, Department of Ecology & Environmental Science, Assam University, silchar-788011

Abstract- S*clerotium rolfsii* Sacc. is known to be a serious pathogen on many crops of economic importance. This pathogen is the major constraint in successful cultivation of most of the cash crops in Barak Valley. The present investigation was carried out to evaluate the potential of six systemic fungicides (i.e. Propiconazole 25% EC, Hexaconazole 5% EC, Mycobutanil 10% WP, Thiophanate Methyl 70% WP, Tebuconazole 25.9 m/m EC & Carbendazim 50%WP); three non-systemic fungicides(i.e. Captaf, Mancozeb 75%WP & Copper oxychloride) and three combo fungicides(i.e. Metalaxyl 8% +Mancozeb 64% , Carbendazim 12% + Mancozeb 63% & Carboxin 37.5% + Thiram 37.5%). These fungicides were evaluated at different concentrations (i.e. 1, 10, 50 and 100 ppm), except copper-oxychloreide (contact) at 0.1%, 0.25%, 0.5% and 1.00% on the growth of *Sclerotium rolfsii in* Potato dextrose agar (PDA) medium using poisoned food technique, *in vitro*. The result shows that the effect of hexaconazole (systemic) has been highly effective in suppressing radial expansion as well as percent inhibition of the fungus at all the concentrations used followed by Carboxin 37.5% + Thiram 37.5%(combo Fungicide) and tebuconazole (systemic). The results suggest that some of these fungicides may be tried against *S. rolfsii* in susceptible crop plants (i.e. brinjal) under field condition.

Index Terms- Fungicides, inhibitory, *in vitro*, radial growth, *Sclerotium rolfsii*

I. INTRODUCTION

Sclerotium rolfsii Sacc.is a devastating soil-borne fungus and infects more than 500 plant species in tropical and subtropical countries of the world (Aycock, 1966; Punja 1985). It can infect seeds, seedlings, mature plants in the field, cause diseases to fresh vegetables and rhizomes, while in storage and transit (Dasgupta and Mandal 1989).

The Barak Valley situated at Southern part of Assam is one of the important regions for both agricultural and horticultural point of view. The economy of the poor families living in this region is always depending on agriculture. The prevailing uncertain climatic condition leads to various plant diseases resulting economic loss of agricultural productivity.

Keeping the above in view the present work was taken up to evaluate some systemic, contact and combo (combination) fungicides against the pathogen *S. rolfsii* in vitro.

II. MATERIALS AND METHODS

Isolation of the target Fungus

Affected brinjal plants showing the typical symptoms of foot rot disease were collected from the farmers' field for isolation of the fungus through standard isolation technique. Affected tissue bits were surface sterilized with 1:1000 mercuric chloride solutions for one minute and then aseptically transferred to Petri plates having potato dextrose agar medium. These plates were then incubated at 25± 2^0C for 72 hours. The fungal growth, which arose through the infected tissues were aseptically transferred to the PDA slants.

In vitro* evaluation of fungicides against *S. rolfsii:

The efficacy of six systemic fungicides(Propiconazole 25% EC, Hexaconazole 5% EC, Mycobutanil 10% WP, Thiophanate Methyl 70% WP, Tebuconazole 25.9 m/m EC & Carbendazim 50% WP); three non-systemic fungicides(Captan 50% WP, Mancozeb 75%WP & Copper oxychloride) and three combo fungicides(Metalaxyl 8% +Mancozeb 64% , Carbendazim 12% + Mancozeb 63% & Carboxin 37.5% + Thiram 37.5%) was evaluated *in vitro* at different concentrations of 1, 10, 50 and 100 ppm, except copper-oxychloride(contact) at 0.1%, 0.25%, 0.5% and 1.00% on the growth of *Sclerotium rolfsii* on Potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal, 1982). Three replications were maintained for each treatment. Potato dextrose agar medium without any of the fungicide served as control. The plates were incubated at 25± 2^0C for recording radial growth of the target fungus *in vitro.*

Growth parameter and analysis of data:

The mean colony diameter in each treatment was recorded by taking diameter of the colony in two directions at 48 hrs after inoculation and subsequent data were recorded at 24 hrs interval. The percent inhibition of the growth over control was calculated at 120 hrs after inoculation by using the formula given by Vincent (1947):

$$I = \frac{C-T}{C} \times 100$$

Where,
I=percent inhibition
C=growth in control
T=growth in treatment

The recorded data were analysed by using Microsoft office Excell-2007, SPSS- 19.2

III. RESULTS AND DISCUSSION

From the results, it can be seen that the lowest radial mycelial growth of *Sclerotium rolfsii* was observed at all the concentrations of Hexaconazole followed by Tebuconazole and highest growth was recorded with Thiophanate Methyl followed by Carbendazim 50WP (Table 1).

All the tested systemic fungicides showed initial inhibitory effect at all the concentrations used as compared to control, except Thiophanate Methyl at 1 ppm. However, Hexaconazole and Tebuconazole exhibited 100% inhibition upto 144 hrs at both 50 and 100 ppm concentration followed by Mycobutanil upto 96 hrs at 100 ppm concentration. Moreover, Hexaconazole at very low concentration had shown satisfactory suppression on the radial growth of S. *rolfsii* i.e. 75.22 % at 1 ppm and 91.33% at 10 ppm as recorded after 120 hrs of incubation following inoculation. Thiophenate methyl had shown less inhibitory effect at all the concentrations tested (Table 1& 4).

Out of the non-systemic fungicides, all the three tested fungicides have shown slight intial inhibitory effect on the test fungus at all the concentration of treatment as compared to control. However, Mancozeb and Captan showed higher inhibitory effect as compared to Copper oxychloride (Table 2, 4).

Among the three combo fungicides, all of them have exhibited initial inhibitory effect on test fungus at all the concentrations used as compared to control. However, Carboxin 37.5% + Thiram 37.5% showed 100% inhibition upto 144 hrs at both 50 and 100 ppm concentration and upto 72 hrs at 10 ppm concentration(Table 3,4).

Hexaconazole (systemic) is found to be the most effective fungicide followed by Carboxin 37.5% + Thiram 37.5%(combo Fungicide) and Tebuconazole (Table 4).

Table 1: Effect of selected systemic fungicide on the radial expansion of S*clerotium rolfsii, in vitro*

Name of fungicides	Treatment	Radial expansion of the test fungus (mm)				
		48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
	Control	29.0±1.11	50.0±1.51	73.0± 2.48	90.0± 0.00	90.0± 0.00
Propiconazole 25% EC	1 ppm	10.5±0.28	27.16±0.92	54.7±1.92	65.5±2.52	83.7±1.85
	10 ppm	7.5±0.50	16.83±0.16	28.3±0.66	40±0.58	49.0±0.76
	50 ppm	0.0±0.00	13.83±0.33	19±0.76	19.7±0.33	20.0±0.28
	100 ppm	0.0±0.00	8.3±0.88	10.3±1.09	10.8±1.36	11.0±1.32
Hexaconazole 5% EC	1 ppm	0.00	14.7±0.72	17.5±0.76	22.3±0.33	28.0±0.57
	10 ppm	0.00	7.7±0.33	8.5±0.57	7.8±0.17	7.8±0.16
	50 ppm	0.00	0.00	0.00	0.00	0.00
	100 ppm	0.00	0.00	0.00	0.00	0.00
Mycobutanil 10% WP	1 ppm	10.0±1.04	18.6 ±0.53	41.6± 0.90	86.0 ±1.8	90.0 ±0.00
	10 ppm	09.0±0.29	16.6± 0.12	34.4 ±0.12	80.0 ±0.50	90.0± 0.00
	50 ppm	0.0± 0.00	0.0± 0.00	19.0 ±0.15	56.3 ±0.36	74.0± 0.40
	100 ppm	0.0 ±0.00	0.0± 0.00	0.0± 0.00	34.3 ±0.30	62.0± 0.87
Thiophanate Methyl 70% WP	1 ppm	29.0±0.92	49.0± 0.98	72.0± 0.83	90.0 ±0.00	90.0± 0.00
	10 ppm	25.3±0.26	48.0 ±1.30	69.8± 1.47	90.0 ±0.00	90.00±0.00
	50 ppm	24.0±0.42	47.8± 0.72	66.7± 0.97	90.0 ±0.00	90.0± 0.00
	100 ppm	18.3±0.69	25.6 ±0.53	41.4± 0.92	72.5 ±2.08	89.0 ±1.06
Tebuconazole 25.9 m/m EC	1 ppm	7.5±0.28	19.2±0.44	36.0±1.75	43.5±0.76	63.3±1.33
	10 ppm	00.00	11±0.29	21.17±2.24	24.7±2.03	28.83±2.58
	50 ppm	0.00	0.00	0.00	0.00	0.00
	100 ppm	0.00	0.00	0.00	0.00	0.00
Carbendazim 50%WP	1 ppm	12.2±1.06	48.7±2.06	61.2±1.63	90.0±0.00	90.0±0.00
	10 ppm	10.6±0.40	44.6±2.40	59.2±0.64	88.8±0.40	90.0±0.00
	50 ppm	10.2±0.30	43.2±0.70	58.4±0.61	88.4±0.42	90.0±0.00
	100 ppm	10.2±0.50	34.2±1.02	51.3±2.02	78.2±1.22	88.0±0.61
	F-test	38.12	22.80	14.50	18.90	14.64

Values are mean ± SE of three replication

Table 2: Effect of selected non systemic(contact) fungicide on the radial expansion of S*clerotium rolfsii, in vitro*

Name of fungicides	Treatment	Radial expansion of the test fungus (mm)				
		48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
	Control	35.3±0.66	63.5±1.35	80±1.90	90±0.00	90.0± 0.00
Captan 50% WP	1 ppm	28.9±0.92	48.6±1.40	61.0±0.72	82.0±1.17	90.0±0.00
	10 ppm	26.0±0.61	44.0±0.83	60.6±0.94	81.3± 0.87	90.0± 0.00

	50 ppm	24.8±0.41	42.0±0.81	57.7± 0.70	80.0± 1.25	84.0± 1.33
	100 ppm	21.3±1.04	39.8±0.81	53.3± 1.42	75.6±1.27	80.2±0.64
Mancozeb 75%WP	1 ppm	28.2±0.92	44.0± 2.02	64.3 ±1.43	86.0± 2.43	90.0± 0.00
	10 ppm	27.0±0.41	43.7 ±0.68	57.5 ±2.07	82.6± 0.99	90.0± 0.00
	50 ppm	24.9±0.93	41.0 ±0.72	54.0 ±1.17	78.3 ±0.47	90.0 ±0.00
	100 ppm	20.8±0.30	38.4± 1.11	48.0 ±0.53	72.0 ±0.99	87.0± 2.10
Copper oxychloride	0.10%	27.80±0.92	48.60±0.50	67.80±0.20	90.00±0.00	90.0±0.00
	0.25%	27.60±0.49	46.40±0.83	64.20±1.35	88.50±0.38	90.0±0.00
	0.50%	24.90±0.74	44.00±0.61	58.40±0.30	87.80±0.50	90.0±0.00
	1.00%	24.00±1.39	42.60±0.40	53.60±0.50	83.20±1.06	90.0±0.00
	F-test:	15.45	51.31	18.66	4.05	2.23

Values are mean ± SE of three replication

Table 3: Effect of selected combo fungicide on the radial expansion (growth) of S*clerotium rolfsii, in vitro*

Name of fungicides	Treatment	Radial expansion of the test fungus (mm)				
		48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
	Control	35.3±0.66	63.5±2.25	80±1.73	90±0.00	90.0± 0.00
Metalaxyl 8% +Mancozeb 64%	1 ppm	26.4±1.13	39.8 ±1.58	54.0 ±1.44	84.0 ±2.03	90.0± 0.00
	10 ppm	24.0±1.47	32.5 ±0.68	48.6 ±0.95	83.3 ±0.64	90.0± 0.00
	50 ppm	21.0±1.01	30.0± 1.11	43.6 ±1.33	73.3± 2.07	83.3± 0.44
	100 ppm	17.6±0.72	26.0± 2.03	31.0± 0.72	57.5 ±1.78	72.0 ±1.06
Carbendazim 12% + Mancozeb 63%	1 ppm	28.4±0.70	41.4±0.94	58.2±1.06	88.2±1.04	90.0±0.00
	10 ppm	24.8±0.72	34.6±0.87	49.8±0.35	86.0±0.47	90.0±0.00
	50 ppm	21.4±0.83	31.0±1.01	44.6±0.99	77.0±0.50	90.0±0.00
	100 ppm	18.6±0.42	28.2±0.61	34.0±0.53	58.4±0.31	74.4±0.92
Carboxin 37.5% + Thiram 37.5%	1 ppm	14.0±0.61	26.8±0.92	44.0±0.70	54.2±1.06	74.2±0.95
	10 ppm	0.0	0.0	8.4±0.31	12.2±0.60	26.6±0.42
	50 ppm	0.0	0.0	0.0	0.0	0.0
	100 ppm	0.0	0.0	0.0	0.0	0.0
	F-test:	5.94	41.66	18.50	17.16	11.20

Values are mean ± SE of three replication

Table 4: Effect of systemic, non systemic (contact) and combo fungicide on the percent inhibition of radial growth of *S. rolfsii* at different concentrations

Name of fungicides	Percent inhibition on radial growth of ***S. rolfsii*** in different concentrations of test fungicides observed at 120 hrs after inoculation			
	1 ppm	10 ppm	50 ppm	100 ppm
Propiconazole 25% EC	27.22	55.55	78.11	87.66
Hexaconazole 5% EC	75.22	91.33	100.00	100.00
Mycobutanil 10% WP	4.44	11.11	37.45	61.89
Thiophanate Methyl 70% WP	0.00	0.00	0.00	19.44
Tebuconazole 25.9 m/m EC	51.66	72.55	100.00	100.00
Carbewndazim 50% WP	0.00	8.66	8.22	3.11
Captan 50% WP	8.89	9.67	11.11	16.00

Mancozeb 75%WP	4.44	8.22	13.00	20.00
Copper oxychloride*	0.00	1.67	2.44	7.55
Metalaxyl 8% +Mancozeb 64%	6.67	7.44	18.56	36.11
Carbendazim12%+Mancozeb 63%	2.00	4.44	14.44	35.11
Carboxin 37.5% + Thiram 37.5%	39.78	86.44	100.00	100.00

S*clerotium rolfsii* is known to be a serious pathogen on many of the crop plants of economic importance (Aycock, 1966). This pathogen is the major constraint in successful cultivation of most of the cash crops in Barak Valley. The extensive field survey through this entire geographic region reveals that the foot rot disease caused by *S. rolfsii* is widely distributed and causes severe damage to many cash crops during all the season(i.e. Kharif and Rabi season).

In the present investigation, six systemic fungicides; three non- systemic and three combo fungicides were evaluated for their potential of inhibition to the growth of the pathogen (*S. rolfsii*) *in vitro*. This was done with poisoned food techniques (Nene and Thapliyal, 1982).

The two fungicides viz Hexaconazole & Tebuconazole among the systemic fungicides, were found to be highly effective at all the concentrations used followed by propiconazole and mycobutnil. Least inhibition was observed with Thiophanate methyl and Bavistin. The result conform with the established findings of earlier workers viz. Prabhu(2003), Choudhury et al. (1998). Manu et. al.(2012) reported that hexaconazole, Tebuconazole & propiconazole were found to be having strong inhibitory effect on the growth of *S. rolfsii* isolated from finger millet at lower concentration.

Out of the three combo fungicides, Carboxin 37.5% + Thiram 37.5% is found to be highly inhibitory on the growth of *S. rolfsii* and it is in accordance with the works of many workers viz. Vyas and Joshi (1977) , Sujatha (1991), Manu et.al.(2012), reported Carboxin was highly effective against *S. rolfsii*..

Systemic fungicides (i.e. benomyl, thiophenate methyl, bavistin) have reported to have given excellent control to the wilt disease of tomato caused by *Verticillium albo-atrum* R. & B (Dutta 1980). Deb and Dutta (1989) reported that nitrofurans caused complete inhibition of *Sclerotium rolfsii* at 1000 µg/ml *in vitro*. The furans have also reduced disease severity to the *S. rolfsii* infected soybean plants under field condition.

Dutta *et al.*(1992) has also reported that some systemic and contact fungicides (i.e. Tridemorph, Hexaconazole, Propiconazole, Cyproconazole, Carbendazim, Carboxin and Copper) applied *in vitro* and as foliar spray gave excellent control of Blister blight disease of tea causing organism *Exobasidium vexans*. It has further reported by Dutta (1994) that some systemic and contact fungicides could control some of the economically important tea diseases caused by *Exobasidium vexans*, *Corticium theae*, *Cephaleuros parasitic* and *Tunstallia acculata* under the agroclimatic condition of Darjeeling district of West Bengal and Barak Valley of Southern Assam respectively.

Based on the result presented above it can be suggested that the fungicides (i.e. Hexaconazole, Carboxin + Thiram (vitavex power), Tebuconazole and Propiconazole) can be recommended to control *S. rolfsii* under field condition for the susceptible crop plants i.e. Brinjal. The experiment under field condition is in progress, the results of the same will be communicated in due course of time.

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AUTHORS

First Author – Nikhil Chandra Das, M.Sc.(Agri) in Plant Pathology, Email: ncdas220266@gmail.com
Second Author – Prof. Biman Kumar Dutta, M.Sc. Ph.D(Wales). F.L.S. (London), Email: bimankdutta@rediffmail.com
Third Author – Prof. Dulal Chandra Ray, M.Sc. Ph.D, Email: raydulal@yahoo.co.in

Correspondence Author – Nikhil Chandra Das, M.Sc.(Agri) in Plant Pathology, Email: ncdas220266@gmail.com