

Original Research Article

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An *In-Vitro* Assay: Employing Different Management Options for Collar Rot Disease of Sunflower Caused by *Sclerotium rolfsii* Sacc. Under Coastal Zone of West Bengal, India

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ABSTRACT

Keywords

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The experiment was conducted to control the *Sclerotium rolfsii* causing of collar rot disease in sunflower *in-vitro* condition. Different fungicides, region specific bio-agents and bio-rationals were screened against the pathogen. Among the fungicides Tebuconazole gave significantly (at 5% level) highest percent inhibition of the growth in all concentrations. Among the seven bio-rationals at 10% concentration, the maximum per cent inhibition was observed in neem (69.26%) wherever at 20% and 30% concentration the maximum per cent inhibition was observed in cow urine (80.74%). In the case of bio agents highest per cent inhibition of the fungal growth was obtained in *T. harzianum* (90.67%).

Introduction

India is one of the leading oilseeds producing countries in the world with 21 % of world's area and 15% of world's production. Oilseeds also form the second largest agricultural product subsequent to cereals sharing 13% of the country's gross cropped area and accounts for nearly 5% of gross national product and 10% of the value of all agricultural products. Sunflower is scientifically known as

Helianthus annuus, derived from the name of Greek sun God Helios. It was originally grown as an ornamental plant for its attractive flowers and photosensitive nature. The total area under sunflower in the world is about 25.34 million hectare with the production of 40.04 million tonnes (USDA, 2012). In India, sunflower occupies the fourth place among oilseed crops in terms of acreage and production. The area under sunflower cultivation in India was 0.72 m ha, with a

total production of 0.50 m t and productivity of 692 kg/ha (Anon., 2013). Collar rot in sunflowers by *Sclerotiumrolfsii*, Mizra and Khokhar (1985).

Sclerotium rolfsii is a soil borne plant pathogen causing root rot, stem rot, collar rot, wilt and foot rot diseases on more than 500 plant species of agricultural and horticultural crops throughout the world (Aycock, 1966). It is one of the most destructive soil inhabiting pathogens so far reported. It has a quite serious status in coastal belt of Sundarbans.

Materials and Methods

Collection of diseased samples and isolation of the pathogen

The infected collar parts of sunflower plant were cut into small bits and washed in running water. These bits were surface sterilized with 0.01% mercuric chloride solution for one minute, were washed thoroughly with sterile distilled water for three times to remove the traces of mercuric chloride if any and then aseptically transferred to Petri-plates containing the sterilized PDA medium.

The plates were incubated at $27\pm 2^{\circ}\text{C}$ for three days. The fungal growth on fourth day, which arose through the infected tissue was taken by inoculation loop and transferred aseptically to the PDA slants.

Maintenance of culture

The pure culture of the fungus was obtained by further growing of the culture on the PDA slants and allowed to grow at $27\pm 2^{\circ}\text{C}$ temperature. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically (15 day interval).

Evaluation of fungicides

The efficacy of seven fungicides was evaluated against *S. rolfsii in-vitro* by poisoned food technique in three concentrations (viz. 100 ppm, 150 ppm and 200 ppm). Required quantity of individual fungicide was added separately into sterilized molten and cooled potato dextrose agar in conical flask so as to get the desired concentration of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelium discs of 5 mm diameter from five days old pure culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. The plate without any fungicide served as control. Three replications were maintained for each concentration. The plates were incubated at room temperature ($27 \pm 2^{\circ}\text{C}$) and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides were expressed as per cent inhibition of mycelial growth over control and calculated by using the formula given by Vincent (1947).

Evaluation of bio rational

Different bio rational (viz. cow urine, neem-*Azadirachta indica*, Tulsi –*Ocimum tenuiflorum*, papaya- *Carica papaya*, Boganvilla – *Boganvilla septabilis*, Datura – *Datura stramonium* and Garlic- *Allium sativum* including control) were evaluated under *in-vitro* conditions by using poison food technique .

Fresh plant samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding a sufficient quantity of sterile distilled water just enough to prepare a 50% w/v standard extract (i.e. stock solution). The extracts were collected by filtering through the two layers

of muslin cloth. Finally, filtrate thus obtained from the leaves were used as stock solution. The stock solution was heated up to 40-50°C for 10 minutes to avoid contamination. To avoid contamination and chemical alteration, the extract was stored at 4°C.

Three different concentrations *i.e.* 10%, 20% and 30% v/v poisoned media for each treatment (plant extract) were prepared by adding required quantity of stock solution separately into sterilized molten and cooled potato dextrose agar medium in conical flask so as to get the desired concentrations. About 20 ml medium of each concentration was poured into 90 mm sterilized Petri plates. Three replications were maintained for each treatment

Cow urine collection and preparation

Fresh cow urine was collected in sterilized vessels from desi cows and was stored in earthen pots. 15 days old cow urine was used for further studies (Sathasivam *et al.*, 2010). A 50% v/v stock solution was prepared by adding sterile water into cow urine. Three different concentrations *i.e.* 10%, 20% and 30% v/v poisoned media for cow urine treatment were prepared by adding required quantity of stock solution of cow urine separately into sterilized molten and cooled potato dextrose agar medium in conical flask so as to get the desired concentration. The media of different concentrations were autoclaved. About 20 ml medium of each concentration was poured into 90 mm sterilized Petri plates. Three replications were maintained for each treatment. Suitable control (without any treatment) was maintained.

To study fungicidal efficacy of the bio-rational, poisoned food technique was followed (Nene and Thapliyal, 1982). The

fungal discs of 5 mm diameter were taken from actively growing 7 day old pure culture by using cork borer and were transferred aseptically on PDA plates poisoned with different treatments. The inoculated plates were incubated at $27 \pm 1^{\circ}\text{C}$ till the growth of the colony touched the periphery in control plate. Mean colony diameter for each treatment was recorded. The efficacy of the treatments were expressed as per cent inhibition of mycelial growth over control by using the formula as given by Vincent (1927).

Continuous and injudicious use of chemicals against the disease causing organisms deteriorates the soil health as well as poses environmental hazards. In this situation organic farming has been encouraged in the field of agriculture. Therefore, in the present study, an eco-friendly management option was carried out by using antagonistic bio-agents.

Evaluation of region specific bio-control agents

Isolates of *Trichoderma* sp. and *pseudomonas* from farmer's field of Kultali block in South 24 Parganas district were screened for their antagonistic ability against the disease collar rot disease of Sunflower caused by *Sclerotium rolfsii* by dual culture technique.

Twenty ml molten PDA medium was poured into sterile Petri plates and allowed to solidify. Five mm mycelial disc was cut from the margin of the actively growing 8 day old culture of *S. rolfsii*, with a sterile cork borer and placed near the periphery, *i.e.* one side of the PDA plate and, antagonistic fungus was placed on the other side of the PDA plate just opposite to the pathogen disc.

Similarly, antagonistic bacterium was streaked on one side of the PDA medium in Petri dishes. Simultaneously, one 5 mm

mycelial disc of *S. rolfsii* were cut from the margin of the actively growing colony with a sterile cork borer and placed on the opposite sides of the bacterial streak. All the plates were incubated at 27° C±1°C for five days. Each treatment replicated five times and appropriate control (without bio control agent) were maintained. The extent of antagonistic activity by fungal and bacterial antagonists was recorded on fifth day by measuring the growth of *S. rolfsii* in dual culture plate and control plate. The per cent inhibition of *S. rolfsii* was calculated as suggested by Vincent (1947).

Results and Discussion

Evaluation of fungicide against the disease

Seven different fungicides in three different concentrations (viz. 100ppm, 150 ppm and 200 ppm) were evaluated with one suitable control.

From the table 1 it was evident that among the seven fungicides with different doses the minimum radial growth was observed in tebuconazole in all three concentrations followed by hezxaconazole, propiconazole and carboxin except in 200ppm where mancozeb showed less radial growth as compared to propiconazole .All the treatments differed among themselves significantly at 5% level except in between tebuconazole and hexaconazole as well as in between propiconazole and carboxin in 150 ppm concentration.

It is also evident from table 2 that maximum per cent decrease in radial growth was found in tebuconazole followed by hexaconazole, propiconazole and carboxin in all three concentrations except in 200ppm where mancozeb showed more per cent decrease in radial growth as compared to propiconazole. The percent inhibition zone for all treatments

were calculated and out of all treatments tebuconazole gave significantly (at 5% level) highest percent inhibition of the growth in all three concentrations as compared to other treatments and control. The second best inhibition obtained in hexaconazole in all three concentrations followed by propiconazole and mancozeb in 100 and 150 ppm and followed by hexaconazole and mancozeb in 200 ppm. (Table 1)

Chowdary (1997) reported that propiconazole at a concentration of 250 ppm was effective in complete inhibition of *S. rolfsii*. In the present study also propiconazole gave more than 80% inhibition at all three concentration used.

Chowdhury *et al.*, (1998) found hexaconazole significantly superior to other fungicides even at lower concentration (50 ppm) in checking the growth of *S. rolfsii*. This supported the present finding where hexaconazole gave more than 94% inhibition at higher concentration (150 and 200 ppm)

Manu *et al.*, (2012) showed that propiconazole, hexaconazole gave 100% inhibition zone as compare to the control however, carbendazim and tricyclazole could not show effective inhibitions of *Sclerotium rolfsii*.

Bhagat and Chakraborty (2013) found mancozeb as least efficient in inhibition of mycelial growth of *Sclerotium rolfsii* among five different fungicides evaluated *in-vitro* in the present study also mancozeb did not show efficient inhibition in lower concentration.

It was reported that among eight fungicides tested *in vitro* against *S. rolfsii*, the maximum (100%) inhibition was observed in carboxin, propiconazole, hexaconazole, difenconazole and carbendazim at all three concentrations viz., 500, 1000 and 1500 ppm followed by captan (79.30, 82.76 and 85.23%) and

triadimenfon (49.13, 60.23 and 65.33%) over control (Begum, *et.al.*, 2014). 100% inhibition might be due to the higher concentration of different chemicals however, in the present study 100% inhibition was found in tebuconazole treatment at a concentration of 200ppm.

Evaluation of bio-rationals against the disease

Seven different biorational in three different concentrations (viz. 10%, 20 % and 30%) were evaluated with one suitable control.

From the table 3 it was evident that among the seven bio-rational at 10% concentration, the minimum radial growth was observed in neem (27.67mm) followed by cow urine (28.67mm), datura (29.67mm) and Tulsi (30.67mm). At 20% concentration the minimum radial growth was observed in cow urine (17.33mm) followed by neem (18.67mm), tulsi (21.33 mm) and datura (23.33mm). At 30% concentration the minimum radial growth was observed in cow urine (0.0 mm) followed by neem (8.67mm), tulsi (13.67 mm) and datura (17.67mm). It is also evident from table 3 at lower concentration (10%) these four biorational did not show significant (at 5%) difference among themselves. However at highest concentration (30%) all treatments differed significantly among themselves.

The percent inhibition zone for all treatments were calculated and from the table Y it was evident that among the seven bio-rationals at 10% concentration, the maximum per cent inhibition was observed in neem (69.26%) followed by cow urine (68.15%), datura (67.04%) and tulsi (65.93%). At 20% concentration the maximum per cent inhibition was observed in cow urine (80.74%) followed by neem (79.26%), tulsi (76.30%) and datura (74.07%). At 30%

concentration the maximum per cent inhibition was observed in cow urine (100%) followed by neem (90.37%), tulsi (84.81%) and datura (80.37%) It is also evident from table 4 that at highest concentration (30%) all treatments differed significantly among themselves.

It is also evident from table 5 that maximum per cent decrease in radial growth was found in cow urine followed by neem, tulsi and datura higher concentrations (20% and 30%). However, at 10% concentration highest per cent decrease in radial growth was found in neem followed by cow urine, datura and tulsi.

It was reported that extracts of *Allium sativum*, *Azadirachta indica* and *Catharanthus roseus* [*Catharanthus roseus*] (all at 10% concentration) were promising against *S. rolfsii*. It was also reported that steam distillates from leaves of *Ocimum gratissimum* completely inhibited the radial growth of *S. rolfsii* (Haralpatil and Raut 2008).

Among eight different plant extracts evaluated *in vitro* against *Sclerotium rolfsii* causing dry root rot in chillies, leaf extract of neem (*Azadirachta indica*) caused maximum inhibition of mycelial growth (80.74%) (Madhavi *et al.*, 2011) which also supported the present findings.

Amin *et al.*, (2013) evaluated the effect of different plants extracts and namely rhizome of turmeric, rhizome ginger, neem leaf, tobacco leaf, tobacco leaf extract in water, tobacco leaf extract in cow's urine, and cow's urine at different concentrations (70%, 60%, 50%, 40% and 30%) on the growth of *Sclerotium rolfsii*. They reported that no growth of the tested fungus was observed in all concentrations of tobacco leaf extract in cow's urine, 70%, 60%, 50%, 40% concentration of cow's urine alone, and 70% and 60% concentration of tobacco leaf

extracts in water. In the present study even lower concentration (30%) of cow urine alone showed 100% growth inhibition of the fungus.

Begum *et al.*, (2014) reported that among different botanicals evaluated against *S. rolfsii*, significantly highest average inhibition was recorded with neem (74.81%), followed by tulsi (67.10%) at 5% and 10 %

concentrations. In the present study neem and tulsi also showed 69.26% and 65.93% inhibition respectively at 10% concentration.

Pawar *et al.*, (2014) reported that neem (*A. indica*), turmeric (*C. longa*) and tulsi (*O. sanctum*) might have some strong toxic principle that directly affects the growth of *Sclerotium rolfsii*.

Table.1 *In-vitro* evaluation of different chemicals against the disease on radial growth

Sl. No.	Fungicides	Radial growth in (mm)		
		100 ppm	150 ppm	200 ppm
1	Tebuconazole 25.9% EC (Folicur)	4.43	3.40	0
2	Hexaconazole 5 % SC (Contaf Plus)	12.60	5.07	4.00
3	Propiconazole 25 % EC (Result)	17.63	14.37	11.57
4	Mancozeb 75% WP (Indofil M-45)	43.67	24.00	7.33
5	Carboxin 75% WP (Vitavax)	18.67	14.67	11.97
6	Carbendazim50 % WP (Bavistin)	89.67	87.33	72.13
7	Tricyclozole 75 % WP (Blastogan)	77.50	75.67	65.37
8	Control	90.00	90.00	90.00
	SE ±(m)	0.79	0.95	2.85
	CD (at 5%)	2.38	2.86	8.56

Table.2 *In vitro* evaluation of different chemicals against the % decrease in Radial growth

Sl.No.	Fungicides	% Decrease in Radial growth		
		100 ppm	150 ppm	200 ppm
1	Tebuconazole 25.9% EC	95.07	96.22	100
2	Hexaconazole 5 % SC	86	94.37	95.56
3	Propiconazole 25 % EC)	80.41	84.03	87.15
4	Mancozeb 75% WP	51.48	73.33	91.85
5	Carboxin 75% WP	79.26	83.70	86.7
6	Carbendazim50 % WP	0.37	2.96	19.85
7	Tricyclozole 75 % WP	13.89	15.93	27.37
8	Control	0	0	0

Table.3 *In-vitro* evaluation of different chemicals against the disease on % of Inhibition zone

Sl. No.	Treatment	% of Inhibition zone		
		100 ppm	150 ppm	200 ppm
1.	Tebuconazole 25.9% EC	95.07(71.9)*	96.22(62.59)	100.00(71.57)
2.	Hexaconazole 5 % SC	86.00(63.82)	94.37(60.86)	95.56(61.95)
3.	Propiconazole 25 % EC)	80.41(60.34)	84.04(54.63)	87.15(56.18)
4	Mancozeb 75% WP	51.48(44.89)	73.33(50.14)	91.85(59.05)
5	Carboxin 75% WP	79.26(59.46)	83.70(54.45)	86.70(55.96)
6	Carbendazim50 % WP	5.69(16.46)	9.24(24.78)	27.87(34.24)
7	Tricyclozole 75 %WP	13.89(23.53)	15.93(28.95)	27.37(34.12)
8	Control	0 (0.01)	0(0.01)	0(0.01)
	SE± (m)	3.36	0.57	0.88
	CD (at 5%)	10.06	1.72	2.64

*Data in parenthesis is angular transformed value

Table.4 *In-vitro* evaluation of different bio-rational against the disease on radial growth

Sl. No.	Bio-rationals	Radial growth in (mm)		
		10%	20%	30%
1	Cow urine	28.67	17.33	0
2	Neem (<i>Azadirachta indica</i>)	27.67	18.67	8.67
3	Tulsi (<i>Ocimum tenuiflorum</i>)	30.67	21.33	13.67
4	Papaya (<i>Carica papaya</i>)	80.00	72.33	42.33
5	Bougainvillea (<i>Bougainvillea septabilis</i>)	69.67	41.67	34.33
6	Datura (<i>Datura stramonium</i>)	29.67	23.33	17.67
7	Garlic (<i>Allium sativum</i>)	39.33	31.67	22.67
8	Control	90	90.00	90.00
	SE ±(m)	1.06	0.90	0.68
	CD (at 5%)	3.18	2.69	2.03

Table.5 *In-vitro* evaluation of different bio-rationals against the disease % Inhibition zone

Sl. No.	Bio-rationals	% Inhibition zone		
		10%	20%	30%
1	Cow urine	68.15(55.65)*	80.74(63.97)*	100.00(90)*
2	Neem (<i>Azadirachta indica</i>)	69.26(56.33)	79.26(62.91)	90.37(71.93)
3	Tulsi (<i>Ocimum tenuiflorum</i>)	65.93(54.30)	76.30(60.87)	84.81(67.09)
4	Papaya (<i>Carica papaya</i>)	11.11(19.41)	19.63(26.26)	52.96(46.70)
5	Bougainvillea (<i>Bougainvillea septabilis</i> L.)	22.59(28.36)	53.70(47.12)	61.85(51.86)
6	Datura (<i>Datura stramonium</i>)	67.04(54.97)	74.07(59.41)	80.37(63.71)
7	Garlic (<i>Allium sativum</i>)	56.30(48.62)	64.81(53.62)	74.81(59.89)
8	Control	0 (0.01)	0(0.01)	0(0.01)
	SE± (m)	0.79	0.67	0.52
	CD at 5%	2.38	2.01	1.57

Table.6 *In-vitro* evaluation of different bio-rationals against the disease % decrease in radial growth over control

Sl. No.	Bio-rationals	% Decrease in Radial growth		
		10%	20%	30%
1	Cow urine	68.15	80.74	100.00
2	Neem leaf extract (<i>Azadirachta indica</i>)	69.26	79.26	91.11
3	Tulsi (<i>Ocimum tenuiflorum</i>)	65.93	76.30	84.44
4	Papaya (<i>Carica papaya</i>)	11.11	19.63	52.22
5	Bougainvillea (<i>Bougainvillea spectabilis</i> L.)	22.59	53.70	63.33
6	Datura (<i>Datura stramonium</i>)	67.04	74.07	82.22
7	Garlic (<i>Allium sativum</i>)	56.30	64.81	76.67
8	Control	0.00	0.00	0.00

Table.7 *In-vitro* evaluation of bio agent against the pathogen

Sl. No.	Bio-control agent	Radial growth in (mm)	Inhibition %	% Decrease in radial growth
1	<i>T. viride</i>	11.30	87.44 (74.00)*	87.44
2	<i>T. harzianum</i>	8.40	90.67 (77.40)	90.67
3	<i>P. fluorescence</i>	65.5	27.22 (31.22)	27.22
4	Control	90	0 (0.015)	0
	SE ±(m)	0.97	1.07	
	CD (at 5%)	2.98	3.31	

*Data in parenthesis is angular transformed value

Evaluation of bio-agents against the disease

To formulate an eco friendly management practices against the disease under study the efficacy of different bio agents were evaluated in the present experiment. It is evident from the table 6 that the minimum radial growth was found in *T. harzianum* (8.4 mm) followed by *T. viride* (11.30 mm). Both the fungal bio agents were found superior (at 5% level) to the bacterial one (*P. fluorescence*) (65.5mm) as well as control. However, both the fungal bio agents did not show significant (at 5% level) difference in between them.

It was also revealed that highest per cent inhibition of the fungal growth was found in

T. harzianum (90.67%) followed by *T. viride* (87.44 %) followed by *P. fluorescence* (27.22%). However, both the fungal bio agents did not show significant (at 5% level) difference in between them. Per cent decrease in radial growth was found highest in in *T. harzianum* followed by *T. viride* followed by *P. fluorescence*.

Dutta and Das (2002) observed the among the antagonism *T. harzianum*, *T. viride* and *T. koningi* against tomato isolate of *S. rolfii* by dual culture technique. Reduced the growth of the pathogen by 61.5, 59.1 and 57.2 per cent, respectively which supported the present study also.

Doley and Jite (2012) observed *T. viride*

significant antifungal activities on the *sclerotiumrolfsii*. *T. viride* significantly inhibited the mycelial radial growth of *S. rolfsii* by 75%.

Manu *et al.* (2012) showed that in evaluation of antagonistic microorganism *T. Harzianum* gave best result to control the disease which was at par with the present evaluation.

Conclusion of the study are as follows:

Evaluation of fungicides

Seven different fungicides in three different concentrations (viz. 100ppm, 150 ppm and 200 ppm) were evaluated with one suitable control. The percent inhibition zone for all treatments were calculated and out of all treatments tebuconazole gave significantly (at 5% level) highest percent inhibition of the growth in all three concentrations as compared to other treatments and control. The second best inhibition obtained in hexaconazole in all three concentrations followed by propiconazole and mancozeb in 100 and 150 ppm and followed by hexaconazole and mancozeb in 200 ppm.

Evaluation of bio-rational

Among the seven bio-rationals at 10% concentration, the maximum per cent inhibition was observed in neem (69.26%) followed by cow urine (68.15%), datura (67.04%) and tulsi (65.93%). At 20% concentration the maximum per cent inhibition was observed in cow urine (80.74%) followed by neem (79.26%), tulsi (76.30%) and datura (74.07%). At 30% concentration the maximum per cent inhibition was observed in cow urine (100%) followed by neem (90.37%), tulsi (84.81%) and datura (80.37%).

Evaluation of bio-agents

Among the bio agents evaluated the highest

per cent inhibition of the fungal growth was obtained in *T. harzianum* (90.67%) followed by *T. viride* (87.44 %) followed by *P. fluorescence* (27.22%). However, both the fungal bio agents did not show significant (at 5% level) difference in between them.

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