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Bio Efficacy of Fungicides, Botanicals, Bioagents and Organic amendments against *S. rolfsii* causing Stem Rot Disease of Groundnut

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ABSTRACT: Groundnut (*Arachis hypogaea* L.) is one of the world's major oil seed crops grown in both tropical and subtropical regions, suffers from many fungal diseases resulting in severe yield loss among those diseases stem rot which is a soil-borne disease caused by *Sclerotium rolfsii* Sacc. is the major yield-limiting factor. The present investigation is on *in vitro* evaluation of different fungicides, botanicals, bioagents and organic amendments against *S. rolfsii* to develop an integrated disease management approach against stem rot disease of groundnut. *In vitro*, studies revealed that Tebuconazole, Nativo, Hexaconazole, and Vitavax were found to be highly effective and completely inhibited (100 %) the mycelial growth of *Sclerotium rolfsii* at all three concentrations tested compared to other fungicides. Among botanicals, neem leaf extract(78.50 % @20%) highly inhibited the mycelial growth of *S. rolfsii,* whereas *Trichoderma harzianum* (71.10 %) exhibited maximum antifungal properties compared to other tested bioagents. Among organic amendments tested neem cake (52.80 %) was found to be effective against the mycelial growth of *S. rolfsii.* Overall, it is suggested that the use of effective fungicides and potential bioagents, botanicals and organic amendments may result in better management of stem rot disease of groundnut.

Keywords: *Sclerotium rolfsii* Sacc., *in vitro,* fungicides, botanicals, bio-agents, organic amendments.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume crop that belongs to the Fabaceae family and is one of the world's major oil seed crops grown in both tropical and subtropical regions. It is known as the 'king of oil seeds (Aycock, 1966). Groundnut crop often suffers from many fungal diseases resulting in severe yield loses among those diseases stem rot which is a soil-borne disease caused by *Sclerotium rolfsii* Sacc. is the major yield-limiting factor that reduces groundnut production which causes more than 50% yield loss (Mayee and Datar 1988). This was the first time reported by Rolfs (1892) as a cause of tomato blight in Florida. Later, Saccardo (1911) named the fungus *S. rolfsii*. It has an extensive host range, prolific growth rate and ability to produce large numbers of sclerotia that may persist in soil for several years (Punja, 1985). The pathogen attacks the germinated seedlings and causes wilt disease in groundnut. Because of the difficulty in dispersing fungicides through the peanut canopy to the soil profile, soilborne diseases are particularly difficult to manage. The pathogen continues to plague groundnut growers and causes significant economic losses. Thus, management approaches for soil-borne diseases include the application of fungicides and some cultural practices. Due to its soil-borne origin and prolonged survival as sclerotial bodies, it is difficult to control with fungicides or resistance breeding (Sarita *et al*., 2018). The application of fungicides although effective, but uneconomical, may affect associated microbiota in soil and become hazardous to the environment. As a result, the current study aims to test the *in-vitro* efficacy of fungicides, botanicals, bioagents and organic amendments against *Sclerotium rolfsii to* develop an environmentally friendly disease management system by integrating chemicals along with bioagents, plant extracts and organic amendments which becomes the best alternative to control the disease.

MATERIALS AND METHODS

Evaluation of fungicides. The efficacy of 13 different fungicides was tested *in vitro* against *S. rolfsii* (GSR7 isolate), at three different concentrations (@1000 ppm, 1500 ppm, 2000 ppm) by applying poisoned food technique by using PDA as basal medium.

Observations on radial mycelial growth were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with the mycelial growth of the test fungus. Percent, inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the following formula (Vincent, 1927).

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

C=growth of test fungus in untreated control plates.

T=growth of the test fungus in treated plates.

Evaluation of botanicals

Fresh extracts from the leaf, bulb (garlic and onion) rhizome (ginger), and fine powder turmeric were used for experiments. Leaf, bulb and rhizome samples of all the tested plants were collected and washed in tap water and then rinsed in sterile distilled water. 100 g of fresh sample was chopped and macerated in a surface-sterilized pestle and mortar by adding 100 ml of 70% ethanol (1:1 w/v). The extract was filtered through a sterilized filter paper disc (Whatman no. 1) measuring 10 mm in diameter, the filtrate thus obtained was used as a stock solution. To study the antifungal mechanism of plant extracts at two different concentrations *viz*., 10 percent and 20 percent were made by adding sterilized distilled water proportionately. The clear extract was used to test the antifungal activity against *S. rolfsii* under *in vitro* by applying the Poisoned food technique (Nene and Thapliyal 1993) and using Potato dextrose agar (PDA) as a basal culture medium.

Observations on radial mycelial growth were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with the mycelial growth of the test fungus. Percent, inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the following formula (Vincent, 1927).

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Evaluation of bioagents.

Dual culture technique. Six fungal antagonists *viz, Trichoderma viride, T. harziamum, T. hamatum* and three bacterial antagonists *viz., Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Raltsonia solanacearum* were evaluated *in vitro* against *S. rolfsii* (GSR7 isolate), applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old cultures of the test bioagents and the test pathogen (*S. rolfsii*) were used for the study, sterilized cork borer was used to cut out culture discs (5 mm dia.) of the test pathogen and bioagents. Then, in Petri plates, two culture discs, one each of the test fungus and bioagent, were aseptically inoculated at equidistance and immediately opposite each other over solidified PDA medium, and the plates were incubated at 28 ± 2°C. Each treatment was replicated five times. PDA plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

Observations on the mycelial growth of the test pathogen and bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with the mycelial growth of the test pathogen. Percent inhibition(I) of the test pathogen by the bioagent over untreated control was calculated by applying the following formula (Arora and Upaddhyay 1978).

$$\text{Percent inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in the control plate}} \times 100$$

Evaluation of organic amendments. Aqueous extracts of 7 organic amendments were evaluated. Except for Vermicompost, the rest of the amendments were crushed to a fine powder with a pestle and mortar and dispensed @ 50 gm in 150 ml sterile distilled water (w/v) and allowed to decompose for 7 days. Later, these extracts were filtered through double-layered muslin cloth and the filtrate obtained was further passed through Whatman No. 1 filter paper, using a funnel and volumetric flasks (100 ml cap.) and autoclaved for 10 min. The final clear extracts/filtrates obtained formed the standard extract of 100% concentration. These aqueous extracts were evaluated (each @ 10% and 20%) *in vitro* against *S. rolfsii*, applying a poisoned food technique (Nene and Thapliyal 1993) and using Potato dextrose agar (PDA) as basal culture medium.

Observations on radial mycelial growth were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with the mycelial growth of the test fungus. Percent, inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the following formula (Vincent, 1927).

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

RESULTS AND DISCUSSIONS

Isolation of the pathogen. Groundnut plants showing typical symptoms of stem rot were used for the isolation of *S. rolfsii* using standard tissue isolation techniques and incubated on a PDA medium at 27±2°C for two days for isolation of the causal organisms. Pure cultures of the

pathogens were obtained by hyphal tip technique and were used for identification and further investigations.

***In vitro* evaluation of fungicides:** Thirteen different fungicides in various concentrations were used to study *in vitro* efficacy of fungicides against the mycelial growth of *S. rolfsii* and results are presented in Table 1 and Fig. 1.

Percent mycelial growth inhibition. At 1000 ppm the percent radial growth inhibition of the test pathogen varies significantly from 0.43 % to 100 % against untreated control. The maximum mycelial inhibition was recorded with the fungicides Tebuconazole, Nativo and Vitavax (100 %) followed by Thiram (90.73 %). Significantly least mycelial growth inhibition was recorded with the fungicide Saaf (0.43 %) followed by Copper-Oxy chloride (1.87 %) Carbendazim (1.87 %) and Thiophenyl methyl (1.87 %).

At 1500 ppm the percent radial growth inhibition of the test pathogen varies significantly from 2.97 % to 100 % against untreated control. The maximum mycelial inhibition was recorded with the fungicides Tebuconazole, Nativo and Vitavax (100 %) followed by Thiram (92.20 %). Significantly least mycelial growth inhibition was recorded with the fungicide Thiophenyl methyl (2.97 %) followed by Copper-Oxy chloride (10.73 %), Carbendazim (12.23%), while Mancozeb (24.43 %) and Saaf (27.40 %).

At 2000 ppm the percent radial growth inhibition of the test pathogen varies significantly from 3.70 % to 100 % against untreated control. The maximum mycelial inhibition was recorded with the fungicides Tebuconazole, Nativo, Hexaconazole and Vitavax (100 %) followed by Thiram (92.93 %) and Propineb (92.57 %). Significantly least mycelial growth inhibition was recorded with the fungicide Thiophenyl methyl (3.70 %) followed by Carbendazim (35.22 %).

All 13 fungicides tested exhibited antifungal activity against *S. rolfsii* at all different concentrations. Tebuconazole, Nativo, Hexaconazole, and Vitavax were found to be highly effective and record cent percent mycelial growth inhibition at all different concentrations tested (each @ 1000, 1500, and 2000 ppm).

Arunasri *et al*. (2011) found that the Triazoles (Propiconazole, Hexaconazole, Difenoconazole) shown highly effective against the radial growth of *Sclerotium rolfsii*. Manu *et al*. (2012) reported that among systemic fungicides Nativo, Avata and Vitavax power were found effective against *Sclerotium rolfsii*, and among contact fungicides mancozeb only at higher concentrations was found effective. Sarita *et al*. (2018) reported that Tebuconazole and Hexaconazole inhibited the mycelial growth maximum at both 500 and 750 ppm concentrations.

Divya Rani *et al.* (2022) found that seed treatment with tebuconazole resulted in the lowest stem rot disease incidence (15.47%).

In vitro* efficacy of botanicals against *S. rolfsii. Aqueous leaf extracts of twenty-two different botanicals in 2 different concentrations (@ 10 % and 20 %) were used to study *in vitro* efficacy of botanicals against mycelial growth of *S. rolfsii* and results are presented in Table 2 and Fig. 2**.**

Results demonstrated that all 22 botanicals at 2 different concentrations significantly inhibited the mycelial growth of *S. rolfsii*. However, the mycelial growth was found to be dramatically reduced with an increase in the concentrations (each @ 10 % and 20 %) of the botanicals tested over the untreated control. While the percent of mycelial inhibition increases with an increase in concentrations of botanicals tested. Among the botanicals tested Neem was found to be more effective and records maximum mycelial growth inhibition (62.20 %, 78.50 %) and no sclerotia development at all different concentrations (each @ 10% and 20%) tested over untreated control. Rest of the botanicals which showed antifungal activity against *S. rolfsii* are Garlic (40.00 %,73.30 %), Aloe Vera (46.67 %, 46.67 %), Calotropis (56.97 %, 70.00 %), Papaya (15.93 %, 74.07 %), Tulasi (41.47 %, 53.33 %) and Parthenium (53.03 %, 61.10 %). The least antifungal activity was observed with the botanicals Lemon grass (87.33 mm; 4.47 % @ 20 %) followed by Eucalyptus, Lantana, Onion, Periwinkle and Asafoetida which records the highest mycelial growth and least mycelial inhibition. The current findings are in agreement with earlier workers. Hanthegowda and Adiver (2001) reported that the mycelial growth of *S.rolfsii* was significantly reduced by 1:20 dilutions of *Azadirachta indica, Polyalthialongifollia* and *Parthenium hysterophorus.*

Sultana *et al.* (2012) tested four plant extracts (@5, 10, and 20%) *in vitro* against *S. rolfsii* and reported that Garlic had the highest percentage of mycelial inhibition (75.18 percent) at 20%, followed by Neem (69.90 percent) at 20%, and Garlic (63.33 percent) at 10%. The plant extracts Ginger @5% was shown to be the least effective, inhibiting mycelial growth by 27.41%, followed by Onion @5% (42.59%) and Ginger @ 10%. (48.52%). Vineela *et al.* (2020) reported that at 20 % concentration, Garlic (*Allium sativum*) was found to be the most effective which records cent percent inhibition of *S. rolfsii*.

***In vitro* evaluation of bioagents:** Three fungal antagonists *Trichoderma viride, Trichoderma harzianum, T. hamatum* and three bacterial antagonists *Raltsonia solanacearum, Pseudomonas florescens, P. aeruginosa* were tested to evaluate their antagonistic potential and type of colony interaction against test pathogen. The results were presented in Table 3 and Fig. 3.

Results demonstrated that all the bio-agents tested effectively inhibited the mycelial growth and sclerotial production of *S. rolfsii.* Among the antagonists evaluated *Trichoderma harzianum* was found to be most effective with significantly least radial growth (13.00 mm) and highest mycelial growth inhibition (71.10 %) of test pathogen followed by *Pseudomonas florescens* and

Trichoderma viride. Whereas, *the* least antagonistic was found to be *Raltsonia solanacearum* withthe least mycelial growth inhibition (30.38 %). Similar results were reported by several workers.

Karthikeyan *et al*. (2006) reported that *Trichoderma viride* Tv1 was the most effective isolate against *S. rolfsii*, inhibiting growth by 69.40%, followed by *P. fluorescens*, which inhibited the growth by 64.40 %. Sclerotial germination was also minimum (16.66%) in the case of Tv1 followed by *P. fluorescens* (27.08%) while in control it was 91.65%. Ganesan *et al*. (2007) reported that *Trichoderma harzianum* showed around 57% of inhibition against *Sclerotium rolfsii*.

Sivakumar *et al.* (2020) tested ten isolates of *Trichoderma viride* and *Pseudomonas fluorescens* against *Sclerotium rolfsii* and discovered that *Trichoderma* isolates Tv3 had the highest growth inhibition at 76.04 % and *Pseudomonas* isolate Pf5 had the highest inhibition zone (13.00 mm).

***In vitro* evaluation of organic amendments:** Seven organic amendments at 2 different concentrations (@ 10 % and 20 %) were used to study *in vitro* efficacy of organic amendments against the growth and sporulation of *S. rolfsii* and results are presented in Table 4 and Fig. 4.

Results revealed that all 7 organic amendments at 2 different concentrations significantly inhibited the mycelial growth and sporulation in *S. rolfsii*. However, the mycelial growth was found to be dramatically reduced with an increase in the concentrations (each @ 10 % and 20 %) of the organic amendments tested over the untreated control. While the percent of mycelial inhibition increases with an increase in concentrations of organic amendments tested. Among the organic amendments tested Neem seed cake was found to be more effective and records maximum mycelial growth inhibition of 52.80 % and minimum mycelial growth of 42.33 mm (@ 20 %) followed by FYM and cottonseed meal. However, groundnut cake was found to be the least effective and recorded maximum mycelial growth (89.00 mm) and minimum mycelial growth inhibition (1.10 %). The results align with earlier workers. The antifungal activity of the organic amendments may be due to the inclusion of antibiotics and phenolic compounds of unknown nature (Jha *et al.*, 2007).

Vineela *et al.* (2020) reported that at 10% concentration, FYM and Groundnut cake were found to be more effective in inhibiting the mycelial growth of *S. rolfsii* by cent percent, followed by Mustard oil cake (88.8%), Neem cake (60.70%). While Vermicompost (59.96%) was found to be the least effective against the mycelial growth of *S. rolfsii.*

Table 1: *In vitro* efficacy of fungicides against *S. rolfsii.*

Treatments	Fungicides	Colony diameter (mm)			% inhibition		
		1000 ppm	1500 ppm	2000 ppm	1000 ppm	1500 ppm	2000 ppm
T1	Propineb 70 WP	42.67	38.33	6.67	52.57 (46.45)	57.40 (49.24)	92.57 (74.22)
T2	Copper-oxy-chloride 50 WP	88.33	80.33	47.00	1.87 (4.56)	10.73 (19.11)	47.87 (43.76)
T3	Vitavax (Carboxin 37.5% + Thiram 37.5% DS)	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T4	Saaf (Carbendazim12% + Mancozeb 63% WP)	89.00	65.33	37.00	0.43 (2.86)	27.40 (31.53)	58.90 (50.11)
T5	Mancozeb 75 WP	78.67	68.00	48.67	12.60 (20.66)	24.43 (29.59)	45.90 (42.63)
T6	Thiophanate methyl 70 WP	88.33	87.33	86.67	1.87 (4.56)	2.97 (8.05)	3.70 (10.49)
T7	Nativo (Tebuconazole 50% + Trifloxystrobin 25% WG)	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T8	Captan 50 WP	24.33	23.00	21.67	72.93 (58.63)	74.43 (59.62)	75.93 (60.60)
T9	Hexaconazole 5EC	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Thiram 75WP	8.33	7.00	6.33	90.73 (72.28)	92.20 (73.75)	92.93 (74.60)
T11	Carbendazim 50 WP	88.33	79.00	58.33	1.87 (4.56)	12.23 (20.32)	35.22 (36.38)
T12	Chlorothalonil 75 WP	48.33	38.33	32.33	46.30 (42.86)	57.33 (49.22)	64.10 (53.18)
T13	Tebuconazole 2% DS	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T14	Control	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	C.D.	3.26	4.09	2.85	3.61	4.53	3.19
	SE(m)	1.12	1.40	0.98	1.24	1.55	1.10

***** Means of three replications; *Figures in parenthesis are arcsine transformed values

Table 2: *In vitro* efficacy of botanicals against *S. rolfsii.*

Treatments	botanicals	Colony diameter (mm)		% Inhibition	
		10 %	20%	10%	20 %
T1	Lemon grass	90.00	86.00	0.00 (0.00)	4.47 (9.97)
T2	Pongamia	73.33	31.33	18.53 (25.48)	68.53 (56.00)
T3	Periwinkle	86.00	65.00	4.43 (12.13)	27.80 (31.80)
T4	Garlic	54.00	24.00	40.00 (39.22)	73.30 (58.87)
T5	Zinger	75.33	43.33	16.27 (23.72)	51.83 (46.03)
T6	Bael	74.67	50.00	17.03 (24.35)	44.43 (41.78)
T7	Ashoka	70.00	47.00	22.20 (28.09)	47.77 (43.70)
T8	Guava	60.67	40.33	32.60 (34.77)	55.17 (47.95)
T9	Onion	78.33	72.33	12.93 (21.02)	19.63 (26.28)
T10	Papaya	75.67	23.33	15.93 (23.44)	74.07 (59.37)
T11	Calotropis	39.33	27.00	56.97 (48.99)	70.00 (56.78)
T12	Nerium	54.33	28.00	39.63 (39.00)	68.90 (56.10)
T13	Tulasi	52.67	42.00	41.47 (40.06)	53.33 (46.89)
T14	Tamarind	72.00	55.00	19.93 (26.47)	38.90 (38.57)
T15	Ber	89.00	45.67	1.10 (3.49)	49.23 (44.54)
T16	Parthenium	41.00	35.00	53.03 (46.72)	61.10 (51.40)
T17	Turmeric	80.00	45.00	10.87 (19.22)	50.00 (44.98)
T18	Lantana	89.00	74.33	1.10 (4.85)	17.40 (24.61)
T19	Asafoetida	87.33	62.33	2.93 (8.01)	30.73 (33.64)
T20	Aloe vera	48.00	42.67	46.67 (43.07)	52.60 (46.47)
T21	Eucalyptus	86.67	68.33	3.70 (10.60)	24.07 (29.35)
T22	Neem	34.00	19.67	62.20 (52.05)	78.50 (62.36)
T23	Control	90.00	90.00	0.00 (0.00)	0.00 (0.00)
	C.D.	4.22	3.96	3.61	5.15
	SE(m)	1.478	1.387	1.26	1.80

***** Means of three replications; *Figures in parenthesis are arcsine transformed values

Table 3: *In vitro* efficacy of bio-agents against *S. rolfsii.*

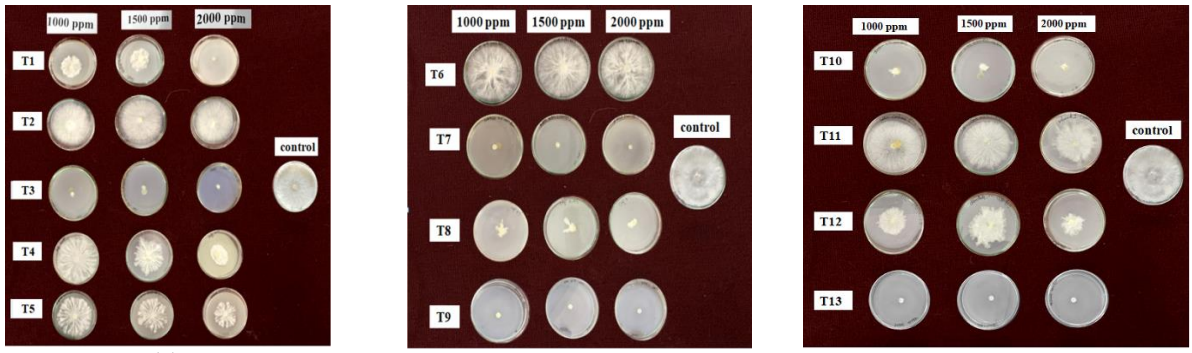
Treatments	Bio-agents	Radial growth (mm)	% Inhibition
T1	*Trichoderma harzianum*	13.00	71.10 (57.47)
T2	*Trichoderma viridae*	16.67	62.93 (52.48)
T3	*Trichoderma hamatum*	18.00	60.00 (50.75)
T4	*Pseudomonas florescens*	15.67	65.17 (53.81)
T5	*P. aeruginosa*	27.33	39.27 (38.79)
T6	*Raltsonia solanacearum*	31.33	30.38 (33.42)
T7	control	45.00	0.00 (0.00)
	C.D.	1.39	3.08
	SE(m)	0.45	1.01

***** Means of five replications; *Figures in parenthesis are arcsine transformed values

Table 4. ***In vitro*** **efficacy of organic amendments against** ***S. rolfsii.***

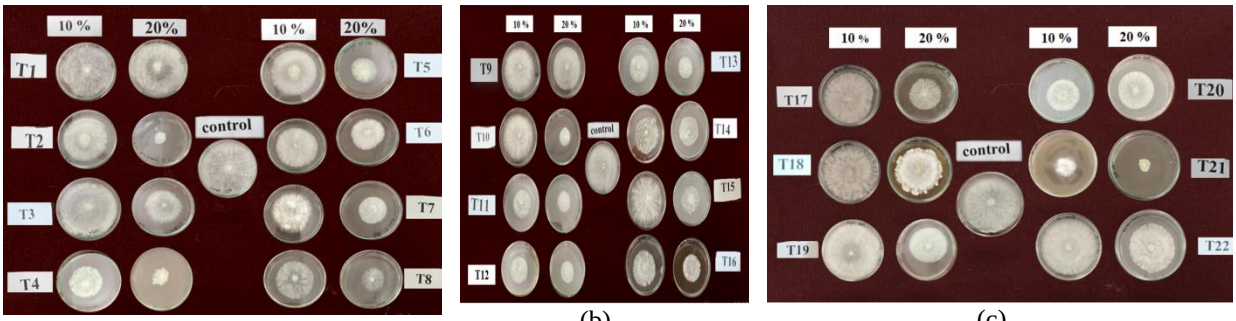
Treatments	Organic amendments	Colony diameter (mm)		% Inhibition	
		10 %	20%	10%	20 %
T1	Neem seed cake	48.33	42.33	46.30 (42.86)	52.80 (46.59)
T2	Groundnut cake	90.00	89.00	0.00 (0.00)	1.10 (6.02)
T3	Castor cake	89.00	84.33	1.10 (6.02)	6.57 (14.83)
T4	Vermicompost	89.67	87.67	0.36 (0.00)	2.57 (8.88)
T5	Cotton seed meal	71.00	58.00	21.10 (27.33)	35.57 (36.59)
T6	Farmyard manure (FYM)	67.67	52.00	23.93 (29.28)	42.20 (40.49)
T7	Mustard seed cake	83.00	79.67	4.43 (12.08)	11.47 (19.75)
T8	Control	90.00	90.00	0.00 (0.00)	0.00
	C.D.	3.38	2.85	1.48	2.41
	SE(m)	1.12	0.94	0.49	0.80

***** Means of five replications; *Figures in parenthesis are arcsine transformed values



(a) (b) (c)

Plate 1. *In vitro* evaluation of fungicides against mycelial growth and inhibition of *S. rolfsii.*



(a) (b) (c)

Plate 2. *In vitro* evaluation of botanicals against mycelial growth and inhibition of *S. rolfsii.*

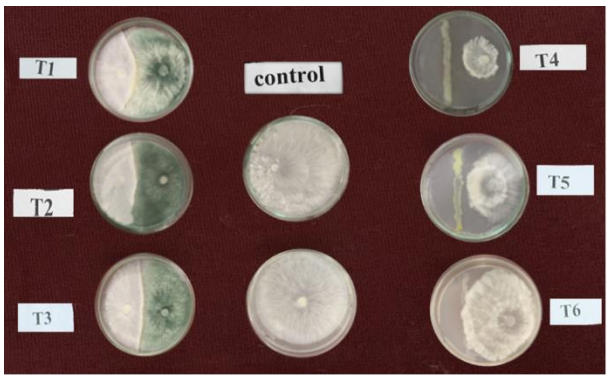


Plate 3. *In vitro* evaluation of bio-agents against mycelial growth and inhibition of *S. rolfsii.*

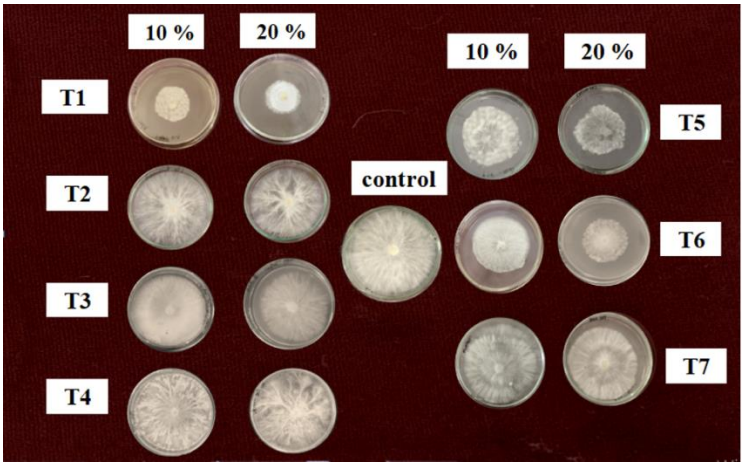


Plate 4. *In vitro* evaluation of organic amendments against mycelial growth and inhibition of *S. rolfsii*.

CONCLUSIONS

The results of the current research reported that among fungicides tested Tebuconazole, Nativo, Hexaconazole, Vitavax were found to be highly effective and completely inhibited mycelial growth of *Sclerotium rolfsii* at all different concentrations tested (each @ 1000, 1500, and 2000 ppm). Neem leaf extract found to have high antifungal properties compared to other botanicals tested irrespective of concentrations. Among bio agents tested *Trichoderma harzianum* highly inhibited the mycelial growth of *S. rolfsii* followed by *Pseudomonas florescens*are found to be efficacious compared to others. Neem seed cake was found to show high inhibition of mycelial growth of *S. rolfsii* followed by FYM in compared to other organic amendments. Fungicide application, while effective, is costly and may harm associated microbiota in soil, making it hazardous to the environment. As a result, the current study intends to to develop an environmentally friendly disease management system by integrating chemicals with bioagents, plant extracts, and organic amendments, which will be the best alternative to control the disease.

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Conflict of Interest. None.

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