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IN VITRO CONTROL OF *Sclerotium rolfsii*, THE CAUSAL AGENT OF COLLAR ROT IN SUNFLOWER USING FUNGICIDES, BOTANICALS AND ORGANIC MATTER

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Abstract

The sunflower (*Helianthus annuus* L.), a vital oilseed crop that significantly reduces production globally, is seriously threatened by collar rot, a disease caused by the soil-borne fungus *Sclerotium rolfsii* reductions. This research sought to assess the effectiveness of various control methods against *S. rolfsii* in laboratory conditions, including nine fungicides, nine plant extracts, and five organic amendments. This study assessed the efficacy of various medicinal plant extracts at different concentrations (5%, 10%, 15%, and 20%) in inhibiting the *in vitro* mycelial growth of *S. rolfsii*. Among the plant extracts tested, garlic demonstrated the most potent antifungal properties, completely suppressing mycelial growth at 10%, 15%, and 20% concentrations. The results indicate that garlic clove extract (98.18-100%) is the most effective at inhibiting *S. rolfsii* growth, with henna (65.92-92.46%) and black cumin extracts (64.80-85.88%) also demonstrating strong efficacy. The fungicide Carboxin and Thiram (Provax 200) were found most effective, achieving total inhibition, followed by Azoxystrobin and Difenoconazole (Amister Top) (91%) and Difenoconazole (Score) (90%). In the case of the extracts of organic amendment material, poultry refuse exhibited the highest inhibition, completely inhibiting the mycelial growth at 20% and 30% concentrations. Among the organic amendments, poultry refuse (94.40-100%), vermicompost (17.55-67.32%), and mustard oilcake (14.88-64.10%), showed strong potential for reducing *S. rolfsii* mycelial growth. The findings of the study suggest that Carboxin and Thiram, poultry refuse, and garlic extract could be effective in managing sunflower collar rot disease.

Keywords: Collar rot, Organic amendment, Plant extract, *Sclerotium rolfsii*.

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Introduction

Sunflower (*Helianthus annuus* L.) is a crucial oilseed crop with considerable potential to decrease the demand for imported vegetable oils. Its oil is considered superior in quality to that of rapeseed and mustard, making it a favored choice among consumers due to its healthy properties. Sunflower ranks fourth globally in vegetable oil production (Pilorgé, 2020), with approximately 30 million hectares harvested and 55 million tons produced (NSA, 2024). This crop is characterized by its short growth cycle (90-110 days), high adaptability, relative drought resistance, and capacity for high yields. Sunflower seeds contain 45-52% edible oil and are rich in protein (23%) (Khan *et al*., 2012). Contributing roughly 13% to global edible oil production (Gabagambi *et al*., 2010), sunflower oil is prized for its nutritional qualities, including its anti-cholesterol properties and high content of essential fatty acids (linoleic acid, 60-73%), making it beneficial for heart health. In Bangladesh, sunflower is a relatively new addition to the oilseed crops, which include mustard, sesame, and groundnut. Although native to the southern United States and Mexico, sunflower cultivation in Bangladesh began on a small scale in 1975 (Habib *et al*., 2017). It has emerged as a promising crop for the dry season in the country's coastal regions. However, its productivity faces threats from both biotic and abiotic factors, with diseases being a significant biotic challenge. One of the major diseases affecting sunflowers is collar rot, caused by the soil-borne fungal pathogen *Sclerotium rolfsii* Sac., which infects over 500 plant species worldwide (Billah *et al*., 2017; Sun *et al*., 2020). This pathogen is particularly harmful to sunflowers, causing rapid wilting and stem lesions that can result in substantial yield losses. Collar rot can cause substantial yield losses in sunflower crops, with the extent of damage influenced by factors such as planting density, soil moisture, and local climate conditions. In severe cases, the disease can decimate entire fields, leading to significant economic losses for farmers. The sunflower plant's susceptibility to collar rot, particularly in its early growth stages, makes early and effective disease management essential. The development of collar rot-resistant sunflower varieties has been slow, partly due to the genetic diversity of *S. rolfsii* and its ability to adapt to environmental conditions.

The characteristic symptoms of collar rot include a brownish lesion at the stem base, which eventually encircles the plant. The infected area becomes covered with white mycelial strands and forms sclerotia, which are small, hard, and resilient structures that allow it to survive in soil for extended periods. The pathogen’s ability to persist in the soil as sclerotia and its wide host range make it challenging to control through traditional crop rotation practices alone. The disease thrives in moist soil conditions, typically appearing within two weeks of planting, and leads to yellowing and eventual plant death. *S. rolfsii* can cause yield losses ranging from 25.60-48.62%, with severe infections resulting in losses of up to 80% (Mehan and McDonald, 1990). The pathogen's persistence in soil and its ability to infect multiple crops make it particularly challenging to control (Sennoi *et al*., 2013).

Managing *S. rolfsii* is complex due to its soil-borne nature, wide host range, ability to produce excessive sclerotia, and the ability to persist in the soil for several years in the form of resistant sclerotia (Billah *et al*., 2017). Traditional methods for controlling collar rot include crop rotation, field sanitation, and the use of fungicides. Crop rotation,

although useful in managing certain pathogens, is less effective against *S. rolfsii* due to its broad host range and ability to persist in the soil. Conventional control methods heavily rely on fungicides, Chemical fungicides, such as Carboxin+thiram, carbendazim, hexaconazole, mancozeb, and thiophanate-methyl, have been widely used to manage collar rot infection in sunflower (Mondal and Khatua, 2013). While effective, the over-reliance on chemical fungicides raises concerns regarding environmental impact and human health risks, and the development of fungicide-resistant strains *of S. rolfsii* has driven research into alternative approaches. Botanicals and organic matter extract present promising alternatives that align with sustainable agriculture practices, offering eco-friendly and potentially more affordable options for collar rot management. Integrating these methods can provide a comprehensive approach to controlling collar rot, improving soil health, and enhancing sunflower plant resilience. Because of the above facts, the present research work was designed to evaluate some fungicides, plant extracts, and extracts of organic amendment materials against collar rot disease of sunflowers.

Materials and Methods

Collection of diseased specimens

Sunflower plants exhibiting collar rot symptoms were collected from agricultural fields across three districts in Bangladesh: Borguna, Potuakhali, and Khulna. The infected samples were brought to the laboratory, cleaned of any soil or debris, and stored in paper bags at 4°C for future analysis. Five samples were collected from each spot to isolate and identify the pathogen responsible for the disease.

Isolation and identification of the pathogen

The pathogen was isolated from the infected sunflower plants using the tissue segment method described by Mian (1995). Infected stem tissues, particularly from the collar area, were thoroughly cleansed, cut into small pieces (4-5 mm), disinfected with 70% ethanol for 5 minutes, washed with sterile distilled water, and dried using sterile absorbent paper. The sanitized tissue fragments were placed on Potato Dextrose Agar (PDA) and kept at 25 ± 1°C for 3- 4 days. Fungal growths that emerged on the PDA plates were transferred to fresh PDA plates and incubated at 25 ± 1°C for 5 days. The extracted pathogen was identified as *Sclerotium rolfsii* based on its morphological characteristics, including white, fluffy mycelia and the formation of brown sclerotia resembling mustard seeds (0.90–1.40 mm in diameter) (Sekhar *et al*., 2017).

Preparation and maintenance of pure culture

Pure culture of *S. rolfsii* was established using the hyphal tip culture method (Alam *et al.*, 2024; Islam *et al*., 2001; Mian, 1995). These cultures were preserved on PDA slants, transferred to fresh media monthly, and stored at 4°C for future use.

Preparation of cold aqueous extracts

Fresh plant components, such as leaves, bulbs, rhizomes, seeds, and cloves, were collected to create botanical extracts. For each extract, 100 g of fresh plant material was blended with 100 mL of sterile distilled water. The mixture was filtered through Whatman No. 1 filter paper, and the resulting liquid was used as the stock solution

(Kamlesh & Gujar, 2002). Extracts from nine plant species viz., ginger, turmeric, onion, garlic, black cumin, henna, neem, tulsi, and eucalyptus were prepared and tested at concentrations of 5%, 10%, 15%, and 20% using PDA as the growth medium (Islam, 2005).

Poisoned food technique

PDA medium was supplemented with botanical extracts at various concentrations (5%, 10%, 15%, and 20%). A mycelial disc of *S. rolfsii* (5 mm diameter) was positioned centrally on each plate and incubated at 25 ± 2°C. Control plates without botanical extracts were also prepared. The fungal colony diameters were recorded daily until complete colonization of control plates occurred, it started one day after inoculation (1 DAI) and continued to four days after inoculation (4 DAI).

In-vitro screening of fungicides against *Sclerotium rolfsii*

The most virulent *Sclerotium rolfsii* isolate (PhaKHaSr1) was tested against nine fungicides encompassing systemic, contact, and combination types, using the poisoned food technique (Islam, 2005) in a grove setting. Fungicide suspensions were prepared following the manufacturer's instructions. For each treatment, 100 ml of PDA was placed in a 250 ml conical flask and autoclaved. The flasks were properly labeled and thoroughly shaken before use. Twenty milliliters of the PDA medium were then poured into 9 cm Petri plates. After solidification, three 5 mm discs were removed from the PDA plate at equal distances from the center using a sterile disc cutter. A measured amount of fungicide (hundred microliters) was placed in each hole, and the plates were refrigerated overnight to allow the fungicide to diffuse into the surrounding medium. The following day, a 5 mm block of a 5-day-old *S. rolfsii* culture grown on PDA was cut with a sterile disc cutter and placed in the center of each plate. The plates were incubated at 25 ± 2°C and monitored daily for fungal growth inhibition. A suitable control was maintained by growing the pathogen on a fungicide-free PDA medium. Four replications were performed for each treatment. Radial mycelial growth of *S. rolfsii* was measured at 24-hour intervals until the colony reached the edge of the Petri dishes in the control plates (Islam *et al.*, 2001; Islam, 2005).

In-vitro screening of the extracts of organic amendment materials

Extracts from five organic amendment materials poultry refuse, vermicompost, mustard oil cake, sawdust, cow dung, and a control were tested against *S. rolfsii* (isolate PhaKHaSr1) at concentrations of 10%, 20%, and 30% using the poisoned food technique (Nene and Thapliyal, 1993). Each amendment was finely ground into powder using a pestle and mortar. For each treatment, 50 grams of powdered material were placed into 250 ml flasks, then 150 ml of sterilized water (w/v) was added and the mixture was left to decompose for 15 days. After 15 days, the mixtures were strained through double-layered muslin cloth, and the filtrates were further filtered using Whatman No. 1 filter paper. The resulting extracts were then autoclaved for 10 minutes to sterilize them, yielding a 100% standard extract solution (Dubey and Patel, 2001). This extract solution was diluted to prepare the required concentrations. PDA plates containing each respective extract concentration were inoculated with 5 mm discs of a 7-day-old *S. rolfsii* culture. Plates without extracts served as controls.

Measurement of radial mycelial growth and growth inhibition

Fungal colony radial growth was measured every 24 hours. The percentage of fungal growth inhibition was determined using the following formula (Hussain *et al*., 2015):

$$Percent\ of\ inhibition\ (PI) = \frac{X-Y}{Y} \times 100$$

Where:

Y = Average radial growth (cm) of *S. rolfsii* in control plates.

Z = Average radial growth (cm) of *S. rolfsii* in treated plates.

Experimental design and statistical analysis

The experiments were conducted in a completely randomized design with four replications per treatment. Data were analyzed using R statistical software, and treatment means were compared using the Least Significant Difference (LSD) test at $P \geq 0.05$.

Results and Discussion

Effect of plant extract on in-vitro colony growth of *Sclerotium rolfsii*

The antifungal potential of various plant extracts against *S. rolfsii* was evaluated, and the results demonstrate significant differences in the efficacy of these natural agents (Table 1 and 2, plate. 1). Among all the tested extracts, garlic clove extract showed the highest antifungal activity after four days post inoculation (dpi). At concentrations of 10%, 15%, and 20%, garlic extract completely inhibited the mycelial growth of *S. rolfsii*. Even at 5%, it significantly initiated the growth to 1.63 mm.

Table 1. Efficacy of botanicals on mycelial growth of *S. rolfsii* under in vitro conditions at different concentrations

Botanicals extracts	Radial mycelial growth (mm)			
	5%	10%	15%	20%
Garlic clove extract	1.63 g	0.00 i	0.00 i	0.00 h
Henna leaf extract	30.50 f	15.12 h	7.75 h	6.75 g
Ginger rhizome extract	66.25 e	48.37 f	40.75 f	19.24 e
Turmeric rhizome extract	67.75 e	53.75 e	45.75 b	21.12 e
Onion bulb extract	66.25 e	49.50 f	41.37 f	19.87 e
Black cumin seed extract	31.50 f	20.50 g	14.12 g	12.62 f
Neem leaf extract	74.0 d	64.25 d	53.62 d	25.0 d
Tulshi leaf extract	82.63 c	78.50 c	65.62 c	59.25 c
Eucalyptus leaf extract	85.62 b	84.37 b	78.75 b	75.50 b
Control	89.50 a	89.50 a	89.50 a	89.50 a
SEm ±	1.0124	0.9082	0.8514	0.7071
LSD ($P \geq 0.05$)	2.93	2.63	2.47	2.05
CV (%)	3.39	3.60	3.89	4.30

Values in a column having the same letter(s) did not differ significantly at the 5% level by LSD.

Table 2. Efficacy of medicinal plant extracts on mycelial growth inhibition of *S. rolfsii* under in-vitro

Botanicals extracts	Mycelial growth inhibition (%)			
	5%	10%	15%	20%
Garlic clove extract	98.18	100.0	100.0	100.0
Henna leaf extract	65.92	83.09	91.34	92.46
Ginger rhizome extract	25.97	45.95	54.46	78.49
Turmeric rhizome extract	24.30	39.95	48.87	76.40
Onion bulb extract	25.99	44.70	53.77	77.80
Black cumin seed extract	64.80	77.09	84.21	85.88
Neem leaf extract	17.31	28.21	40.08	72.07
Tulshi leaf extract	7.67	12.27	26.66	33.79
Eucalyptus leaf extract	4.34	5.71	12.02	15.64

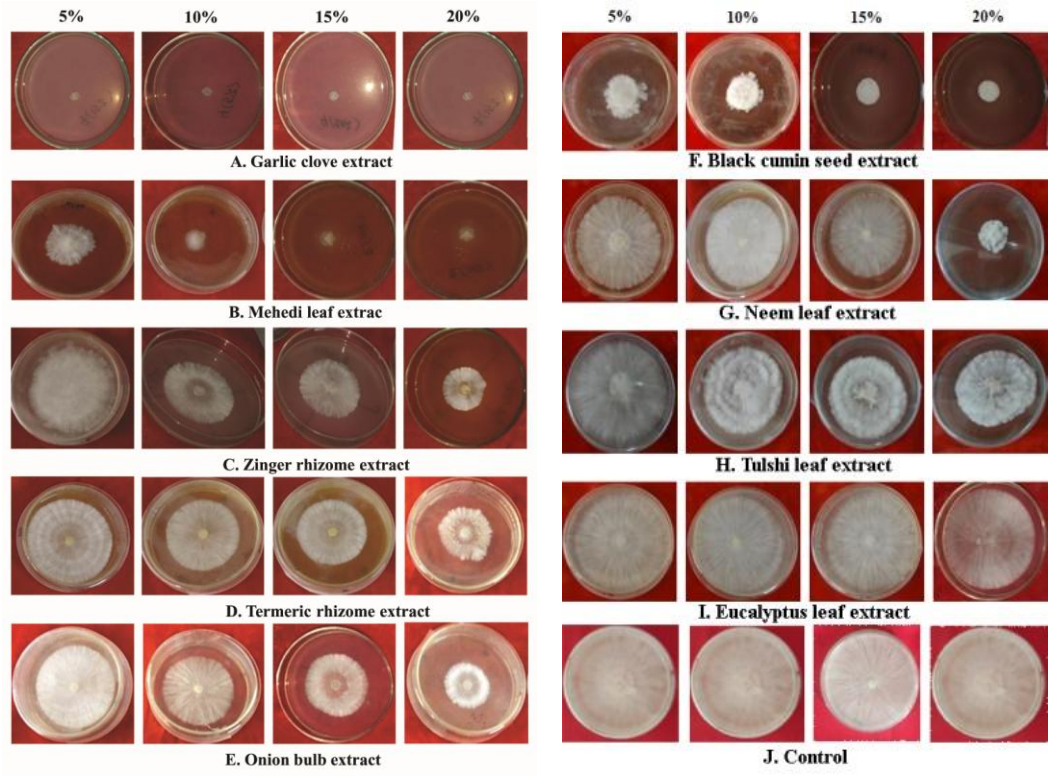

Fig. 1. (a). Pictorial view of radial mycelial growth of *S. rolfsii* in Petri plates containing PDA amended with botanicals (A) Garlic clove extract, (B) Henna leaf extract, (C) Zinger rhizome extract, (D) Turmeric rhizome extract, (E) Onion bulb extract, (F) Black cumin seed extract, (G) Neem leaf extract, (H), Tulshi leaf extract, (I) Eucalyptus leaf extract, and (J) Control at 4 days after inoculation

This profound inhibition is likely due to the presence of sulfur-containing compounds, such as allicin, which has been widely recognized for its antimicrobial properties. Previous studies have shown that garlic has strong inhibitory effects against a wide range of plant pathogens due to its ability to disrupt microbial cell walls and inhibit enzyme activity essential for fungal survival (Benkeblia, 2004). Eucalyptus leaf extract was not as effective as garlic, but still demonstrated significant inhibitory effects. Radial mycelial growth was reduced from 85.62 mm at 5% to 75.50 mm at 20% concentration. Eucalyptus contains bioactive compounds like eucalyptol and flavonoids, which have been documented for their antimicrobial activities. The antifungal mechanism of eucalyptus may involve interfering with fungal cell wall integrity and inhibition of spore germination (Batish *et al*., 2008). Tulsi showed moderate antifungal activity, with mycelial growth decreasing from 82.63 mm at 5% to 59.25 mm at 20% concentration. Tulsi's antifungal properties are attributed to its essential oils, particularly eugenol, which has been found to inhibit fungal growth by disrupting cell membranes and inhibiting enzymatic processes. Previous research supports the moderate antifungal action of Tulsi extracts against a variety of soil-borne pathogens (Prakash and Gupta, 2005). Black cumin seed extract significantly inhibited *S. rolfsii* growth, with a reduction from 31.50 mm at 5% to 12.62 mm at 20% concentration. This suggests that Black cumin has strong potential as a botanical fungicide. Similarly, Henna leaf extract demonstrated substantial inhibition, reducing mycelial growth from 30.50 mm at 5% to 6.75 mm at 20% concentration, suggesting its role as a strong antifungal agent. Other plant extracts such as ginger, onion, neem, and turmeric exhibited moderate inhibition. While garlic, Black cumin, and Henna extracts showed complete inhibition or significant reduction at higher concentrations, these extracts reduced mycelial growth by approximately 85.88-100% at 20% concentration. The antimicrobial properties of these plants are well-documented, with studies attributing their antifungal action to active compounds like curcumin in turmeric, gingerol in ginger, and azadirachtin in neem (Gupta and Sharma, 2017; Tewari *et al*., 2014). In terms of percentage inhibition, garlic extract showed near-complete inhibition (98.18%) at 5%, and complete inhibition at concentrations above 10%. Black cumin and Henna extracts also demonstrated strong antifungal efficacy, achieving 85.88% and 92.46% inhibition, respectively, at 20% concentrations. However, Tulshi and eucalyptus leaf extracts showed comparatively low inhibition rates, indicating limited efficacy against *S. rolfsii*. Tulshi achieved 33.79% inhibition at 20%, and eucalyptus only reached 15.64% at the same concentration. While these plants contain antifungal compounds such as eugenol and eucalyptol, respectively, their concentrations in these extracts may be insufficient for effective suppression of *S. rolfsii*. Their limited efficacy suggests they may not be suitable as primary antifungal agents for managing this pathogen but could be explored in combination with other stronger extracts (Chakrapani *et al*., 2020). These findings align with previous reports indicating variable antifungal efficacy among different plant extracts, depending on their phytochemical composition and mode of action (Pandey *et al*., 2010).

Effects of fungicides on *in-vitro* colony growth of *S. rolfsii*

The efficacy of different fungicidal treatments on the radial mycelial growth of *S, rolfsii* over four days after inoculation was presented in Table 3 and Plate 2. Ipridion

50WP (Rovral) at 0.2% concentration, reduced fungal growth to 70.38 mm by day 4, achieved 21.81% inhibition compared to the control, and demonstrated moderate effectiveness throughout the study period. Metalaxyl and Mancozeb (Newben), applied at 0.25%, showed greater inhibition (36.94%) by day 4, limited growth to 56.75 mm. Similarly, Metalaxyl and Mancozeb were observed as a protective treatment under moderate disease pressure (Ganguly and Banik, 2010). Azostrobin and Difenoconazole (Amistar Top 325 SC), at 0.10% concentration, proved highly effective, restricted the growth to 8.13 mm by day 4 with 90.97% inhibition, ranked it among the most potent treatments in the trial. These fungicides, both of which belong to the triazole class, inhibit the biosynthesis of ergosterol, a vital component of fungal cell membranes, thereby preventing fungal growth (Lamb *et al*., 2000). Carboxin and Thiram (Provax-200 WP) (0.20%) emerged as the most effective, completely restricted fungal growth at all times, and achieved 100% inhibition. These results corroborate earlier studies showing that Carboxin and Thiram are highly effective against soil-borne pathogens like *S. rolfsii* and can be an essential tool in chemical disease management programs (Mahapatra *et al*., 2016). Bavistin, at 0.10%, limited growth up to 65.13 mm by day 4, resulting in 27.64% inhibition, yielded moderately but less effectively than that of Azostrobin and Difenoconazole or Carboxin and Thiram restricted fungal growth to 60.25 mm by day 4, with 33.06% inhibition, yielded moderate results.

Table 3. In vitro efficacy of fungicides on controlling mycelial growth of *Sclerotium rolfsii* in poisoned food technique after 1, 2, 3, and 4 days after incubation (DAI)

Treatments	Dose (%)	Mean radial growth of the fungus (mm) after				% inhibition of mycelial growth over control at 4 DAI
		1 DAI	2 DAI	3 DAI	4 DAI	
Rovral (Iprodion 50% WP)	0.2	20.25b	41.38c	55.88c	70.38bc	21.81
Newben (Metalaxyl 8% + Mancozeb 64%)	0.25	11.62f	29.00e	45.13e	56.75f	36.94
Amistar Top 325 SC (Azostrobin + Difenoconazole)	0.10	2.50g	4.00f	6.63f	8.13g	90.97
Provax-200 WP (Carboxin 37.5% + Thiram 37.5%)	0.20	0.00h	0.00g	0.00g	0.00h	100
Bavistin 50% WP (Carbendazim)	0.10	16.37d	34.63d	49.50d	65.13d	27.64
Cynil 72% WP (Cymoxanil 8% + Mancozeb 64%)	0.25	13.87e	33.00d	45.63e	60.25e	33.06
Oxycob (Copper oxychloride 50% WP)	0.20	20.50b	44.38b	60.00b	72.50b	19.44
Dithen M-45 (Mancozeb 75% WP)	0.25	17.87c	40.13c	53.75c	68.00cd	24.44
Score (Difenoconazole 25% EC)	0.20	2.63g	5.25f	7.50f	9.13g	89.86
Control	-	25.88a	54.25a	75.63a	90.00a	-

Treatments	Dose (%)	Mean radial growth of the fungus (mm) after				% inhibition of mycelial growth over control at 4 DAI
		1 DAI	2 DAI	3 DAI	4 DAI	
SEm ±	-	0.811	0.788	0.981	1.0124	-
LSD (P ≥ 0.05)	-	1.17	2.28	2.84	2.93	-
CV (%)	-	6.19	5.51	4.90	4.04	-

Means followed by the same letter/letters do not significantly differ at the 5% level tested by LSD.

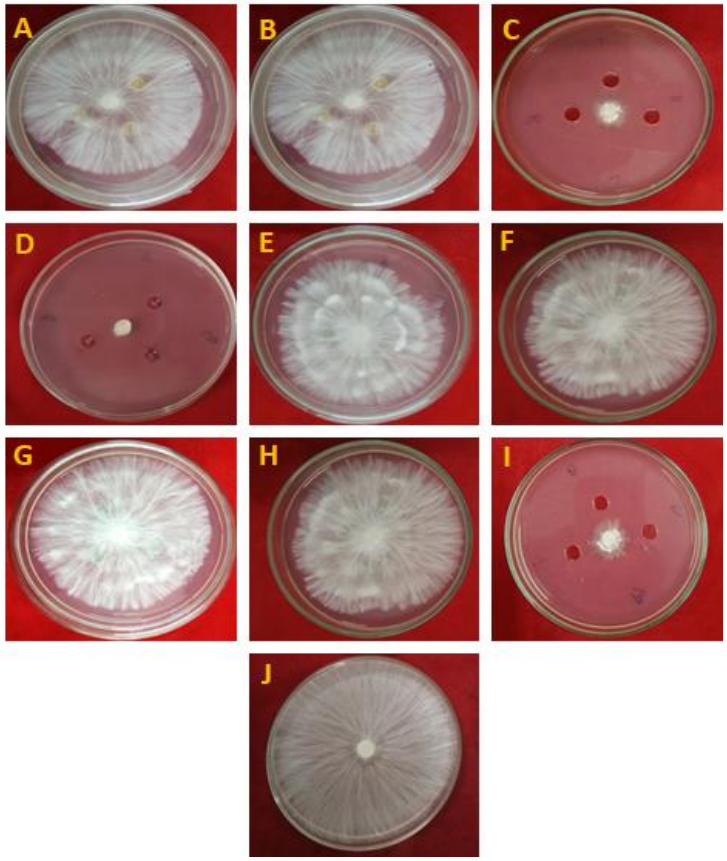

Fig. 2. Radial mycelial growth of *Sclerotium rolfsii* against (A) Rovral 50 WP, (B) Newben (C) Amistar Top, (D) Provax 200 WP, (E) Bavistin, (F) Cynil 72WP, (G) Oxycob, (H) Dithan M-45, (I) Score, and (J) Control at 4 days after inoculation.

Oxycob at 0.20% concentration allowed growth up to 72.50 mm by day 4, with 19.44% inhibition, it was one of the less effective treatments. Mancozeb (Dithane M-45), at 0.25%, reduced fungal growth up to 68.00 mm by day 4, with 24.44% inhibition, demonstrating a moderate inhibitory effect. Difenoconazole (Score 25EC 0.20%) exhibited high efficacy, inhibited growth up to 9.13 mm by day 4, with 89.86%

inhibition, comparable to Azostrobin and Difenoconazole in effectiveness. Similarly, Suneeta *et al*. (2017) conducted an *in vitro* evaluation of different fungicides against Collar Rot of Gerbera caused by *S. rolfsii*. Difenoconazole 25% EC recorded 100 percent inhibition of pathogen at 500, 1000, and 1500 ppm concentrations. The study concludes that Provax-200 is the most potent fungicide for the growth inhibition of *S. rolfsii*, while Azostrobin Difenoconazole, and Difenoconazole also demonstrate strong efficacy in controlling fungal growth.

In-vitro effects of different extracts of organic amendment materials

An in vitro assessment was conducted to evaluate the effectiveness of organic amendment material extracts on mycelial growth inhibition of *S. rolfsii* and sclerotia production. The study included three concentrations (10%, 20%, and 30%), with results given in Table 4, Table 5, and Fig. 3. Poultry refuse extract demonstrated superior efficacy in inhibiting mycelial growth, reduced it up to 6.75 mm at 10% concentration and completely inhibited growth at 20% and 30%. It was also highly effective against sclerotia production, allowed only 32.25 sclerotia at 10% and entirely suppressed formation of sclerotia at higher concentrations. Vermicompost extract showed moderate effectiveness in controlling mycelial growth, with 73.38 mm, 42.38 mm, and 29.25 mm at 10%, 20%, and 30% concentrations, respectively. The inhibition percentage increased from 17.55% to 67.32% as concentration increased. It also substantially decreased sclerotia production, with counts of 123.50, 88.25, and 73.25 at increasing concentrations, corresponding to inhibition rates between 80.16% and 88.92%.

Table 4. Effect of various organic amendment extracts on the mycelial growth and growth inhibition of *S. rolfsii* under *in-vitro* condition

Treatments	Mycelial growth (mm) of *S. rolfsii* at different concentrations			Percent inhibition at different concentrations		
	10%	20%	30%	10%	20%	30%
Poultry refuse extract	6.75e	0.00e	0.00f	92.40	100.00	100.00
Vermi compost extract	73.38d	42.38d	29.25e	17.55	52.44	67.32
Mustard oilcake extract	75.75cd	44.25c	32.13d	14.88	50.34	64.10
Saw dust extract	77.63c	45.50c	34.88c	12.77	48.94	61.03
Cowdung extract	81.38b	75.75b	72.13b	8.57	15.00	19.40
Control	89.0a	89.13a	89.50a	-	-	-
SEm ±	0.908	0.591	0.5	-	-	-
LSD ($P \geq 0.05$)	2.73	1.78	1.507	-	-	-
CV (%)	2.69	2.39	2.32	-	-	-

Values in a column having the same letter(s) did not differ significantly at the 5% level by LSD.

Table 5. Effect of various organic amendments on Sclerotia production of *S. rolfsii* under *in-vitro* condition

Treatments	No. of mature sclerotia of *S. rolfsii*			Percent inhibition at different concentrations		
	10%	20%	30%	10%	20%	30%
Poultry manure extract	32.25e	0.00e	0.00e	94.82	100.00	100.00
Vermi compost extract	123.50d	88.25d	73.25d	80.16	86.04	88.92
Mustard oilcake extract	130.0d	95.75d	79.75d	79.11	84.86	87.96
Saw dust extract	331.0c	228.50c	183c	46.83	63.87	72.35
Cowdung extract	496.5 b	473.25b	356b	20.24	25.17	46.20
Control	622.50a	632.50a	661.75a	-	-	-
SEm ±	2.958	2.783	3.464	-	-	-
LSD ($P \geq 0.05$)	8.916	8.391	10.441	-	-	-
CV (%)	2.04	2.20	3.07	-	-	-

Values in a column having the same letter(s) did not differ significantly at the 5% level by LSD

Mustard oilcake extract exhibited similar efficacy to vermicompost in restricting mycelial growth, with 75.75 mm, 44.25 mm, and 32.13 mm at 10%, 20%, and 30% concentrations, respectively. Inhibition percentages ranged from 14.88% to 64.10%. Sclerotia production was also significantly reduced, with counts of 130.0, 95.75, and 79.75 at increasing concentrations, resulting in inhibition rates from 79.11% to 87.96%. Sawdust extract had a lesser impact on mycelial growth, with measurements of 77.63 mm, 45.50 mm, and 34.88 mm at 10%, 20%, and 30% concentrations, respectively. Inhibition percentages ranged from 12.77% to 61.03%. Its effect on sclerotia reduction was limited, with counts of 331.0, 228.50, and 183.0 at increasing concentrations, corresponding to inhibition rates between 46.83% and 72.35%. Cowdung extract was found least effective in inhibiting mycelial growth, with measurements of 81.38 mm, 75.75 mm, and 72.13 mm at 10%, 20%, and 30% concentrations, respectively. Inhibition percentages were minimal, ranging from 8.57% to 19.40%. Its impact on sclerotia production was also limited, with counts of 496.5, 473.25, and 356 at increasing concentrations, resulting in inhibition rates between 20.24% and 46.20%.

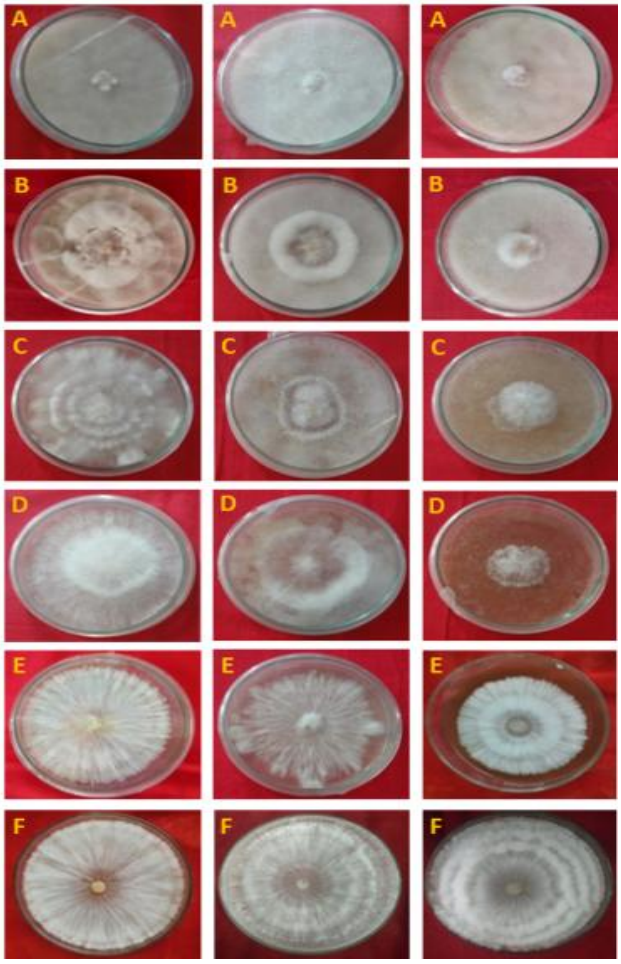

Fig. 3. Radial mycelial growth of *S. rolfsii* in Petri dishes containing PDA amended with an organic amendment at different concentrations, where (A) Poultry manure, (B) Vermicompost, (C), Mustard oil cake (D) Sawdust, (E), Cowdung, and (F) Control

The study concluded that poultry refuse extract demonstrated the highest efficacy in inhibiting both mycelial growth and sclerotia production of *S. rolfsii*, followed by vermicompost and mustard oilcake extract. According to different researchers, organic soil amendments such as Poultry manure, farm yard manure (FYM), Mustard oil cake, compost, at 4 days after inoculation. vermicompost saw dust etc. as a source for inhibition of the fungal growth of *S. rolfsii* (Saha *et al*., 2008). 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*. Further field studies are recommended to validate the *in vitro* control of *S. rolfsii*, the causal agent of collar rot in sunflowers, using fungicides, botanicals, and organic matter extracts.

Conclusion

Collar rot poses a serious threat to sunflower production, highlighting the need for effective and sustainable management strategies. While, chemical fungicides are effective, they have limitations due to their environmental impact, the risk of resistance development, and associated costs. Botanicals and organic matter extracts offer promising, eco-friendly alternatives that align with sustainable agriculture practices and may also be more affordable for collar rot management. Integrating these methods creates a comprehensive approach to collar rot control, enhancing soil health and boosting sunflower resilience. While challenges exist in standardizing and consistently applying these alternative methods, ongoing research can promote the adoption of integrated disease management strategies. By combining the strengths of fungicides, botanicals, and organic amendments, farmers can more effectively control collar rot, reduce reliance on chemical inputs, and support sustainable sunflower production. Future research should focus on refining these combinations and optimizing their application timing under varying field conditions to achieve consistent, long-term disease management.

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Author's contribution

The conception, planning, and design of the experiments, as well as the statistical methodology and data analysis, were carried out collaboratively by NAS, NAI, and MSI. All authors equally contributed to the preparation of the manuscript. NAS and MMA conducted the in-vitro culture. All authors actively contributed to the article and have approved the final version for submission.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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