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Evaluation of selected fungicides and botanicals against *Sclerotium rolfsii* causing Southern blight disease of spine gourd (*Momordica dioca*Roxb.) *in vitro*

KESHAV NARAYAN PAI[1] AND M. CHANDRA[2]

[1]Department of Applied Botany, and [2]Department of Biosciences, Mangalagangotri, Mangalore University, Mangalore, Dakshina Kannada - 5741991, Mangalore

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Southern blight disease in spine gourds caused by *Sclerotium rolfsii* leads to severe yield loss in the major spine gourd fields of coastal Karnataka. Symptoms include formation of water-soaked lesions at soil-stem interface followed by yellowing of leaves and loss of firmness in the stem which eventually leads to death of the plant. Therefore, investigation was carried out to manage the pathogen with chemical fungicides and plant extracts. Five chemical fungicides and 20 selected plant extracts were evaluated against the fungal pathogen under *in vitro* conditions using poison food technique. Results of the study showed that the fungicides Score and Master were able to show the maximum inhibition at 0.1% concentration. Among the botanicals tested, *Allium sativum* (56.81% and 54.19%) and *Sapindus trifoliatus* (56.13% and 52.68%) showed inhibition at 4% and 2%, respectively. Whereas, *Agave americana* showed inhibition (60.68%) at 4% concentration. The plants selected for the study are potential and eco-friendly to treat many fungal pathogens.

Keywords : Botanicals, fungicides, Poison food technique, *Sclerotium rolfsii*, Southern blight, spine gourd.

INTRODUCTION

Vegetable crops are majorly grown in temperate to humid tropical regions in India. Vegetables are rich sources of minerals such as calcium, iron, proteins, and carbohydrates. They also contain high amounts of riboflavin, niacin, thiamine, and vitamin A, B, and C (Tejaswini *et al*. 2022). Spine gourd (*Momordica dioca*) or kantol is a dioecious, perennial vegetable crop that belongs to family Cucurbitaceae and originated from the Indo-Malayan region (Rashmi and Negi, 2022). It has high demand in the market due to its high nutritive and medicinal value (Talukdar and Hossain, 2014). It also exhibits anti-malarial, anti-cancer, and anti-inflammatory properties (Rupachandra and Sarada, 2013; Nagarani *et al*. 2014).*S. rolfsii* is a necrotrophic phytopathogen that infects a wide range of economically important crops. It produces sclerotia on the plant tissues and can survive in the soil for years without the host (Ridge and Shrew, 2014).

The abundance of *S. rolfsii in* warmer regions of the world proves that high temperature is a favourable condition for the formation and development of its sclerotia. While biological methods of controlling plant diseases are effective, use of chemical fungicides is considered to be the most effective (Das and Sarma, 2023). Fungicides are used in an excessive, unreasonable, and indiscriminate manner that has caused problems not only for the safety of consumers but also for the environment (Goswami *et al*. 2018).

In ancient Indian agriculture, plant extracts are used in prevention of plant diseases. Plant metabolites and plant-based fungicides are

*Correspondence:keshavpai2805@gmail.com

expected to have minimal environmental impact and danger to consumers in contrast to chemical fungicides. Plant extracts contain numerous bioactive metabolites that cause physiological effects on the pathogens. As plant extracts are eco-friendly and antagonistic to many diseases, it can be considered as a replacement of chemical fungicides to manage plant diseases. Hence, the present study aims to evaluate plant extracts and chemical fungicides against the *S. rolfsii* that causes Southern blight disease in spine gourd.

MATERIALS AND METHODS

Isolation and identification of fungal pathogen

Infected plant parts of spine gourd were collected from the spine gourd field of coastal Karnataka, India. The plant parts were sterilized thoroughly and inoculated onto Petri plates containing Potato dextrose agar medium and Chloramphenicol and incubated at room temperature. A pathogenicity test was conducted to satisfy Koch's postulate. Identification of the fungal pathogen was done based on the morphological and molecular characteristics.

Screening of chemical fungicides against pathogenic fungi

The sensitivity of *Sclerotium rolfsii* against chemical fungicides was evaluated in the in vitro condition using the poison food technique (Nene and Thapliyal, 1979). Total 5 chemical fungicides (Table1) were weighed separately at respective concentrations, dissolved in the distilled water, and mixed with 20 ml of the PDA medium to make the concentrations 0.1%, 0.05%, 0.025%, and 0.0125% respectively in a test tube. The PDA medium with fungicides was subjected to an autoclave and poured into the Petriplate in the laminar airflow aseptically. The pathogenic mycelial disc (5 mm diameter) was inoculated on the PDA medium containing chloramphenicol. The plates containing distilled water were served as a control. The experimental plates were incubated for 7 days at room temperature. The experiment was conducted in triplicates and the mean of the readings was calculated. The

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

percentage of inhibition was calculated by the following formula (Vincent, 1947).

T=Radius of the pathogen in treatment plate.
C = Radius of the pathogen in control plate,

Selection of the botanicals and preparation of the plant extract

Based on the traditional knowledge, botanicals available in coastal Karnataka were selected for evaluation of their anti-microbial property. Selected plants and their parts used in the study are mentioned in Table 2. A total of 20 plant samples were collected and were washed in sterile water. 100 g of each botanical was added with 100 ml of distilled water and was subjected to homogenization using mortar and pestle. Macerated biomass was kept overnight at 4°C for exudation of the biochemicals. Biomass was filtered with the muslin cloth followed by Whatman No. 1 filter paper. The filtrate was stored at 4°C and considered as 100% stock solution.

Evaluation of the plant extract against fungal pathogen S. rolfsii.

The fungicidal activity of the plant extracts was tested against the isolated fungal pathogen by poison food technique (Nene and Thapliyal, 1979). Stock solution (100%) of plant extract was mixed with 20 ml of PDA medium to make the concentration 4% in a test tube. The PDA medium with plant extract was autoclaved, amended with antibiotic chloramphenicol (120 mg/L), and poured onto the petriplate under sterile conditions. The pathogenic mycelial disc (5 mm diameter) was inoculated on the center of the PDA medium. The PDA medium with distilled water and fungicides (Score) serves as a negative and positive control respectively. The experimental plates were incubated for 7 days at room temperature. After incubation, the mycelial growth was measured using a ruler, and the values were recorded. The mean values were calculated and the percentage of inhibition was determined using the following formula (Vincent, 1947).

RESULTS AND DISCUSSION

Isolation and Identification

The isolated fungal pathogen showed white, fluffy colonies with globoid to irregular-shaped sclerotia. Initially, the sclerotia were whitish but eventually turned into dark brownish colour. The pathogenicity test showed similar symptoms on naturally and artificially infected spine gourd plants. Based on the morphological, microscopic, and molecular characteristics, the fungal pathogen was identified as *Sclerotium rolfsii* (*Agroathelia rolfsii*) (Pai and Chandra, 2023).

Screening of chemical fungicides against fungal pathogen S. rolfsii.

The efficacy of chemical fungicides was tested against the pathogenic fungus *S. rolfsii* at various concentrations (0.1%, 0.05%, 0.025%, and 0.0125%). The fungicides score exhibited complete inhibition (100%) at 0.1% and 0.05% concentrations. Master fungicide showed complete inhibition (100%) against S. rolfsii at 0.1% concentration, followed by 63.65% and 62.07% of inhibition at 0.05% and 0.025% respectively. Indofil M-45 and Saaf showed inhibition of 94.80% and 84.38% at 0.1% concentration. Kavach was least effective in inhibiting the mycelial growth of *S. rolfsii* (Table 3).

Similar results were observed by Sahana *et al*. (2020) who evaluated 12 fungicides against S. rolfsii and found difenoconazole (Score) to show complete inhibition of mycelial growth. El-Naggar and Yassin (2023) observed that difenoconazole (25%) is very effective against *S. rolfsii* causing sugar beet root rot disease. Mahato *et al*. (2014) reported a negligible effect of Kavach (Chlorothalonil 75%) in reducing the mycelial growth of *S. rolfsii*. Wankhade *et al*. (2019) evaluated various fungicides against *S. rolfsii* causing collar rot disease in betel vine and found maximum inhibition by mancozeb. Results showed that the efficacy of fungicides increases with an increase in the fungicidal concentration. Most of the fungicides showed high inhibition of fungal colony at higher concentrations of fungicides which matches with the observations of Rakholiya (2015) on *S. rolfsii* causing stem rot of groundnut.

Table 1: List of fungicides evaluated *invitro* against *S. rolfsii* isolated from *Momordica dioca*

Trade Name	Common Name
Saaf	Carbendazim 12% +Mancozeb 63%
Indofil M-45	Mancozeb 63%
Kavach	Chlorothalonil 75%
Master	Metalaxyl 8% + Mancozeb 64%
Score	Difenoconazole 25%

Table 2: List of plants and parts used against fungal pathogen *S.rolfsii*.

Name of the plants	Parts used
Agave americana L.	Leaves only
Lantana camera L.	Young twigs with flowers
Catharanthus roseus (L.) G.Don.	Young twigs with flowers
Polyalthia longifolia (Sonn.) Thwaites	Young twigs without fruits
Moringa oleifera L.	Young twigs with flowers
Azadirachta indica A. Juss.	Young twigs without fruits
Sapindus trifoliatus L.	Fruits only
Murrayya koenigii L.	Young twigs without fruits
Averrhoa bilimbi L.	Fruits only
Ocimum sanctum L.	Young twigs with flowers
Duranta repens L.	Young twigs without fruits
Capsicum frutescens L.	Young twigs
Cassia occidentalis L.	Young twigs with flowers
Nerium oleander L.	Young twigs with flowers
Gliricidia sepium L.	Young twigs with flowers
Pongamia pinnata L.	Young twigs without flowers
Chromolaena odorata L.	Young twigs with flowers
Bougainvillea spectabilis Willd	Young twigs with flowers
Zingiber officinale Rosc	Rhizome
Allium sativum L.	Bulb scales

Fig. 1: a- Pure culture of the *Sclerotium rolfsii*; b-Irregular shaped sclerotia

Table 3: Efficacy of fungicides against mycelial growth of *S. rolfsii.*

Type of fungicides	Concentration (%)	Mycelial growth	Inhibition of mycelial growth (%)
Saaf	0.1	0.700±0.20	84.38
	0.05	2.333±0.64	48.07
	0.025	4.000±0.36	10.78
	0.0125	4.133±0.152	8.073
Indofil M-45	0.1	0.233±0.23	94.80
	0.05	1.200±0.88	73.29
	0.025	2.300±1.352	48.69
	0.0125	4.067±0.321	9.541
Kavach	0.1	3.733±0.15	16.72
	0.05	4.166±0.288	7.27
	0.025	4.433±0.57	1.11
	0.0125	4.466±0.57	0.66
Master	0.1	00.00 ± 0.00	100
	0.05	1.633±0.321	63.65
	0.025	1.700±0.435	62.07
	0.0125	3.50±0.100	22.15
Score	0.1	00.00 ± 0.00	100
	0.05	00.00±0.00	100
	0.025	0.300±0.100	93.30
	0.0125	0.733±0.152	83.69
Control	0.1	4.483±0.28	00
	0.05	4.493±0.11	00
	0.025	4.483±0.288	00
	0.0125	4.496±0.0058	00

Evaluation of the plant extract against Sclerotium rolfsii

Among the 20 plant extracts used for the evaluation, 6 showed significant inhibition of the mycelial growth of *S. rolfsii*. *Agave americana* extract showed the highest inhibition percentage (60.68%) followed by *Allium sativum* (56.81%), *Sapindus trifoliatus* (56.13%), *Azadirachta indica* (51.59%). *Pongamia pinnata* and *Nerium oleander* exhibited the least inhibition at 9.09% and 9.09% respectively. Here, the 50% fungal mycelial growth inhibition was taken into consideration (Table 4).

Among 20 plant extracts, only 6 showed promising fungal mycelial inhibition and were further evaluated for 1% and 2% concentration. The plants selected for the preparation of an aqueous solution of 1% and 2% concentration were *Agave americana, Azadirachta indica, Sapindus trifoliatus, Polyalthia longifolia, Allium sativum*, and *Murrayya koenigii* (Table5).

Table 4. Fungal mycelial growth inhibition of plant extracts against *S. rolfsii.*

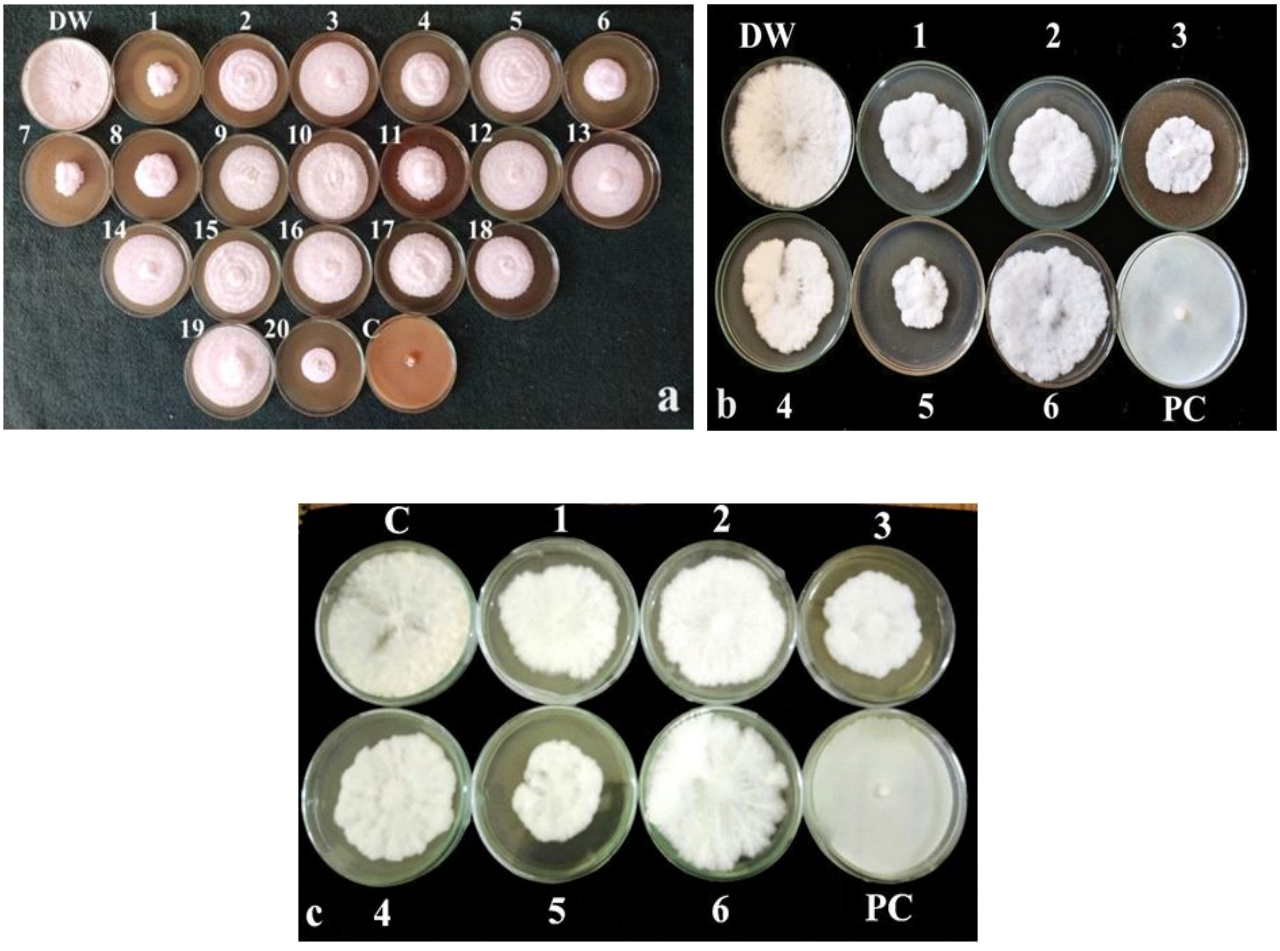
Name of the plants	Colony Diameter	% of Inhibition
Agave americana L.	1.73±0.057	60.68
Lantana camera L.	3.16±0.057	28.18
Catharanthus roseus (L.) G.Don.	3.76±0.057	14.54
Polyalthia longifolia (Sonn.) Thwaites	2.63±0.057	40.22
Moringa oleifera L.	3.70±0.1	15.90
Azadirachta indica A. Juss.	2.13±0.057	51.59
Sapindus trifoliatus L.	1.93±0.057	56.13
Murrayya koenigii L.	2.23±0.057	49.31
Averrhoa bilimbi L.	3.36±0.057	23.63
Ocimum sanctum L.	3.73±0.152	15.22
Duranta repens L.	3.06±0.152	30.45
Capsicum frutescens L.	3.63±0.057	16.81
Cassia occidentalis L.	3.70±0.1	15.90
Nerium oleander L.	4.00±0.1	9.09
Gliricidia sepium L.	3.70±0.1	15.90
Pongamia pinnata L.	4.00±0.1	9.09
Chromolaena odorata L.	3.36±0.152	23.63
Bougainvillea spectabilis Willd	3.3±0.115	25.0
Zingiber officinale Rosc	3.66±0.57	16.81
Allium sativum L.	1.90±0.1	56.81
Distilled water (Negative control)	4.4±0	0
Metaxyl-M4%+ Mancozeb 64% (Positive control)	0±0	100

4% Extract was used in all cases

Among the 6 plant extracts, *Allium sativum* and *Sapindus trifoliatus* showed highest fungal growth inhibition of 54.19% and 52.67% respectively at 2% concentration. *Murrayya koenigii* showed lowest inhibition of 14.49% at 2% concentration. At 1% concentration, *Allium sativum* and

Table 5: Antifungal activity of selected plant extracts against the *S. rolfsii*

Name of the plants	1% Extract		2% Extract	
	Colony Diameter	% of Inhibition	Colony diameter	% of inhibition
Agave americana L.	3.43±0.55	22.92	2.36±0.32	45.81
Azadirachta indica A. Juss.	3.60±0.10	19.10	2.40±0.10	45.03
Sapindus trifoliatus L.	2.73±0.41	38.65	2.06±0.57	52.68
Polyalthia longifolia (Sonn.) Thwaites	3.63±0.15	18.42	3.43±0.57	21.37
Allium sativum L.	2.40±0.10	46.06	2.00±0.10	54.19
Murrayya koenigii L.	4.23±0.20	4.94	3.73±0.15	14.50
Distilled water (Negative control)	4.45±0.05	0	4.36±0.58	0
Score fungicide (Positive control)	0	100	0	100



PC – Positive control, DW- Distilled water

Fig. 3: Antifungal activity of plant extracts at 4% (a), 2% (b), and 1% (c) against *S. rolfsii.*

Sapindus trifoliatus showed the highest inhibition of 46.06% and 38.65% respectively. *Murrayya koenigii* exhibited least inhibition of 4.94% (Table 5, Fig. 3).

The results obtained in the present study aligned with Mahato *et al*. (2018) who observed *Allium sativum* to exhibit maximum inhibition of *S. rolfsii* infecting tomato. Kiran *et al*. (2006) screened 30 plant extracts against *S. rolfsii* associated with ground nut stem rot disease. They observed *Allium sativum* (2.5%) and *Agave americana* (2.5%) showed inhibition of 60% and 70% respectively. Nimbalkar *et al*. (2023) assessed the antifungal properties of 13 plant extracts against *S. rolfsii* and found *Allium sativum* (100%) to exhibit inhibition at different concentrations. Valvi *et al*. (2019) evaluated the fruit extract of *Sapindus trifoliatus* at 10% concentration against *Alternaria brassicae* and found an inhibition of 80.56%. Lindsey and Staden (2004) observed the inhibition of fungal pathogens such as *Botrytis cinerea, Pythium ultimum,* and *Rhizoctonia solani* against *Allium sativum* extract.

CONCLUSION

No studies have yet been undertaken in India to control *S. rolfsii* infecting spine gourd chemically or biologically. This is the first report on the management of *S. rolfsii* infecting spine gourd. The chemical fungicide Difenoconazole (Score) and Master (chemical fungicide) and plant extracts viz., *Agave americana, Azadirachta indica, Sapindus trifoliatus*, and *Allium sativum* used in this study showed maximum inhibition of fungal mycelial growth of *S. rolfsii*. Thus, it can be concluded that these plants are eco-friendly and can safely be used to manage the fungal disease in the field condition.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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