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Comparative *in vitro* evaluation of fungicides against collar rot pathogen, *Sclerotium rolfsii* in elephant foot yam

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ABSTRACT

Amorphophallus or elephant foot yam (*Amorphophallus paeoniifolius*) popularly known as the 'King of tubers' is one of the major tuber crops grown in India and other parts of the world. Collar rot disease, caused by *Sclerotium rolfsii* is one of the primary obstacles to tuber cultivation which can significantly reduce yield up to 100%. Saprophytic nature, broad host range, and ability to produce resting structures make it difficult to manage. In this experiment, 10 isolates of *S. rolfsii* were isolated by the surveying Thiruvananthapuram, Kollam and Alappuzha districts of Kerala, proved the pathogenicity by inoculating the pathogen cultured in sand- maize meal media (9:1). The isolate I_3 obtained from Thiruvananthapuram district was found to be the most virulent based on the days taken for symptom development (4 days). Six fungicides (copper oxy chloride (50% WP), mancozeb (75% WP), propiconazole (25% EC), carbendazim (50% WP), trifloxystrobin (25%) + tebuconazole (50%) 75 WG, and carbendazim (12%) + mancozeb (63%) WP @ 50, 100, 250, 500, 1000 and 2000 ppm were evaluated *in vitro* for their efficacy in inhibiting the fungal mycelial growth and sclerotia formation in isolate I_3. All fungicides except carbendazim 50% WP and copper oxy chloride 50% WP, considerably reduced the mycelial development of *S. rolfsii.* Mancozeb 75%WP and trifloxystrobin (25%) + tebuconazole (50%) 75WG, showed complete inhibition of mycelial growth even at a lower concentration of 50 ppm.

Keywords: Collar rot, Elephant foot yam, Fungicides, *In vitro*, *Sclerotium rolfsii*.

INTRODUCTION

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.)), commonly known as the "King of tubers," belongs to the Araceae family, is regarded as one of the most significant among the tropical tuber crops which is valued for its therapeutic and nutritional qualities. It serves as a food security and remunerative cash crop [1]. It grows in well-drained sandy loam soil at a temperature of 25 to 35 °C and requires an annual rainfall of 1000 to 1500mm.

Similar to any horticultural crops, elephant foot yam is incited with several diseases such as collar rot (*Sclerotium rolfsii*), foot rot (*Rhizoctonia solani*), leaf spot (*Cornyspora cassiicola*), anthracnose (*Colletotrichum gloeosporioides*), bacterial leaf spot (*Xanthomonas campestris* pv *amorphophalli*), and mosaic (*Elephant foot yam mosaic virus*) [2]. Of all the fungal diseases, collar rot caused by *S. rolfsii* is the primary obstacle to its cultivation in India resulting in a yield loss of up to 100% [3].

In those fields where collar rot disease is primarily an impeding factor affecting elephant foot yam cultivation, several chemical fungicides are recommended for management to reduce yield loss

Therefore, the current study aims to explore the effectiveness of various fungicidal compounds on the mycelial growth and sclerotia formation of S. *rolfsii* under *in vitro* conditions.

MATERIALS AND METHODS

Collection of infected plant samples

As part of the study, samples of elephant foot yam plants affected by collar rot were collected from different districts viz., Thiruvananthapuram, Kollam, and Alappuzha in Kerala during the 2022–2023 cropping season based on the symptoms [5].

Isolation and pathogenicity testing

The pathogen was isolated from the elephant foot yam plants affected with collar rot by standard procedure [6]. A small (5 mm) piece of tissue from diseased stems, roots, or corms was removed along

with healthy tissue using a sterile scalpel. The tissues were surface sterilized with 0.1 percent mercuric chloride for one minute and rinsed thrice in sterile distilled water to remove the traces. Excess moisture was removed by using sterilized blotting paper. The bits were transferred aseptically onto the Potato Dextrose Agar (PDA plates. The inoculated plates were incubated at 28 ± 1 °C and growth was recorded daily for seven days. The fungi were then sub-cultured on PDA slants and incubated at 28 ± 1 °C. The pure culture of each isolate was stored and maintained separately in PDA slants at 4°C.

Pathogenicity test was done by artificially inoculating the pathogen into the soil where two-month-old elephant foot yam plants were grown. Mass multiplication of each isolate of *S. rolfsii* was done using sterilized sand maize meal medium taken in a 250 ml conical flask (90 g of washed sand, 10 g of maize meal, and 15 ml of distilled water) and sterilized by autoclaving at 121ºC for 15minutes [7]. The medium was inoculated with two discs of 1.0 cm diameter (three days old culture of the pathogen on PDA plate) and incubated at 28±2°C. After 10-15 days, the media containing mycelia and sclerotia were applied to the soil near the collar region of plants. The pathogen was reisolated later from inoculated plants onto a PDA plates and isolated cultures were compared with the original isolates. Observations on the number of days taken for symptom development and plant death were recorded for all isolates.

Cultural characterization of different isolates

All the ten isolates were grown individually on PDA medium. Fifteen millilitre of the medium was poured into sterile Petri plates for solidification under aseptic conditions and 5 mm mycelial discs of seven-day old fungal culture was inoculated at the centre of Petri dish and incubated at room temperature (28 ± 1^0 C). Observations on colour and nature of mycelia, days taken for sclerotial formation and arrangement of sclerotia were recorded.

Morphological characterization of different isolates

The morphological characters of different isolates viz., size of hypha, septation in hypha, anastomosis, clamp connection, shape and size of sclerotium were studied by preparing slide culture of all ten isolates and stained them with lactophenol cotton blue. The slides were observed under microscope with 40X magnification.

Virulence rating of different isolates

Virulence rating was undertaken to identify the most virulent isolate of the fungus by artificial inoculation of two-month-old elephant foot yam plants using the pathogen inoculum, mass multiplied on maize meal sand medium (1:9). The virulent isolate was identified considering the minimum duration taken for infecting the plant along with cultural characters.

***In vitro* evaluation on the effectiveness of fungicides against the pathogen**

The effectiveness of two contact fungicides, two systemic fungicides), and two combination fungicides were assessed against the most virulent isolate of *S. rolfsii* using the poisoned food technique [8].

The following fungicides @2000, 1000, 500, 250, 100, and 50 ppm were selected for the experiment.

T_1- copper oxychloride (50% WP)

T_2 - mancozeb (75% WP)

T_3 - carbendazim (50% WP)

T_4 - propiconazole (25% EC

T_5 - trifloxystrobin (25%) + tebuconazole (50%) 75 WG

T_6 - carbendazim (12%) + mancozeb (63%) WP

50 ml each of fungicidal solution and double-strength PDA were mixed to get final concentrations of 50, 100, 250, 500, 1000, and 2000 ppm. In 9 cm petri dishes, 20 ml of this medium was plated. The test fungus (seven-day-old, actively growing pure culture) was inoculated aseptically into the centre of each plate containing PDA. PDA medium without any fungicide served as control. The inoculated plates were incubated at 28 ± 1 °C. The experiment was designed in CRD and with treatments replicated thrice. Observations taken on radial mycelial growth and the percentage of inhibition was calculated by using the formula [9]

$$I = [(C-T)/ C] \times 100$$

Where,

I= Percent inhibition of mycelial growth (diameter in cm of *S. rolfsii*)

C = Mycelial growth (diameter in cm) of *S. rolfsii* in control

T = Mycelial growth (diameter in cm) of *S. rolfsii* in various treatments

RESULTS

Collection of infected plant samples

A survey was conducted during 2022-2023 covering two agroecological units (AEU) of Kerala, viz., Southern laterites AEU 8 and Southern central laterite AEU 9. The surveyed districts include Thiruvananthapuram, Kollam, and Alappuzha. Ten plant samples infected with collar rot were collected from the surveyed locations. The collected samples belong to the local variety. All growing stages such as seedling, vegetative, and harvesting stages were susceptible to the pathogen. Symptoms such as peeling off the bark, brown to black discoloration, rotting of roots, and yellow to pink discoloration of leaves, followed by shedding of leaves and finally drying up of the plants were observed. The soil around the affected plants also exhibited a white mycelial mass with brown mustard seed-like sclerotia. The affected plants were also seen to have white profuse mycelial growth with brown sclerotia at the collar area (Figure1, Table 1).

Isolation and pathogenicity testing

The fungal pathogen from each sample collected from different locations was isolated on PDA medium under aseptic conditions and incubated for mycelial growth and the pure culture was maintained for further studies. Pathogenicity testing of the isolates obtained from each area was undertaken artificially by soil inoculation of pathogen multiplied on sterilized sand maize meal medium (9:1). The pathogenicity test revealed the same symptom as that of the original. The morphological and cultural characteristics of mycelia and sclerotia of all the re-isolated fungal isolates were found to be the same as those of the original ones. The isolate I_3 obtained from Thiruvananthapuram district was found to be the most virulent as it had taken only a minimum of four days for symptom development and ten days for complete death of the plant (Table 4, Figure 2) while the other cultures took more days than I_3.

Cultural characterization of different isolates

Out of ten fungal isolates obtained, eight (I_1, I_2, I_3, I_4, I_5, I_7, I_8, and I_9) appeared to be white coloured and fan shaped whereas isolate I_6 showed white fluffy mycelial growth while isolate I_{10} produced white coloured mycelia which was sparse towards the centre of the petri plate. The sclerotia of all the samples appeared to be light cream in the beginning to light and dark brown colour in advance stages except isolate I_6 where no sclerotial production was observed. The mycelium of all the isolates appeared hyaline under 40X magnification. There

was variation in the duration of sclerotial formation on PDA medium among the isolates. Isolate I_3 took the least duration (6 days) for the formation of sclerotia on PDA medium whereas the longest duration (12 days) for sclerotial formation was taken by isolate I_{10}. The pattern of sclerotia formation in the PDA plates are also showed some variability. It appeared as scattered (I_7, I_8), more sclerotia found to be distributed at the peripheral side of petri plate (I_1, I_3, I_4, I_9), ring formation at centre (I_5), scattered at centre and periphery (I_2, I_{10}) and no sclerotia production found in the isolate I_6 (Table 2).

Morphological characterization of different isolates

Morphological characteristics of all the isolates were studied in detail. The variation in the size of hyphae among the isolates ranged from 1.14 to 3.68 µm. The hypha was septate and sclerotium was round in the case of all the isolates. The size of sclerotium was least in isolate I_7 (1.20 mm) whereas, it was highest in isolate I_3 (1.96 mm) (Table 3).

Virulence rating of different isolates

The ten isolates were compared for their virulence to identify the most virulent isolate for the *in vitro* evaluation of the efficacy of fungicides against the pathogen. Isolate I_3 recorded the least number of days for symptom development (4 days) and plant death (10 days) during pathogenicity testing as also it took the least number of days for sclerotial production in Petri plate (6 days). Hence, I_3 was identified as the most virulent isolate among the ten isolates and was used for further studies.

***In vitro* evaluation of the efficacy of fungicides against the pathogen**

For *in vitro* evaluation, contact, systemic, and combination fungicides were used against the most virulent pathogen isolate (I_3). Mycelial growth of the pathogen varied in its sensitivity towards the fungicides tested at different concentrations. Compared to control, all fungicides suppressed the growth of pathogen at all tested concentrations, except copper oxychloride (50%WP) and carbendazim (50%WP). At all tested concentrations, contact fungicide mancozeb (50%WP) and combination fungicide trifloxystrobin (25%) + tebuconazole (50%) 75 WG showed 100% suppression of mycelial growth (Table 5, Figure 3, 4). At 2000, 1000, 500, and 250 ppm, propiconazole and combination fungicide carbendazim (12%) + mancozeb (63%) WP exhibited 100% inhibition. At lower concentrations of 100 and 50 ppm, propiconazole showed 65.55 % and 41.85% inhibition respectively. Carbendazim (12%) + mancozeb (63%) WP showed absence of any inhibition at the above doses, whereas carbendazim 50% WP and copper oxy chloride 50% WP showed least inhibition.

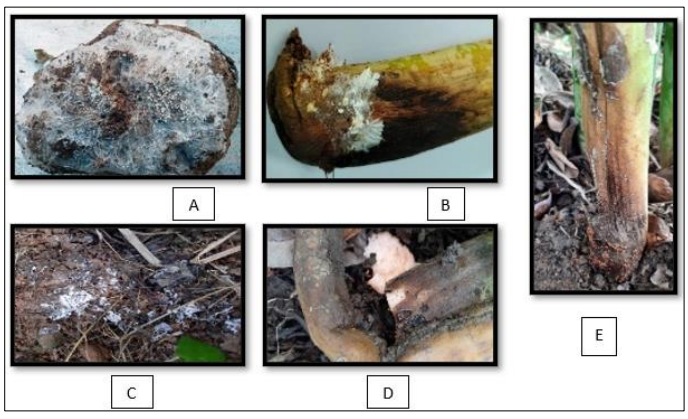

Figure 1: Symptoms of collar rot in elephant foot yam. **(A)** White mycelial growth and sclerotia on the surface of corm; **(B)** White mycelia on collar region**; (C)** Mycelia spread to the soil; **(D)** Rotting of collar part; **(E)** Brown sclerotia on collar region

Table 1: Details of collar rot infection in different locations

District	Location	AEU	Isolate	Stage of crop
Thiruvananthapuram	Pallichal	8	I_1	Harvesting
	Maranalloor	8	I_2	Vegetative
	Vengannoor	8	I_3	Vegetative
	Kalliyoor	8	I_4	Harvesting
	Kottukal	8	I_5	Vegetative
Kollam	Chadayamangalam	9	I_6	Harvesting
	Nilamel	9	I_7	Vegetative
	Kottarakara	9	I_8	Corm in storage
	Sasthamkotta	9	I_9	Vegetative
Alappuzha	Cheriyanad	9	I_{10}	Vegetative

Figure 2: Virulent isolate I_3 grown in PDA medium

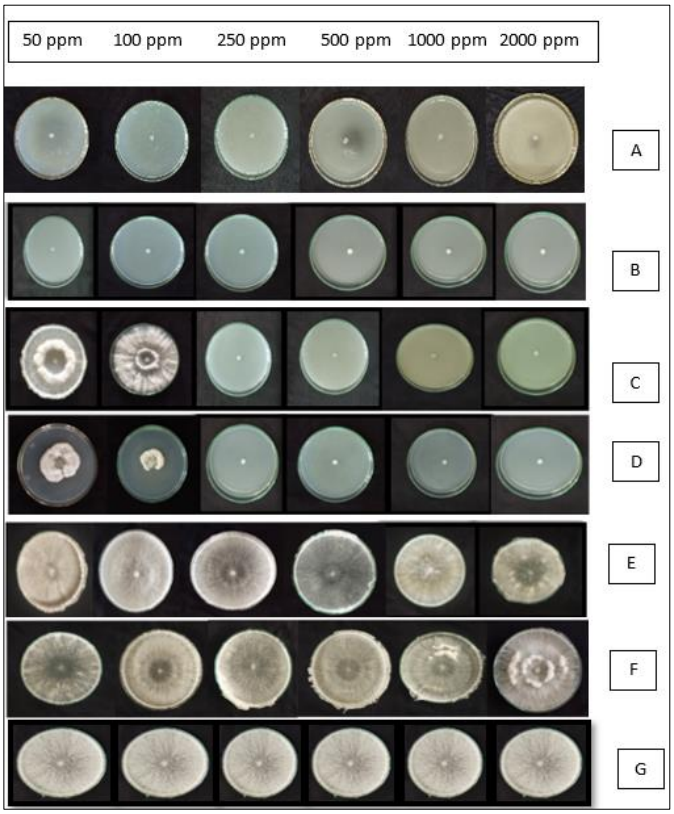

Figure 3: *In vitro* evaluation of efficacy of fungicides against *Sclerotium rolfsii* at 50, 100, 250, 500, 1000 and 2000 ppm. **(A)** Mancozeb 75% W **(B)**Trifloxystrobin (25% +Tebuconazole (50%) 70 WG; **(C)**Carbendazim 12% + Mancozeb (63%) WP; **(D)**Propiconazole 25% EC; **(E)** Carbendazim 50% WP; **(F)** Copper oxychloride 50% WP; **(G)** Control

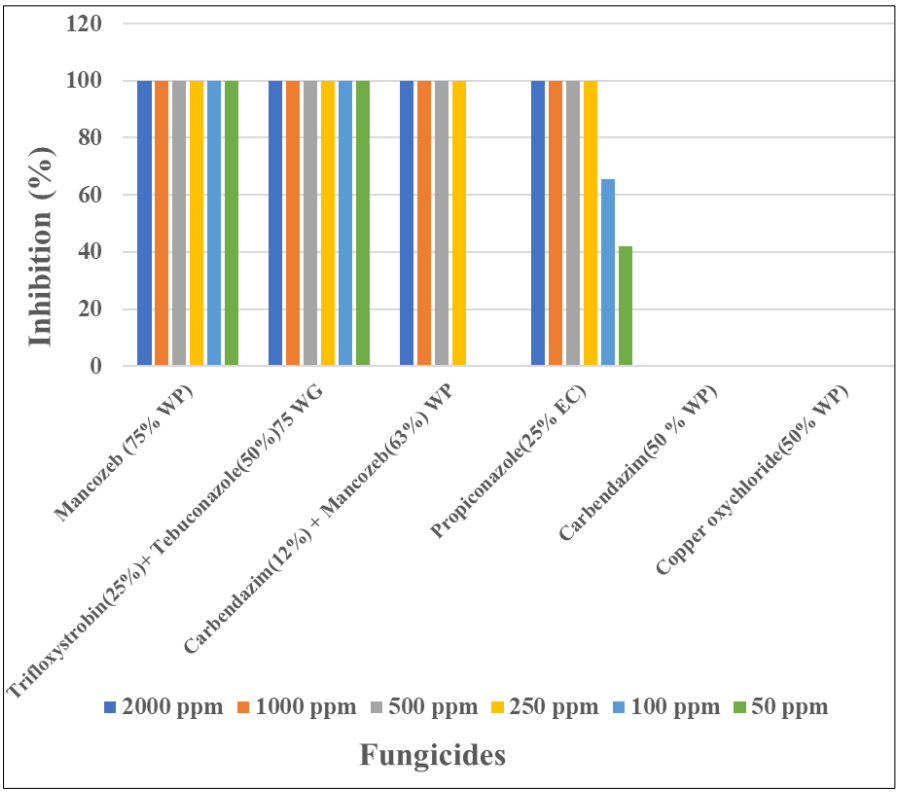

Figure 4: Percentage inhibition of different fungicides against *S. rolfsii*

Table 2: Characteristics of isolates of collar rot pathogen collected from different locations

Isolate	Nature of mycelium	Colour of sclerotium	Days taken for completing mycelial growth in petri plates	Days for formation of sclerotium in petri plates
I_1	Fan shaped	Light cream to dark brown	5	11
I_2	Fan shaped	Light cream to dark brown	4	8
I_3	Fan shaped	Light cream to dark brown	3	6
I_4	Fan shaped	Light cream to dark brown	3	8
I_5	Fan shaped	Light cream to dark brown	4	9
I_6	Fluffy	-	3	-
I_7	Fan shaped	Light cream to dark brown	3	8
I_8	Fan shaped	Light cream to light and dark brown	4	9
I_9	Fan shaped	Light cream to light and dark brown	4	9
I_{10}	Sparse towards the centre	Light cream to light and dark brown	4	12

Table 3: Morphological characteristics of isolates of collar rot pathogen collected from different locations

Isolate	Size of hypha (µm)	Size of sclerotium (mm)
I_1	3.15	1.82
I_2	2.44	1.42
I_3	3.68	1.96
I_4	2.10	1.72
I_5	1.14	1.84
I_6	1.88	-
I_7	2.76	1.20
I_8	3.10	1.36
I_9	2.18	1.60
I_{10}	2.91	1.30

Table 4: Virulence rating of isolates of collar rot pathogen collected from different locations

Isolate	Days taken for symptom development	Days taken for death of the plant
I_1	9	21
I_2	7	15
I_3	4	10
I_4	6	13
I_5	8	17
I_6	8	17
I_7	7	14
I_8	7	15
I_9	9	18
I_{10}	9	19

Table 5: *In vitro* evaluation of fungicides against mycelial growth of *S. rolfsii* isolate I_3

Treatment	Mycelial growth (diameter in cm) at different dose*					
	50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	2000 ppm
Mancozeb 75% WP	0.00	0.00	0.00	0.00	0.00	0.00
Trifloxystrobin (25%) + tebuconazole (50%) 70 WG	0.00	0.00	0.00	0.00	0.00	0.00
Carbendazim (12%) + Mancozeb (63%) WP	9.00	9.00	0.00	0.00	0.00	0.00
Propiconazole 25 % EC	5.23	3.10	0.00	0.00	0.00	0.00
Carbendazim 50% WP	9.00	9.00	9.00	9.00	9.00	9.00
Copper Oxychloride 50 % WP	9.00	9.00	9.00	9.00	9.00	9.00

The treatment of propiconazole at 50 ppm is found to be significantly different from 0 (t value=59.34, p value=0.00**)

The treatment of propiconazole at 100 ppm is found to be significantly different from 0 (t value= 53.69, p value=0.00**)

*Mean of 3 replications

** Indicates a significant difference at 1 % level [10]

DISCUSSION

Elephant foot yam even though it is affected by many diseases including fungal and viral diseases, collar rot caused by *Sclerotium rolfsii* results in significant yield losses. Hence, the present study was undertaken during 2023- 24 to characterize the collar rot pathogen of elephant foot yam and develop a suitable management strategy using chemical fungicides. Collar rot samples were collected through survey and the pathogen was isolated on potato dextrose agar (PDA) medium. Pathogenicity testing of each isolate was undertaken artificially by inoculating the pathogen cultured on maize meal- sand medium (1:9) on collar portion of two-month-old elephant foot yam plants which revealed the same symptom expression as that of the original samples collected from the ten locations. Similar symptoms of the pathogen were observed which included white fungal strands (mycelia or hyphae) around collar region of the infected plant parts and on the soil surrounding the plant upon artificial inoculation of lentil [11].

The cultural characteristics of all the isolates obtained from different locations were carefully examined. The mycelial growth of isolates I_1, I_2, I_3, I_4, I_5, I_7, I_8, and I_9 appeared to be white coloured and fan-shaped. In contrast, isolate I_6 was observed to have white coloured fluffy mycelial growth, and isolate I_{10} had sparse mycelial growth towards the center. Paparu *et al.*, 2020 reported the occurrence of variations in mycelial growth of *S. rolfsii* infecting common beans in which all isolates formed white cultures with fluffy, fibrous, or compact mycelia with different growth rate among isolates in different agroecological zones [12].

In the present study, the sclerotia developed on the infected samples appeared to be light cream in colour initially and later turned to light brown and dark brown. Also, variation in duration and pattern of sclerotial formation was observed in nine isolates of *S. rolfsii* on PDA medium. Prasad *et al*., 2012 reported that isolates of *S. rolfsii* varied in growth parameters such as colony morphology, mycelial growth rate, colony colour, sclerotial production, number and size of sclerotia [13], while Xie *et al.*, 2014 observed that there were, significant differences in the average number and size of sclerotia produced *in vitro.* Most isolates produced sclerotia that were tan in colour but a few isolates formed light-brown, brown, or dark-brown sclerotia. The average sclerotia size was 0.56 to 1.91 mm in diameter [14].

The morphology of all the isolates was studied in detail. The mycelia of all the isolates were hyaline having a width ranging from 1.14 to 3.68 µm with septations and the presence of clamp connections. The size of the sclerotia ranged from 1.20 to 1.96 mm. Arya *et al*., 2021, observed that the mycelium of *S. rolfsii* to be hyaline, and much branched and hyphae were thin-walled and septate. The colonies appeared as pure white to dull white mycelial growth and sclerotial bodies were formed after 6-7 days of incubation [11]. The authors indicated that the variations in the cultural characteristics and sclerotia formation may be due to variations in nutrient requirements as well as environmental factors.

All the isolates showed variations concerning pathogenicity and virulence. I_3 was proved to be the most virulent isolate which produced symptoms and caused death of the plant within 4 and 10 days of inoculation respectively and formed sclerotia within 6 days *in vitro* compared to others. *S. rolfsii* isolates with high growth rates also had high virulence characterized by symptoms of water soaking, root colonization, and seedling infection (Punja *et al.*, 1985) [15]. The positive association between the growth rate of mycelia and aggressiveness was observed by Patra *et al*., 2023[16] and this can be due to variability among the different isolates [17].

The fungicidal potential of selected chemicals at six different concentrations ie., 50, 100, 250, 500, 1000, and 2000 ppm on the growth of the pathogen was evaluated. The present study revealed that, even at a lower dose of 50 ppm, the combination fungicide trifloxystrobin (25%) + tebuconazole (50%) 75WG and the contact fungicide mancozeb 75%WP were very efficient at inhibiting the growth of fungal mycelium and sclerotium of *S. rolfsii.* Mancozeb 75% WP functions by inactivating the sulfhydryl groups of amino acids thereby interrupting the enzymatic activities within the fungal cell. This action disrupts lipid metabolism and respiration and also lead to partial or complete degradation of the cell wall [18] whereas trifloxystrobin (25%) + tebuconazole (50%) 70 WG operates through a synergestic mode of action, combining the effects of its two active ingredients, Trifloxystrobin and Tebuconazole. Trifloxystrobin, belonging to the strobilurin class of fungicides, disrupts the mitochondrial respiration of fungal pathogens by binding to the Qo site of the cytochrome b complex in their electron transport chain thereby inhibiting ATP synthesis, curtailing fungal growth and propagation. Tebuconazole, a triazole fungicide, acts as a demethylation inhibitor, hindering the biosynthesis of ergosterol in the fungal cell membranes. It disrupts the production of ergosterol by blocking the enzyme CYP51, leading to membrane instability, cell leakage, and eventual pathogen death [19].

The results were all in agreement with similar works in the management of the pathogen. Johnson *et al.*, 2008 observed that propiconazole inhibited the growth of *S. rolfsii* at all tested concentrations (500, 700, and 1000 ppm) [20]. Experiments of Manu

and Nagarajan (2012), Arya *et al*., 2021 and Nath and Patel, 2023 on the effectiveness of systemic and contact fungicides against *S. rolfsii* revealed that the pathogen was completely inhibited by tebuconazole 50% + trifloxystrobin 25% at all tested concentrations [21,11,22]. Copper oxychloride and carbendazim were the two compounds that inhibited the mycelial development of *S. rolfsii* to its lowest level [23]. Propiconazole (500 and 1000 ppm) and mancozeb (1000 and 2000 ppm) could completely suppress the pathogen [24].

CONCLUSION

The objective was to identify the most virulent isolate of *S. rolfsii* causing collar rot in elephant foot yam and to find out the most effective fungicide for inhibiting the mycelial growth and sclerotia production of the pathogen. The most virulent isolate of the pathogen obtained was I_3. All concentrations of mancozeb 75% WP, and trifloxystrobin (25%) + tebuconazole (50%) 75 WG, showed 100% efficacy in suppressing the pathogen. Even at a lower concentration of 50 ppm, they prevented the mycelial proliferation and sclerotial formation under *in vitro* conditions. Thus, the findings of the present study suggested that the fungicides mentioned above can be recommended for further field-level investigations.

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Conflict of interest

The authors declare that they have no conflict of interest.

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