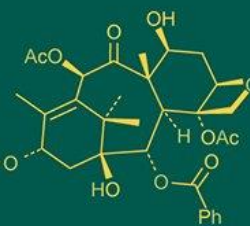
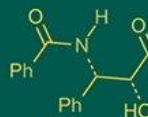


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In vitro evaluation of botanicals and fungicides against *Sclerotium oryzae* causing stem rot of paddy

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Abstract

Sclerotium oryzae is the causal organism of rice stem rot and it is one of the important diseases affecting rice production. Laboratory experiment was carried out to evaluate the effectiveness of botanicals and fungicides over stem rot pathogen under *in vitro* conditions. Seven botanical extracts and eight fungicide molecules were tested by poisoned food technique. Among the botanical extracts tested, turmeric (*Curcuma longa*) exhibited the maximum suppression of mycelial growth, with an inhibition of 88.61%, followed by Eucalyptus (*Eucalyptus globulus*-83.80%), Ginger (*Zingiber officinale*-83.70%) and Tulasi (*Ocimum sanctum*-82.22%). Mancozeb 50% + Carbendazim 25% WS was the most effective fungicide, producing complete inhibition of mycelial growth at 60 ppm. In contrast, Trifloxystrobin 25% + Tebuconazole 50% WG and Metiram 55% + Pyraclostrobin 5% WG required higher concentrations to achieve complete inhibition. Significant differences were observed among the treatments and concentrations tested, which indicates that effective botanicals and fungicides can be used as potential components in developing sustainable management strategies for rice stem rot.

Keywords: Rice stem rot, *Sclerotium oryzae*, botanical extracts, fungicides, poisoned food technique

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crops, serves as the principal food for more than half of the world's population. Rice is the most consumable food grain in India and contributes nearly 43% of the total caloric intake of the population. Besides ensuring food and nutritional security, rice plays a significant role in the country's economy by providing livelihood to millions of farming households and contributing substantially to Agricultural GDP. Sustaining rice productivity is therefore essential to meet the increasing food demand of the growing population.

Rice cultivation is constrained by several biotic stresses, among which fungal diseases are considered the most destructive because of their widespread occurrence and ability to cause severe yield and quality losses. Rice blast, sheath blight, brown spot, false smut, bacterial leaf blight, sheath and stem rot are of major economic importance. Under favourable environmental conditions, these diseases significantly reduce grain yield and adversely affect grain quality, particularly in intensive irrigated rice ecosystems (Martinez, 2024) ^[11]. Stem rot, caused by *Magnaporthe salvinii* (Catt.) (Krause and Webster1972) ^[7] (anamorph: *Sclerotium oryzae* Catt.) is an important soil-borne disease of rice. The disease has emerged as a serious production constraint, particularly in high-input rice cultivation where intensive nitrogen fertilization, continuous rice cropping, high plant density and poor field drainage favour disease development (Gowdar *et al.*, 2021) ^[4]. In India, stem rot has been reported from several rice-growing states and is prevalent in the districts of Mahbubnagar, Nalgonda, Warangal, Khammam, Nellore, East Godavari and West Godavari of Telangana and Andhra Pradesh (Gopika *et al.*, 2011) ^[3]. The disease is initially characterized by small, irregular, dark brown to black lesions that develop on the leaf sheath near the water level. As infection progresses, lesions enlarge and coalesce, extending to the culm where they weaken stem tissues. Numerous black sclerotia develop within and on infected sheaths, serving as survival structures of the pathogen. Severely infected plants exhibit poor tillering, incomplete grain filling, lodging and premature drying, ultimately resulting in considerable yield losses (Quintana *et al.*, 2022) ^[12].

Chemical fungicides remain an effective option for reducing pathogen growth, but their repeated and indiscriminate use may lead to environmental contamination, development of fungicide resistance, disruption of beneficial microorganisms and increased production costs. Botanical extracts have received considerable attention as environmentally safe alternatives because they contain diverse bioactive compounds with antifungal properties and are biodegradable, inexpensive and relatively safe to non-target organisms.

Considering the increasing importance of stem rot in rice and the need for eco-friendly management strategies, this study assessed the efficacy of selected botanical extracts and fungicides under *in vitro* conditions to identify effective management options for suppressing rice stem rot.

2. Materials and Methods

2.1 Sample collection, isolation and characterization of *S. oryzae*

Rice plants exhibiting stem rot symptoms were collected from the major rice-growing districts of Telangana during the Kharif 2025, later isolation was carried out. The isolated pathogen was identified based on its cultural and morphological characteristics following the descriptions provided by Barnett and Hunter (1972)^[1]. Pathogenicity test was conducted for all the collected isolates to prove the Koch's postulates on the Susceptible cultivar BPT-5204 and to identify the virulent isolate. The virulent isolate was used for efficacy studies of botanicals and fungicides.

2.2 Preparation of botanical extracts

Freshly collected plant materials (leaves, bulbs and

rhizomes) were collected and washed thoroughly with tap water followed by sterile distilled water. The cleaned plant materials were shade dried and separately macerated with sterile distilled water in a 1:1 (w/v) ratio using mortar and pestle. Later homogenised extract was filtered through a four layered muslin cloth and centrifuged at 4000 rpm for 5 minutes. The clear supernatant was filtered through Whatman No. 1 filter paper and used as the 100% stock extract solution for further studies (Khadka 2024)^[6].

2.3 *In vitro* screening of botanical extracts against *S. oryzae*

The required concentrations of botanical extracts were prepared by using the calculated quantity of stock solution into sterilized PDA media. Approximately 20 mL of the amended PDA medium was dispensed into each sterilized petri plate and allowed to solidify. The control plates were maintained without the addition of botanical extracts. A 5 mm mycelial disc from a 3 day old culture of test pathogen was aseptically placed at the middle of the solidified PDA plate. Each treatment was replicated three times. The inoculated petri plates were incubated at 27±2 °C for 3 days, after which the radial growth of the pathogen was measured. The percentage inhibition of mycelial growth was determined using the formula proposed by Vincent (1947):

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

Where,

C = Mycelial growth of the pathogen in control

T = Mycelial growth of the pathogen in the respective treatment

Table 1: Details of botanical extracts and concentrations used for *in vitro* evaluation

Treatments	Common name	Botanical name	Plant part used	Concentration used
T ₁	Neem	<i>Azadirachta indica</i>	Leaf	1%, 3%, 5%
T ₂	Onion	<i>Allium cepa</i>	Bulb	1%, 3%, 5%
T ₃	Ginger	<i>Zingiber officinale</i>	Rhizome	1%, 3%, 5%
T ₄	Garlic	<i>Allium sativum</i>	Bulb	1%, 3%, 5%
T ₅	Eucalyptus	<i>Eucalyptus globulus</i>	Leaf	1%, 3%, 5%
T ₆	Tulasi	<i>Ocimum sanctum</i>	Leaf	1%, 3%, 5%
T ₇	Turmeric	<i>Curcuma longa</i>	Rhizome	1%, 3%, 5%
T ₈	Control	-	-	-

2.4 *In vitro* screening of fungicides against *S. oryzae*

The *in vitro* efficacy of selected fungicides against *S. oryzae* was evaluated using the poisoned food technique. The required concentrations of fungicides were prepared by adding the calculated quantity of fungicide formulations into sterilized PDA media. The amended PDA was thoroughly mixed and 20 ml of each treatment media was poured into sterile petri plates and allowed to solidify. PDA plates without fungicide amendment served as the untreated control. A five mm diameter mycelial disc obtained from an actively growing culture of test pathogen was aseptically placed at the centre of each treated plate. Each treatment was maintained with three replications. The inoculated plates were incubated at 27±2 °C for 3 days. The radial growth of the pathogen was recorded and the percent inhibition of mycelial growth over control was calculated using the following formula (Vincent 1947)^[14]:

The antifungal activity of selected fungicides against *S. oryzae* was screened under *in vitro* conditions using the poisoned food technique. Required concentrations of each fungicide were incorporated into sterilized PDA medium by

adding the appropriate quantity of fungicide formulation. The amended medium was mixed thoroughly and 20 mL of each treatment medium was dispensed into sterile petri plates and allowed to solidify. PDA medium without fungicide served as the untreated control. A 5 mm diameter mycelial disc taken from an actively growing culture of the pathogen was aseptically placed at the middle of each treated plate. Each treatment was maintained with three replications. The inoculated plates were incubated at 27±2 °C for 3 days and the radial growth of the pathogen was measured. The percentage inhibition of mycelial growth over the control was calculated using the formula proposed by Vincent (1947):

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

Where,

C = Mycelial growth of the pathogen in control

T = Mycelial growth of the pathogen in the respective treatment

Table 2: Different fungicides and concentrations used for *in vitro* evaluation

Treatments	Details of the fungicide	Concentrations used
T ₁	Trifloxystrobin 25% + Tebuconazole 50% WG	30, 60, 120 and 240 ppm
T ₂	Azoxystrobin 11% + Tebuconazole 18.3% (w/w) SC	30, 60, 120 and 240 ppm
T ₃	Hexaconazole 4% + Zineb 68% WP	30, 60, 120 and 240 ppm
T ₄	Metiram 55% + Pyraclostrobin (5%) WG	30, 60, 120 and 240 ppm
T ₅	Hexaconazole 5% EC	30, 60, 120 and 240 ppm
T ₆	Propiconazole 25% EC	30, 60, 120 and 240 ppm
T ₇	Validamycin 3% L	30, 60, 120 and 240 ppm
T ₈	Mancozeb 50% + Carbendazim 25% WS	30, 60, 120 and 240 ppm
T ₉	Control	-

2.5 Experimental design and statistical analysis

The study was carried out in a Completely Randomized Design (CRD) with three replications. The data recorded on radial mycelial growth and percent inhibition were subjected to analysis of variance (ANOVA). The critical difference (CD) values were calculated at the 5 percent level of significance.

3. Results and Discussion

3.1 Evaluation of botanical extracts against *Sclerotium oryzae*

The *in vitro* screening of seven botanical extracts showed variations in their capacity to suppress the mycelial growth

of the test pathogen. The inhibitory effect increased with increasing concentration of botanical extracts. Turmeric leaf extract was the most effective among the tested botanicals recording the highest mycelial growth inhibition (88.61%) at 5% concentration, followed by Eucalyptus (83.80%), Ginger (83.70%) and Tulasi (82.22%). The untreated control recorded maximum radial growth with no inhibition (Table 3). Turmeric showed superior antifungal activity is due to the presence of curcumin, phenolic compounds and essential oils which inhibits fungal growth and development and the inhibitory effects of eucalyptus and ginger is due to their rich content of phenolics, flavonoids and terpenoids possessing antimicrobial properties (Gurjar *et al.*, 2012)^[5].

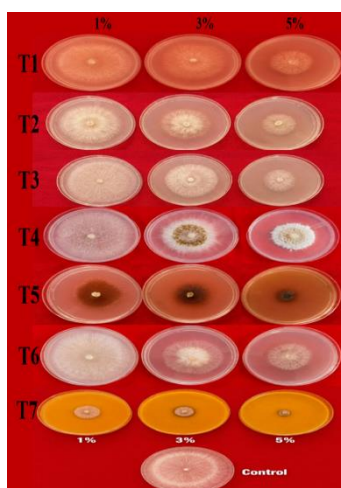
Table 3: Effect of botanical extracts on mycelial growth inhibition of *S. oryzae*

S.no	Treatment	% inhibition of radial growth*		
		1%	3%	5%
T ₁	Neem	32.22 (34.59)	47.22 (43.41)	74.63 (59.76)
T ₂	Onion	38.43 (38.31)	47.22 (43.41)	74.63 (59.76)
T ₃	Ginger	28.80 (32.45)	67.59 (55.30)	83.70 (66.19)
T ₄	Garlic	61.94 (51.91)	58.15 (49.69)	62.87 (52.46)
T ₅	Eucalyptus	59.17 (50.28)	67.50 (55.24)	83.80 (66.26)
T ₆	Tulasi	59.91 (50.71)	80.93 (64.10)	82.22 (65.06)
T ₇	Turmeric	72.50 (58.37)	84.44 (66.77)	88.61 (70.28)
T ₈	Control	0.00	0.00	0.00
Factors		F (A)	F (B)	F (A×B)
CD		3.304	2.02	5.72
SE(d)		1.638	1.00	2.84
SE(m)		1.158	0.71	2.01

() values in the parenthesis are transformed values, *mean of three replications

Turmeric and other botanical extracts exhibited strong inhibitory activity against the mycelial growth of *Sclerotium rolfsii* under *in vitro* conditions, greater inhibition observed at higher concentrations. The antifungal potential of neem and other botanicals against has been widely reported, and

the inhibitory activity of botanicals has been attributed to the presence of bioactive secondary metabolites such as phenolics, flavonoids and terpenoids (Madhavi *et al.*, 2011; Gurjar *et al.*, 2012; Chopra *et al.*, 2018; Mahato 2018)^[9, 5, 2, 10].

**Fig 1:** Effect of different concentrations of botanical extracts on mycelial growth of *Sclerotium oryzae* by poisoned food technique

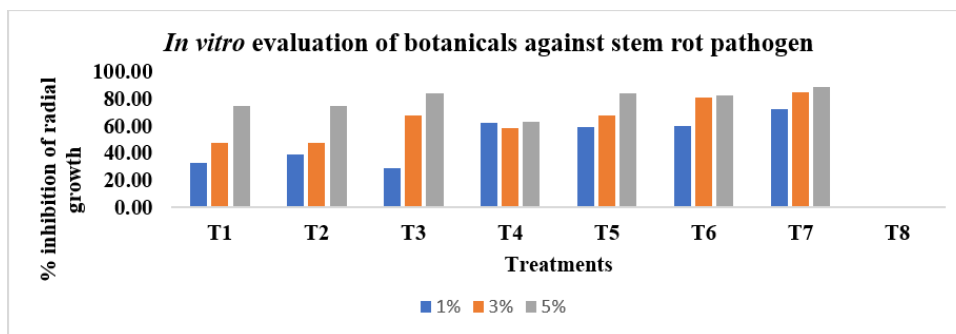


Fig 2: Effect of treatments of botanicals on radial growth of *Sclerotium oryzae* in vitro

3.2 In vitro assessment of fungicides against *S. oryzae*

Fungicides markedly reduced the mycelial growth of *S. oryzae*, with inhibition increasing as the concentration increased. Among the fungicides tested, Mancozeb 50% + Carbendazim 25% WS was the most effective, completely reducing the fungal growth from 60 ppm onwards. Trifloxystrobin 25% + Tebuconazole 50% WG and Metiram 55% + Pyraclostrobin 5% WG also produced complete inhibition at 120 and 240 ppm, whereas Propiconazole 25% EC and Validamycin 3% L completely suppressed the pathogen only at 240 ppm. Hexaconazole 4% + Zineb 68% WP and Hexaconazole 5% EC were also highly effective, recording more than 96% inhibition at higher concentrations (Table 4). Higher effectiveness of Mancozeb + Carbendazim

observed in the present study was supported by reports of reducing *S. oryzae* growth by carbendazim-based and mancozeb + carbendazim combinations under laboratory conditions. Systemic triazole fungicides, including propiconazole and hexaconazole, have been reported to exhibit effective suppression of *S. oryzae*. Fungicides combining strobilurin and triazole groups, such as trifloxystrobin + tebuconazole, have also shown promising antifungal activity against various fungal pathogens owing to their distinct modes of action. These findings highlight the potential of systemic and combination fungicides as effective methods for managing disease (Rangarani *et al.*, 2018; Gopika *et al.*, 2011; Kumar *et al.*, 2003) [15, 3, 8].

Table 4: Inhibitory effect of botanical extracts on the radial growth of *S. oryzae*

Treatment	Concentration	Radial growth (cm)	% Growth inhibition
T ₁	30 PPM	3.11	65.46 (54.01)
	60 PPM	1.68	81.39 (64.44)
	120 PPM	0.00	100.00 (90.00)
	240 PPM	0.00	100.00 (90.00)
T ₂	30 PPM	1.38	84.63 (66.92)
	60 PPM	1.12	87.59 (69.37)
	120 PPM	0.80	91.11 (72.65)
	240 PPM	0.48	94.72 (76.72)
T ₃	30 PPM	1.33	85.28 (67.44)
	60 PPM	0.89	90.09 (71.65)
	120 PPM	0.21	97.69 (81.25)
	240 PPM	0.23	97.41 (80.73)
T ₄	30 PPM	1.94	78.43 (62.32)
	60 PPM	1.58	82.41 (65.20)
	120 PPM	0.00	100.00 (90.00)
	240 PPM	0.00	100.00 (90.00)
T ₅	30 PPM	0.97	89.26 (70.87)
	60 PPM	0.54	93.98 (75.80)
	120 PPM	0.43	95.19 (77.33)
	240 PPM	0.34	96.20 (78.76)
T ₆	30 PPM	1.54	82.87 (65.55)
	60 PPM	0.75	91.67 (73.22)
	120 PPM	0.55	93.89 (75.69)
	240 PPM	0.00	100.00 (90.00)
T ₇	30 PPM	2.28	74.63 (59.76)
	60 PPM	3.13	65.19 (53.84)
	120 PPM	0.94	89.54 (71.13)
	240 PPM	0.00	100.00 (90.00)
T ₈	30 PPM	2.46	72.68 (58.49)
	60 PPM	0.00	100.00 (90.00)
	120 PPM	0.00	100.00 (90.00)
	240 PPM	0.00	100.00 (90.00)
T ₉	Control	9.00	
	Factor (A)	Factor (B)	Factor (A X B)
C.D.	3.754	2.654	7.508
SE(d)	1.875	1.326	3.749
SE(m)	1.326	0.937	2.651

() values in the parenthesis are transformed values, *mean of three replications

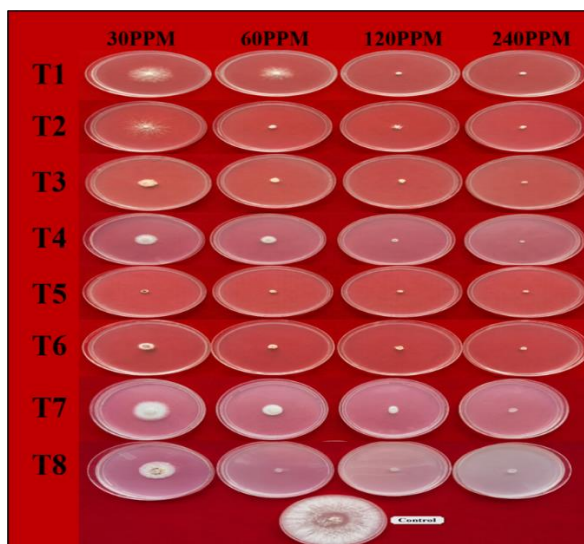


Fig 3: Effect of different concentrations of fungicide molecules on mycelial growth of *Sclerotium oryzae* under poisoned food technique

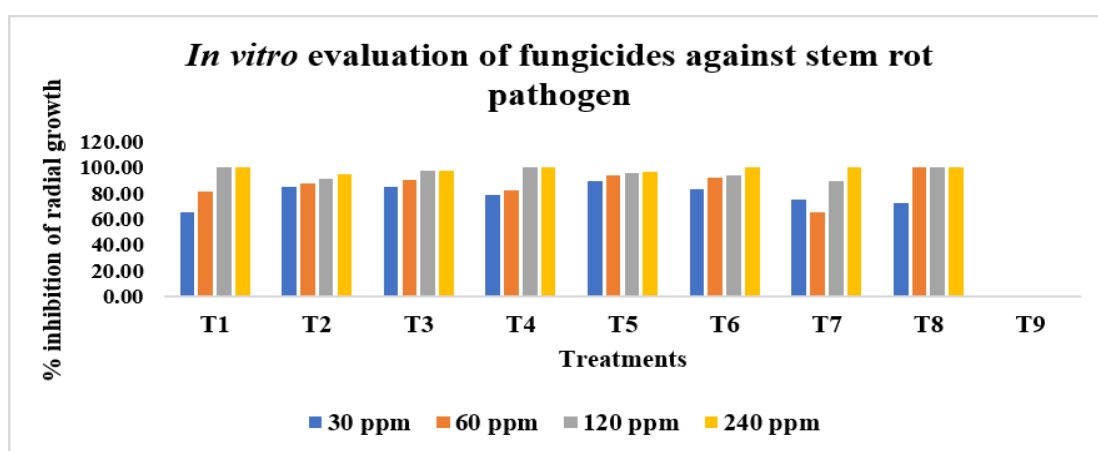


Fig 4: Effect of treatments of fungicides on radial growth of *Sclerotium oryzae* under *in vitro* conditions

4. Conclusions

This study showed that botanicals and fungicides were efficient in inhibiting the growth of pathogen. *Curcuma longa* showed the maximum suppressive effect against the pathogen, indicating its potential as an eco-friendly option for disease management. Mancozeb 50% + Carbendazim 25% WS, Trifloxystrobin 25% + Tebuconazole 50% WG and Metiram 55% + Pyraclostrobin 5% WG were highly effective. Combination of effective botanicals with suitable fungicides leads to development of a better and sustainable management strategy for rice stem rot.

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