

## ***In vitro* Efficacy of Essential Oils, Fungicides and Botanicals Against *Bipolaris oryzae* Causing Brown Spot of Rice**

**Basavaraj K\*, Prashanth Pandi, Jasudasu GS, Prakasam V, Ladhalakshmi D, Kannan C, Laha GS and Srinivas Prasad M**

ICAR- Indian Institute of Rice Research (IIRR), Hyderabad

\*Corresponding author E mail \*[basavaraj4229@gmail.com](mailto:basavaraj4229@gmail.com)

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### **Abstract**

*Bipolaris oryzae*, the causal agent of rice brown spot, is a major constraint to rice production, necessitating effective eco-friendly and chemical management options. A series of *in vitro* studies were conducted to evaluate the essential oils, fungicides and botanical extracts against *B. oryzae* using the poisoned food technique. Among essential oils, clove oil showed the highest inhibition (75.6%) at 500 ppm concentration, followed by lemon grass oil (64.9%). Among fungicides, azoxystrobin 23% SC was most effective, recording 67.3% mean inhibition and 88.9% inhibition at 500 ppm, while difenconazole 25% EC and propiconazole 25% EC showed 84.4% and 81.5% inhibition, respectively. Fifteen botanical extracts were tested at 1-3% concentrations, of which soap nut extract was most effective, causing 61-86% inhibition, followed by garlic, datura and ocimum whereas turmeric and ginger were ineffective up to 3% concentration. The study highlights clove and lemongrass oils, soapnut extract and effective fungicides (azoxystrobin, difenconazole and propiconazole) as promising options for managing rice brown spot.

**Keywords:** Rice brown spot, *Bipolaris oryzae*, essential oils, fungicides, *Oryza sativa* and *Oryza glabberima*

### **Introduction**

Rice (*Oryza sativa*- Asian rice; less commonly *Oryza glabberima* – African rice) second most important cereal crop and is staple food for over more than half of the world's human population. World's rice production is challenged by many biotic stresses. Brown spot of rice caused by *Bipolaris oryzae* (syn-*Helminthosporium oryzae*; teleomorph-*Cochliobolus miyabeanus*) is second important leaf spot disease after blast and causes substantial quantitative and qualitative losses in grain yield (Savary *et al.*, 2000). The disease of historic importance, responsible for two major epidemics in India; one in 1918-19 in Godavari delta areas and other being Great Bengal famine in 1943 leading to death of nearly 1.5 million people in present day areas belonging to Bengal in India and Bangladesh (Padmanabhan, 1973). In present times, brown spot occurs widely in all the rice-growing

tracts of India causing severe yield loss from 4 to 52% (Barnwal *et al.*, 2013). There are evidences that disease becoming more severe in direct seeded rice (Savary *et al.*, 2000) and in areas of water scarcity combined with soil nutritional deficiency (Barnwal *et al.*, 2013). The symptoms of brown spot include small, oval to spindle-shaped lesions on leaves. The lesions are typically brown in colour and surrounded by a yellowish halo (Sunder *et al.*, 2014). The disease occurs at different of crop growth stages; glume blotch phase results in poor seed germinability and seedling death while at booting stage affects the number of tillers, total photosynthetic leaf area and lowers the quality and weight of individual grains when pathogen reaches panicle (Sunder *et al.*, 2014).

At present, rice cultivars with an adequate level of resistance are not available in India, which is partly attributed to limitations in reliable and precise



screening protocols (Sunder *et al.*, 2014), as well as the specific nutritional and light requirements of *Bipolaris oryzae* that are essential for consistent and adequate sporulation (Basavaraj *et al.*, 2023). Although cultural and nutrient-management practices can reduce disease intensity, traditional management of rice brown spot has heavily relied on synthetic fungicides. In recent years, several new fungicide molecules have been identified to be effective against brown spot disease (Lore *et al.*, 2007; Poudel *et al.*, 2019; Baite *et al.*, 2025; Balgude *et al.*, 2017; Balgude and Gaikwad, 2016); while many of the fungicides reported earlier have become outdated. This situation necessitates in identifying the most effective molecules against the pathogen. In recent years, attention has been given towards alternative and sustainable approaches, including botanicals and essential oils derived from plant sources (Nikiema *et al.*, 2017; Akila and Mini, 2020). Extracts from medicinal and aromatic plants (*Lippia multiflora*) have shown significant *in vitro* antifungal activity against *B. oryzae* by inhibiting mycelial growth at low concentrations (Nikiema *et al.*, 2017). Plant essential oils have shown strong potential in disease management. *In vitro* studies demonstrated that essential oils of *Callistemon citrinus* and *Cymbopogon citratus* completely inhibited the mycelial growth of *Bipolaris oryzae*, while field experiments reported a 20–80% reduction in brown spot severity in rice (Nguefack *et al.*, 2013). Amaredra Kumar *et al.*, (2020) reported citronella oil and lemon grass oils were effective in reducing the brown spot severity under field conditions. Plant extracts from *Lawsonia inermis* and other botanicals have demonstrated strong inhibitory effects against *B. oryzae* in poisoned food and diffusion assays, offering environmentally benign alternatives to chemical fungicide (Akila and Mini, 2020). In this perspective, the present study has been conducted with objective to test the *in vitro* efficacy of selected fungicides, essential oils and botanicals against *B. oryzae*.

## Materials and Methods

*In vitro* experiments were conducted in the Department

of Plant Pathology, ICAR- Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad during 2020-2021.

### Isolation of the brown spot pathogen *Bipolaris oryzae*

The infected leaves showing typical brown leaf spot symptoms were collected from naturally infected rice fields of ICAR-IIRR farm. Small tissue segments were excised from the lesion margins, surface-sterilized in 0.1% sodium hypochlorite for 1 min, rinsed thrice with sterile distilled water, and blotted dry. The tissues were aseptically placed on Potato Dextrose Agar (PDA) supplemented with streptomycin (50 mg L<sup>-1</sup>) and incubated at 26 ± 1°C. Emerging fungal colonies were sub-cultured using the hyphal-tip method to obtain pure cultures. The pathogen was identified as *Bipolaris oryzae* based on cultural and microscopic characteristics, and cultures were maintained on PDA at 4°C for further studies.

### *In vitro* evaluation of essential oils against *Bipolaris oryzae*

The essential oils of Citronella (*Cymbopogon winterianus*), Eucalyptus (*Eucalyptus globules*), Cedar wood (*Cedrus atiantica*), Nirgundi (*Vitex negundo*), Lemon grass (*Cymbopogon citratus*), Clove (*Syzygium aromaticum*) and Neem (*Azadirachta indica*) obtained from different parts of the plants were collected from open market as 100 per cent pure and reliable commercial preparations. Different concentrations of the essential oil viz., 100, 200, 300, 400 and 500 ppm were prepared in ethylene glycol and tested for their efficacy against *B. oryzae* by following the standard protocol (Nene and Thapliyal, 2000). Different concentrations of essential oils were prepared by mixing in sterile Tween 20 (0.1% w/v) solution and added to a 60 ml lukewarm PDA in flasks to obtain the required concentrations. After solidification of the medium, about 8 mm diameter of 5-day old fungal culture (*B. oryzae*) was taken with the help of sterile cork borer and placed in the petridishes with PDA media with the help of sterile

inoculation needle and incubated at  $26 \pm 1^\circ\text{C}$ . After 10 days of incubation, when the growth of the fungus in the control plates fully covered, the radial growth of the fungus (cm) was measured in different treatments. The percentage inhibition of the radial growth of fungus was calculated using the formula  $I = ((C-T)/C) \times 100$  where C and T are the growth of fungal colony (cm) in the control and treated plates, respectively. The experiment was replicated three times, essential oil free PDA medium, containing only SDW and Tween 20 (0.5% v/v), was used as a control (Mounira *et al.*, 2011).

### ***In vitro* fungicides against *Bipolaris oryzae***

The efficacy of six fungicides *viz.*, hexconazole 5% EC, difenconazole 25% EC, prochloraz 45% EC, azoxystrobin 23% SC, propiconazole 25% EC and metiram 70% WG were tested at 10, 50, 100, 250 and 500 ppm concentrations against the *B. oryzae* using poison food technique. Required quantity of individual fungicide was added to cooled potato dextrose agar to get the desired concentration of the fungicide. Later, 20 ml of the poisoned medium was poured into Petri plates. Mycelial disc of 5 mm in diameter was placed at the centre of the poisoned agar plate. The medium without any fungicide served as control. Inoculated plates were incubated at  $26 \pm 1^\circ\text{C}$  and colony diameter was measured in different treatments after 10 days when mycelial growth covered the entire Petri plates in control. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth, which was calculated by using the formula  $I = ((C-T)/C) \times 100$  where C and T are the growth of fungal colony (cm) in the control and treated plates, respectively given by Vincent (1947).

### ***In vitro* evaluation of different plant extracts against *B. oryzae***

Fifteen plant extracts prepared from neem (*Azadiracta indica*), tulasi (*Ocimum sanctum*), aloe vera (*Aloe barbadensis*), soapnut (*Sapindus mukorossi*), datura (*Datura stramonium*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), garlic (*Allium*

*sativum*), marigold (*Tagetes erecta*), clove (*Syzygium aromaticum*), pepper (*Piper nigrum*), curry leaf (*Murraya koenigii*), lemon grass (*Cymbopogon citratus*), lemon (*Citrus limon*) and jamun (*Syzygium cumini*) were used in the study. Fresh and healthy plant parts were thoroughly washed and aseptically macerated in sterile distilled water in a 1:1 (w/v) ratio to obtain aqueous extracts. The homogenate was filtered through double-layered muslin cloth followed by membrane filtration using bacterial filter (0.45  $\mu\text{m}$ ) to obtain a clear filtrate, which was considered as the aqueous stock solution. The aqueous plant extracts were evaluated at three concentrations (1, 2 and 3%) using the poisoned food technique on potato dextrose agar. Mycelial discs (5 mm) from actively growing cultures of the pathogen were placed at the centre of the plates and incubated at  $25 \pm 1^\circ\text{C}$ . Radial mycelial growth was colony diameter was measured in different treatments when mycelial growth covered the entire Petri plates in control, and per cent inhibition of mycelial growth was calculated using the formula of Vincent (1947).

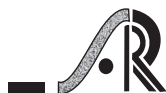
### **Statistical analysis**

Statistical package of program OPSTAT was used for analysis of variance (ANOVA) in analysing the *in vitro* data. To ensure homogeneity of variances and normality of the distribution of each variable, data in percentages were arcsine transformed.

## **Results and Discussion**

### ***In vitro* evaluation of essential oils**

Seven essential oils along with emulsifier (tween 20) were tested for their ability to inhibit the mycelial growth *in vitro* against *Bipolaris oryzae* at concentrations ranging from 100 to 500 ppm. It was found, all the essential oils tested were effective in inhibiting the growth of mycelium at higher concentration (500 ppm) but not at lower concentration. Citronella oil (*Cymbopogon winterianus*) recorded a progressive reduction in radial mycelial growth from 6.0 cm at 100 ppm to 3.4 cm at 500 ppm, corresponding to the highest mean inhibition of 44.5%. Clove



oil (*Syzygium aromaticum*) followed Lemongrass oil (*Cymbopogon citratus*) and Citronella oil (*Cymbopogon winterianus*) were highly effective at 500 ppm concentration showing mycelial inhibitions of 75.6%, 64.8% and 61.9% respectively (**Table 1 and Figure 1**). Eucalyptus oil (*Eucalyptus globulus*) and nirgundi oil (*Vitex negundo*) showed comparatively lower mycelial inhibition at 500 ppm (21.9 and 25.2% respectively). Overall, the inhibitory effect increased with concentration for all essential oils, with clove, citronella and lemongrass oils emerging as the most potent against *B. oryzae*. Essential oils are complex mixtures typically composed of terpenoids, phenolics

biosynthesized by plants as part of their defense and metabolic systems and known to have potential antifungal action against plant pathogens (Kaur *et al.*, 2025). Nikiama *et al.*, (2017) reported that, essential oil of *Lippia multiflora* at 0.01% concentration significantly reduced the growth of the *B. oryzae* to 62.64%, and the concentration of 0.1% completely inhibited its growth with 100% efficiency. *In vitro* studies demonstrated that essential oils of *Callistemon citrinus* and *Cymbopogon citratus* completely inhibited the mycelial growth of *Bipolaris oryzae*, while field experiments reported a 20-80% reduction in brown spot severity in rice (Nguefack *et al.*, 2013).

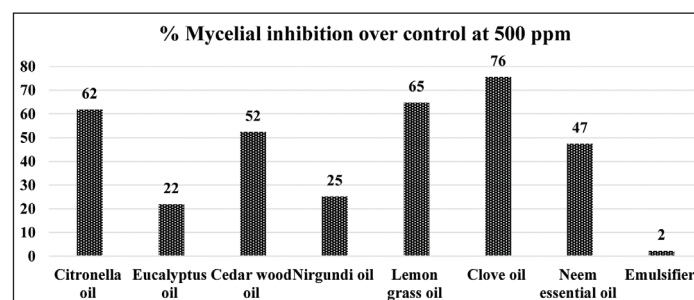
**Table 1: *In vitro* efficacy of different essential oils on the mycelial growth of *B. oryzae***

Essential oil	Radial growth of fungus (cm)						Per cent mycelial inhibition					
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	Mean	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	Mean
Citronella oil ( <i>Cymbopogon winterianus</i> )	6.0	5.9	5.2	4.4	3.4	5.0	33.0 (35.0)*	34.1 (35.7)	42.2 (40.5)	51.5 (45.8)	61.9 (51.9)	44.5 (41.9)
Eucalyptus oil ( <i>Eucalyptus globulus</i> )	8.7	8.1	7.3	7.3	7.0	7.7	3.7 (11.1)	10.4 (18.8)	19.3 (26.0)	19.3 (26.0)	21.9 (27.9)	14.9 (22.7)
Cedar wood oil ( <i>Cedrus atiantica</i> )	7.6	5.2	4.9	4.6	4.3	5.3	15.6 (23.2)	42.6 (40.7)	45.9 (42.7)	48.5 (44.2)	52.4 (46.4)	41.0 (39.8)
Nirgundi oil ( <i>Vitex negundo</i> )	7.9	7.4	7.5	7.3	6.7	7.4	12.6 (20.8)	17.4 (24.7)	17.0 (24.4)	18.9 (25.8)	25.2 (30.1)	18.2 (25.3)
Lemon grass oil ( <i>Cymbopogon citratus</i> )	8.3	6.9	6.2	4.4	3.2	5.8	7.8 (16.2)	23.0 (28.6)	31.5 (34.1)	50.7 (45.4)	64.8 (53.6)	35.6 (36.6)
Clove oil ( <i>Syzygium aromaticum</i> )	7.8	6.8	5.2	4.1	2.2	5.2	13.3 (21.4)	24.1 (29.4)	42.2 (40.5)	54.4 (47.5)	75.6 (60.4)	41.4 (40.4)
Neem essential oil ( <i>Azadirachta indica</i> )	6.5	5.9	5.6	5.1	4.7	5.6	28.1 (32.0)	34.4 (35.9)	37.8 (37.9)	43.3 (41.2)	47.4 (43.5)	38.2 (38.2)
Emulsifier (tween 20)	9.0	8.9	8.8	8.7	8.8	8.8	0.4 (3.5)	1.1 (6.1)	2.2 (8.6)	3.0 (9.9)	2.2 (8.6)	1.8 (7.7)
Control	9.0	9.0	9.0	9.0	9.0	9.0	-	-	-	-	-	
C.D.	0.26	0.30	0.26	0.31	0.35							
CV	1.82	2.47	2.27	2.92	3.73							

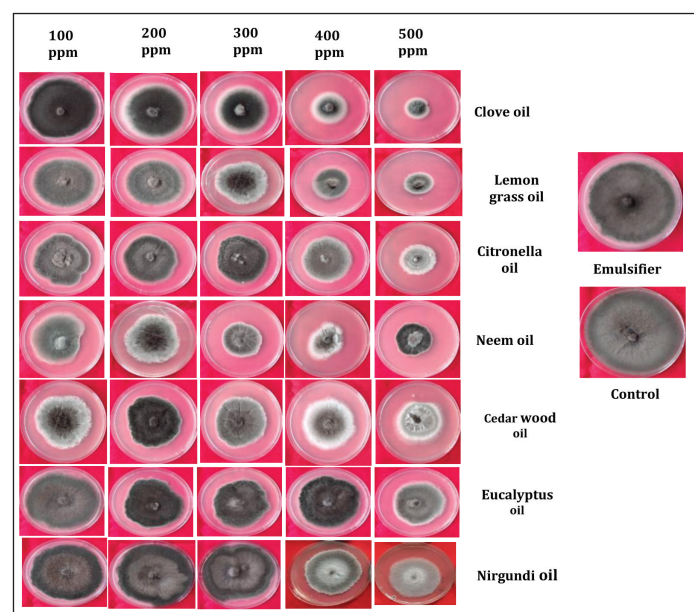
\*values in parenthesis are arcsine-transformed values



Amaredra Kumar *et al.* (2020) reported citronella oil and lemon grass oils were effective in reducing the brown spot severity under field conditions (**Figure 2**). Zerumbone, an isolated constituent from *Zingiber zerumbet* rhizomes has the antifungal potential against three major rice fungi: *Bipolaris oryzae*, *Fusarium moniliforme*, and *Rhizoctonia solani* (Kaur *et al.*, 2025).



**Figure 1: Effect of different essential oils on the percent mycelial growth inhibition at 500 ppm concentration**



**Figure 2: Effect of different essential oils on the inhibition of mycelial growth of *B. oryzae***

### ***In vitro* evaluation of fungicides**

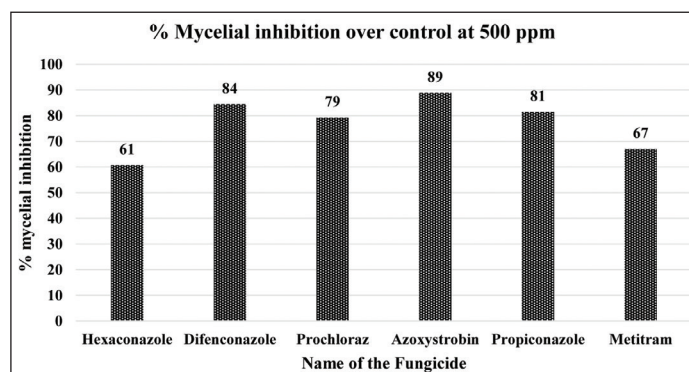
Six fungicides were tested *in vitro* against *B. oryzae* for their ability to inhibit the mycelial growth at five different concentrations (10 ppm, 50 ppm, 100 ppm, 250 ppm, and 500 ppm). Significant variation in inhibitory effects was observed across concentrations,

and all tested fungicides showed their effectiveness at the highest concentration (500 ppm). Azoxystrobin was found as most effective fungicide with lowest mean radial growth (2.9 cm) and the highest mean inhibition (67.3%), with inhibition reaching 88.9% at 500 ppm (**Table 2 and Figures 3 and 4**). This was followed by difenconazole, propiconazole and prochloraz which showed 84.4%, 81.5% and 79.3% inhibition respectively at 500 ppm concentration. The non-systemic fungicide metiram showed the least effectiveness at lower concentrations; however, its inhibitory activity increased substantially with increasing dose, reaching 67.0% inhibition at 500 ppm. In contrast, the untreated control recorded complete mycelial growth with a radial expansion of 9.0 cm. Overall, all fungicides exhibited a clear dose dependent suppression of fungal growth, with azoxystrobin, followed by difenconazole and propiconazole emerging as the most effective molecules against *B. oryzae* under *in vitro* conditions. The results of the present study are in accordance with observations of several earlier workers. In the past, several workers have tested the *in vitro* efficacy of different fungicides and reported some effective molecules against *B. oryzae*. Channakeshava and Pankaj (2018) reported that azoxystrobin could inhibit the mycelial growth up to 46% at 250 ppm concentration, however, in our study the same molecule showed maximum effectiveness (69.6%) at same concentration. The effectiveness of propiconazole in inhibiting the growth of *B. oryzae* aligns with earlier reports against *B. oryzae* (Karan *et al.*, 2021; Channakeshava and Pankaja, 2018; Baite *et al.*, 2025). Baite *et al.*, (2025) had identified tebuconazole, tricyclazole, and propiconazole as effective molecules in inhibition of *B. oryzae* growth under *in vitro* conditions. Gupta *et al.*, (2013) made similar observation of propiconazole as most effective with maximum inhibition of 97% at 250 ppm concentration. Propiconazole is a triazole group of fungicide inhibit the action of 14-  $\alpha$ - sterol demethylase which is a precursor of ergosterol.

**Table 2: *In vitro* efficacy of selected fungicides on the mycelial growth of *B. oryzae***

Sl No.	Fungicide	Radial growth of fungus (cm)						Per cent inhibition					
		10 ppm	50 ppm	100 ppm	250 ppm	500 ppm	Mean	10 ppm	50 ppm	100 ppm	250 ppm	500 ppm	Mean
1	Hexconazole 5% EC	6.6	6.5	6.3	5.1	3.5	5.6	26.3 (30.9)*	27.4 (31.6)	30.0 (33.2)	43.3 (41.2)	60.7 (51.2)	37.6 (37.8)
2	Difenconazole 25% EC	6.2	5.8	4.6	3.9	1.4	4.4	31.1 (33.9)	35.6 (36.6)	49.3 (44.6)	56.3 (48.6)	84.4 (66.8)	51.3 (45.8)
3	Prochloraz 45% EC	4.5	4.0	3.5	3.2	1.9	3.4	50.4 (45.2)	55.9 (48.4)	61.1 (51.4)	64.1 (53.2)	79.3 (62.9)	62.1 (52.0)
4	Azoxystrobin 23% SC	4.0	3.6	3.5	2.7	1.0	2.9	55.9 (48.4)	60.4 (51.0)	61.5 (51.6)	69.6 (56.6)	88.9 (70.5)	67.3 (55.1)
5	Propiconazole 25% EC	6.5	5.9	5.3	4.9	1.7	4.9	27.4 (31.6)	34.1 (35.7)	41.5 (40.1)	45.6 (42.5)	81.5 (64.5)	46.0 (42.7)
6	Metiram 70% WG	8.6	8.4	7.5	5.3	3.0	6.6	4.4 (12.2)	6.7 (15.0)	16.7 (24.1)	40.7 (39.7)	67.0 (55.0)	27.1 (31.4)
7	Control	9.0	9.0	9.0	9.0	9.0	9.0	-	-	-	-	-	-
	C.D.	0.33	0.24	0.37	0.37	0.15							
	CV	3.0	2.4	4.0	4.8	4.1							

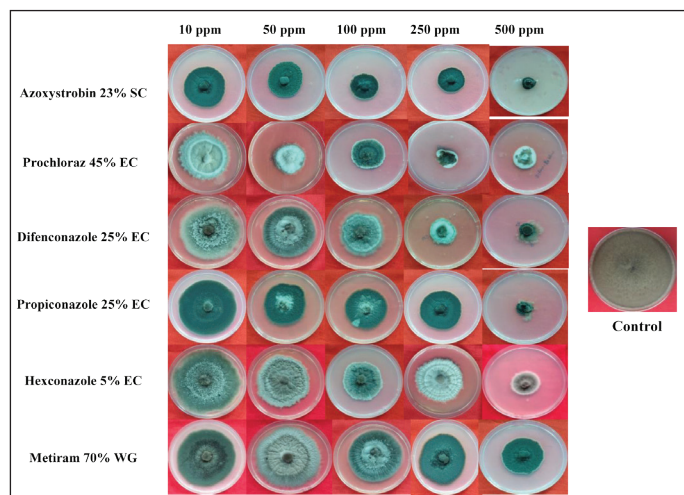
\*values in parenthesis are arcsine-transformed values



**Figure 3: Effect of fungicides on the inhibition of mycelial growth of *B. oryzae* at 500 ppm concentration**

### Effect of different plant extracts against *Bipolaris oryzae* *in vitro*

Antifungal activity of 15 plant extracts tested *in vitro* following poison food technique, showed considerable variation in antifungal activity across plant species and concentration levels. Soapnut (*Sapindus mukorossi*) exhibited the strongest inhibitory activity, recording the lowest mean mycelial growth (2.5 cm) and the highest mean inhibition (72.6%), with suppression increasing from 61.5% at 1% to 85.9% at 3%. Garlic (*Allium sativum*) also showed substantial antifungal potential, achieving a mean inhibition



**Figure 4: Effect of fungicides on the inhibition of mycelial growth of *B. oryzae***

of 56.0% and its highest inhibition of 64.4% at 3% concentration. Moderate inhibition was recorded with Ocimum (*Ocimum sanctum*) and datura (*Datura stramonium*), with mean inhibition values of 43.6% and 48.5%, respectively. Lemon grass (*Cymbopogon citratus*), marigold (*Tagetes erecta*), jamun (*Syzygium cumini*), pepper (*Piper nigrum*), and clove (*Syzygium aromaticum*) extracts displayed low to moderate activity. In contrast, neem, aloe vera, turmeric, ginger, and curry leaf extracts exhibited minimal or no

inhibition, with turmeric and ginger showing complete ineffectiveness at all concentrations (**Table 3 and Figures 5 and 6**). The untreated control maintained full radial growth (9.0 cm). Overall, soapnut and garlic emerged as the most effective plant-based inhibitors of *B. oryzae* under *in vitro* conditions. Several studies in the past have shown effectiveness

of plant based extracts in inhibition of mycelial growth of *B. oryzae*. The effectiveness of garlic in our study corroborate with the findings of Channakeshava and Pankaja (2018) wherein they reported, clove and garlic extracts as most effective plant extracts against *B. oryzae*. Plant extracts from *Lawsonia inermis* and other botanicals have demonstrated strong inhibitory

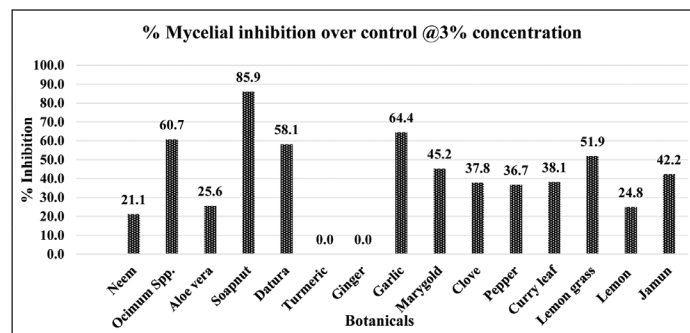
**Table 3: Effect of different plant extracts (botanicals) on the radial growth of *Bipolaris oryzae***

Sl. No	Botanical name	Plant part used	Radial growth of fungus (cm)				Per cent inhibition			
			1%	2%	3%	Mean	1%	2%	3%	Mean
1	Neem ( <i>Azadiracta indica</i> )	Leaf	9.0	8.1	7.1	8.1	0.0 (0.0)	9.6 (18.1)	21.1 (27.4)	10.2 (18.7)
2	Ocimum Spp. ( <i>Ocimum sanctum</i> )	Leaf	6.9	4.8	3.5	5.1	23.7 (29.1)*	46.3 (42.9)	60.7 (51.2)	43.6 (41.3)
3	Aloe vera ( <i>Aloe barbadensis</i> )	Leaf	9.0	8.7	6.7	8.1	0.0 (0.0)	3.7 (11.1)	25.6 (30.4)	9.8 (18.2)
4	Soapnut ( <i>Sapindus mukorossi</i> )	Nuts	3.5	2.7	1.3	2.5	61.5 (51.6)	70.4 (57.0)	85.9 (68.0)	72.6 (58.4)
5	Datura ( <i>Datura stramonium</i> )	Leaf	6.2	3.9	3.8	4.6	31.1 (33.9)	56.3 (48.6)	58.1 (49.7)	48.5 (44.2)
6	Turmeric ( <i>Curcuma longa</i> )	Powder	9.0	9.0	9.0	9.0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
7	Ginger ( <i>Zingiber officinale</i> )	Rhizome	9.0	9.0	9.0	9.0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
8	Garlic ( <i>Allium sativum</i> )	Bulb	4.5	4.2	3.2	4.0	50.0 (45.0)	53.7 (47.1)	64.4 (53.4)	56.0 (48.5)
9	Marigold ( <i>Tagetes erecta</i> )	Leaf	5.9	5.7	4.9	5.5	34.4 (35.9)	36.7 (37.3)	45.2 (42.2)	38.8 (38.5)
10	Clove ( <i>Syzygium aromaticum</i> )	Flower buds	9.0	6.7	5.6	7.1	0.0 (0.0)	25.2 (30.1)	37.8 (37.9)	21.0 (27.3)
11	Pepper ( <i>Piper nigrum</i> )	Pepper berries	8.0	7.1	5.7	6.9	11.5 (19.8)	21.1 (27.4)	36.7 (37.3)	23.1 (28.7)
12	Curry leaf ( <i>Murraya koenigii</i> )	Leaf	9.0	8.0	5.6	7.5	0.0 (0.0)	10.7 (19.1)	38.1 (38.1)	16.3 (23.8)
13	Lemon grass ( <i>Cymbopogon citratus</i> )	Leaf	6.2	5.2	4.3	5.3	30.7 (33.7)	42.2 (41.5)	51.9 (46.2)	41.6 (40.2)
14	Lemon ( <i>Citrus limon</i> )	Leaf	7.5	7.2	6.8	7.2	17.0 (24.4)	19.6 (26.3)	24.8 (29.9)	20.5 (26.9)
15	Jamun ( <i>Syzygium cumini</i> )	Leaf	6.5	5.9	5.2	5.9	27.4 (31.6)	34.8 (36.2)	42.2 (40.5)	34.8 (36.2)
16	Control		9.0	9.0	9.0	9.0				
			<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>					
	Factor(A-Botanicals)		0.151	0.076	0.054					
	Factor(B-Concentrations)		0.065	0.033	0.023					
	Factor(A X B)		0.262	0.132	0.093					

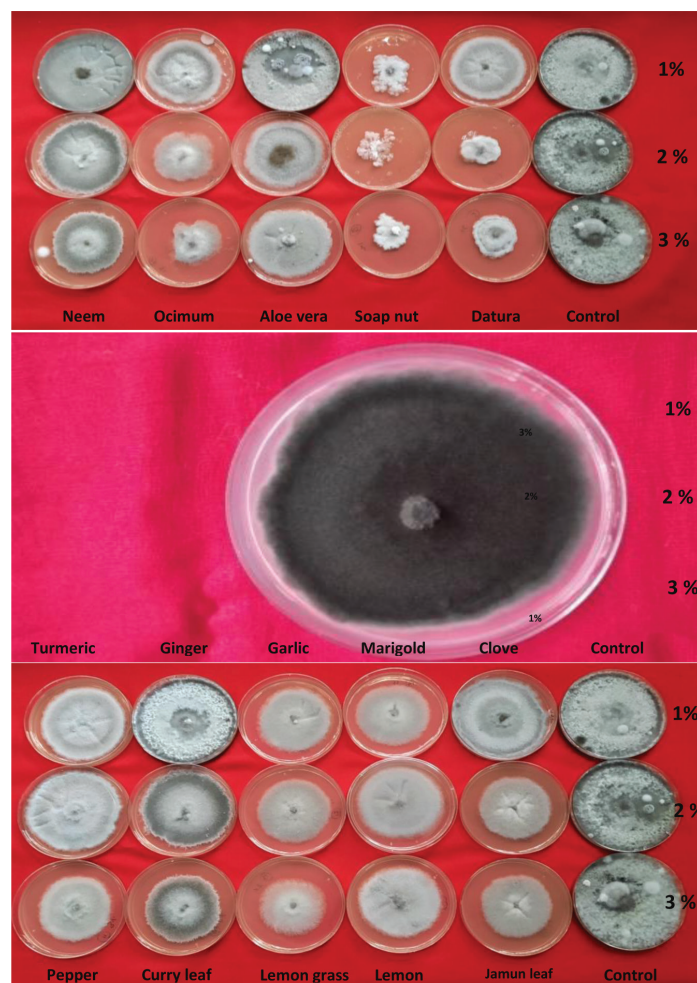
\*values in parenthesis are arcsine-transformed values



effects against *B. oryzae* (Akila and Mini, 2020). Bhat *et al.* (2024) demonstrated that extracts of several medicinal plants possess strong antifungal activity against *Bipolaris oryzae*, with methanolic extract of *Syzygium aromaticum* and *Inula racemose* showing mycelial inhibition 100% and 90 % respectively at 4000 ppm concentration under *in vitro* testing.



**Figure 5: Effect of different plant extracts (botanicals) on the radial growth of *Bipolaris oryzae* at 3% concentration**



**Figure 6: Effect of different plant extracts (botanicals) on the radial growth of *Bipolaris oryzae***

## Conclusion

The present study demonstrated distinct *in vitro* antifungal efficacy of essential oils, fungicides, and botanical extracts against *Bipolaris oryzae*. Clove oil, followed by lemongrass oil, showed the highest inhibitory activity among essential oils, highlighting their potential as eco-friendly disease management options. Among fungicides, azoxystrobin, difenoconazole, and propiconazole were highly effective, particularly at higher concentrations, confirming their strong activity against *B. oryzae*. Soapnut extract was the most effective botanical, while garlic, *Datura*, and *Ocimum* extracts also exhibited moderate inhibition. Overall, the findings identify promising chemical and non-chemical agents that may be integrated into brown spot management programs; however, field-level evaluation is necessary to validate their efficacy and consistency under natural conditions.

## References

- Akila R and Mini ML. 2020. Solvent extraction and antifungal assay of *Lawsonia inermis* Linn. against the brown spot fungus *Bipolaris oryzae*. *Journal of Pharmacognosy and Phytochemistry*, 9(1): 05-08.
- Baite, MS, Kumar, P, Rathinam, PKS, Shivappa, R, Umapathy, K and Dash, D. 2025. Evaluation of the efficacy of fungicides in controlling brown spot (*Bipolaris oryzae*) disease in rice. *Discover life*, 55(3):1-9. <https://doi.org/10.1007/s11084-025-09678-5>.
- Balgude, YS and Gaikwad, AP. 2016. Evaluation on the efficacy of modern fungicides against rice diseases. *Journal of Rice Research*, 9(1): 53-57.
- Balgude, YS, Gaikwad AP and Kshirsagar CR. 2017. *Pseudomonas fluorescens*, a potential bioagent for effective management of diseases in organic rice production. *Journal of Rice Research*, 10(2): 80-84.
- Basavaraj, K, Jasudasu, GS, Prakasam, V, Ladhakshmi, D, Kannan, C, Krishnaveni, D, Laha, GS, Ch Suvana Rani and Prasad, MS. 2023. Enhanced sporulation by different light



- and nutritional sources in *Bipolaris oryzae* causing brown spot disease of rice. *Journal of Environmental Biology* 44(3): 380–389.
- Barnwal MK, Kotasthane A, Magculia N, Mukherjee PK, Savary S, Sharma AK, Singh HB, Singh US, Sparks AH, Variar M and Zaidi N. 2013. A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. *European Journal of Plant Pathology*, 136: 443-457.
- Bhat S, Rather M, Gani S, Nabi A, Ganai SA, Shah MD, Sofi P, Jeelani F, Hussain A, Ashraf S, Anwar A, Iqbal I, Un Nisa T, Summuna B and Banday S. 2024. Identification of plant-based potential antifungal compounds against BMK-1 protein of *Bipolaris oryzae* using molecular docking approach. *Scientific Reports*, 14: 15665. <https://doi.org/10.1038/s41598-024-61431-6>.
- Channakeshava C and Pankaja NS. 2018. In vitro evaluation of fungicides, plant extracts and biocontrol agents against brown leaf spot of paddy. *International Journal of Current Microbiology and Applied Sciences*, 7(05): 127-132.
- Gupta V, Shamas N, Razad VK, Sharma BC, Sharma R, Kaur K, Singh I, John D and Kumar A. 2013. Foliar application of fungicides for the management of brown spot disease in rice (*Oryza sativa* L.) caused by *Bipolaris oryzae*. *African Journal of Agricultural Research*, 8(25): 3503-3309.
- Karan R, Renganathan P and Balabaskar P. 2021. In vitro evaluation of selected fungicides against *Bipolaris oryzae* causing brown leaf spot of rice. *Journal of Agricultural Science*, 12(5): 1583-1584.
- Kaur N, Kaur R, Bhardwaj U and Sharma P. 2025. Isolation and derivatization of bioactive compounds from *Zingiber zerumbet* essential oil with antifungal activity against rice pathogens. *Discover Plants*, 2: 249. <https://doi.org/10.21203/rs.3.rs-6116554/v1>
- Kumar A, Choudhary SK, Kumar U, Singh RN and Haque M. 2020. Eco-friendly management of brown spot disease of rice by the application of essential oils in Bihar. *Journal of Pharmacognosy and Phytochemistry*, 9(4): 285-289.
- Lore JS, Thind TS, Hunjum MS and Goel RK. 2007. Performance of different fungicides against multiple diseases of rice. *Indian Phytopathology*, 60(3): 296-301.
- Mounira S, Ibtissem S, Mouna K, Boujemaa K and Boughalleb N. 2011. Downy mildew of sunflower in Tunisia and evaluation of four fungicides. *Research in Plant Biology*, 1(5): 27-32.
- Nene YL and Thapliyal PN. 2000. *Fungicides in Plant Disease Control*. 3<sup>rd</sup> ed. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 531-533.
- Nguefack J, Nguikwie SK, Fotio D, Dongmo B, Leth V and Nkengfack AE. 2007. Fungicidal potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* to control *Alternaria padwickii* and *Bipolaris oryzae*. *Journal of Essential Oil Research*, 19(6): 581-587.
- Nikiema WF, Koita K, Zida EP and Kabore KB. 2017. In vitro management of *Bipolaris oryzae*, the causal pathogen of brown spot of rice, by plant extracts. *International Journal of Sciences*, 6(12): 42-47.
- Padmanabhan SY. 1973. The great Bengal famine. *Annual Review of Phytopathology*, 11( ): 11-26.
- Poudel NS, Bharatee P and Acharya M. 2019. Influence of different chemical fungicides against rice brown leaf spot disease caused by *Bipolaris oryzae*. *International Journal of Current Microbiology and Applied Sciences*, 8(01): 441-446.
- Savary S, Willocquet L, Elazegui FA, Castilla N and Teng PS. 2000. Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. *Plant Disease*, 84(3): 357-369.
- Sunder S, Singh R and Agarwal R. 2014. Brown spot of rice: an overview. *Indian Phytopathology*, 67(3): 201-215.
- Vincent JM. 1947. Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*, 150: 850.