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Efficacy of new fungicide molecules against *Colletotrichum siamense* causing arecanut leaf spot

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Abstract
Leaf spot disease is a major constraint to arecanut (*Areca catechu* L.) production in different growing regions of the country, resulting in considerable yield losses. Although the disease can be managed through cultural practices, chemical control remains an essential component as cultural methods alone are often inadequate. In this study, the fungitoxic effect of different fungicides against the leaf spot pathogen was evaluated under *in vitro* conditions. A total of 20 fungicides, comprising eight systemic, six contact, and six combi formulations, were assessed against *Colletotrichum siamense* using the poisoned food technique. All the fungicides tested significantly inhibited the radial mycelial growth of the pathogen compared to the untreated control. Among them, Captan 50% WP and Chlorothalonil 75% WP recorded high mean mycelial growth inhibition (93.21%) at the lowest concentrations tested. This was followed by Carbendazim 50% WP (93.70%), Propiconazole 25% EC (93.45%), Tebuconazole 25% EC (90.49%), and Tricyclazole 45% + Hexaconazole 10% WG (90.74%). These fungicides exhibited consistent performance across concentrations and were significantly superior to the control in suppressing the growth of *C. siamense*.

Keywords: Arecanut, Leafspot, Colletotrichum, fungicides, *in vitro*, inhibition

Introduction

Arecanut (*Areca catechu* L.) is a commercially important plantation crop of the family Arecaceae, widely grown in the tropical regions of South and Southeast Asia. In India, it plays a crucial role in supporting small and marginal farmers while contributing significantly to domestic trade and export earnings. The crop also holds social, cultural, and religious importance and has traditionally been used for oral hygiene and digestive purposes, with recent studies reporting antimicrobial activity against oral pathogens. India leads global arecanut production, with cultivation exceeding 1.01 million hectares and a production of about 1.44 million tonnes during 2024-25, of which Karnataka contributes the major share (ANON., 2025) [1]. However, productivity is severely affected by several diseases caused by fungal and bacterial pathogens, among which leaf spot disease has recently re-emerged as a major constraint in Karnataka. The disease causes necrotic lesions, premature leaf drying, and significant yield loss, and is now predominantly associated with *Colletotrichum siamense*. Since cultural practices alone are insufficient under favourable conditions, fungicidal management remains essential, with fungicides such as Captan, Chlorothalonil, Carbendazim, Propiconazole, Tebuconazole, and Tricyclazole + Hexaconazole showing effective inhibition of the pathogen.

Material and Methods

A total of 20 fungicides, comprising six contact, eight systemic and six combi products, were assessed for their efficacy against the leaf spot pathogen by following the poisoned food technique. The isolate *Colletotrichum siamense* (CsHn2) was utilized for *in vitro* studies based on its sporulation and virulence. The PDA medium was prepared and amended with the appropriate quantity of fungicides as per the concentration. Twenty ml of media amended with fungicides was added to sterile Petri plates and was inoculated with 9 mm mycelial disc of the pathogen. The control plates were maintained without fungicides. The inoculated plates were maintained at 27 °C in a BOD incubator.

The growth of the pathogen in fungicide amended plates were measured when the pathogen growth in control plates attained full growth. The radial growth and percent inhibition in each concentration was calculated as per Vincent (1947). Inhibition percent = [C-T/C] X100, Where, I = Percent inhibition, C = Growth of test fungus in control plate (mm) and T = Growth of the test fungus in treatment plate (mm).

Results and Discussion

The screening of the fungicides revealed that all the test fungicides significantly inhibited the radial mycelial growth of *Colletotrichum siamense* at various tested concentrations. Among the fungicides evaluated, carbendazim 50% WP, propiconazole 25% EC and tebuconazole 25% EC consistently recorded the highest mycelial growth inhibition across all concentrations (100-200 ppm), with inhibition exceeding 90 percent. Difenoconazole 25% EC and hexaconazole 75% WG showed moderate efficacy, while tricyclazole 75% WP and thiophanate methyl 70% WP were comparatively less effective. Azoxystrobin 23% SC exhibited the lowest inhibition at all concentrations, even at 200 pp. The superior performance of triazole fungicides is attributed to their inhibition of sterol biosynthesis, whereas carbendazim acts by disrupting mitosis through inhibition of β-tubulin assembly. These results are in agreement with Burgute and Magar (2019), who reported high mycelial growth inhibition of *Colletotrichum* fungi by carbendazim, hexaconazole and difenoconazole under *in vitro* conditions.

Among the contact fungicides, Captan 50% WP and chlorothalonil 75% WP consistently recorded the highest mycelial growth inhibition across concentrations (500-1000 ppm), with inhibition exceeding 93 percent. Propineb 70% WP and mancozeb 75% WP showed moderate efficacy, whereas zineb 75% WP was comparatively less effective. Copper oxychloride 50% WP recorded the lowest inhibition at all concentrations. The high efficacy of chlorothalonil is attributed to its multisite contact action, while captan disrupts thiol-containing enzymes, impairing fungal metabolism. These findings are in agreement with Anwar *et al.* (2017) [4].

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Among the combi-fungicides evaluated, tricyclazole 45% + hexaconazole 10% WG consistently recorded the highest mycelial growth inhibition (90.74-92.96%) across all concentrations. This was followed by azoxystrobin 11% + tebuconazole 18.3%, which showed high efficacy, particularly at higher concentrations. Tebuconazole 50% + trifloxystrobin 25% WG and azoxystrobin 18.2% + difenoconazole 11.4% SC exhibited moderate inhibition, whereas carbendazim 12% + mancozeb 63% WP and hexaconazole 4% + zineb 68% WP were comparatively less effective.The superior efficacy of azoxystrobin-based combinations is attributed to their QoI-mediated inhibition of fungal respiration at Complex III, while triazole-based combinations act as demethylation inhibitors. These results are in agreement with the findings of Mahesh *et al.* (2020) [5].

In vitro evaluation of fungicides provides effective and preliminary information about fungicide efficacy against pathogens in a short period, and thus serves as a model for field testing. In the present investigation, the poison food technique was used for testing fungicide efficacy. Eight systemic, six contacts and six combi fungicides were screened for their efficacy against *Colletotrichum siamense*.

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Table 1: Effect of systemic fungicides on the growth of *Colletotrichum siamense*

Sl. No.	Treatments (Fungicides)	Percent inhibition ppm			
		100 ppm (A)	**150 ppm (A)**	**200 ppm (A)**	**Mean (B)**
1	Propiconazole 25 % EC	93.45 (76.86)	94.07 (77.30)	91.48 (73.19)	93.00 (75.78)*
2	Difenoconazole 25 % EC	85.18 (67.29)	87.53 (69.53)	90.86 (72.37)	87.86 (69.73)
3	Hexaconazole 75 % WG	80.98 (64.21)	81.85 (65.00)	82.96 (65.93)	81.93 (65.05)
4	Tebuconazole 25 % EC	90.49 (72.27)	94.07 (77.30)	93.94 (77.17)	92.83 (75.58)
5	Tricyclazole 75 % WP	56.05 (48.37)	69.75 (57.97)	83.45 (68.00)	69.75 (58.11)
6	Thiophanate Methyl 70 % WP	47.53 (43.59)	78.39 (62.28)	82.46 (67.80)	69.46 (57.89)
7	Carbendazim 50 % WP	93.70 (77.02)	93.33 (76.78)	93.45 (76.86)	93.49 (76.89)
8	Azoxystrobin 23 % SC	44.93 (42.10)	49.63 (44.67)	56.17 (48.56)	50.24 (45.11)
	Mean (A)	74.04 (61.84)	81.07 (66.73)	84.35 (68.98)	
		S.Em. ±		C.D. (p = 0.01)	
	CD (B)	0.22		0.63	
	CD (A)	0.13		0.38	
	CD (BXA)	0.38		1.09	

*Figures in the parenthesis indicates the arcsine values

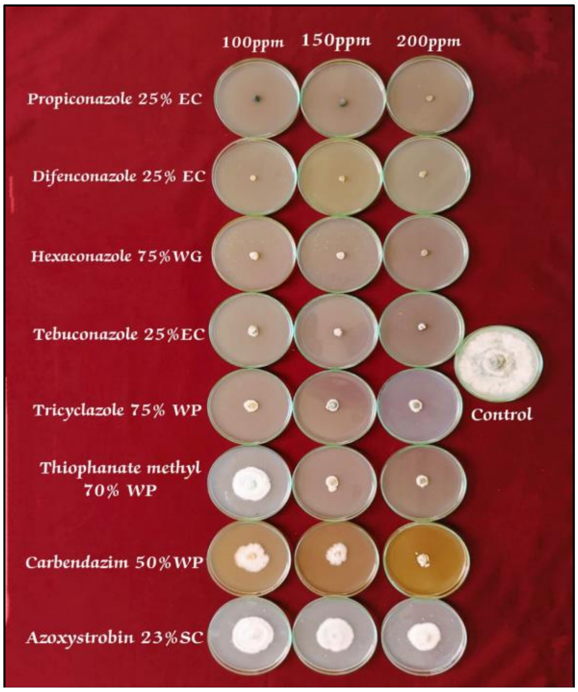

Plate 1: Screening of systemic fungicides under *in vitro* against *Colletotrichum siamense*

Table 2: Effect of contact fungicides on the growth of *Colletotrichum siamense*

Sl. No.	Treatments (Fungicides)	Percent inhibition			
		ppm			
		500 ppm (A)	750 ppm (A)	1000 ppm (A)	Mean (B)
1	Propineb 70 % WP	54.07 (46.67)	68.03 (56.01)	74.32 (60.10)	65.47 (54.26)*
2	Copper Oxychloride 50 % WP	32.71 (33.96)	44.19 (41.67)	54.94 (47.00)	43.95 (40.88)
3	Captan 50 % WP	93.21 (76.89)	93.82 (77.38)	93.33 (76.79)	93.45 (77.02)
4	Chlorothalonil 75 % WP	93.21 (76.89)	92.22 (75.83)	93.45 (77.02)	92.96 (76.58)
5	Zineb 75 % WP	45.19 (42.22)	59.75 (50.82)	93.45 (77.02)	66.13 (56.69)
6	Mancozeb 75 % WP	52.71 (46.61)	54.44 (47.45)	92.71 (76.28)	66.62 (56.78)
	Mean (A)	61.85 (53.54)	68.74 (59.21)	83.70 (69.70)	
		S.Em. ±		C.D. (p = 0.01)	
	CD (B)	0.28		0.806	
	CD (A)	0.198		0.57	

*Figures in the parenthesis indicates the arcsine values

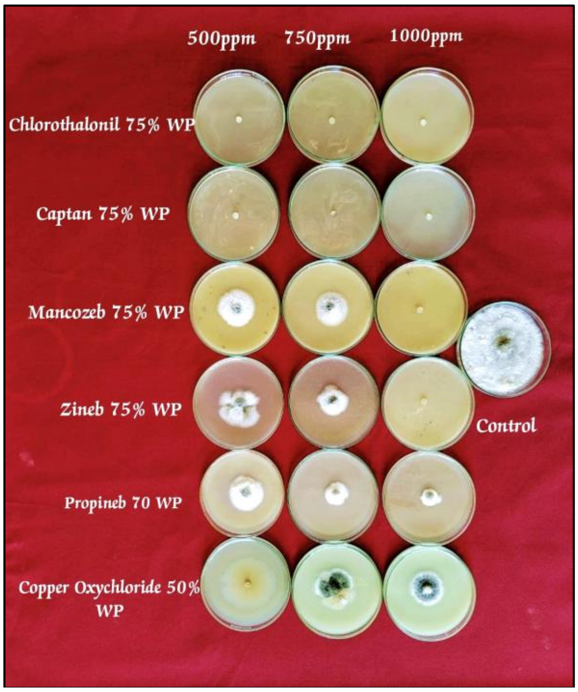

Plate 2: Screening of contact fungicides under *in vitro* against *Colletotrichum siamense*

Table 3: Effect of combi fungicides on the growth of *Colletotrichum siamense*

Sl. No.	Treatments (Fungicides)	Percent inhibition ppm			
		200 ppm (A)	300 ppm (A)	500 ppm (A)	Mean (B)
1	Tricyclazole 45 % + Hexaconazole 10 % WG	90.74 (72.49)	91.48 (73.19)	92.96 (74.56)	91.72 (73.41)*
2	Azoxystrobin 11 % + Tebuconazole 18.3 %	84.69 (67.03)	89.63 (71.25)	92.83 (74.45)	89.05 (70.91)
3	Tebuconazole 50 % + Trifloxystrobin 25 % WG.	81.97 (64.81)	85.92 (67.90)	89.63 (71.25)	85.84 (68.02)
4	Azoxystrobin 18.2% + Difenoconazole 11.4 % SC	81.35 (64.29)	83.20 (65.82)	84.69 (67.03)	83.08 (65.71)
5	Carbendazinm1 2 % |+Mancozeb 63 % WP	67.41 (55.19)	75.31 (60.34)	75.43 (60.43)	72.71 (58.65)
6	Hexaconazole 4 % + Zineb 68 % WP	62.83 (52.41)	65.55 (54.22)	69.63 (56.65)	66.00 (54.43)
	Mean (A)	78.16 (62.37)	81.85 (65.45)	84.19 (67.73)	
		S.Em. ±		C.D. (p = 0.01)	
	CD (B)	0.45		1.31	
	CD (A)	0.32		0.92	
	CD (BXA)	0.79		2.27	

*Figures in the parenthesis indicates the arcsine values

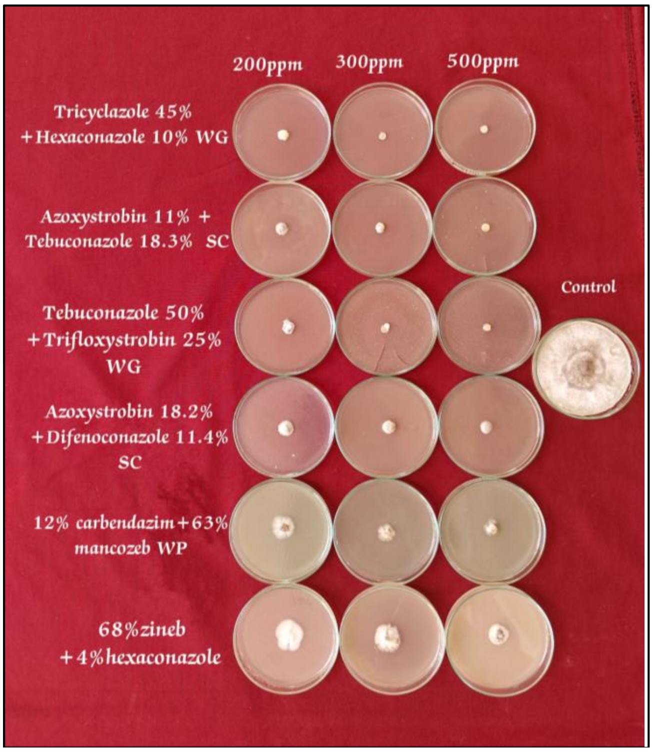

Plate 3: Screening of combi fungicides under *in vitro* against *Colletotrichum siamense*

Conclusion

All the tested fungicides significantly inhibited the mycelial growth of *Colletotrichum siamense* under *in vitro* conditions. The *in vitro* evaluation revealed that triazole fungicides such as tebuconazole and propiconazole were highly effective against *Colletotrichum siamense* due to their inhibition of sterol biosynthesis, while carbendazim effectively suppressed mycelial growth by disrupting mitosis through inhibition of β-tubulin assembly. Contact fungicides like chlorothalonil and captan also showed high efficacy owing to their multisite action and interference with thiol-containing enzymes, respectively. Combi-fungicides, particularly tricyclazole + hexaconazole and azoxystrobin + tebuconazole, exhibited superior inhibition due to the combined action of demethylation inhibition and QoI-mediated disruption of fungal respiration. The effectiveness of these fungicides and validating *in vitro* screening is a reliable preliminary tool for selecting fungicides for field evaluation in leaf spot management.

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