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# COMBATING ALTERNARIA BLIGHT OF CUMIN: *IN VITRO* EVALUATION OF NON-SYSTEMIC, SYSTEMIC AND READY-MIX FUNGICIDES FOR DISEASE MANAGEMENT

### H.A. Shekhada<sup>1\*</sup>, D.S. Kelaiya<sup>2</sup>, I.B. Kapadiya<sup>3</sup> and C.M. Bhaliya<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India 
<sup>2</sup>College of Agriculture, Junagadh Agricultural University, Mota Bhandariya (Amreli), Gujarat, India 
<sup>3</sup>Wheat Research Station, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India 
\*Corresponding author E-mail: shekhadahiren123@gmail.com

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### ABSTRACT

Alternaria blight, caused by Alternaria burnsii, poses a significant threat to cumin (Cuminum cyminum L.) cultivation, particularly in the arid and semi-arid regions of India. The development of effective disease management strategies is crucial due to the economic importance of the crop and the increasing resistance of pathogens to single-site fungicides. The present study aimed to evaluate the efficacy of nonsystemic, systemic and ready-mix fungicides against A. burnsii under in vitro conditions using the poisoned food technique. Six fungicides from each category were tested with respective four concentrations and mycelial growth inhibition was recorded ten days after inoculation. Among nonsystemic fungicides, copper oxychloride 50% WP showed the highest mean mycelial inhibition (93.07%). Systemic fungicides propiconazole 25% EC and tebuconazole 25.9% EC exhibited maximum inhibition (99.61%), remaining statistically at par across all concentrations. Among ready-mix formulations, propiconazole 13.9% + difenoconazole 13.9% EC demonstrated the most consistent and highest inhibition (99.61%). Interaction analysis revealed a significant concentration-dependent response, particularly in systemic and ready-mix treatments. The findings underscore the superior efficacy of triazole based fungicides and highlight the potential of ready-mix formulations for managing A. burnsii, offering valuable input for integrated disease management strategies in cumin cultivation. Keyword: Cumin, Alternaria blight, A. burnsii, Fungicides, Management

#### Introduction

India, often referred to as the "Home of Spices," is globally recognized for producing high-quality spices and holds a prominent position as one of the leading producers, consumers and exporters of seed spices. Among these, cumin (*Cuminum cyminum* L.), commonly known as 'Jeera' or 'Jiru', is a major spice crop of considerable economic importance. Cumin is a globally important aromatic spice crop valued for its distinctive flavor and aroma, primarily due to the presence of essential oils, antioxidants and various bioactive compounds. It holds significant culinary and medicinal relevance and is extensively cultivated in arid and semi-arid regions, particularly in India. Despite its economic and therapeutic importance,

cumin production is severely constrained by several phytopathological challenges. Among the major diseases affecting this crop, blight, wilt and powdery mildew are the most prevalent, with blight being the most destructive.

Alternaria blight, caused by *Alternaria burnsii* Uppal, Patel & Kamat (1938), is a widespread and recurrent disease across cumin-growing regions. The pathogen affects aerial parts of the plant, including leaves, stems and seeds, leading to rapid disease progression under conducive environmental conditions particularly high humidity and cloudy weather. Historical reports indicate yield losses of up to 80% due to blight (Gemawat and Prasad, 1972). The absence of resistant cultivars further exacerbates the

problem, as none of the currently cultivated cumin genotypes have demonstrated effective resistance against the pathogen. A. burnsii continues to pose a critical threat to cumin productivity, underscoring the urgent need for integrated disease management strategies and the development of resistant genotypes. A study by Wadud et al. (2021) reported blight incidence as high as 98%, with disease severity reaching up to 88%, leading to substantial yield losses. Various strategies have been explored for managing cumin blight, including the application of synthetic fungicides, botanical extracts and biological control agents (Jagani et al., 2023).

Although botanicals and microbial biocontrol agents are considered environmentally benign and sustainable, their inconsistent efficacy under field conditions limits their practical utility in managing aggressive pathogens like A. burnsii (Yadav et al., 2022). In contrast, fungicides continue to be integral to effective disease management practices. chemically synthesized compounds are specifically designed to inhibit fungal growth and development, making them indispensable in controlling severe fungal infections in cumin (Kakraliya et al., 2021). While several non-systemic, systemic and ready-mix fungicides have demonstrated in vitro efficacy against Alternaria burnsii, the emergence of newer chemistries necessitates comparative evaluation of their effectiveness. Assessing the relative in vitro efficacy of both established and novel fungicidal formulations is for developing robust, evidence-based management strategies for cumin blight.

### **Materials and Methods**

The inhibitory effect of different fungicides, including six non-systemic fungicides (Table 1), systemic fungicides (Table 2) and ready-mix fungicides (Table 3), along with four concentrations, was evaluated against the *A. burnsii* under *in vitro* conditions using the poisoned food technique as originally described by Nene and Thapliyal (1979). The basal medium used throughout the study was

PDA. The experiment was conducted during the Rabi season of 2022-23 at the PG Laboratory, Department of Plant Pathology, Junagadh Agricultural University, Junagadh, Gujarat. The required quantity of each fungicide was added to a conical flask containing 100 mL of melted PDA medium to achieve the required concentration. The flask containing the poisoned medium was shaken well to facilitate a uniform mixture of the fungicides and 20 mL of the medium was poured into sterilized petri plates. Upon solidification of the medium, the plate was inoculated at the center by placing a 5 mm diameter mycelial disc of a seven-day-old culture of the A. burnsii using a sterilized cork borer. Three repetitions were maintained for each concentration of the respective fungicides and the plates were incubated at  $25 \pm 1$  °C. A control was also maintained where the medium was not supplemented with any fungicides. The experiment was laid out in a Factorial Completely Randomized Design (FCRD) with six treatments and three repetitions for each concentration of the respective fungicides.

Quantification of the inhibitory effect of different fungicides on the growth of *A. burnsii* was carried out by determining the percentage of mycelial growth inhibition at all concentrations. The colony diameter was measured at the point of maximum growth of the pathogen on the control plate. The recorded observations were then used to calculate the percentage of pathogen growth inhibition using the formula by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent growth inhibition

C = Average diameter of mycelial colony in control plate (mm)

T = Average diameter of mycelial colony in treated plate (mm)

Table 1: Details of non-systemic fungicides evaluated against A. burnsii under in vitro condition

Tr. No.	Non-systemic fungicides	Concentration (ppm)				
	Non-systemic fungicides	1	2	3	4	
$T_1$	Mancozeb 75% WP	1000	1500	2000	2500	
$T_2$	Propineb 70% WP	1000	1500	2000	2500	
$T_3$	Copper oxychloride 50% WP	1000	1500	2000	2500	
$T_4$	Thiram 75% WP	1000	1500	2000	2500	
$T_5$	Zineb 75% WP	1000	1500	2000	2500	
$T_6$	Wettable sulphur 80% WP	1000	1500	2000	2500	

|--|

Tr. No.	Systemic fungicides	Concentration (ppm)				
11.10.	Systemic rungicides	1	2	3	4	
$T_1$	Carbendazim 50% WP	100	250	500	1000	
$T_2$	Azoxystrobin 23% SC	100	250	500	1000	
$T_3$	Propiconazole 25% EC	100	250	500	1000	
$T_4$	Picoxystrobin 22.52% SC	100	250	500	1000	
$T_5$	Tebuconazole 25.9% EC	100	250	500	1000	
$T_6$	Thiophanate methyl 70% WP	100	250	500	1000	

**Table 3:** Details of ready-mix fungicides evaluated against *A. burnsii* under *in vitro* condition

Tr. No.	Ready-mix fungicides	Concentration (ppm)				
11.140.	Ready-mix rungicides	1	2	3	4	
$T_1$	Captan 70% + Hexaconazole 5% WP		250	500	1000	
$T_2$	Pyraclostrobin 133 g/l + Epoxiconazole 50 g/l SE		250	500	1000	
$T_3$	Carbendazim 12% + Mancozeb 63% WP		250	500	1000	
$T_4$	Propiconazole 13.9% + Difenoconazole 13.9% EC		250	500	1000	
$T_5$	Metriram 55% + Pyraclostrobin 5% WG		250	500	1000	
$T_6$	Chlorothalonil 40.0% + Difenoconazole 4.0% SC	100	250	500	1000	

#### **Results and discussion**

## Evaluation of non-systemic fungicides against A. burnsii under in vitro condition

A comprehensive *in vitro* evaluation was conducted to determine the efficacy of six non-systemic fungicides against *Alternaria burnsii*, employing the poisoned food technique across four concentrations (1000, 1500, 2000 and 2500 ppm). Observations on mycelial growth inhibition were recorded ten days post-inoculation and the results were statistically analyzed to discern treatment effects (Table 4, Fig. 1 and Plate 1).

The findings revealed a highly significant inhibitory effect of all fungicides over the untreated control. Among the treatments, copper oxychloride 50% WP emerged as the most potent fungicide, registering the highest mean mycelial inhibition of 93.07%. This was closely followed by mancozeb 75% WP with 87.26%, and propineb 70% WP, which exhibited a moderate efficacy of 80.14%. Thiram 75% WP also showed considerable suppression of fungal growth (74.44%), whereas zineb 75% WP (48.61%) and wettable sulphur 80% WP (39.07%) were markedly less effective.

A clear dose-dependent response was observed across all treatments, with higher concentrations significantly enhancing fungicidal activity. The maximum inhibition (99.61%) was achieved by mancozeb 75% WP at 2500 ppm, while the lowest inhibition (20.54%) was noted for wettable sulphur 80% WP at 1000 ppm.

The interaction effect between fungicides and concentrations (F × C) demonstrated a highly significant enhancement in mycelial growth inhibition at higher concentrations, particularly in the case of copper oxychloride 50% WP and mancozeb 75% WP, underscoring their robust fungicidal efficacy. The superior performance of copper oxychloride can be attributed to its high copper content, which interferes with fungal metabolism by disrupting critical enzymatic activities. Conversely, mancozeb, a broadspectrum dithiocarbamate fungicide, exerts its action by inhibiting multiple enzyme systems within the fungal cell, making it highly effective against a wide range of phytopathogens. Despite the strong performance of both fungicides, copper oxychloride was found to be statistically superior across all concentrations tested.

These findings are in close agreement with the results reported by Shekhada et al. (2023), who evaluated four non-systemic fungicides using the poisoned food technique at 1000 and 2000 ppm. Their study revealed that copper oxychloride 50% WP exhibited the highest mycelial inhibition (72.96% at 1000 ppm and 80.37% at 2000 ppm), significantly outperforming other treatments. Likewise, Sudani (2023) assessed the efficacy of non-systemic fungicides at varying concentrations and identified copper oxychloride 50% WP as the most effective, recording a mean inhibition of 83.27%, thus reinforcing its consistent antifungal activity. Mancozeb 75% WP also ranked second in effectiveness in both referenced studies, aligning closely with the current investigation's outcomes.

Tr.	Non-systemic fungicides		Mean			
No.		1000 ppm#	1500 ppm#	2000 ppm#	2500 ppm#	inhibition
$T_1$	Mancozeb 75% WP	50.34	75.27	79.58	86.44	72.91
11	Wallcozeo 75% WF	(59.26)*	(93.54)	(96.73)	(99.61)	(87.26)
т	Broningh 70% WD	54.63	57.72	69.88	76.38	64.65
$T_2$	Propineb 70% WP	(66.49)	(71.48)	(88.17)	(94.45)	(80.14)
$T_3$	Copper oxychloride 50% WP	67.97	73.23	78.38	83.93	75.88
13	Copper oxychionde 50% WP	(85.93)	(91.67)	(95.94)	(98.88)	(93.07)
$T_4$	Thiram 75% WP	53.95	59.39	61.88	63.84	59.77
14	Thiram 75% WP	(65.38)	(74.08)	(77.78)	(80.56)	(74.44)
т	Zineb 75% WP	38.91	44.04	45.96	47.80	44.19
$T_5$		(39.44)	(48.33)	(51.67)	(55.00)	(48.61)
т	Wettable sulphur 80% WP	26.95	33.67	40.85	52.08	38.39
$T_6$	wettable sulphul 80% wr	(20.54)	(30.74)	(42.78)	(62.22)	(39.07)
Mean		48.79	57.22	62.75	68.42	
		(56.60)	(70.69)	(79.04)	(86.47)	-
		Fungicide (F)		Concentration (C)		$\mathbf{F} \times \mathbf{C}$
S.Em. ±		0.26		0.21		0.53
C.D. at 5%		0.7	0.75		61	1.50
C.V.%		1.54				•

Table 4: Evaluation of non-systemic fungicides against A. burnsii under in vitro condition

#Mean of three repetitions

<sup>\*</sup>Data outside the parentheses are arcsine transformed, whereas inside are retransformed values

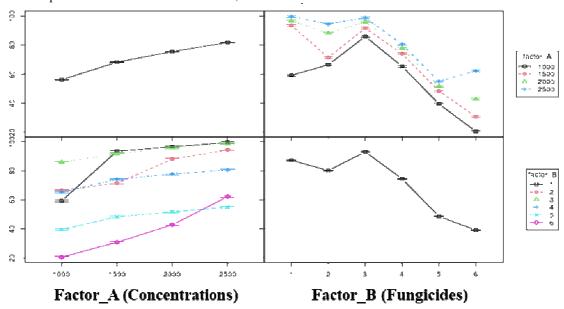


Fig. 1: Comparative analysis of non-systemic fungicides against A. burnsii in vitro

# Evaluation of systemic fungicides against A. burnsii under in vitro condition

An *in vitro* evaluation of six systemic fungicides was conducted against *A. burnsii* using the poisoned food technique. The fungicides were tested at four concentrations (100, 250, 500 and 1000 ppm), along with an untreated control, and the results are presented in Table 5, Figure 2 and Plate 2. Significant variation was observed in mycelial growth inhibition among the treatments and the interaction effect between

fungicides and concentrations ( $F \times C$ ) played a pivotal role in determining their efficacy.

Among the systemic fungicides tested, propiconazole 25% EC and tebuconazole 25.9% EC consistently exhibited the highest inhibition of mycelial growth, reaching 99.61% across all concentrations. Both fungicides were statistically at par with each other, underscoring their potent antifungal activity. Picoxystrobin 22.52% SC and azoxystrobin 23% SC showed moderate efficacy, with mean

inhibitions of 47.41% and 40.00%, respectively; however, picoxystrobin was significantly more effective than azoxystrobin. Thiophanate methyl 70%

WP demonstrated limited efficacy (34.35%), while carbendazim 50% WP was the least effective, recording a mean inhibition of only 17.36%.

Table 5: Evaluation of systemic fungicides against A. burnsii under in vitro condition

Tr.	Systemic fungicides		Mean inhibition			
No.		100 ppm#	250 ppm#	500 ppm#	1000 ppm#	Mean initibilion
$T_1$	Carbendazim 50% WP	8.38	15.78	24.79	40.52	22.37
		(2.12)*	(7.39)	(17.58)	(42.22)	(17.36)
$T_2$	Azoxystrobin 23% SC	29.75	37.04	43.62	45.85	39.07
12	Azoxystroom 25% SC	(24.63)	(36.29)	(47.59)	(51.48)	(40.00)
$T_3$	Propiognazola 25% EC	86.44	86.44	86.44	86.44	86.44
13	Propiconazole 25% EC	(99.61)	(99.61)	(99.61)	(99.61)	(99.61)
$T_4$	Picoxystrobin 22.52% SC	35.71	38.36	45.85	54.07	43.50
14		(34.07)	(38.52)	(51.48)	(65.56)	(47.41)
$T_5$	Tebuconazole 25.9% EC	86.44	86.44	86.44	86.44	86.44
15		(99.61)	(99.61)	(99.61)	(99.61)	(99.61)
$T_6$	Thiophanate methyl 70% WP	28.25	33.21	39.01	42.34	35.70
16		(22.40)	(30.00)	(39.63)	(45.37)	(34.35)
Mean		45.83	49.54	54.36	59.28	
		(51.45)	(57.90)	(66.05)	(73.90)	-
		Fungicide (F)		Concentration (C)		$\mathbf{F} \times \mathbf{C}$
S.Em. ±		0.21		0.18		0.43
C.D. at 5%		0.61 0.50		.50	1.22	
C.V.%		1.42				

#Mean of three repetitions

The interaction analysis reinforced the superior performance of propiconazole and tebuconazole, which maintained maximum inhibition across all tested concentrations. These triazole fungicides function as demethylation inhibitors (DMIs), disrupting ergosterol biosynthesis an essential component of fungal cell membranes and thereby impeding fungal growth. Their consistent effectiveness across concentrations highlights their suitability for managing *A. burnsii* under controlled conditions.

The present findings are strongly supported by earlier studies. Thaware *et al.* (2010) reported complete

inhibition of *A. alternata* causing cowpea leaf blight with propiconazole (0.05%). Similarly, Pamrao (2017) documented 100% inhibition of *A. alternata* infecting mungbean at 0.1% propiconazole. Rani *et al.* (2018) also confirmed complete inhibition of *Alternaria* spp. at 0.1-0.3% concentrations. In alignment with the current study, Shekhada *et al.* (2023) observed total inhibition of *A. burnsii* by propiconazole 25% EC at 500 ppm, while Sudani (2023) reported similar efficacy for propiconazole (94.47%) and hexaconazole (94.36%), further validating the superior performance of triazole fungicides against *A. burnsii*.

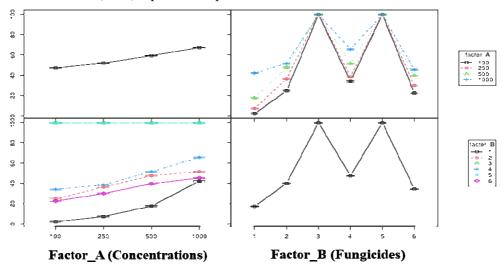


Fig. 2: Comparative analysis of systemic fungicides against A. burnsii in vitro

<sup>\*</sup>Data outside the parentheses are arcsine transformed, whereas inside are retransformed values

## Evaluation of ready-mix fungicides against A. burnsii under in vitro condition

The emergence of fungicide resistance in several phytopathogens, particularly to single site mode of action fungicides, has necessitated the development and evaluation of novel formulations. Ready-mix fungicides combining two active ingredients with different modes of action offer a promising alternative, enhancing efficacy through synergistic interactions and reducing the risk of resistance development. In this context, the present study aimed to assess the efficacy of six ready-mix fungicides against *A. burnsii*, the

causal agent of Alternaria blight in cumin, under in vitro conditions.

Fungicidal efficacy was evaluated against A. burnsii using the poisoned food technique at concentrations of 100, 250, 500 and 1000 ppm. The mycelial growth inhibition (%) was recorded ten days after inoculation. Data were statistically analyzed at a 5% significance level, with the interaction between fungicides and concentrations (F  $\times$  C) further confirming treatment differences (Table 6, Fig. 3 and Plate 3).

**Table 6:** Evaluation of ready-mix fungicides against A. burnsii under in vitro condition

Tr.	Ready-mix fungicides		Mean			
No.	Ready-Illix Tuligicides	100 ppm#	250 ppm#	500 ppm#	1000 ppm#	inhibition
$T_1$	Captan 70% + Hexaconazole 5%	78.93	86.44	86.44	86.44	84.56
	WP	(96.31)*	(99.61)	(99.61)	(99.61)	(98.78)
$T_2$	Pyraclostrobin 133 g/l +	75.49	86.44	86.44	86.44	83.70
	Epoxiconazole 50 g/l SE	(93.72)	(99.61)	(99.61)	(99.61)	(98.14)
$T_3$	Carbendazim 12% + Mancozeb	50.88	56.34	69.55	86.44	65.80
	63% WP	(60.19)	(69.28)	(87.79)	(99.61)	(79.21)
$T_4$	Propiconazole 13.9% +	86.44	86.44	86.44	86.44	86.44
	Difenoconazole 13.9% EC	(99.61)	(99.61)	(99.61)	(99.61)	(99.61)
$T_5$	Metriram 55% + Pyraclostrobin	60.75	67.82	72.66	74.64	68.97
	5% WG	(76.12)	(85.75)	(91.12)	(92.98)	(86.48)
$T_6$	Chlorothalonil 40.0% +	48.30	54.62	63.05	73.33	59.82
	Difenoconazole 4.0% SC	(55.74)	(66.48)	(79.46)	(91.77)	(73.36)
	Maria		73.02	77.43	82.29	
Mean		(84.48)	(91.47)	(95.26)	(98.20)	-
		Fungicide (F)		Concen	F × C	
S.Em. ±		0.21		(	0.41	
C.D. at 5%		0.59		0.48		1.18
	C.V.%	0.96				

<sup>#</sup>Mean of three repetitions

Among the tested fungicides, propiconazole 13.9% + difenoconazole 13.9% EC exhibited the most consistent and highest inhibition (99.61%) across all concentrations. This treatment (T<sub>4</sub>) was significantly superior to all other fungicides at every concentration level, maintaining its effectiveness even at the lowest dose of 100 ppm. It was statistically at par with captan 70% + hexaconazole 5% WP (98.78%) and pyraclostrobin 133 g/L + epoxiconazole 50 g/L SE (98.14%), the latter two also showing excellent inhibition, particularly at higher concentrations. These three treatments formed the most effective group, with statistically significant differences from the remaining fungicides.

Metiram 55% + pyraclostrobin 5% WG followed with a mean inhibition of 86.48%, indicating good efficacy. Carbendazim 12% + mancozeb 63% WP

demonstrated moderate effectiveness, with inhibition values increasing progressively from 60.19% at 100 ppm to 99.61% at 1000 ppm, suggesting a clear concentration dependent response. The least effective fungicide was chlorothalonil 40% + difenoconazole 4% SC, with a mean inhibition of 73.36%, significantly lower than all other treatments.

The interaction analysis (F  $\times$  C) revealed that increasing concentrations consistently enhanced mycelial inhibition. Treatment  $T_4$  maintained superior performance across all concentration levels.  $T_1$  and  $T_2$  also showed high efficacy, especially at higher doses, while  $T_3$  and  $T_5$  exhibited concentration-dependent improvement.  $T_6$  remained the least effective at all tested concentrations.

<sup>\*</sup>Data outside the parentheses are arcsine transformed, whereas inside are retransformed values

These findings are consistent with earlier research. Adhikari (2023) reported strong antifungal activity of propiconazole 13.9% + difenoconazole 13.9% EC against Alternaria spp., while Harsoda (2021) found complete inhibition of *A. alternata* with propiconazole 25% EC at 100-500 ppm, followed by

high efficacy of difenoconazole 25% EC (84.25%). The present study further validates the potent inhibitory effect of propiconazole and difenoconazole combinations, emphasizing their suitability for integrated management of *A. burnsii*.

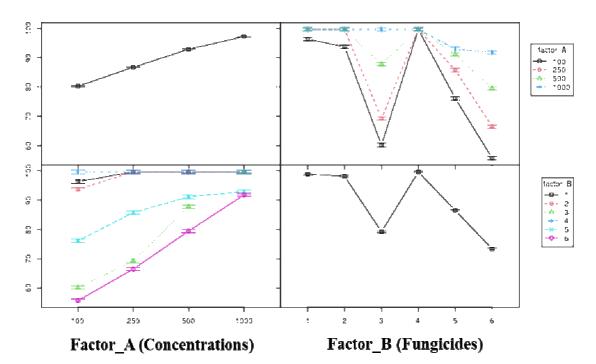


Fig. 3: Comparative analysis of ready-mix fungicides against A. burnsii in vitro

### Conclusion

The present in vitro investigation demonstrated significant differences in the efficacy of various fungicides against Alternaria burnsii, the causal agent of Alternaria blight in cumin. Among the non-systemic fungicides, copper oxychloride 50% WP emerged as the most effective, with 93.07% mycelial growth inhibition. In the systemic category, propiconazole 25% EC and tebuconazole 25.9% EC exhibited the highest inhibition (99.61%), highlighting their potent fungicidal properties as demethylation inhibitors (DMIs). Notably, the ready-mix formulation propiconazole 13.9% + difenoconazole 13.9% EC also achieved 99.61% inhibition, demonstrating consistent and superior performance across all tested concentrations.

These findings emphasize the potential of triazole based fungicides both solo and in combination for effective management of *A. burnsii*. The enhanced efficacy of ready-mix formulations further suggests a

promising approach for resistance management and improved disease control strategies. The study provides a scientific basis for selecting fungicides in integrated disease management programs aimed at mitigating Alternaria blight in cumin cultivation.

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### **Conflict of interest**

The authors declare that they have no conflict of interest.

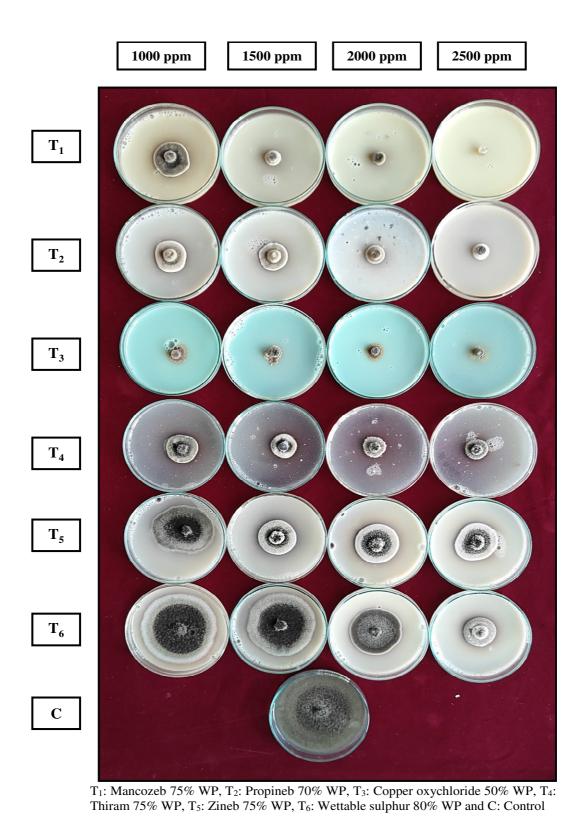
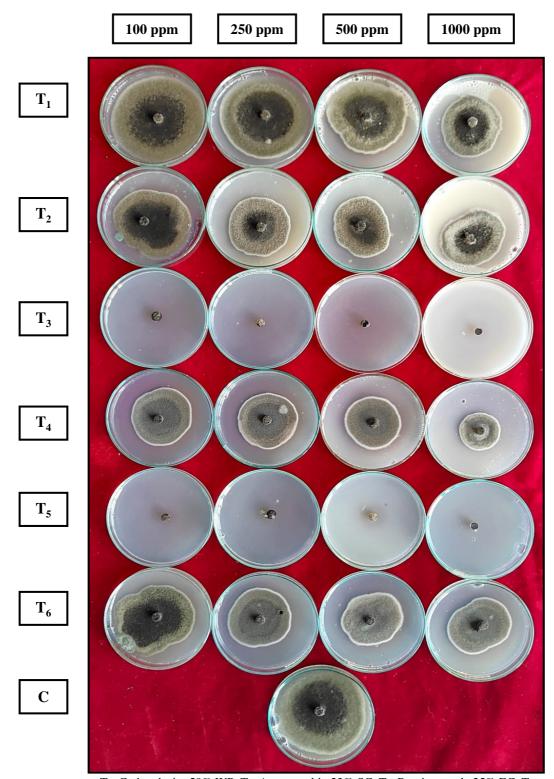
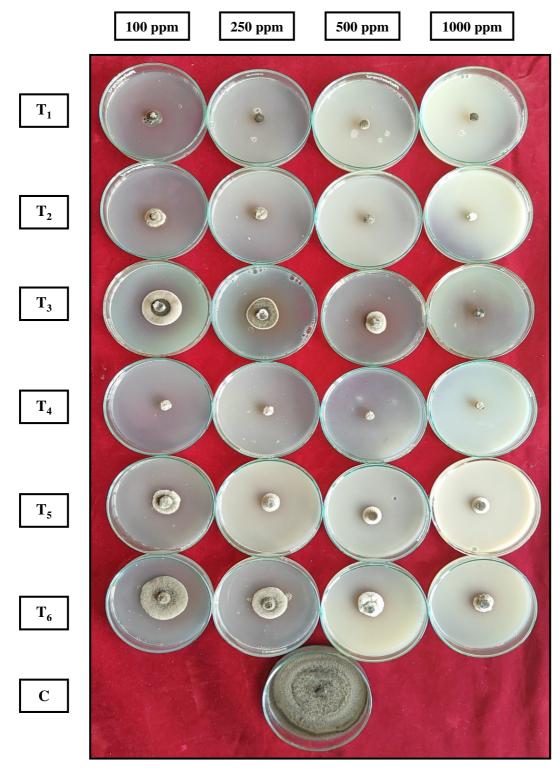


Plate 1: Evaluation of non-systemic fungicides against A. burnsii under in vitro condition



T<sub>1</sub>: Carbendazim 50% WP, T<sub>2</sub>: Azoxystrobin 23% SC, T<sub>3</sub>: Propiconazole 25% EC, T<sub>4</sub>: Picoxystrobin 22.52% SC, T<sub>5</sub>: Tebuconazole 25.9% EC, T<sub>6</sub>: Thiophanate methyl 70% WP and C: Control **Plate 2:** Evaluation of systemic fungicides against *A. burnsii* under *in vitro* condition



T1: Captan 70% + Hexaconazole 5% WP, T2: Pyraclostrobin 133 g/l + Epoxiconazole 50 g/l SE, T3: Carbendazim 12% + Mancozeb 63% WP, T4: Propiconazole 13.9% + Difenoconazole 13.9% EC, T5: Metriram 55% + Pyraclostrobin 5% WG, T6: Chlorothalonil 40.0% + Difenoconazole 4.0% SC and C: Control

Plate 3: Evaluation of ready-mix fungicides against A. burnsii under in vitro condition

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