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In-vitro evaluation of fungicides against *Alternaria burnsii* (Uppal, Patel and Kamat) causing blight of cumin (*Cuminum cyminum* L.)

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ABSTRACT

The present investigation aimed to determine the per cent growth inhibition of different fungicides against *Alternaria burnsii*, the causal agent of cumin blight. The study was conducted during 2020-21 at the Experiential Unit of Plant Pathology, College of Agriculture, SKRAU, Bikaner. Using the poisoned food technique, eleven fungicides were tested at varying concentrations (100, 200, 300, and 500 ppm). After seven days of incubation, the radial growth and per cent growth inhibition of *A. burnsii* were measured. The results revealed that increasing the fungicide concentration led to greater inhibition of mycelium growth. Among the tested fungicides, Tebuconazole 50% + Trifloxystrobin 20% WG exhibited the highest mean inhibition (76.94%), followed by Tebuconazole 2DS (65.09%) and Pyraclostrobin 13.30% + Epoxiconazole 5% SE (58.75%). Notably, Tebuconazole 50% + Trifloxystrobin 20% WG at concentrations of 300 ppm and 500 ppm, as well as Tebuconazole 2DS at 500 ppm, demonstrated the highest effectiveness with cent per cent growth inhibition. On the other hand, Chlorothalonil showed the least mean growth inhibition (22.96%). The results demonstrated that as the fungicide concentration increased, there was a corresponding increase in the inhibition of *A. burnsii* growth. These findings highlight the potential effectiveness of selected fungicides, particularly Tebuconazole 50% + Trifloxystrobin 20% WG and Tebuconazole 2DS for managing cumin blight caused by *A. burnsii*.

Introduction

Cumin (*Cuminum cyminum*) is an aromatic spice widely used in culinary applications and known for its distinctive flavour and aroma. It is a popular ingredient in various cuisines worldwide and has been valued for its medicinal properties for centuries. Cumin seeds contain essential oils, antioxidants, and bioactive compounds contributing to their flavour, aroma, and potential health benefits. Cumin, a widely cultivated crop, faces various diseases that pose significant challenges to its productivity. Among the most prominent diseases affecting cumin are blight, wilt, and powdery mildew, which result in substantial yield losses (Dange, 1995). Blight, in particular, is a widespread and devastating problem that occurs annually in many cumin-growing regions. This disease spreads rapidly during humid and cloudy weather conditions, lacking effective control measures and causing yield losses of up to 80% (Gemawat and Prasad, 1972). Unfortunately, none of the existing cumin varieties exhibits resistance to blight, making it a quick-spreading and destructive threat that affects all parts of the plant, including the seeds. The cumin blight-causing pathogen *Alternaria burnsii* was first identified in India (Uppal *et al.*, 1938) and subsequently reported in Pakistan (Shakir *et al.*, 1995). Research studies have demonstrated the significant impact of blight on cumin crops. In a study conducted by Kakraliya *et al.,* (2021) in Rajasthan, it was observed that

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cumin crops faced a substantial setback under favorable conditions, experiencing 62.88 per cent decrease in yield due to the presence of Alternaria blight. In another study conducted by Wadud *et al.*, 2021, it was found that blight of cumin caused a drastic yield loss, with an incidence rate of nearly 98 per cent and a disease severity of up to 88 per cent.

There can be many approaches to managing cumin blight, which includes different fungicides, plant extract, or bioagents (Jagani *et al.,* 2023). While management through biological agents and botanicals is considered an eco-friendly approach for disease control, their effectiveness against the pathogen is often unsatisfactory, resulting in poor disease management outcomes (Yadav *et al.,* 2022). The evaluation of fungicides plays a crucial role in effectively managing cumin blight, as it is a severe fungal disease. Fungicides are chemical compounds specifically formulated to control fungal diseases and can serve as effective tools in combating blight in cumin crops (Kakraliya *et al.,* 2021). While several broad-spectrum fungicides have been previously reported for their *in-vitro* efficacy against Alternaria blight of cumin, it is vital to assess their relative effectiveness compared to new-generation fungicides. This evaluation will contribute to a better understanding of the *in-vitro* efficacy of both individual fungicides and new combinations, aiming for more effective management of cumin blight.

Material and Methods

A total of eleven fungicides (Table 1) were evaluated for their effectiveness against *Alternaria burnsii* using the poisoned food technique (Nene and Thapaliyal, 1993) at the Experiential Unit of Plant Pathology, College of Agriculture, SKRAU, Bikaner during 2020-21. Four different doses of fungicides (100, 200, 300 and 500 ppm) were used.

The fungicides were accurately measured to achieve concentrations of 100, 200, 300, and 500 ppm. These fungicides were thoroughly mixed with molten Potato Dextrose Agar (PDA) medium, which was then poured into sterilized 90 mm Petri plates. Each plate received 20 ml of the fungicide-amended medium and was allowed to solidify, ensuring uniform distribution.

For the experiment, three replications of each treatment were selected. Actively growing cultures of *A. burnsii*, aged seven days, were used. From the periphery of the cultures, 5 mm diameter discs were aseptically cut using a cork borer. The Petri plates containing the fungicide-amended medium were then aseptically inoculated with these 5 mm discs of *A. burnsii*. Control plates without fungicide treatment were also prepared for comparison purposes. The experimental design followed a completely randomized design (CRD) with three replications for each treatment.

Incubation of the inoculated Petri plates took place at a temperature of 28 ± 2 °C in BOD. After a period of seven days, when the control plates exhibited significant fungal growth, the colony diameters were measured. The control and treatment plates were measured to accurately determine the colony diameters. To evaluate the efficacy of the fungicides, the per cent inhibition of mycelial growth was calculated using Bliss's formula (1934):

$$\textbf{Per cent inhibition (I)} = \frac{(C - T)}{C} X100$$

Where,
I = Per cent inhibition
C = Colony diameter in control
T = Colony diameter in treatment

Table 1: Fungicides used in lab condition

S.N	Name of fungicide
1.	Tebuconazole 50% +Trifloxystrobin 20% WG
2.	Tebuconazole 2DS
3.	Pyraclostrobin 13.3%+ Epoxiconazole 5% SE
4.	Carbendazim 12% + Mancozeb 63% WP
5	Carbendazim 25% + Iprodione 25% WP
6.	Captan 70% + Hexaconazole 5% WP
7.	Mancozeb 75% WP
8.	Hexaconazole 5% EC
9.	Propiconazole 25% EC
10.	Pyraclostrobin 20 WG + Azoxystrobin 23% SC
11.	Chlorothalonil 75% WP

Results and Discussion

The data presented in Table 2 demonstrates the radial growth and percentage growth inhibition of various fungicides at different concentrations against *Alternaria burnsii*. In the present study, statistical analysis was conducted using analysis of variance (ANOVA) with a significance level of 0.05 (α=0.05). Post-hoc multiple comparison tests were performed to assess significant differences between the treatments. The critical difference (CD) at $P \leq 0.05$ and the coefficient of variation (CV%) were calculated as measures of the level of significance and variation, respectively.

According to the results of the statistical analysis (Table 2), significant differences ($P \leq 0.05$) were observed in the mycelial growth inhibition of *Alternaria burnsii* among different fungicides and their concentrations. The mean radial growth and per cent growth inhibition values were used to compare the effectiveness of each fungicide treatment. The results reveal that an increase in the concentration of fungicides led to greater inhibition of the mycelial growth of the fungus. Among the tested fungicides, Tebuconazole 50% + Trifloxystrobin 20% WG exhibited superior performance in inhibiting the growth of *Alternaria burnsii* at all four concentrations, with a mean percentage inhibition of 76.94%, followed by Tebuconazole 2DS (65.09%) and Pyraclostrobin 13.3% + Epoxiconazole 5% SE (58.7%). Conversely, the fungicides Propiconazole 25% EC (29.81%) and Pyraclostrobin 20 WG + Azoxystrobin 23% SC (25.56%) demonstrated comparatively less effectiveness in inhibiting mycelial growth. Besides this, Chlorothalonil recorded the least per cent inhibition of mycelium growth at all the concentrations, with a (22.96%) mean growth inhibition.

Analyzing the individual concentrations, at 100 ppm, Tebuconazole 50% + Trifloxystrobin 20% WG showed the maximum percentage inhibition (52.23%), followed by Tebuconazole 2DS (48.14%), with Chlorothalonil recording the lowest percentage inhibition (11.12%). At 200 ppm, Tebuconazole 50% + Trifloxystrobin 20% WG displayed 55.56% growth inhibition, followed by Tebuconazole 2DS (52.23%), while Chlorothalonil 75% WP demonstrated the least percentage inhibition (13.70%). At 300 ppm, Tebuconazole 50% + Trifloxystrobin 20% WG exhibited complete growth inhibition, followed by Tebuconazole 2DS (60.00%), and Chlorothalonil showed the least percentage inhibition (18.14%). At 500 ppm, both Tebuconazole 50% + Trifloxystrobin 20% WG and Tebuconazole 2DS displayed complete inhibition, with Pyraclostrobin 13.3% + Epoxiconazole 5% SE showing a high per cent inhibition (83.34%), and Chlorothalonil recording the lowest (48.89%). It is of significance to observe that all the examined fungicides effectively inhibited the mycelial growth of *Alternaria burnsii.*

Among all the fungicides, Tebuconazole 50% + Trifloxystrobin 20% WG demonstrated the highest efficacy in inhibiting the growth of *A. burnsii* at all concentrations, followed by Tebuconazole 2DS. A comparable investigation pertaining to Alternaria blight caused in cumin and other crop was undertaken by several researchers, and their findings have exhibited consistent agreement.

For instance, Rajvanshi *et al.,* (2020) evaluated the efficacy of six different fungicides against *Alternaria brassicae in vitro* and found Tebuconazole 50% + Trifloxystrobin 20% WG and Tebuconazole 250 EC to be the most effective, resulting in complete inhibition of mycelium growth. Saha *et al.,* (2018) conducted comprehensive *in vitro* and *in vivo* studies on *Alternaria brassicae* and observed that Tebuconazole 50% + Trifloxystrobin 20% WG at a concentration of 300 ppm exhibited the most significant inhibition of the pathogen, with its lower dose at 250 ppm also demonstrating notable inhibitory effects. These findings highlight the potential of the Trifloxystrobin-Tebuconazole combination as an effective treatment option for managing *Alternaria* sp.

Shekhawat *et al.,* (2013) investigated the efficacy of four different fungicides against five *Alternaria burnsii* isolates and found that Tebuconazole exhibited the highest efficacy in inhibiting mycelium growth at various concentrations (250, 500, and 1000 ppm). Similarly, Pipliwal *et al.,* (2017) assessed the efficacy of 14 different fungicides *in vitro* and observed that Tebuconazole, Hexaconazole, and Propiconazole demonstrated complete growth inhibition at various concentrations (50, 100, 250, and 500 ppm).

Table 2: Effect of fungicides on mycelial growth inhibition of *Alternaria burnsii* after seven days of incubation

S N	Name of fungicide	Radial Growth (mm)				Per cent Growth Inhibition				
		100 PPM	200 PPM	300 PPM	500 PPM	100 PPM	200 PPM	300 PPM	500 PPM	Mean
1.	Tebuconazole 50% +Trifloxystrobin 20% WG	43.00	40.00	0.00	0.00	52.23 (46.25) *	55.56 (48.17)	100 (90.00)	100 (90.00)	76.94
2.	Tebuconazole 2DS	46.67	43.00	36.00	0.00	48.14 (43.92)	52.23 (46.25)	60.00 (50.74)	100 (90.00)	65.09
3.	Pyraclostrobin 13.3%+ Epoxiconazole 5% SE	49.00	44.67	40.00	15.00	45.55 (42.43)	50.37 (45.19)	55.56 (48.17)	83.34 (65.88)	58.70
4.	Carbendazim 12% + Mancozeb 63% WP	56.00	48.00	43.00	18.67	37.70 (37.90)	46.67 (43.07)	52.23 (46.25)	79.25 (62.88)	53.98
5.	Carbendazim 25% + Iprodione 25% WP	65.67	62.00	47.00	21.67	27.03 (31.28)	31.12 (33.88)	47.78 (43.70)	75.92 (60.36)	45.37
6	Captan 70% + Hexaconazole 5% WP	70.67	68.67	55.34	24.67	21.48 (27.59)	23.70 (29.12)	38.51 (38.34)	72.59 (58.40)	39.07
7.	Mancozeb 75% WP	72.00	69.66	60.67	27.34	20.00 (26.53)	22.59 (28.33)	32.59 (34.78)	69.62 (56.53)	36.20
8.	Hexaconazole 5% EC	75.67	72.66	65.67	31.00	15.92 (23.50)	19.25 (26.01)	27.03 (31.31)	65.55 (54.04)	31.94
9.	Propiconazole 25% EC	77.34	73.34	67.34	34.67	14.07 (22.00)	18.51 (25.46)	25.18 (30.09)	61.48 (51.61)	29.81
10.	Pyraclostrobin 20 WG + Azoxystrobin 23% SC	78.67	76.00	71.67	41.67	12.59 (20.77)	15.56 (23.19)	20.37 (26.68)	53.70 (47.10)	25.56
11.	Chlorothalonil 75% WP	80.00	77.67	73.67	46.00	11.12 (19.44)	13.70 (21.71)	18.14 (25.20)	48.89 (44.34)	22.96
12.	Control	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00	0.00
	S Em (±) CD (P≤0.05) CV (%)					0.60 1.77 3.67	0.57 1.67 3.20	0.85 2.51 3.82	0.38 1.13 1.18	

***Values in parenthesis are angular transformed value**

Additionally, Pranaya *et al.,* (2021) conducted an *in vitro* study to assess the effectiveness of five systemic fungicides, including Tebuconazole and Propiconazole, against *Alternaria* sp., with both fungicides demonstrating complete inhibition of mycelium radial growth at concentrations of 500 and 1000 ppm. The findings presented by Jia *et al.,* (2022) further corroborate the significance of TFS-TBZ (Trifloxystrobin-Tebuconazole) as a practical and potent means to combat *Alternaria* sp. Trifloxystrobin-Tebuconazole has emerged as a widely employed fungicide, combining specific ratios of Trifloxystrobin and Tebuconazole. Through their synergistic action, TFS-TBZ has demonstrated remarkable effectiveness in countering *Alternaria* sp., a prevalent fungal pathogen with significant implications for crop health and productivity. Trifloxystrobin-Tebuconazole operates through a combined mode of action, leveraging 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. This interference inhibits ATP synthesis, curtailing fungal growth and propagation. Tebuconazole, a triazole fungicide, acts as a demethylation inhibitor, impeding the biosynthesis of ergosterol in the fungal cell membranes. By blocking the enzyme CYP51, tebuconazole disrupts the production of ergosterol, leading to membrane instability, cell leakage, and eventual pathogen death. The synergistic interaction between Trifloxystrobin and Tebuconazole amplifies their individual effects, augmenting the overall fungicidal activity of Trifloxystrobin-Tebuconazole and providing a potent tool for combating a wide range of fungal diseases while reducing the risk of resistance development. While Tebuconazole based fungicides demonstrated promising results, it is essential to consider the efficacy of other fungicides as well. For instance, Mancozeb 75% WP and Carbendazim 12% + Mancozeb 63% WP also showed effectiveness against *Alternaria burnsii* with mean growth inhibitions of 36.20% and 53.98%, respectively. However, it is worth noting that broad-spectrum fungicides like Mancozeb, Carbendazim, and Copper oxychloride have limitations due to their limited persistence on foliage and the potential development of pathogen resistance. In light of these limitations, the use of newer fungicides, such as Trifloxystrobin-Tebuconazole or Tebuconazole-based fungicides with newer modes of action, should be considered to ensure effective disease management and minimize the risk of resistance development. Further research and field evaluations are necessary to determine the long-term efficacy and sustainability of these newer fungicide options in managing *Alternaria burnsii* and other similar pathogens.

Conclusion

The findings highlight the potential efficacy of selected fungicides, particularly Tebuconazole 50% + Trifloxystrobin 20% WG and Tebuconazole 2DS, for managing cumin blight caused by *A. burnsii*. To ensure effective disease management and sustainability, it is crucial to explore newer fungicide options with different modes of action, such as Trifloxystrobin-Tebuconazole or Tebuconazole-based fungicides. Further research and field evaluations are warranted to assess the long-term efficacy and practical applicability of these newer fungicide combinations in managing cumin blight and similar fungal pathogens.

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Conflict of interest

The authors declare that they have no conflict of interest.

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