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In vitro screening of fungicides against *Colletotrichum truncatum* causing anthracnose of dragon fruit (*Hylocereus* spp.)

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ABSTRACT

The present study was conducted to evaluate the efficacy of selected fungicides against *Colletotrichum truncatum*, the causal pathogen of anthracnose of dragon fruit (*Hylocereus* spp.), under In vitro conditions. Seven fungicides, namely Chlorothalonil 75% WP, Mancozeb 75% WP, Copper Hydroxide 61.41% WG, Metiram 55% + Pyraclostrobin 5% WG, Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 50% + Trifloxystrobin 25% WG and Captan 70% + Hexaconazole 5% WP, were evaluated using the poisoned food technique. The experiment was conducted in a Completely Randomized Design with three replications. After seven days of incubation, significant variation was observed in the inhibition of mycelial growth among the fungicides. Metiram 55% + Pyraclostrobin 5% WG recorded the highest inhibition of mycelial growth (94.44%) with the minimum colony diameter of 5.00 mm, followed by Tebuconazole 50% + Trifloxystrobin 25% WG (92.96%) and Carbendazim 12% + Mancozeb 63% WP (90.74%). Mancozeb 75% WP recorded 86.66% inhibition, while Copper Hydroxide 61.41% WG, Captan 70% + Hexaconazole 5% WP and Chlorothalonil 75% WP recorded 75.18, 66.30 and 54.07 per cent inhibition, respectively. The untreated control recorded a colony diameter of 90.00 mm. The study indicated that Metiram 55% + Pyraclostrobin 5% WG was the most effective fungicide against *C. truncatum* under In vitro conditions.

Keywords: Dragon fruit, Anthracnose, *Colletotrichum truncatum*, Fungicides, Poison food technique, Mycelial inhibition

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1. Introduction

Dragon fruit (*Hylocereus* spp.) is an important tropical and subtropical fruit crop that has gained considerable importance because of its attractive appearance, nutritional value and increasing consumer demand. However, the productivity and quality of dragon fruit are affected by several fungal diseases, among which anthracnose is an important disease causing lesions, necrosis and deterioration of infected plant tissues. Different species of *Colletotrichum* have been associated with anthracnose of dragon fruit in different production regions, indicating the importance of effective disease management strategies (Masyahit *et al.*, 2009).

Colletotrichum truncatum has been reported as an important causal pathogen of anthracnose of dragon fruit. Earlier studies from China documented *C. truncatum* associated with anthracnose of *Hylocereus undatus*, while its occurrence on dragon fruit has also been reported from India. The identification of *C. truncatum* as a pathogen of dragon fruit highlights the need for suitable management measures to reduce pathogen growth and disease development (Guo *et al.*, 2014).

Chemical fungicides remain an important component of fungal disease management because they can provide rapid suppression of pathogenic fungi and are particularly useful when disease pressure is high. Fungicides belonging to different chemical groups and combinations of active ingredients may differ considerably in their ability to inhibit mycelial growth of *Colletotrichum* species. Therefore, screening available fungicides under *In vitro* conditions provides useful preliminary information for identifying effective molecules or combinations for further disease-management studies.

The poisoned food technique is widely used for evaluating the antifungal activity of fungicides by incorporating the test fungicide into a suitable culture medium and measuring the subsequent growth of the pathogen. The reduction in colony growth compared with an untreated control provides an estimate of the inhibitory effect of the tested fungicide. Such laboratory screening helps in comparing the relative efficacy of different fungicides before their further evaluation under greenhouse or field conditions (Nene *et al.*, 1993) [1].

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Considering the importance of anthracnose caused by *C. truncatum* in dragon fruit, the present investigation was undertaken to screen selected fungicides for their inhibitory effect against the pathogen under *In vitro* conditions. Seven fungicides representing different active ingredients and combinations were evaluated using the poisoned food technique, and their efficacy was assessed based on colony diameter and percentage inhibition of mycelial growth.

2. Materials and Methods

2.1 Preparation of PDA medium and fungicide solutions

Potato Dextrose Agar (PDA) was used as the basal culture medium for evaluating the efficacy of selected fungicides against *Colletotrichum truncatum*. The required quantities of each fungicide were incorporated aseptically into molten PDA to obtain the desired concentrations. Streptomycin was added to the medium to avoid bacterial contamination. The fungicides were evaluated at their respective half and recommended dose concentrations.

2.2 Poisoned food technique

The efficacy of the fungicides was assessed using the Poisoned Food Technique as described by Nene *et al.* (1993) [1]. Fungicide-amended PDA medium was dispensed at 20 ml per sterile 90 mm Petri plate

and allowed to solidify at room temperature. PDA without fungicide was maintained as the untreated control.

2.3 Inoculation of *C. truncatum*

A 5 mm diameter mycelial disc was cut from the actively growing margin of a seven-day-old culture of *C. truncatum* using a sterilized cork borer. The disc was placed at the centre of each fungicide-amended PDA plate in an inverted position to ensure proper contact with the medium.

2.4 Incubation and observation

Three plates were maintained for each treatment along with three untreated control plates. The inoculated Petri plates were incubated at 25 ± 2 °C. Radial growth of the pathogen was observed and colony diameter was recorded after 7 days of incubation.

2.5 Assessment of fungicidal efficacy

The colony diameter of *C. truncatum* was measured in millimetres using a scale. The percentage inhibition of mycelial growth was calculated in comparison with the untreated control using the formula given by Arora *et al.* (1978) [2]:

$$\text{Percent growth inhibition (I)} = \left[\frac{(C - T)}{C} \right] \times 100$$

Where, C = radial growth of the pathogen in untreated control (mm)
T = radial growth of the pathogen in treatment (mm)

2.6 Experimental design and statistical analysis

The experiment consisted of eight treatments with three replications and was conducted in a Completely Randomized Design (CRD). The experimental data were statistically analysed, and standard error (S.E.) and critical difference (C.D.) at the 5 per cent level of significance were calculated for comparison of treatments.

3. Results

3.1 In vitro screening of fungicides against *Colletotrichum truncatum*

The efficacy of seven fungicides against *Colletotrichum truncatum* causing anthracnose of dragon fruit was evaluated under *In vitro* conditions using the poisoned food technique. Considerable variation was observed among the fungicides in their ability to suppress the mycelial growth of the pathogen. The colony diameter ranged from

5.00 to 41.33 mm, while the percentage inhibition of mycelial growth ranged from 54.07 to 94.44 per cent. All fungicidal treatments recorded lower colony growth than the untreated control, which recorded a maximum colony diameter of 90.00 mm.

Among the fungicides tested, Metiram 55% + Pyraclostrobin 5% WG (T_4) recorded the minimum colony diameter of 5.00 mm and the highest mycelial growth inhibition of 94.44 per cent. It was followed by Tebuconazole 50% + Trifloxystrobin 25% WG (T_6), which recorded 6.33 mm colony diameter and 92.96 per cent inhibition. Carbendazim 12% + Mancozeb 63% WP (T_5) recorded 8.33 mm colony diameter with 90.74 per cent inhibition.

Mancozeb 75% WP (T_2) recorded 12.00 mm colony diameter with 86.66 per cent inhibition. Copper Hydroxide 61.41% WG (T_3) recorded 22.33 mm colony diameter and 75.18 per cent inhibition. Captan 70% + Hexaconazole 5% WP (T_7) recorded 30.33 mm colony diameter with 66.30 per cent inhibition, whereas Chlorothalonil 75% WP (T_1) recorded 41.33 mm colony diameter and 54.07 per cent inhibition.

Table 1. Treatment details of fungicides evaluated against *Colletotrichum truncatum*

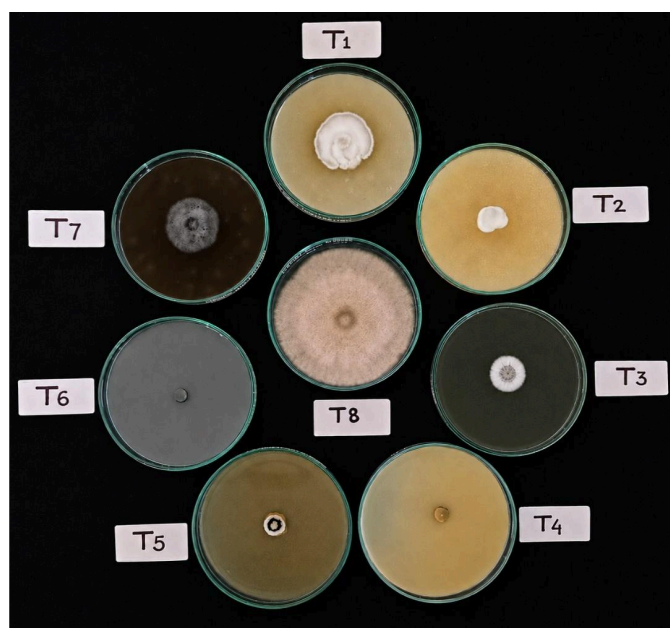
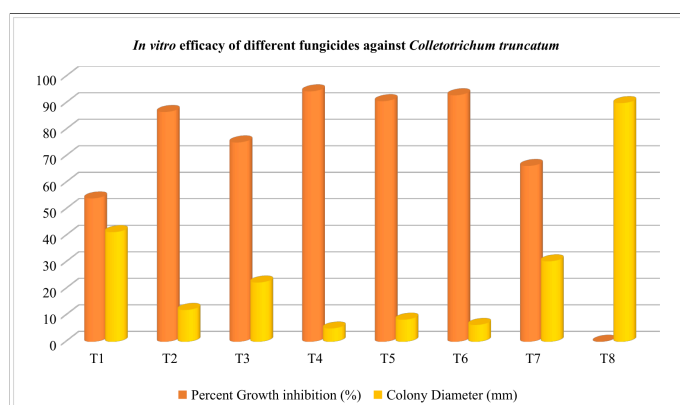
Treatment	Fungicide	Recommended dose (ppm)	Half dose (ppm)
T_1	Chlorothalonil 75% WP	1500	750
T_2	Mancozeb 75% WP	2500	1250
T_3	Copper Hydroxide 61.41% WG	1750	875
T_4	Metiram 55% + Pyraclostrobin 5% WG	2000	1000
T_5	Carbendazim 12% + Mancozeb 63% WP	1500	750
T_6	Tebuconazole 50% + Trifloxystrobin 25% WG	1000	500
T_7	Captan 70% + Hexaconazole 5% WP	2000	1000
T_8	Control	—	—

The untreated control (T_8) recorded the maximum colony diameter of 90.00 mm with 0.00 per cent inhibition. The statistical analysis

showed a S.E. (m) $\pm$ of 0.48 mm and C.D. (5%) of 1.45 mm.

Table 2. *In vitro* screening of fungicides against *Colletotrichum truncatum*

Tr. No.	Treatments/Fungicides	Colony diameter of the pathogen* (mm)	Growth inhibition (%)
T ₁	Chlorothalonil 75% WP	41.33	54.07
T ₂	Mancozeb 75% WP	12.00	86.66
T ₃	Copper Hydroxide 61.41% WG	22.33	75.18
T ₄	Metiram 55% + Pyraclostrobin 5% WG	5.00	94.44
T ₅	Carbendazim 12% + Mancozeb 63% WP	8.33	90.74
T ₆	Tebuconazole 50% + Trifloxystrobin 25% WG	6.33	92.96
T ₇	Captan 70% + Hexaconazole 5% WP	30.33	66.30
T ₈	Control	90.00	0.00
	S.E. (m) ±	0.48	
	C.D. (5%)	1.45	
Mean of three replications			

**Figure 1.** *In vitro* screening of fungicides against *Colletotrichum truncatum***Figure 2.** Effect of *In vitro* screening of fungicides against *Colletotrichum truncatum*

4. Discussion

The present investigation demonstrated considerable variation in the efficacy of the fungicides evaluated against *Colletotrichum truncatum* under *In vitro* conditions. The fungicide treatments differed in their ability to suppress the radial growth of the pathogen, with inhibition ranging from 54.07 to 94.44 per cent. The higher efficacy observed with fungicide combinations indicated their strong inhibitory activity against the pathogen under laboratory conditions. Among the tested fungicides, Metiram 55% + Pyraclostrobin 5% WG recorded the highest inhibition of mycelial growth (94.44%), fol-

lowed by Tebuconazole 50% + Trifloxystrobin 25% WG (92.96%) and Carbendazim 12% + Mancozeb 63% WP (90.74%). The higher activity of these combination fungicides may be associated with the presence of active ingredients having different modes of action, resulting in stronger suppression of fungal growth. Similar effectiveness of fungicide combinations against *Colletotrichum* spp. has been reported by Jagtap *et al.* (2013) [3] and Mahesh *et al.* (2020) [4].

Mancozeb 75% WP also showed considerable inhibition of *C. truncatum*, with 86.66 per cent inhibition of mycelial growth. Similar inhibitory effects of fungicides against *Colletotrichum* species have been reported by Vani *et al.* (2018) [5]. Bagade *et al.* (2020) [6] also reported variation in the efficacy of different fungicides against *Colletotrichum* causing anthracnose, supporting the observed differences among fungicidal treatments in the present study.

The comparatively lower inhibition recorded by Copper Hydroxide 61.41% WG, Captan 70% + Hexaconazole 5% WP and Chlorothalonil 75% WP indicated that their inhibitory activity against *C. truncatum* was lower than that of the highly effective fungicide combinations. Similar variation in fungicidal efficacy against anthracnose-causing *Colletotrichum* species has been reported in earlier *In vitro* studies (Golakiya *et al.*, 2020) [7].

Overall, the present findings indicated that Metiram 55% + Pyraclostrobin 5% WG, Tebuconazole 50% + Trifloxystrobin 25% WG and Carbendazim 12% + Mancozeb 63% WP were highly effective in suppressing the mycelial growth of *C. truncatum* under *In vitro* conditions. These findings provide preliminary information for selecting promising fungicides for further evaluation under greenhouse and field conditions.

5. Conclusion

The present study demonstrated significant variation in the efficacy of fungicides against *Colletotrichum truncatum* causing anthracnose of dragon fruit under *In vitro* conditions. Among the tested fungicides, Metiram 55% + Pyraclostrobin 5% WG recorded the highest inhibition of mycelial growth (94.44%), followed by Tebuconazole 50% + Trifloxystrobin 25% WG (92.96%) and Carbendazim 12% + Mancozeb 63% WP (90.74%). These fungicides showed strong inhibitory activity against *C. truncatum* and can therefore be considered promising candidates for further evaluation under greenhouse and field conditions for the management of dragon fruit anthracnose.

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