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In vitro Evaluation of Botanicals and Fungicides against Mango Anthracnose Caused by *Colletotrichum gloeosporioides*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Mango (*Mangifera indica L.*) is one of the most important fruit crops in tropical and subtropical regions, but its productivity is severely constrained by anthracnose disease caused by *Colletotrichum gloeosporioides*. The present study was undertaken to evaluate the efficacy of selected fungicides and botanical extracts against *C. gloeosporioides* under in vitro conditions using the poisoned food technique. Diseased mango samples were collected and the pathogen was isolated and purified on Potato Dextrose Agar (PDA). Four fungicides, namely carbendazim, mancozeb, copper oxychloride, and carbendazim + mancozeb, were tested at three concentrations (0.1%, 0.2%, and 0.3%), while two botanicals, garlic (*Allium sativum*) and onion (*Allium cepa*), were

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evaluated at 5%, 10%, and 15% concentrations. Percent inhibition of mycelial growth was calculated and statistically analyzed. Among the fungicides tested, carbendazim + mancozeb exhibited the highest inhibition of mycelial growth (up to 87.28%), followed by mancozeb (90.00%) at higher concentrations. Carbendazim showed moderate inhibition, whereas copper oxychloride was comparatively less effective. Among botanicals, garlic extract recorded the highest inhibition (90.00%) at 15% concentration, while onion extract showed moderate antifungal activity. The results indicated a clear dose-dependent increase in inhibition for both fungicides and botanicals. The study concludes that fungicides were more effective than botanicals under in vitro conditions; however, garlic extract demonstrated promising antifungal potential and could be integrated into sustainable disease management strategies for mango anthracnose.

Keywords: Mango anthracnose; Colletotrichum gloeosporioides; fungicides; botanicals; poisoned food technique.

1. Introduction

Mango (*Mangifera indica L.*) is one of the most economically important fruit crops cultivated in tropical and subtropical regions, particularly in India, which is the world's largest producer (Kumar et al., 2024). Despite its commercial importance, mango productivity and fruit quality are severely constrained by several biotic and abiotic stresses occurring at different stages of crop growth, from nursery to post-harvest handling. Among biotic factors, fungal diseases pose a major threat to mango cultivation by reducing yield, market value, and shelf life (Koulotioloma et al., 2025, Cornago et al., 2011).

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph Glomerella cingulata), is considered the most destructive and widespread disease of mango worldwide (Choudhary et al., 2024). The disease affects leaves, panicles, twigs, and fruits, leading to flower blight, fruit drop, and severe post-harvest losses. Typical symptoms include irregular dark necrotic lesions on leaves, blossom blight during flowering, and sunken black spots on fruits during ripening. Under favourable environmental conditions, the pathogen produces abundant conidia, facilitating rapid disease spread and persistence in orchards.

Management of mango anthracnose largely relies on the application of chemical fungicides (Jeevanantham et al., 2024; Bhagwat et al., 2016). Systemic fungicides such as carbendazim and contact fungicides like mancozeb and copper oxychloride are widely used for effective disease suppression (Inayat et al., 2024). Combination fungicides have gained importance due to their enhanced efficacy and reduced risk of resistance development through multiple modes of action. However, excessive and indiscriminate use of fungicides has raised concerns related to fungicide resistance, environmental pollution, and food safety, emphasizing the need for alternative or supplementary disease management approaches.

Botanical extracts have emerged as eco-friendly and cost-effective alternatives for managing plant diseases (Niazi et al., 2022). Several plant-derived products possess antifungal properties due to the presence of bioactive compounds such as phenolics, flavonoids, and sulfur-containing compounds. Garlic (*Allium sativum*) extract has been reported to exhibit strong antifungal activity against a wide range of phytopathogenic fungi, including *Colletotrichum species*, owing to compounds such as allicin and ajoene (Kumar et al., 2024, Umamaheswari et al., 2022). Onion (*Allium cepa*) extract has also shown moderate antifungal potential, though its efficacy is generally lower than that of garlic.

In vitro evaluation of fungicides and botanicals using the poisoned food technique provides a reliable method to assess their antifungal efficacy before field application (Ranjitha et al., 2019). Such studies help in identifying effective chemicals and plant-based products that can be incorporated into integrated disease management strategies.

In view of the economic importance of mango and the destructive nature of anthracnose disease, the present study was undertaken to evaluate the efficacy of selected fungicides and botanical extracts against *Colletotrichum gloeosporioides* under in vitro conditions. The findings aim to identify effective treatments that could contribute to sustainable management of mango anthracnose.

2. Materials and Methods

2.1 Experimental Site and Sample Collection

Diseased mango leaves showing typical symptoms of anthracnose were collected from mango orchards at Lara Green Fields, Vignan's Foundation for Science, Technology and Research. The samples were placed in clean polythene bags, properly labeled, and transported to the Plant Pathology Laboratory present in Vignan's Foundation for Science, Technology and Research for further investigation.

2.2 Isolation and Purification of the Pathogen

The collected diseased samples were thoroughly washed under running tap water to remove adhering debris. Small segments (approximately 5–7 mm) from the infected portions were cut using a sterile scalpel. These segments were surface sterilized with 70% ethanol for 30 seconds, followed by rinsing twice with sterile distilled water to remove traces of the sterilant. The sterilized pieces were blot-dried on sterile filter paper.

Potato Dextrose Agar (PDA) medium was prepared, sterilized by autoclaving at 121°C and 15 psi for 15 minutes, and poured into sterilized Petri plates under aseptic conditions. The surface-sterilized leaf segments were aseptically transferred onto PDA plates and incubated at 25 ± 2°C for 7–10 days.

After incubation, fungal growth emerging from the tissues was observed. Subculturing was carried out by transferring actively growing mycelial tips onto fresh PDA plates to obtain pure cultures. The purified culture of *Colletotrichum gloeosporioides* was maintained on PDA slants and used for further experimentation.

2.3 Preparation of Botanical Extracts

Fresh plant materials used for botanical extracts included garlic (*Allium sativum*) cloves and onion (*Allium cepa*) bulbs (Table 1). The collected plant parts were thoroughly washed with distilled water and crushed separately using a sterile mortar and pestle to obtain a fine paste.

An equal volume of sterile distilled water was added to the paste, and the mixture was filtered through Whatman No. 45 filter paper to obtain a clear aqueous extract. The filtrate was considered as 100% stock solution and further diluted with sterile distilled water to obtain required concentrations of 5%, 10%, and 15% for antifungal evaluation.

2.4 Fungicides used

Four fungicides commonly used for the management of mango anthracnose were evaluated in the present study. These included one systemic fungicide and two contact fungicides, along with a combination fungicide (Table 1). The fungicides were tested at three concentrations, namely 0.1%, 0.2%, and 0.3%, using the poisoned food technique.

2.5 *In vitro* Evaluation of Fungicides and Botanicals (Poisoned Food Technique)

The poisoned food technique was employed to assess the antifungal efficacy of fungicides and botanical extracts against *Colletotrichum gloeosporioides*. The required quantity of each fungicide or botanical extract was incorporated into sterilized molten PDA medium to obtain the desired concentrations. The medium was thoroughly mixed and poured into sterilized Petri plates.

Plates without any fungicide or botanical extract served as control. After solidification, a 5 mm diameter mycelial disc cut from the periphery of a 7-day-old actively growing culture of C. gloeosporioides was placed at the center of each plate. All treatments were replicated thrice.

The inoculated plates were incubated at 25 ± 2°C, and observations on radial mycelial growth were recorded once the control plates attained full growth.

Calculation of percent inhibition:

The percent inhibition of mycelial growth was calculated using the formula:

Percent inhibition (%)= $\frac{c-t}{c} \times 100$

Where:

C = radial growth of the pathogen in control (mm)

T = radial growth of the pathogen in treated plate (mm)

Table 1. List of fungicides used in experiment.

Fungicide	Concentration	Mode of action
Carbendazim	0.1%, 0.2%, 0.3%	Systemic
Mancozeb	0.1%, 0.2%, 0.3%	Contact
Copper oxychloride	0.1%, 0.2%, 0.3%	Contact
Carbendazim + Mancozeb	0.1%, 0.2%, 0.3%	Systemic

Table 2. Efficacy of fungicides on pathogen control

Treatment	Concentration	Percent inhibition
Control		0.000
Carbendazim	0.10%	45.555
	0.20%	59.757
	0.30%	63.422
Carbendazim + Mancozeb	0.10%	69.737
	0.20%	73.540
	0.30%	87.279
Mancozeb	0.10%	64.137
	0.20%	74.367
	0.30%	90.000
Copper oxy chloride	0.10%	52.122
	0.20%	53.709
	0.30%	57.817
C.D.		2.734
SE(m)		0.935
SE(d)		1.323
C.V.		2.661

2.6 Statistical Analysis

The experiment was conducted using a Completely Randomized Design (CRD). The data obtained were statistically analyzed to determine the significance of treatments. Critical Difference (CD) at 5% probability level, Standard Error of Mean [SE(m)], Standard Error of Difference [SE(d)], and Coefficient of Variation (CV) were calculated to assess the reliability and precision of the results.

3. Results

Effect of fungicides on mycelial growth of *Colletotrichum gloeosporioides* under *in vitro* conditions: The *in vitro* evaluation of different fungicides against *Colletotrichum gloeosporioides* revealed significant variation in their efficacy in inhibiting mycelial growth when compared to the untreated control (Table 3). The untreated control recorded 0.00% inhibition, indicating unrestricted growth of the pathogen under laboratory conditions.

Among the fungicides tested, mancozeb proved to be the most effective at higher concentrations, recording the maximum percent inhibition of 90.00% at 0.30% concentration, followed by carbendazim + mancozeb, which showed 87.28% inhibition at 0.30% concentration. At lower concentrations, carbendazim + mancozeb recorded 69.74% inhibition at 0.10% and 73.54% at 0.20% concentration, indicating its consistent efficacy across concentrations.

Carbendazim alone exhibited moderate antifungal activity with a gradual increase in percent inhibition as the concentration increased. It recorded 45.56% inhibition at 0.10%, 59.76% at 0.20%, and 63.42% at 0.30% concentration.

Copper oxychloride showed comparatively lower efficacy among the fungicides tested. The percent inhibition recorded was 52.12% at 0.10%, 53.71% at 0.20%, and 57.82% at 0.30% concentration, indicating limited effectiveness even at higher doses.

Statistical analysis indicated that the differences among treatments were significant at the 5% level. The calculated CD (2.734), SE(m) (0.935), SE(d) (1.323), and CV (2.661%) values confirmed the reliability and precision of the experimental data.

Effect of botanicals on mycelial growth of *Colletotrichum gloeosporioides*: The antifungal activity of selected botanicals against *Colletotrichum gloeosporioides* was evaluated at three concentrations (5%, 10%, and 15%) using the poisoned food technique (Table 3). All botanical treatments significantly reduced mycelial growth compared to the untreated control, which recorded 0.00% inhibition.

Among the botanicals tested, garlic extract exhibited the highest antifungal activity. The percent inhibition recorded was 56.77% at 5%, 58.25% at 10%, and 90.00% at 15% concentration, indicating a strong dose-dependent response and high antifungal potential at higher concentrations.

Onion extract showed moderate inhibition of mycelial growth. The percent inhibition values were 43.26% at 5%, 56.98% at 10%, and 60.43% at 15% concentration, which were lower compared to garlic extract at corresponding concentrations.

The statistical parameters revealed significant differences among treatments. The CD value (1.791), SE(m) (0.585), SE(d) (0.827), and CV (1.938%) indicated consistency and reliability of the experimental results.

Table 3. Efficacy of botanicals on pathogen control

Treatment	Concentration	Percent inhibition
Control		0.000
Garlic	5%	56.772
	10%	58.247
	15%	90.000
Onion	5%	43.261
	10%	56.976
	15%	60.433
C.D.		1.791
SE(m)		0.585
SE(d)		0.827
C.V.		1.938

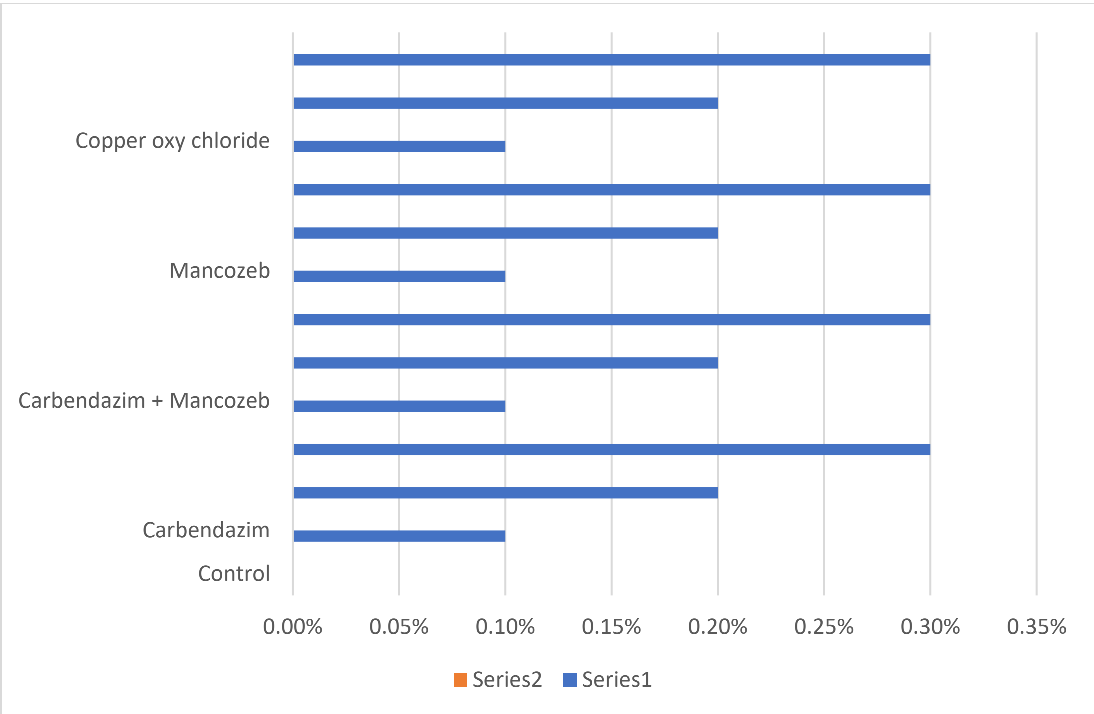

Fig. 1. Effect of fungicides on mycelial growth inhibition of *Colletotrichum gloeosporioides*

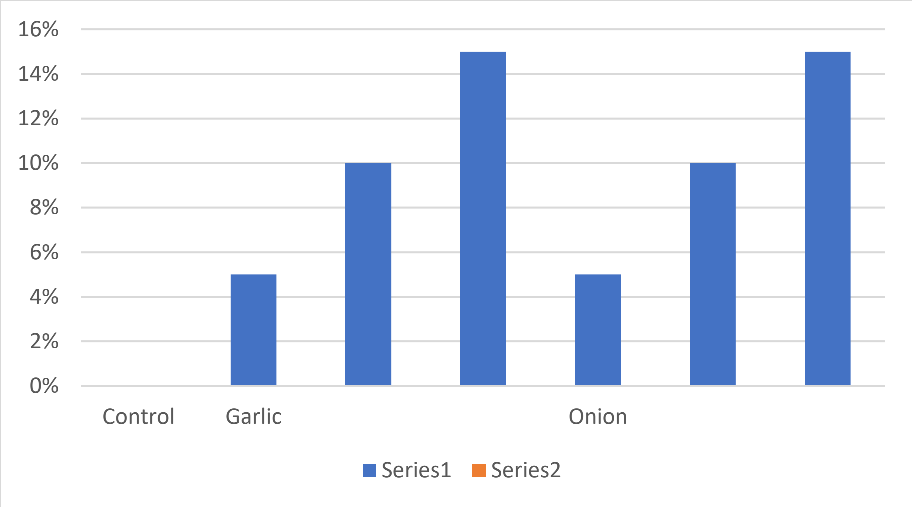

Fig. 2. Effect of botanical extracts on mycelial growth inhibition of *Colletotrichum gloeosporioides*

The experimental results clearly demonstrated significant variation among treatments in suppressing the mycelial growth of *Colletotrichum gloeosporioides* under in vitro conditions. All fungicides and botanical extracts exhibited measurable inhibitory effects compared to the untreated control, with increased concentrations resulting in higher percent inhibition. Among fungicides, mancozeb and the combination of carbendazim + mancozeb recorded the highest inhibition at 0.30% concentration, whereas copper oxychloride showed comparatively lower inhibition across all concentrations. Botanical extracts also reduced mycelial growth in a concentration-dependent manner, with garlic extract showing markedly higher inhibition than onion extract, particularly at 15% concentration. The statistical parameters indicated that the observed differences among treatments were significant and the experimental data were reliable.

4. Discussion

The present investigation demonstrated that fungicides were more effective than botanicals in suppressing the mycelial growth of *Colletotrichum gloeosporioides* under in vitro conditions. Among the fungicides tested, the combination of carbendazim + mancozeb was the most effective, achieving near-complete inhibition at higher concentrations. Similar findings have been reported earlier, where combination fungicides provided superior control of anthracnose pathogens due to their dual mode of action.

The strong efficacy of carbendazim observed in the present study corroborates earlier reports that documented significant inhibition of *C. gloeosporioides* due to interference with fungal mitosis and nuclear division. Mancozeb also exhibited high antifungal activity, particularly at higher concentrations, which may be attributed to its multisite inhibition of fungal enzymes, reducing the likelihood of resistance development.

Copper oxychloride, though effective, showed comparatively lower inhibition. This reduced efficacy may be due to its contact mode of action and limited penetration into fungal tissues, as reported earlier.

Among botanicals, garlic extract exhibited the highest antifungal activity. The strong inhibitory effect of garlic extract observed in the present study is in agreement with earlier findings that reported significant suppression of phytopathogenic fungi due to sulfur-containing compounds such as allicin and ajoene. These compounds are known to disrupt cell membrane integrity and inhibit essential metabolic pathways in fungi.

Onion extract showed moderate antifungal activity, which may be attributed to the presence of phenolic and flavonoid compounds, though in lower concentrations compared to garlic. The comparatively lower efficacy of onion extract observed in the present study aligns with earlier reports.

The dose-dependent increase in inhibition observed for both fungicides and botanicals emphasizes the importance of concentration in effective disease management. Higher concentrations consistently resulted in greater suppression of mycelial growth, highlighting the need for optimized application rates.

Overall, the findings suggest that while fungicides remain the most effective means of controlling mango anthracnose, botanicals-particularly garlic extract-can play a supportive role in integrated disease management strategies aimed at reducing chemical load and promoting sustainable agriculture.

The variation in antifungal efficacy observed among the tested treatments can be attributed to differences in their modes of action and concentration levels. Combination fungicides exhibited enhanced inhibitory effects due to the synergistic action of systemic and contact components, which may have limited pathogen growth more effectively than single fungicides. The comparatively lower performance of copper oxychloride suggests restricted penetration and reliance on surface action, whereas higher inhibition by mancozeb reflects its broad-spectrum, multisite activity. The strong antifungal response of garlic extract highlights the potential of plant-based products in disease suppression, likely due to bioactive sulfur compounds that interfere with fungal metabolism. The consistent dose-dependent increase in inhibition across treatments emphasizes the importance of optimizing concentrations for effective disease management under laboratory conditions.

5. Conclusion

The present investigation clearly demonstrated that *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose, can be effectively suppressed under in vitro conditions through the use of fungicides and botanical extracts. Among the fungicides evaluated, the combination fungicide carbendazim + mancozeb proved to be the most effective, followed by mancozeb alone, indicating the advantage of combined systemic and contact modes of action. Carbendazim showed moderate efficacy, while copper oxychloride was comparatively less effective in inhibiting mycelial growth.

Among the botanical treatments, garlic extract exhibited strong antifungal activity, particularly at higher concentrations, whereas onion extract showed moderate inhibition. The results revealed a clear concentration-dependent response for both fungicides and botanicals, emphasizing the importance of optimized dosage in disease management.

Although chemical fungicides provided superior control under laboratory conditions, the promising performance of garlic extract highlights its potential as an eco-friendly alternative or supplementary component in integrated disease management programs. The incorporation of botanicals along with judicious fungicide use may help reduce chemical dependency and promote sustainable management of mango anthracnose. Further studies under field conditions are recommended to validate the effectiveness of these treatments.

In conclusion, the present study confirms that *Colletotrichum gloeosporioides* can be effectively inhibited under in vitro conditions using both chemical fungicides and botanical extracts. The superior performance of combination and contact fungicides at higher concentrations highlights their effectiveness in managing mango anthracnose, while the notable antifungal activity of garlic extract demonstrates the potential of botanicals as eco-friendly alternatives. The concentration-dependent response observed across treatments emphasizes the need for optimized application rates. These findings support the integration of effective botanicals with judicious fungicide use as a sustainable approach to disease management, warranting further validation under field conditions.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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