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ECO-FRIENDLY MANAGEMENT OF *COLLETOTRICHUM GLOEOSPORIOIDES* CAUSING MANGO ANTHRACNOSE

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ABSTRACT

Mango (*Mangifera indica* L.), popularly known as the "king of fruits," is an important tropical fruit crop but suffers severe post-harvest losses due to anthracnose caused by *Colletotrichum gloeosporioides*, leading to reduced fruit quality, storage life and marketability. The present study evaluated the antifungal efficacy of selected essential oils and botanical extracts against *C. gloeosporioides* under *in vitro* conditions. Among eight essential oils tested at 0.5%, 1.0% and 2.0% concentrations, basil and lemongrass oils caused complete inhibition of fungal growth at all concentrations. Cinnamon and mentha oils achieved complete inhibition at 1.0%, while clove and peppermint oils were fully effective at 2.0%. Mentha oil also showed high efficacy at 0.5% concentration with 93.52% mycelial inhibition. In addition, botanical extracts evaluated at 2.5%, 5% and 10% concentrations showed significant variation in antifungal activity. *Eucalyptus globulus* leaf extract was the most effective, recording 42.59%, 45.00% and 75.37% inhibition, respectively. Garlic (*Allium sativum*) extract exhibited 35.26% inhibition at 2.5%, while onion (*Allium cepa*) extract recorded 42.96% inhibition at 5%. Custard apple (*Annona squamosa*) leaf extract was the least effective, with 17.33%, 22.52% and 30.19% inhibition at 2.5%, 5% and 10%, respectively. Overall, the findings highlight the strong antifungal potential of specific essential oils and botanical extracts, particularly basil, lemongrass and eucalyptus, as eco-friendly alternatives for the management of mango anthracnose.

Keywords **:** Mango, Anthracnose, *Colletotrichum*, Post-harvest, Essential oil.

Introduction

Mango (*Mangifera indica* L.), revered as the "king of fruits," is one of the most important tropical fruit crops cultivated extensively in India and other mango-growing countries owing to its high nutritional value, unique flavour and significant economic importance. India is the largest producer of mango in the world; however, productivity and marketability are severely constrained by several diseases occurring during pre and post-harvest stages. Among these, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is considered the most destructive disease of mango, particularly under warm and humid conditions (Prakash & Rao, 1997; Dodd *et al*., 1997).

Anthracnose affects leaves, panicles, twigs and fruits, but its impact is most severe on fruits during ripening and storage, where latent infections develop into characteristic black necrotic lesions, leading to heavy post-harvest losses, reduced shelf life and poor market acceptability (Ploetz, 2003; Freeman *et al*., 1998). Conventional management of mango anthracnose relies largely on synthetic fungicides; however, their indiscriminate use has raised concerns regarding fungicide resistance, environmental pollution, pesticide residues on fruits and risks to human health (Brent and Hollomon, 2007). In this context, plant derived botanicals and essential oils have gained considerable attention as safe, biodegradable and eco-friendly alternatives to chemical fungicides. Several essential oils such as basil, lemongrass,

cinnamon, clove and mint oils are reported to possess strong antifungal properties due to the presence of bioactive compounds like citral, eugenol, cinnamaldehyde and menthol, which disrupt fungal cell membranes and metabolic processes (Burt, 2004; Tripathi *et al*., 2008). Similarly, botanical extracts from plants such as eucalyptus, garlic and onion are known to inhibit fungal growth through phenolics, sulfur-containing compounds and other secondary metabolites (Dubey *et al*., 2011; Singh and Singh, 2012).

Material and Methods

Isolation of the pathogen

Mango leaves showing typical anthracnose symptoms were collected from a mango orchard and used for the isolation of the pathogen. Infected leaf portions along with a small area of healthy tissue were cut into small bits and surface sterilized with 1 per cent sodium hypochlorite solution for 60 seconds. The leaf bits were subsequently rinsed three times with sterile distilled water to remove any residual sterilant and blotted dry under aseptic conditions. The sterilized leaf bits were aseptically placed on potato dextrose agar (PDA) medium in sterilized petri plates inside a laminar air-flow chamber. The plates were incubated at 25 ± 2 °C. After 72 hours of incubation, fungal colonies emerging from the leaf bits were transferred to fresh PDA plates to obtain pure cultures. The cultures were periodically observed for mycelial growth and sporulation. Identification of the pathogen was carried out based on morphological characteristics of the mycelium and conidia using microscopic examination.

Identification of the fungus

The pathogen was identified based on its mycelial and conidial characteristics as described by Barnett and Hunter (1972). After identification, the cultures were transferred to fresh potato dextrose agar (PDA) slants and incubated at 25 ± 2 °C for further use. The fungus was sub-cultured on PDA slants and allowed to grow for seven days at 25 ± 2 °C. The actively growing cultures were preserved in a refrigerator at 5 °C for maintenance. Sub-culturing was carried out at monthly intervals to maintain culture viability and these cultures were used throughout the course of the study.

In vitro evaluation of botanicals against *Colletotrichum gloeosporioides* (Cg-1)

The antifungal efficacy of selected botanical extracts was evaluated under *in vitro* conditions against *Colletotrichum gloeosporioides*. Freshly prepared plant extracts were incorporated into the culture medium to assess their fungitoxic effects on mycelial growth of the pathogen.

Preparation of plant extracts

Fresh plant parts such as leaves, bulbs, cloves and rhizomes were collected from the field and thoroughly washed with distilled water to remove adhering soil and surface contaminants. The plant materials were air dried under shade prior to extraction. For each botanical, 100 g of plant material was macerated using a sterile mortar and pestle and homogenized with 100 mL of sterilized distilled water, maintaining a 1:1 (w/v) ratio. The homogenate was squeezed through sterilized muslin cloth and further filtered through Whatman No. 1 filter paper. The filtrate obtained was considered as the 100 per cent (stock) extract. From this stock solution, required concentrations of 2.5%, 5% and 10% were prepared by appropriate dilution with sterilized distilled water and used for further studies.

Evaluation of antifungal activity

The poisoned food technique (Ranjitha *et al* 2019) was employed to assess the antifungal activity of the botanical extracts against *C. gloeosporioides*. Required quantities of potato dextrose agar (PDA) medium and plant extracts were autoclaved separately and allowed to cool. To obtain 2.5%, 5% and 10% concentrations, 2.5, 5.0 and 10.0 mL of the respective plant extracts were mixed thoroughly with 97.5, 95.0 and 90.0 mL of molten PDA, respectively. The amended medium was then poured into sterilized 90 mm Petri plates and allowed to solidify. A 5 mm mycelial disc cut from the actively growing margin of a seven days old culture of *C. gloeosporioides* was aseptically placed at the centre of each petri plate. Plates containing PDA without botanical extract served as the control. Each treatment was replicated three times. The inoculated plates were incubated at 25 ± 2 °C for seven days in a BOD incubator.

In vitro evaluation of essential oils against *Colletotrichum gloeosporioides* (Cg-1)

The essential oils with reported antifungal activity in different crops were selected for the present study and procured from the local market. Their efficacy against *Colletotrichum gloeosporioides* (Cg-1) was evaluated under *in vitro* conditions using the poisoned food technique. The required quantity of each essential oil was aseptically incorporated into molten potato dextrose agar (PDA) after cooling to obtain concentrations of 0.5%, 1.0% and 2.0% (v/v) in a final volume of 100 mL. The amended medium was thoroughly mixed and 20 mL was poured into sterile petri plates and allowed to solidify. PDA plates without essential oils served as the control. A 5 mm mycelial

disc cut from the actively growing margin of a seven days old culture of *C. gloeosporioides* was aseptically placed at the centre of each petri plate. Each treatment was replicated three times. The inoculated plates were incubated at room temperature (25 ± 2 °C), and radial mycelial growth was recorded at regular intervals.

Observations were recorded by calculating per cent inhibition of mycelial growth over control by following formula stated below given by Baratta (1998).

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

Where,

I= Per cent reduction in growth of the test isolate

C=radial growth (mm) in control

T= radial growth in treatment

Result and Discussion

In vitro evaluation of botanicals against *Colletotrichum gloeosporioides* (Cg-1)

The *in vitro* evaluation of different botanical extracts against *Colletotrichum gloeosporioides* (Cg-1) revealed significant variation in their ability to inhibit mycelial growth. Among the botanicals, *Eucalyptus globulus* leaf extract was the most effective across all concentrations. It recorded the highest mycelial growth inhibition of 42.59%, 45.00% and 75.37% at 2.5%, 5% and 10% concentrations, respectively (Table 1). The inhibitory effect of eucalyptus increased with increasing concentration and was found to be statistically superior over other botanical treatments at all levels. Garlic (*Allium sativum*) extract was the next best treatment at 2.5% concentration, showing 35.26% inhibition of mycelial growth. At 5% concentration, onion (*Allium cepa*) extract exhibited comparatively higher inhibition (42.96%) than other botanicals at the same concentration (Table 1; Fig. 1 & 3). In contrast, custard apple (*Annona squamosa*) leaf extract was the least effective among the botanicals tested. It recorded only 17.33%, 22.52% and 30.19% inhibition at 2.5%, 5% and 10% concentrations, respectively (Fig.1).

The present findings are in close agreement with earlier reports highlighting the antifungal potential of botanicals against *Colletotrichum gloeosporioides* Similarly, Ajay Kumar G. (2014) demonstrated significant inhibition of mycelial growth by extracts of *Allium sativum*, *Lantana camara*, *Azadirachta indica* and *Allium cepa*, with inhibition levels comparable to those recorded in the present study, while eucalyptus extracts showed inhibition around 41-42 per cent. The superior performance of eucalyptus observed in the present study is also consistent with the findings of Amelu *et al*. (2014), who reported the highest mycelial growth inhibition with eucalyptus extracts across all concentrations. Likewise, Pandey *et al*. (2009) observed maximum growth inhibition of *C. gloeosporioides* with leaf extracts of *Morus alba* and *Azadirachta indica*. Collectively, these studies corroborate the strong fungitoxic potential of botanicals and support their use as eco-friendly alternatives for managing mango anthracnose.

In vitro evaluation of essential oils against *Colletotrichum gloeosporioides* (Cg-1)

The *in vitro* evaluation of different essential oils against *Colletotrichum gloeosporioides* (Cg-1). Most of the essential oils exhibited strong antifungal activity and several treatments resulted in complete inhibition of mycelial growth. Among the essential oils tested, basil oil (*Ocimum canum*) and lemongrass oil (*Cymbopogon flexuosus*) were the most effective, exhibiting 100 per cent inhibition of mycelial growth at all concentrations, including the lowest dose of 0.5% (Table 2). This indicates their strong fungitoxic nature and high efficacy even at lower concentrations. Cinnamon oil (*Cinnamomum verum*) and mentha oil (*Mentha arvensis*) were also highly effective against the pathogen. Both oils recorded complete inhibition (100%) at 1% and 2% concentrations, while maintaining high levels of inhibition at 0.5%, with 93.15% and 93.52% inhibition, respectively (Table 2; Fig. 2 & 4). Clove oil (*Syzygium aromaticum*) showed a progressive increase in antifungal activity with increasing concentration, recording 88.33%, 90.56% and 100% inhibition at 0.5%, 1% and 2%, respectively. Neem oil (*Azadirachta indica*) exhibited moderate antifungal activity, with inhibition values of 55.19%, 60.74% and 66.30% at 0.5%, 1% and 2%, respectively, indicating a dose-dependent response (Fig. 2). In contrast, castor oil (*Ricinus communis*) was the least effective among the oils tested, showing only 19.44%, 29.44% and 41.11% inhibition at 0.5%, 1% and 2%, respectively.

The results of the present study are well supported by earlier findings on the antifungal efficacy of essential oils against *Colletotrichum gloeosporioides*. Paudel *et al*. (2022) reported strong bioactivity of cinnamon oil, which significantly inhibited both spore germination and mycelial growth of the pathogen. Similarly, Darshan *et al*. (2019) observed complete inhibition of mycelial growth with cinnamon and thyme oils across low concentrations, while clove oil showed slightly lower but comparable efficacy. Duong *et al*. (2024) further demonstrated the effectiveness of cinnamon leaf and bark oils, alone or in combination

with lemongrass oil, indicating the potential for synergistic use in mango anthracnose management. Supporting these observations, Rabari *et al*. (2018) reported high sensitivity of *C. gloeosporioides* to cinnamon oils, evidenced by large zones of inhibition, while Perumal *et al*. (2016) confirmed complete growth suppression of mango pathogens by thyme, clove and cinnamon oils. Overall, essential oils differed significantly in their antifungal activity against *C. gloeosporioides*, with basil, lemongrass, cinnamon, mentha and clove oils completely inhibiting mycelial growth at low concentrations, indicating their strong potential for eco-friendly management of mango anthracnose

Table 1 : *In vitro* efficacy of different botanicals against *C. gloeosporioides* (Cg-1) at different concentrations.

Tr. No.	Name of botanicals	Botanical Name	Plant part	Con. 2.5 (%)		Con. 5 (%)		Con. 10 (%)	
				Mean colony diameter at 7th DAI (mm)	Per cent growth inhibition (%)	Mean colony diameter at 7th DAI (mm)	Per cent growth inhibition (%)	Mean colony diameter at 7th DAI (mm)	Per cent growth inhibition (%)
T_1	Ginger	*Zingiber officinale*	Rhizome	63.33	29.63	60.50	32.78	56.33	37.41
T_2	Garlic	*Allium sativum*	Clove	58.27	35.26	54.83	39.07	42.83	52.41
T_3	Neem	*Azadirachta indica*	Leaves	72.50	19.44	67.83	24.63	10.50	88.33
T_4	Ghaneri	*Lantana camera*	Leaves	72.17	19.81	68.10	24.33	57.17	36.48
T_5	Jamun	*Syzygium cumini*	Leaves	67.50	25.00	62.63	30.41	35.17	60.93
T_6	Onion	*Allium cepa*	Bulb	63.57	29.37	51.33	42.96	43.67	51.48
T_7	Custard Apple	*Annona squamosa*	Leaves	74.40	17.33	69.73	22.52	62.83	30.19
T_8	Eucalyptus	*Eucalyptus globulus*	Leaves	51.67	42.59	49.50	45.00	22.17	75.37
T_9	Drumstick	*Moringa oleifera*	Leaves	64.77	28.04	54.27	39.70	41.60	53.78
T_{10}	Aloe vera	*Aloe vera*	Leaves	69.33	22.96	66.20	26.44	45.38	49.58
T_{11}	Control			90.00		90.00		90.00	0.00
SE ± (m)				0.70		0.53		0.73	
CD (P=0.01)				2.80		2.12		2.91	

Table 2 : *In vitro* efficacy of different essential oils against *C. gloeosporioides* (Cg-1) at different concentrations.

Tr. No.	Name of essential oils	Botanical name	Con. 0.5 (%)		Con. 1 (%)		Con. 2 (%)	
			Mean colony diameter at 7th DAI (mm)	Per cent growth inhibition (%)	Mean colony diameter at 7th DAI (mm)	Per cent growth inhibition (%)	Mean colony diameter at 7th DAI (mm)	Per cent growth inhibition (%)
T_1	Basil oil	*Ocimum canum*	0.00	100.00	0.00	100.00	0.00	100.00
T_2	Castor oil	*Ricinus communis*	72.50	19.44	63.50	29.44	53.00	41.11
T_3	Clove oil	*Szygium aromatium*	10.50	88.33	8.50	90.56	0.00	100.00
T_4	Cinnamom oil	*Cinnamomum verum*	6.17	93.15	0.00	100.00	0.00	100.00
T_5	Lemongrass oil	*Cymbopogan flexuosus*	0.00	100.00	0.00	100.00	0.00	100.00
T_6	Neem oil	*Azadirachta indica*	40.33	55.19	35.33	60.74	30.33	66.30
T_7	Mentha oil	*Mentha arvens*	5.83	93.52	0.00	100.00	0.00	100.00
T_8	Peppermint oil	*Mentrha piperita* L.	25.50	71.67	19.33	78.52	0.00	100.00
T_9	Control		90.00		90.00		90.00	
SE ± (m)			0.58		0.37		0.53	
CD (P=0.01)			2.35		1.50		2.16	

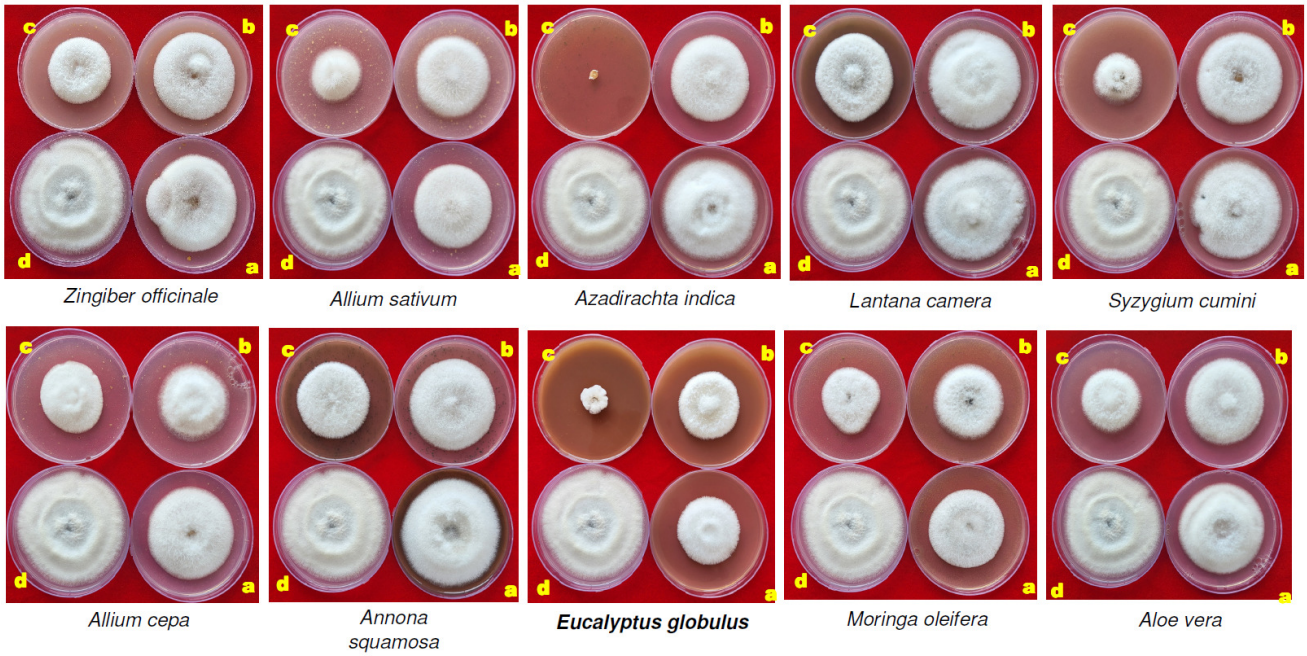

Fig. 3. ***In vitro*** **efficacy of different botanicals against** ***C. gloeosporioides*** **(Cg-1) at different concentrations. a) 2.5% b). 5% c)**

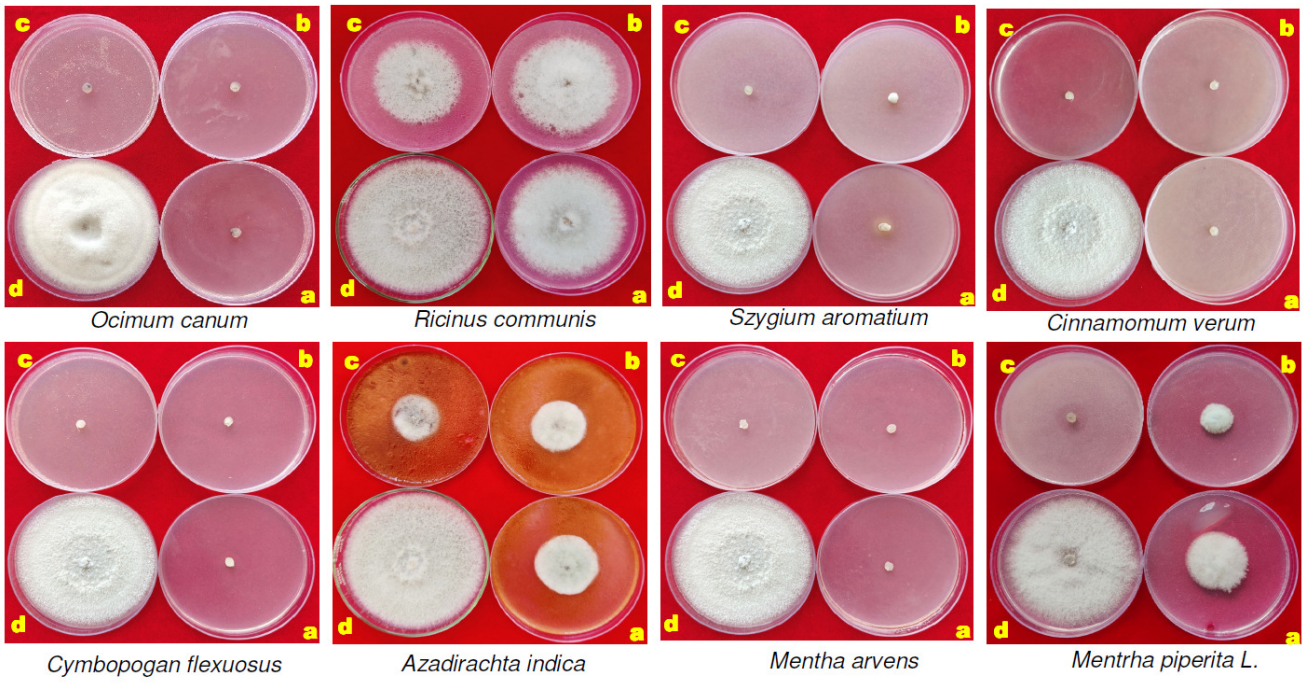

Fig. 4 ***In vitro*** **efficacy of different essential oils against** ***C. gloeosporioides*** **(Cg-1) at different concentrations a) 0.5 %, b) 1%, c) 2% and d) control.**

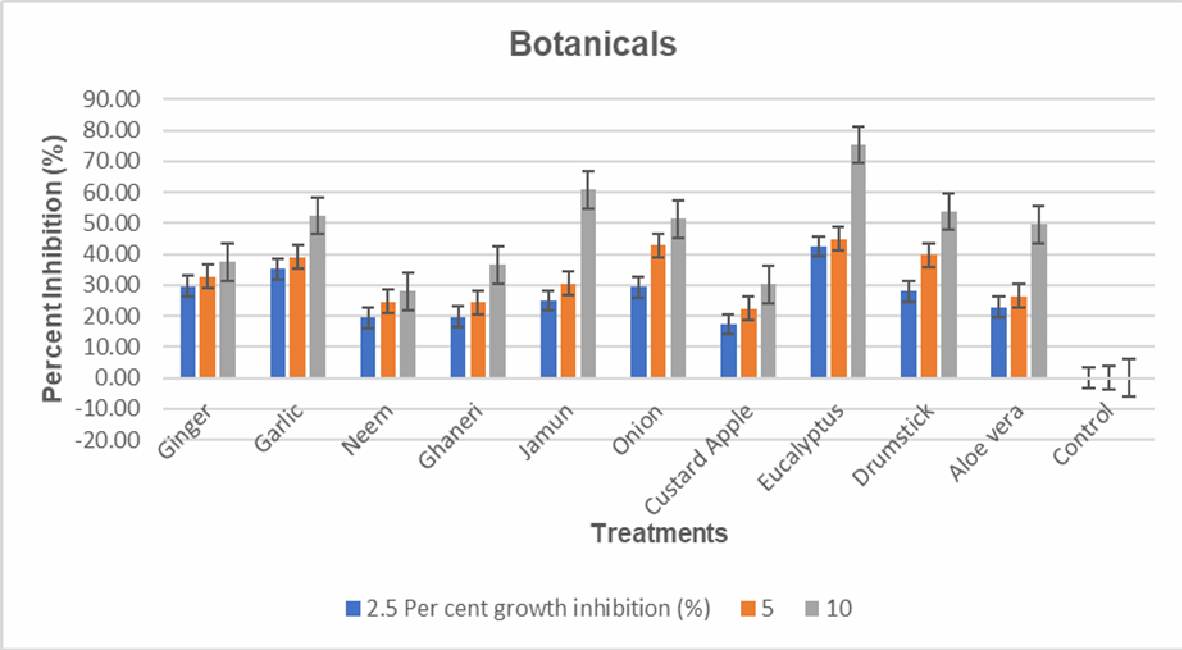

Fig. 1 : *In vitro* efficacy of different botanicals against *C. gloeosporioides* (Cg-1)

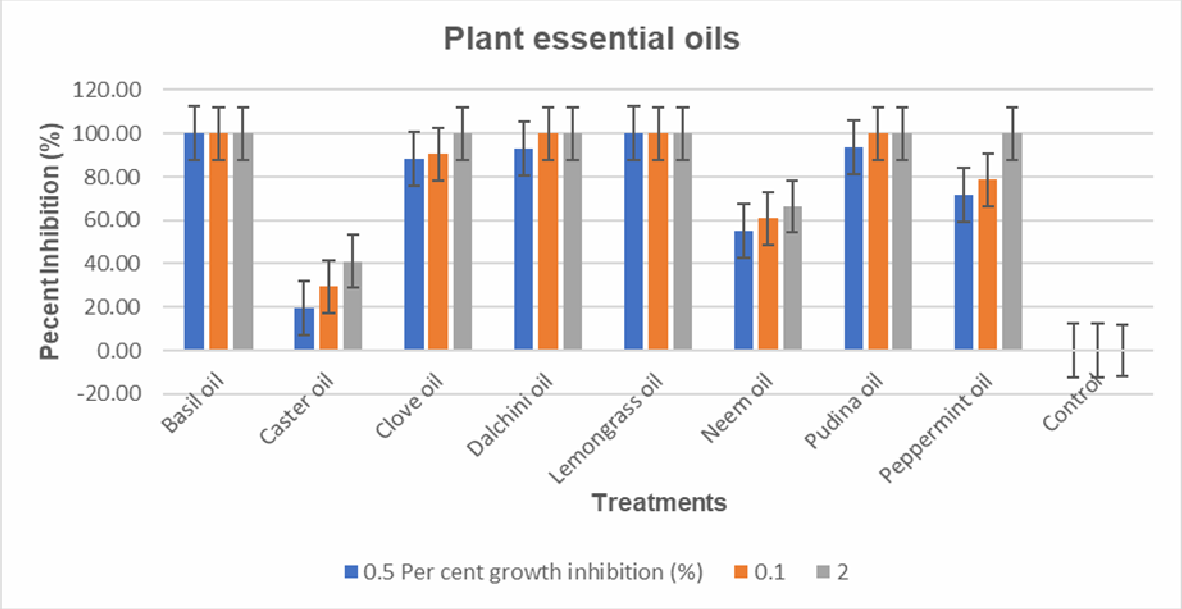

Fig. 2 : *In vitro* efficacy of different essential oils against *C. gloeosporioides* (Cg-2)

Conclusion

Botanical extracts and essential oils showed significant antifungal activity against *Colletotrichum gloeosporioides*. *Eucalyptus globulus* was the most effective botanical, while garlic and onion showed moderate inhibition. Among essential oils, basil and lemongrass completely suppressed mycelial growth even at low concentrations, followed by cinnamon, mentha and clove, whereas neem and castor oils were less effective. These results highlight the potential of eucalyptus, basil and lemongrass as eco-friendly alternatives for managing mango anthracnose.

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