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RESEARCH ARTICLE

Evaluation of antifungal bioactive compounds in essential oils for eco-friendly management of mango anthracnose

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Abstract

Mango anthracnose, caused by *Colletotrichum gloeosporioides*, is an important disease responsible for considerable yield and quality losses both in the field and postharvest conditions. Currently, synthetic fungicides are used to manage anthracnose; however, growing concerns over chemical residues, environmental impact and consumer health have driven the search for safer and more sustainable alternatives. The use of essential oils (EOs) is recognized as a safe and environmentally sustainable alternative to synthetic fungicides. Hence, the *in vitro* study assessed the antifungal effects of selected EOs against *C. gloeosporioides*. A poisoned food bioassay was carried out to evaluate the antifungal effect of EOs (citronella, clove, ginger, ocimum, lemon grass, garlic, cinnamon, black cumin, black pepper, onion). Bioassays consisted of EOs at 0.025, 0.050, 0.075, 0.10, 0.15 and 0.20 % concentration with an untreated control and three replicates. Complete inhibition of mycelial growth was recorded with citronella and cinnamon EOs at 0.050 %, whereas lemon grass and clove EOs required 0.075 % for complete suppression against *C. gloeosporioides,* causing mango anthracnose. Gas chromatography mass spectrometry (GC-MS) analysis revealed key bioactive compounds attributed to antifungal activity: citronellal and D-Limonene in citronella; eugenol in clove; cinnamaldehyde in cinnamon; and neral and geranial in lemon grass essential EOs. These compounds are known to disrupt fungal membranes and enzymatic functions. The concentration-dependent inhibition indicated that these four EOs, rich in specific antifungal constituents, could be effective natural alternatives for managing mango anthracnose.

Keywords: anthracnose; antifungal activity; bioactive compounds; *Colletotrichum gloeosporioides*; GC-MS analysis; mango

Introduction

Mango (*Mangifera indica* L.) is one of the most widely cultivated and economically significant tropical fruit crops in India. India is the world's largest mango producer, contributing over 40 % of the global supply (1). Mango is highly valued for its rich nutritional content and unique flavour, making it one of the most important tropical fruits. It also plays a vital role in supporting the livelihoods of millions of farmers and stakeholders involved in cultivation, postharvest handling, processing and export. Several major diseases, including powdery mildew, bacterial black spot and anthracnose, constrain mango production. Powdery mildew, caused by *Oidium mangiferae*, reduces fruit set through severe flower and fruitlet drop, while bacterial black spot, caused by *Xanthomonas citri* pv. *mangifera indicae* produces dark lesions on leaves, stems and fruits, lowering yield and market value. Among these, the most prominent is anthracnose, a highly destructive disease caused by the fungal pathogen *C. gloeosporioides* (2). This disease primarily affects mango during the flowering, fruit development and postharvest phases, resulting in tissue breakdown and rapid decay during ripening, storage and transit.

Colletotrichum gloeosporioides exhibits a hemibiotrophic lifestyle, characterized by a brief biotrophic phase followed by a shift to necrotrophy, which enables successful colonization of mango tissues (3, 4). The pathogen develops appressoria, melanized infection structures that generate turgor pressure to penetrate the host cuticle and epidermis, facilitating invasion (5). High humidity, rainfall and warm temperatures strongly favour anthracnose epidemics, since conidial germination and appressorium formation are moisture-dependent processes (6, 7). *Colletotrichum gloeosporioides* persists on infected debris, alternate hosts and latently infected tissues, which act as reservoirs for inoculum and sources of reinfection (8, 9). The pathogen displays considerable genetic and pathogenic variability, reflected in differences in virulence, fungicide sensitivity and host adaptation, which complicates its management (10).

Furthermore, quiescent infections are a key feature of postharvest anthracnose: the fungus establishes latent infections during fruit development and resumes activity during ripening, causing severe postharvest losses (6, 11). Postharvest losses of

fresh mango fruits in India have been reported to range between 25 and 40 % (12). Under humid conditions, approximately 25 to 30 % of total mango production is lost due to the incidence of anthracnose and stem-end rot (13). The conventional approach to managing anthracnose has primarily involved the use of synthetic fungicides such as mancozeb, carbendazim, thiophanate-methyl and copper oxychloride. Although these chemicals offer effective short-term control, their prolonged use has led to several concerns, particularly the development of resistant pathogen strains and environmental pollution. In addition, the accumulation of chemical residues on fruits poses risks to human health and food safety (14). Moreover, the increasing demand for safer and more sustainable food production practices has accelerated the search for alternative disease control strategies that are both effective and environmentally friendly.

Among the promising alternatives, plant-derived EOs have garnered attention due to their broad-spectrum antimicrobial properties and low environmental footprint (15). Essential oils are volatile, aromatic secondary metabolites produced by plants, consisting mainly of terpenes, aldehydes, phenols, alcohols and esters. These bioactive compounds exhibit potent antifungal activity against a wide range of phytopathogens, including *Colletotrichum* species (16). The mechanism of action of EOs involves multiple biological targets, making them particularly effective as antifungal agents. Their lipophilic nature allows them to integrate into and disrupt fungal cell membranes, leading to increased membrane permeability, ion leakage and eventual cell lysis (17). It also inhibits critical biosynthetic pathways, including ergosterol production, which is essential for maintaining fungal membrane structure and function (18). In addition, some essential oil components impair mitochondrial activity, generate oxidative stress and trigger programmed cell death in fungal cells (19). Essential oils such as cinnamon (*Cinnamomum zeylanicum*) and oregano (*Origanum vulgare*), as well as their active compounds cinnamaldehyde and carvacrol, inhibited the growth of *Aspergillus flavus* and *Aspergillus parasiticus* and reduced aflatoxin production, specifically AFB_1 and AFB_2 by *A. flavus* and AFB_1, AFB_2, AFG_1 and AFG_2 by *A. parasiticus* (20).

In addition to their efficacy, EOs possess several desirable attributes for postharvest disease management. They are biodegradable, generally recognized as safe (GRAS) and leave minimal residues on treated produce. Their multi-site action reduces the risk of resistance development, a growing problem with conventional fungicides (21). These characteristics make EOs attractive alternatives for integration into sustainable disease management programs. Therefore, this study aims to evaluate the antifungal efficacy of selected plant-derived EOs against *C. gloeosporioides*. In addition, the chemical composition of these Eos will be profiled using chromatographic techniques to correlate specific bioactive constituents with antifungal activity, with an emphasis on their potential application in both pre- and postharvest management of mango anthracnose. This study not only advances the development of eco-friendly strategies for managing mango anthracnose but also offers a sustainable alternative to conventional chemical-based approaches in fruit production systems.

Materials and Methods

Isolation and molecular confirmation of the fungal pathogen

Mango fruits showing typical anthracnose symptoms were collected from an orchard in Dharmapuri district, Tamil Nadu, India (12.39289777 °N, 78.10293899 °E). Symptomatic pericarp tissues (~1 cm^2) were excised from the advancing margins of lesions under aseptic conditions and surface-sterilized using 1 % sodium hypochlorite (NaOCl) for 30 sec, followed by triple rinsing with sterile distilled water. The tissues were blotted dry in a laminar airflow chamber using sterile Whatman No. 1 filter paper and sectioned into four quadrants. The sterilized segments were plated on potato dextrose agar (PDA) medium amended with 100 mg/L streptomycin sulphate to suppress bacterial contamination. Plates were incubated at 28±2 °C with a 12 hr photoperiod for 5 to 7 days. Mycelial outgrowths were subcultured onto two fresh PDA plates using single-tip isolation to obtain a genetically uniform fungal culture. Pathogenicity of the isolated fungus was confirmed by artificially inoculating healthy, surface-sterilized mango fruits with a mycelial plug (5 mm) from an actively growing culture. Inoculated fruits were incubated under humid conditions at 25 ± 2 °C. Typical anthracnose symptoms appeared within 7-10 days. Disease severity

Table 1. Disease scale (0-5) for scoring the percent fruit infection of anthracnose disease on mango fruits

Grade	Description
0	No infection
1	<1 % fruit surface infected
2	1-5 % fruit surface infected
3	6-25 % fruit surface infected
4	26-50 % fruit surface infected
5	>50 % fruit surface infected

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of observations} \times \text{Maximum rating}} \times 100 \quad \text{(Eqn.1)}$$

was assessed using a 0-5 disease scale (Table 1) based on lesion coverage on fruit surface and the percent disease index (PDI) was calculated using the given formula (Eqn. 1) (22). The fungus was re-isolated from symptomatic tissues using the same protocol as described above to fulfil Koch's postulates.

Genomic deoxyribonucleic acid (DNA) was extracted from actively growing mycelia using the cetyltrimethylammonium bromide (CTAB) method. Polymerase chain reaction (PCR) amplification of the internal transcribed spacer ITS region was carried out using universal primers ITS1 and ITS4. Species-specific primers CgF and CgR were used in a separate PCR to target *C. gloeosporioides* (23). The amplified products were visualized on a 1.2 % agarose gel stained with ethidium bromide. The ITS PCR product was purified and sequenced and the resulting sequence was submitted to NCBI GenBank.

Screening of essential oils against *C. gloeosporioides*

The EOs used in this study were procured from a commercial supplier. These included citronella (*Cymbopogon winterianus*; leaves and stems), clove (*Syzygium aromaticum*; flower buds), ginger (*Zingiber officinale*; rhizomes), basil (*Ocimum basilicum*; leaves and flowering tops), lemon grass (*Cymbopogon citratus*; leaves), garlic (*Allium sativum*; bulbs), cinnamon bark (*Cinnamomum cassia*; bark), black cumin (*Nigella sativa*; seeds), black pepper (*Piper nigrum*; dried unripe fruits) and onion (*Allium cepa*; bulbs). The oils were used as received without further purification.

A total of ten botanically derived EOs were screened against *C. gloeosporioides* using the poisoned food technique at different concentrations, viz., 0.025, 0.050, 0.075, 0.10, 0.15 and 0.20 % (v/v) (24). The required volumes of EOs were aseptically amended into autoclaved PDA supplemented with Tween-20 as an emulsifier, previously cooled to 45 °C. Precisely 20 mL of amended media was poured into sterile 90 mm Petri plates under laminar airflow conditions. Actively growing cultures of *C. gloeosporioides* were used as inoculum, from which 5-mm diameter mycelial discs were excised from the periphery of 7-day-old colonies and centrally placed on the EO-infused PDA plates. Negative control plates (untreated check) with PDA and Tween-20 alone were also maintained. Plates were tightly sealed with parafilm to prevent volatilization of EO constituents and incubated at 25 ± 2 °C. Mycelial growth was recorded daily for seven days and antifungal activity was quantified using the formula (25).

$$\text{Percent inhibition} = [(C - T) / C] \times 100 \quad \text{(Eqn.2)}$$

Where, C = mycelial growth of the pathogen in the control plate and T = mycelial growth of the pathogen in the treatment plate. All the treatments were laid out in a completely randomized design (CRD) with three replicates per treatment per concentration. The data were statistically analyzed using OPSTAT software to assess those response relationships and determine the most effective essential oil and minimum inhibitory concentration (MIC).

Gas chromatography - mass spectrometry profiling of selected essential oils

Comprehensive chemical profiling of the selected four EOs was performed at QIMA Quality Inspections, India Pvt. Ltd., Tirupur District, Tamil Nadu, employing a high-resolution gas chromatograph mass spectrometer system comprising of Agilent 8890GC unit interfaced with a 5977C inert MSD single quadrupole mass spectrometer equipped with a 7693A automatic liquid sampler (Agilent Technologies USA). Chromatographic separation of volatile constituents was achieved using a DB-5ms silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness; J&W Scientific, USA), coated with a 5 % phenyl metal polysiloxane stationary phase, providing optimal resolution of semi-volatile and nonpolar compounds. High-purity helium 99.999 % served as a carrier gas maintained at a consistent linear velocity of 1 mL min^{-1} under electronically controlled flow conditions. A splitless injection mode was employed with an inlet temperature of 250 °C, with 1 µL of each essential oil sample diluted in HPLC-grade n-hexane to ensure complete volatilization and minimal thermal degradation. The woven temperature program was optimized to provide maximal component elution efficiency. Initial hold at 50 °C, for 1 min, ramped at 10 °C per min to 300 °C, followed by a final hold for 1 min, facilitating the illusion of both early and late retaining compounds. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with Mass Spectra acquisition in full scan ranging from m/z 40 to 1100, allowing for detailed molecular fragmentation analysis. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively, while the transfer line was maintained at 280 °C to prevent analyte condensation. Compound identification was executed by matching the academic Spectra against NIST 20 Mass Spectral Reference Library using Agilent Mass Hunter workstation software (version B.09.00), employing a reverse match factor ≥80 % as the identification threshold. Where applicable, retention indices RI were calculated related to the homologous series of C7-C30 n-alkanes run under identical conditions to validate compound assignments. Only peaks with a signal-to-noise ratio ≥3:1 and relative abundance >0.5 % were considered for analysis.

Results

Isolation and molecular confirmation of the fungal pathogen

Mango fruits showing typical anthracnose symptoms were collected from an orchard in the Dharmapuri district, Tamil Nadu. Fungal colonies resembling *Colletotrichum* species developed within 5-7 days of incubation. A pure culture was obtained and used for further studies. Based on morphological characteristics, the colony appeared white and fluffy. The conidia were cylindrical with a central oil globule (Fig. 1). Pathogenicity test confirmed the fungus as the causal agent, as typical anthracnose lesions with a maximum PDI of 65.04 % developed on inoculated mango fruits within 7 to 10 days and the same fungus was successfully re-isolated. PCR amplification using ITS1 and ITS4 primers produced a single band of approximately 550 bp and the ITS sequence was submitted to GenBank under the accession number [GenBank PV915532] after sequencing. Further confirmation using species-specific primers CgF and CgR, which have been previously reported in the literature, resulted in a clear band at 450 bp, indicating the species as *C. gloeosporioides.*

Screening of essential oils against *C. gloeosporioides*

In the present study, among the ten different EOs tested, citronella, clove, lemon grass and cinnamon significantly inhibited the

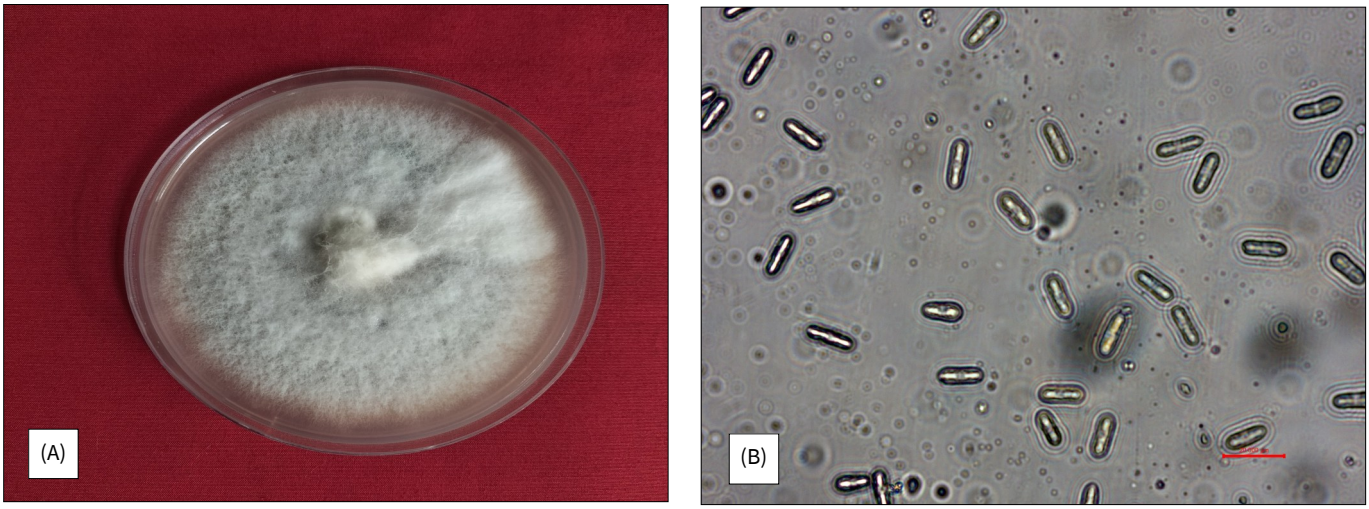
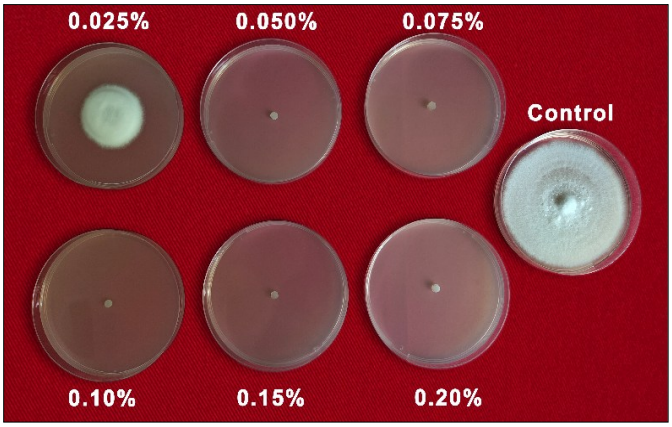
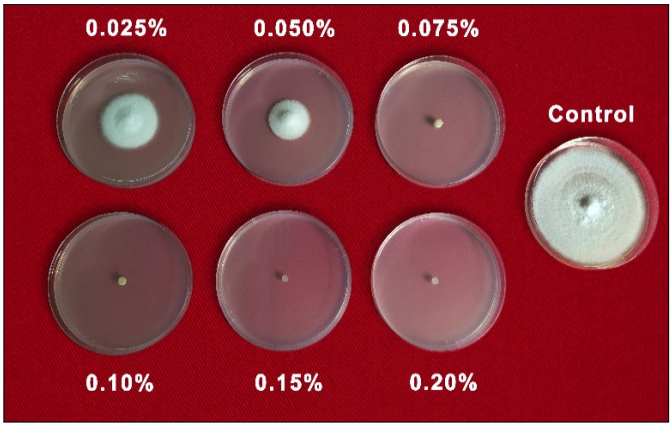


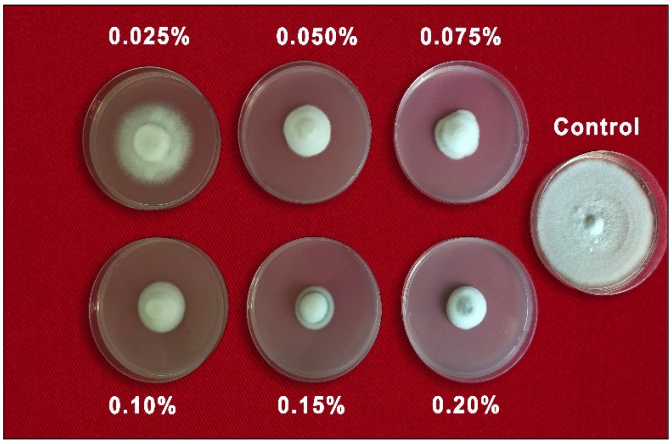
Fig. 1. Cultural characteristics of *C. gloeosporioides.* A) White, fluffy and cottony colony; B) Cylindrical spores with a central oil globule.



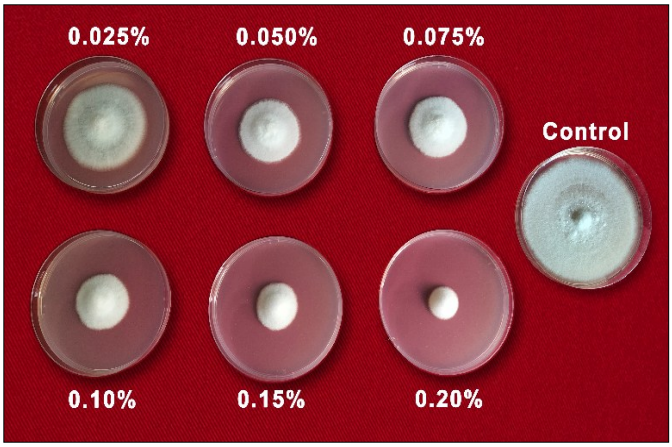
(A) Citronella



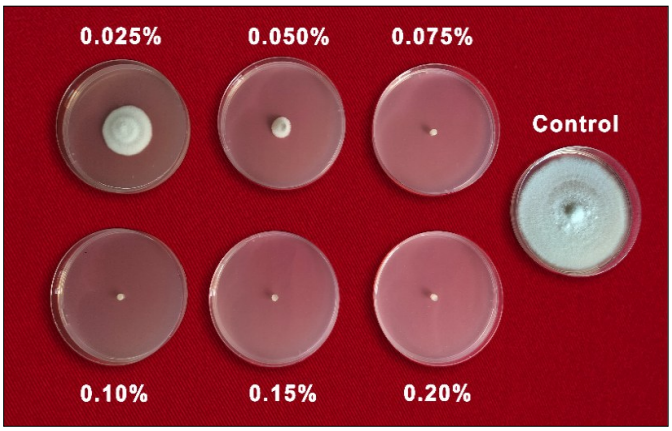
(B) Clove



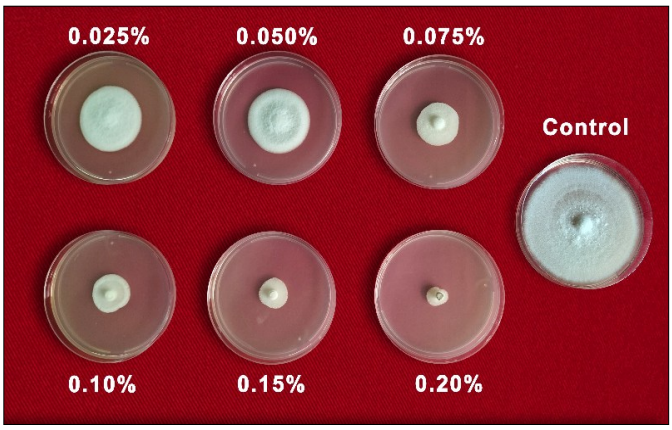
(C) Ginger



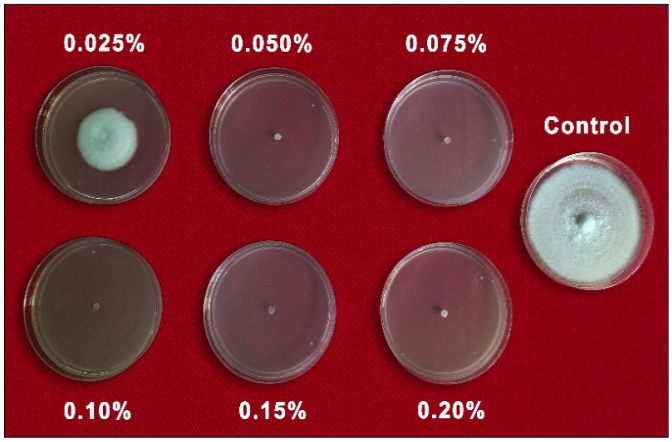
(D) Ocimum



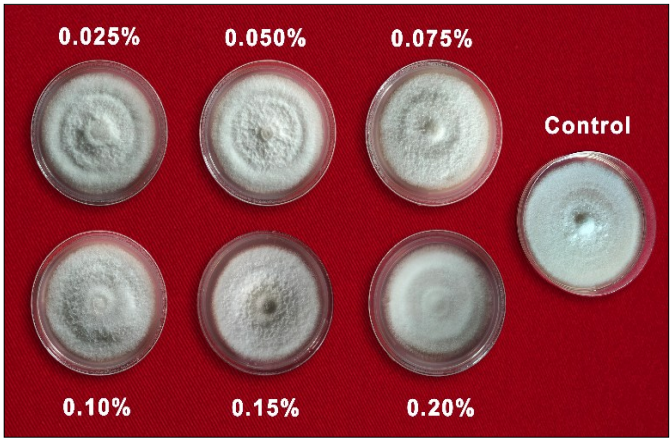
(E) Lemon grass



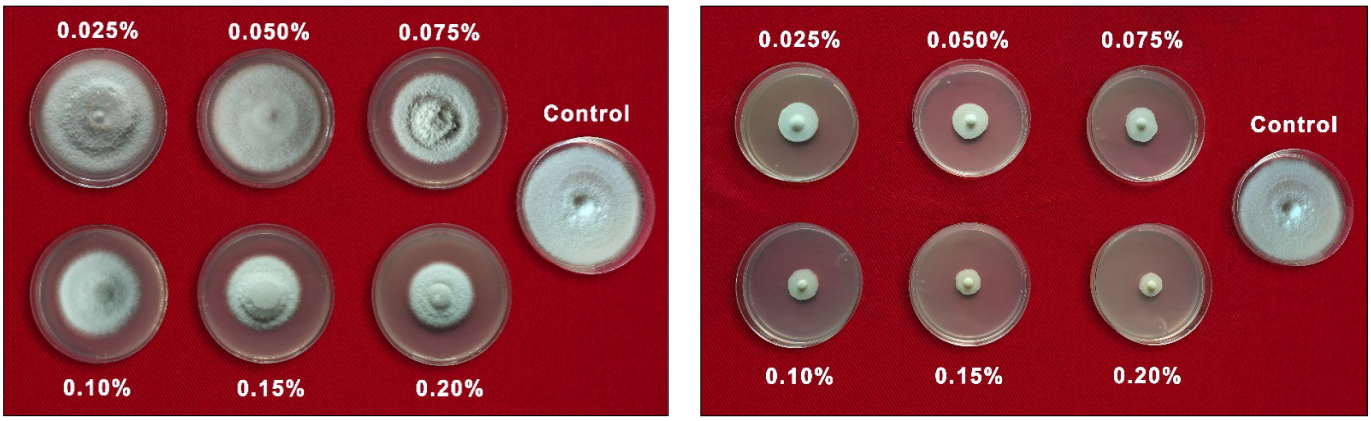
(F) Garlic



(G) Cinnamon



(H) Black cumin



(I) Black pepper **(J) Onion**

Fig. 2. Percent inhibition in mycelial growth of *C. gloeosporioides* when treated with (A) Citronella; (B) Clove; (C) Ginger; (D) Ocimum; (E) Lemon grass; (F) Gralic; (G) Cinnamom; (H) Black cumin; (I) Black pepper; (J) Onion.

Table 2. Screening of different essential oils against the mycelial growth of *C. gloeosporioides*

S. No.	Treatments	Mean mycelial growth (mm)						Percent inhibition over control (%)					
		0.025 %	0.050 %	0.075 %	0.10 %	0.15 %	0.20 %	0.025 %	0.050 %	0.075 %	0.10 %	0.15 %	0.20 %
1.	Citronella	30.00^{a} (5.56)	0.03^{a} (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	66.67	100.00	100.00	100.00	100.00	100.00
2.	Clove	37.00^{b} (6.16)	27.00^{c} (5.29)	0.03^{a} (1.02)	0.03 (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	58.89	70.00	100.00	100.00	100.00	100.00
3.	Ginger	50.00^{e} (7.14)	37.00^{e} (6.16)	34.00^{b} (5.92)	34.00^{c} (5.92)	26.00^{c} (5.20)	25.00^{d} (5.10)	44.44	58.89	62.22	62.22	71.11	72.22
4.	Oscimum	70.00^{f} (8.43)	50.00^{g} (7.14)	48.00^{c} (6.99)	43.00^{d} (6.63)	38.00^{d} (6.25)	30.00^{e} (5.57)	22.22	44.44	46.67	52.22	57.78	66.67
5.	Lemon grass	45.00^{d} (6.78)	23.00^{b} (4.89)	0.03^{a} (1.02)	0.03^{a} (1.015)	0.03^{a} (1.02)	0.03^{a} (1.02)	50.00	74.44	100.00	100.00	100.00	100.00
6.	Garlic	45.00^{d} (6.78)	45.00^{f} (6.78)	34.00^{b} (5.91)	27.00^{b} (5.29)	21.00^{b} (4.69)	15.00^{b} (4.00)	50.00	50.00	62.22	70.00	76.67	83.33
7.	Cinnamon	40.00^{c} (6.40)	0.03^{a} (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	0.03^{a} (1.02)	55.56	100.00	100.00	100.00	100.00	100.00
8.	Black cumin	78.00^{g} (8.88)	76.00^{i} (8.76)	76.00^{f} (8.77)	76.00^{f} (8.77)	75.00^{f} (8.72)	75.00^{g} (8.72)	13.33	15.56	15.56	15.56	16.67	16.67
9.	Black pepper	76.00^{g} (8.77)	70.00^{h} (8.43)	61.00^{e} (7.87)	56.00^{e} (7.55)	51.00^{e} (7.21)	48.00^{f} (7.00)	15.56	22.22	32.22	37.78	43.33	46.67
10.	Onion	38.00^{b} (6.24)	35.00^{d} (5.99)	35.00^{b} (6.00)	26.00^{b} (5.20)	22.00^{b} (4.79)	22.00^{c} (4.79)	57.78	61.11	61.11	71.11	75.56	75.56
11.	Control	90.00^{g} (9.54)	90.00^{j} (9.54)	90.00^{g} (9.54)	90.00^{g} (9.54)	90.00^{g} (9.54)	90.00^{h} (9.54)	0.00	0.00	0.00	0.00	0.00	0.00
	CD	3.041	2.323	2.323	2.076	2.576	1.638						
	SE (d)	1.457	1.113	1.113	0.994	1.234	0.785						

Figures in parentheses are square root transformed values. In a column, means followed by a common letter are not significantly different at the 5 % level according to DMRT.

mycelial growth of *C. gloeosporioides* compared with the other EOs tested (Table 2; Fig. 2). Citronella and cinnamon EOs completely inhibited the mycelial growth of *C. gloeosporioides* (0.00 mm) at 0.050 % concentration, while clove and lemon grass oils achieved complete inhibition (0.00 mm) at 0.075 % concentration, with the same effect observed in all three replications. Garlic, onion, ginger and *Ocimum* EOs exhibited moderate inhibition of mycelial growth at 0.20 %, while black cumin and black pepper EOs had little or no effect. All three control replicates reached the maximum colony diameter of 90 mm.

Gas chromatography-mass spectrometry profiling of selected essential oils

The chemical profile of citronella essential oil indicated the presence of several bioactive compounds. These included 6-octen-1-ol, 3,7-dimethyl-, acetate (93.04 % area, match score 90.6), 2,6-octadien-1-ol, 3,7-dimethyl-, acetate, (Z)- (65.93 % area, match score 86.6), phenol, 2-methoxy-3-(2-propenyl)- (64.40 % area, match score 82.5), 2-cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)- (56.17 % area, match score 68.4), 6-octenal, 3,7-dimethyl-, (R)- (55.62 % area, match score 93.8), 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (Z, E)- (32.99 % area, match score 86.3), eucalyptol (28.89 % area, match score 58.0), D-limonene (15.23 % area, match score 97.8), citronellal (10.92 % area, match score 87.2) and carbonic acid, hexadecyl phenyl ester (8.76 % area, match score 73.7). The complete list of identified constituents is presented in Table 3.

The chemical profile of clove essential oil indicated the presence of several bioactive compounds. These included benzyl mandelate (94.89 % area, match score 96.6), 2-(1-benzyloxy-2-bromoethyl) oxirane (83.24 % area, match score 21.2), formic acid, (3-methyl-2-nitrophenyl) methyl ester (77.51 % area, match score 93.9), 1-(2',2'-dimethylcyclopropylideno)-2-chloroethylene (70.81 % area, match score 2.1), caryophyllenyl alcohol (66.25 % area, match score 31.2), benzyl alcohol (60.75 % area, match score 17.1), phenol, 2-methoxy-4-(2-propenyl)-, acetate (59.79 % area, match score 92.2), 1,5-heptadien-3-yne (59.87 % area, match score 15.6), eugenol (54.67 % area, match score 18.5) and benzene propanoic

Table 3. GC-MS analysis of citronella essential oil

Compound name	Retention time (RT)	Area % (M)	Match score
6-Octen-1-ol, 3,7-dimethyl-, acetate	12.1663	93.04	90.6
2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-	11.3470	56.17	68.4
6-Octenal, 3,7-dimethyl-, (R)-	8.8052	55.62	93.8
Phenol, 2-methoxy-3-(2-propenyl)-	12.3375	64.40	82.5
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	12.4831	65.93	86.6
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z, E)-	12.3885	32.99	86.3
Citronellal	8.8854	10.92	87.2
Carbonic acid, hexadecyl phenyl ester	8.9072	8.76	73.7
D-Limonene	6.7114	15.23	97.8
Eucalyptol	11.6165	28.89	58.0

acid, 4-hydroxy-.alpha.-phenyl-, methyl ester (51.42 % area, match score 59.2). Additional constituents detected included benzene, nitroso- (45.61 % area, match score 78.5), caryophyllene (44.02 % area, match score 12.2), 3H-pyrazole, 5-ethynyl-3,3-dimethyl- (43.19 % area, match score 29.1), p-cresol (38.87 % area, match score 27.1), 1,4,7-cyclododecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (37.45 % area, match score 95.1), bicyclo [5.2.0] nonane, 2-methylene-4,8,8-trimethyl-4-vinyl- (30.46 % area, match score 9.8), 3-allyl-6-methoxyphenol (18.34 % area, match score 30.4), phenol, 3-methyl- (19.60 % area, match score 4.5), 4-chromanol (13.95 % area, match score 29.2), 1,5-hexadiyne (5.81 % area, match score 86.6) and caryophyllene oxide (3.44 % area, match score 90.9). The complete list of identified constituents is presented in Table 4.

Gas chromatography-mass spectrometry analysis of cinnamon essential oil revealed several bioactive compounds. These included 2-propenal, 3-phenyl- (98.69 % area, match score 77.2), acetic acid, cinnamyl ester (72.90 % area, match score 77.1), cinnamaldehyde, (E)- (63.76 % area, match score 88.7), benzene, 1-methyl-3-(1-methylethyl)- (62.33 % area, match score 33.1), eucalyptol (56.13 % area, match score 77.1), 2-propen-1-ol, 3-phenyl-, (E)- (35.68 % area, match score 28.1), benzene, 1,3-hexadienyl- (35.85 % area, match score 11.6), cinnamaldehyde dimethyl acetal (31.10 % area, match score 32.5) and α-terpineol (31.44 % area, match score 50.9). Other compounds detected included 2-propenal, 2-methyl-3-phenyl-, 3-phenyl-1-propanol, acetate, cyclohexanol derivative, hexa-2,4-dienylbenzene and linalool. Details of all identified compounds are provided in Table 5.

The chemical profile of lemon grass essential oil indicated the presence of several bioactive compounds. These included 2,6 -octadiene-1,8-diol, 2,6-dimethyl- (81.80 % area, match score 86.1), 1,3,6-octatriene, 3,7-dimethyl-, (Z)- (80.74 % area, match score 89.6), bromoacetic acid, dodecyl ester (90.83 % area, match score 24.0), D-limonene (72.82 % area, match score 36.8), trans-Isoeugenol (64.52 % area, match score 17.4), geraniol (63.35 % area, match score 53.6). Other minor compounds detected included

Table 4. GC-MS analysis of clove essential oil

Compound name	Retention time (RT)	Area % (M)	Match score
1,4,7, -Cyclododecatriene, 1,5,9,9-tetramethyl-, Z, Z, Z-	7.3199	37.45	95.1
1,5-Heptadien-3-yne	1.5599	59.87	15.6
1,5-Hexadiyne	6.0112	5.81	86.6
1-(2',2'-Dimethylcyclopropylideno)-2-chloroethylene	9.6991	70.81	2.1
2-(1-Benzyloxy-2-bromoethyl) oxirane	1.8182	83.24	21.2
3-Allyl-6-methoxyphenol	5.2476	18.34	30.4
3H-Pyrazole, 5-ethynyl-3,3-dimethyl-	6.1185	43.19	29.1
4-Chromanol	3.6636	13.95	29.2
Benzene, nitroso-	1.9967	45.61	78.5
Benzene propanoic acid, 4-hydroxy-. alpha. -phenyl-, methyl ester	0.4645	51.42	59.2
Benzyl alcohol	0.6505	60.75	17.1
Benzyl mandelate	8.0840	94.89	96.6
Bicyclo [5.2.0] nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	6.8423	30.46	9.8
Caryophyllene	4.9518	44.02	12.2
Caryophyllene oxide	2.5878	3.44	90.9
Caryophyllenyl alcohol	5.2007	66.25	31.2
Eugenol	9.6958	54.67	18.5
Formic acid, (3-methyl-2-nitrophenyl) methyl ester	8.9483	77.51	93.9
Phenol, 2-methoxy-4-(2-propenyl)-, acetate	0.8849	59.79	92.2
Phenol, 3-methyl-	3.2533	19.60	4.5
p-Cresol	8.2874	38.87	27.1

Table 5. GC-MS analysis of cinnamon essential oil

Compound name	Retention time (RT)	Area % (M)	Match score
2-Propen-1-ol, 3-phenyl-, (E)-	5.4270	35.68	28.1
2-Propenal, 2-methyl-3-phenyl-	0.7455	14.09	80.2
2-Propenal, 3-phenyl-	1.9872	98.69	77.2
3-Phenyl-1-propanol, acetate	7.0686	0.55	81.5
Acetic acid, cinnamyl ester	0.7404	72.90	77.1
Benzene, 1,3-hexadienyl-	8.6310	35.85	11.6
Benzene, 1-methyl-3-(1-methylethyl)-	0.6356	62.33	33.1
Cinnamaldehyde dimethyl acetal	7.2961	31.10	32.5
Cinnamaldehyde, (E)-	4.7221	63.76	88.7
Cyclohexanol, 1-methyl-4-(1-methylethylidene)-	7.6079	11.96	71.3
Eucalyptol	4.9380	56.13	77.1
Hexa-2,4-dienylbenzene	0.2542	52.27	42.8
Linalool	6.3641	10.79	3.1
α-Terpineol	9.0757	31.44	50.9

Table 6. GC-MS analysis of lemon grass essential oil

Compound name	Retention time (RT)	Area % (M)	Match score
(-)-cis-Isopiperitenol	7.5555	24.93	41.0
(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3methylenetricyclo[4.4.0.0^2,7]decane-rel-	2.8975	22.88	7.7
(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta [1,3] cyclopropa [1,2] benzen-3-ol	8.0812	16.12	93.0
(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa [1,2]benzen-3-ol	8.0367	63.34	87.1
(E)-1-Methyl-4-(6-methylhept-5-en-2-ylidene)cyclohex-1-ene	5.3934	18.66	89.3
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	3.1800	80.74	89.6
2,3-Dehydro-1,8-cineole	4.2711	11.01	22.8
2,6-Octadiene-1,8-diol, 2,6-dimethyl-	0.0695	81.80	86.1
2,7-Dimethyl-2,7-octanediol	2.2211	51.07	41.7
2-((3,3-Dimethyloxiran-2-yl)methyl)- 3-methylfuran	9.4291	11.99	33.8
3,7-Dimethyloct-6-ene-1,2,3-triol	7.0302	32.32	51.9
3-Nitro-2-methyl propene	9.6245	36.36	97.2
3-Pentyn-1-ol	3.0088	25.18	49.7
4-Nonanone	6.0956	28.48	3.7
5-Hepten-2-one, 6-methyl-	2.7865	50.27	5.1
Bromoacetic acid, dodecyl ester	1.4489	90.83	24.0
Camphene	2.4206	48.95	98.6
Cyclopropanemethanol, 2,2-dimethyl-3-(2-methyl-1propenyl)-	2.3764	67.21	76.2
D-Limonene	6.3231	72.82	36.8
Geraniol	0.9029	63.35	53.6
Naphthalene, octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	1.8652	83.53	32.1
Neral	6.7756	4.08	59.1
Octanal	2.2650	1.66	51.2
trans-Isoeugenol	6.9094	64.52	17.4
trans-p-Mentha-2,8-dienol	1.3752	38.67	93.7
trans-β-Ocimene	9.2469	34.11	11.3
α-Pinene	5.1381	1.31	98.6
β-Pinene	6.0230	1.02	77.2

camphene, 4-nonanone, neral, α-pinene, β-pinene and octanal. Details of all identified compounds are provided in Table 6.

Discussion

The pathogen isolated from symptomatic mango fruits was identified as *C. gloeosporioides* based on morphology, pathogenicity and molecular assays. The colony and conidial features observed in this study were in agreement with earlier descriptions of the species (26, 27). Pathogenicity tests reproduced typical anthracnose symptoms, thereby fulfilling Koch's postulates, while PCR amplification using ITS and species-specific primers confirmed the identity as *C. gloeosporioides*, consistent with previous reports on mango anthracnose (28, 29).

The present study reveals the significant antifungal activity of selected EOs against *C. gloeosporioides*, the causal agent of mango anthracnose. Among the tested EOs, citronella, cinnamon, lemon grass and clove oils showed potent inhibitory effects on mycelial growth, with citronella and cinnamon achieving complete inhibition at 0.050 % concentration and lemon grass and clove at 0.075 %. These findings are in agreement with several prior studies showing the antifungal efficacy of EOs against *Colletotrichum* spp. and other plant pathogens (30, 31). Essential oils may affect the metabolic pathways of microorganisms (32). The hydrophobic nature of EOs and their components enables them to penetrate fungal cell membrane lipids and mitochondria, leading to the accumulation of these compounds in the cell membrane of pathogens and resulting in energy depletion (33). Similar mechanisms have been proposed, suggesting that the effectiveness of EOs is due to their ability to disrupt membrane structure and function, ultimately causing cell death (34).

Citronella oil exhibited a strong inhibitory effect on the conidia of *Aspergillus niger* by rupturing the cell wall and affecting the protoplasm (35). Similarly, EOs of thyme, lemon grass and citronella completely suppressed the mycelial growth of *Colletotrichum* sp. on onion seeds at 2000 ppm (36). In the present study, GC-MS analysis identified citronellal (10.92 % area, match score 87.2) and D-limonene (15.23 % area, match score 97.8) as the major antifungal constituents of citronella oil. These monoterpenoids are known to disrupt fungal cell membranes and interfere with metabolic processes, causing leakage of intracellular contents and ultimately cell death (37, 38). Citronellal, in particular, has been reported to inhibit spore germination and mycelial elongation in *C. gloeosporioides* and other phytopathogenic fungi. At the same time, D-limonene enhances this effect through synergistic disruption of membrane integrity (39). Moreover, citronella oil containing citronellal and geraniol exerts strong antifungal activity by altering membrane permeability and interfering with metabolic processes, with geraniol shown to induce apoptosis-like cell death in fungi (40, 41).

The potent antifungal activity of cinnamon oil is primarily attributed to its high content of cinnamaldehyde, which disrupts fungal cell membrane integrity, leading to leakage of cellular contents and inhibition of growth (42, 43). Cinnamaldehyde has been widely reported to inhibit mycelial growth and spore germination in several phytopathogenic fungi, including *Fusarium*

spp. and *Colletotrichum* (43, 44). In the present study, cinnamon oil completely inhibited the mycelial growth of *C. gloeosporioides* at the lowest concentration tested (0.50 %). Gas chromatography-mass spectrometry analysis confirmed cinnamaldehyde as the dominant bioactive compound in cinnamon oil, accounting for 63.76 % of the total composition with a high match score of 88.7, underscoring its key role in the oil's potent antifungal activity. Minor constituents, such as eucalyptol and cinnamaldehyde derivatives, may further enhance this antifungal potential through additive or synergistic effects (45). The high concentration of cinnamaldehyde and other bioactive compounds explains the strong inhibitory effect observed in the mycelial growth assay, highlighting cinnamon oil and, particularly, cinnamaldehyde as a promising natural antifungal agent.

Clove oil, rich in eugenol, exhibited significant inhibition of *C. gloeosporioides*, primarily by disrupting the fungal cell membrane and causing leakage of intracellular contents (16). Eugenol has been widely reported to inhibit fungal enzyme activity, alter membrane permeability and reduce spore germination, contributing to its potent antifungal activity (18, 46). Gas chromatography-mass spectrometry analysis of the clove oil used in this study identified eugenol (54.67 % area, match score 18.5) and β-caryophyllene (44.02 % area, match score 12.2) as the major bioactive compounds. β-Caryophyllene acts synergistically with eugenol, reinforcing membrane disruption and interfering with metabolic pathways, thereby enhancing the overall antifungal effect (47).

Lemon grass oil exhibited significant antifungal activity, achieving complete inhibition of *C. gloeosporioides* at 0.75 % concentration. Gas chromatography-mass spectrometry analysis identified D-limonene (72.82 % area, match score 36.8), geraniol (63.35 % area, match score 53.6) and neral (4.08 % area, match score 59.1) as the major bioactive compounds. Geraniol and neral are known to interfere with fungal enzyme systems and cell wall synthesis, while D-limonene disrupts membrane integrity, collectively leading to effective inhibition of mycelial growth (38, 40, 48). The high content of these monoterpenoids in lemon grass oil accounts for its antifungal effects. However, garlic and onion oils exhibited moderate inhibition, due to sulfur-containing compounds such as allicin, which are known to interfere with fungal respiration and enzyme function (49). Previous studies (50, 51) have also reported the strong antimicrobial and antifungal effects of allicin and related sulfur volatiles.

The extent of inhibition across all tested oils was clearly concentration-dependent, indicating the necessity of optimizing EO dosages for effective fungal control. These findings are consistent with earlier reports suggesting that the efficacy of EOs against *C. gloeosporioides* depends not only on their chemical composition but also on their formulation and application rate (52). Furthermore, the synergistic effects between EO components and their mode of application (e.g., fumigation, emulsification, or encapsulation) may significantly influence their antifungal potential (53).

Conclusion

The present study confirms the antifungal potential of selected EOs against *C. gloeosporioides*, the pathogen responsible for mango anthracnose. Among the oils tested, citronella, cinnamon, lemon grass and clove showed significant mycelial growth inhibition at concentrations as low as 0.075 %. These results highlight the potential of specific EOs as natural, eco-friendly alternatives to chemical fungicides. Further studies are needed to develop suitable formulations of these EOs and to evaluate their efficacy under field conditions. Additionally, assessing the safety of these products for humans and the environment, along with determining the optimal modes of application, is essential to ensure their practical and effective use in mango disease management.

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Authors' contributions

SM carried out laboratory experiments and wrote the article. SV designed the experiment and made corrections in the manuscript. VG over-viewed the manuscript and corrected the article. SP and KPR reviewed the manuscript. VS made corrections in the manuscript. GV overviewed the manuscript. All the authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

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