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Efficacy of plant-derived essential oils in post-harvest management of anthracnose disease on mango fruits

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Abstract

The harmful effects of chemical pesticides have elicited the evolvement of eco-friendly natural products in plant disease management. This study evaluated the antifungal activity of six plant-based essential oils (EOs) for the management of anthracnose disease on infected mango fruits. The pre-characterized EOs were assessed at seven concentrations which varied between 50 and 2000 ($\mu L/L^{-1}$ against mycelial growth of *Colletotrichum gloeosporioides in vitro* and *in vivo*. Mycelial growth inhibition (MGI), minimum complete inhibition (MCI) and percent conidia germination (PCG) were determined based on standard procedures. The *in vivo* trial tested the ability of EOs to mitigate anthracnose disease development on inoculated but treated fruits. The EOs of clove (*Syzygium aromaticum*), and thyme (*Thymus vulgaris*) had 100% MGI, attained MCI at 1000 $\mu L/L^{-1}$ and had zero PCG. These were significantly ($P<0.05$) higher than the other treatments including metalaxyl fungicide, the positive control. Although absolute disease control could not be achieved using the EOs *in vivo*, anthracnose incidence was, however, significantly reduced to between 62.8 and 92.5% in artificially inoculated and treated mango fruits. EOs from *S. aromaticum* and *T. vulgaris* at a concentration of 2000 $\mu L/L^{-1}$ were more effective than metalaxyl fungicide in reducing anthracnose disease development on inoculated mango fruits. They recorded percentage control of 87.5% and 92.5%, respectively. These findings imply that the use of clove and thyme EOs as antifungal agents have potential of replacing the indiscriminate application of metalaxyl and other synthetic fungicides in mango orchards and postharvest preservation against anthracnose disease.

Key words: Anthracnose incidence, *Colletotrichum gloeosporioides*, essential oil, fungicide, orchard

Introduction

Mango (*Mangifera indica* L.) is an important fruit crop which is widely cultivated across the tropical and sub-tropical regions of the world. Its fresh fruits are important sources of vitamins A, C, K and beta carotene (Angasu *et al*., 2014). However, its yield and harvest quality are often constrained by abiotic and biotic factors. Some of these factors influence fruit shelf life. Fruit diseases are among the major biotic limitations to its production, especially during post-harvest period. They are largely characterized by relatively high moisture and nutrient content that predispose them to quick deterioration by plant pathogens during ripening and post-harvest (Tucho *et al*., 2014).

Anthracnose caused by the fungus, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc, is one of the most important diseases of mango (Gava *et al*., 2018; Konsue *et al*., 2020). The pathogen has a wide host range. Fungi in the *Colletotrichum* genus can infect the whole plant, including the below and above ground parts. In mango, it infects flowers, leaves, branches and fruits (Corkidi *et al*., 2006). *Colletotrichum gloeosporioides* is widely recognized as the most important field and post-harvest disease of mango worldwide (Akem, 2006; Rungjindamai, 2016; Zakaria *et al*., 2020). The disease is associated with an economic loss of about $2000/ton in the international mango trade (Alvindia *et al*., 2020).

Hitherto, anthracnose disease incidence had been predominantly managed under field conditions through application of conventional fungicides with impressive results (Sundravadana *et al*., 2007; Hameed *et al*., 2016). However, disease control using conventional chemicals is now increasingly unattractive in the international trade on fruits including mango because of public concerns about residues exceeding WHO limits which may be harmful to the consumers (Ji *et al*., 2018). In addition, indiscriminate use of fungicides encourages emergence of pathogenic strains and alters existing plant-pathogen equilibrium (Perumal *et al*., 2017). Therefore, bioassay of natural products with fungicidal potential are increasingly becoming invaluable in disease management as they minimize the harmful effects of synthetic pesticides on the environment.

The application of essential oils (EOs) is an eco-friendly method of managing post-harvest diseases of fruits (Sivakumar and Bautista-Banos, 2014; Sarkhosh *et al*., 2017) and a safer alternative to synthetic fungicides (Calo *et al*., 2015). Plant-derived EOs exhibit medicinal properties making them better management option, mainly due to having a wide range of action mechanisms, biodegradability and very low toxicity levels (Raja, 2014). Further, they are less likely to induce pathogen resistance (Scariot *et al*., 2020). Mango fruits are perishable and highly susceptible to

anthracnose disease. Although attempts at controlling the disease in mango orchards using non-systemic chemicals have previously been made, such efforts were mainly concentrated on biological control using resident antagonists (Zhou *et al*., 2018; Kosue *et al*., 2020) and physical approach with hot water treatment (Angasu *et al*., 2014; Sripong *et al*., 2015). Biological control is often fraught with issues of safety concerns (Marikunte *et al*., 2011; Bautista Rosales *et al*., 2014; Da Cunha *et al*., 2014), especially on edible fruits, while hot water treatment could impair fruit quality and shelf life (Sripong *et al*., 2015; Seid *et al*., 2017). Owing to these shortfalls, it has become expedient to explore the use of essential oils in anthracnose disease management. Although bioactivity of some EOs against post-harvest pathogens of mango had been reported, most of them are *in vitro* assays. The objective of this study was to evaluate the effects of varying concentrations of EOs from thyme, lemon grass, cassia, ginger, clove and eucalyptus on *in vitro* growth inhibition of *C. gloeosporioides* and to determine their fungicidal potential in managing mango fruit decay incited by the fungus.

Materials and methods

Source of essential oils and fungal isolate

Six plant-extracted essential oils were tested in this study. They included-thyme (*Thymus vulgaris* L.), lemon grass (*Cymbopogon citratus* (de Candolle) Stapf, cassia (*Senna siamea* (Lam) H.S. Irwin & Barneby, ginger (*Zingiber officinale* (L.) H. Karst), clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry and eucalyptus (*Eucalyptus globulus* Labill.). These were purchased from Bett Organics, Lagos, Nigeria (Table 1). The pathogenic *C. gloeosporiodes* NCG-19 isolate used in this study was obtained from the Plant Pathology Culture Collection of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

Experimental design and study treatments

The experiments including *in vitro* and *in vivo* bioassays were laid out in a 6×7 factorial in a completely randomized design. The factors included the six essential oils and their concentrations. Seven EO concentrations (50, 100, 300, 600, 1000, and 1500, and 2000 $\mu L/L^{-1}$) were tested. The experiment was replicated thrice.

In vitro assay of essential oils against mango fruit rot pathogen

Bioactivity of the EOs was evaluated against the test pathogen using modified agar diffusion method described by Yooussef *et al.* (2016). The different EO concentrations i.e. 50, 100, 300, 600, 1000, and 1500, and 2000 $\mu L/L^{-1}$ were amended with 0.05% polysorbate 80 (Tween 80) emulsifier before being dispensed into Petri dishes containing 12 ml potato dextrose agar (PDA) with chloramphenicol (100 mg L^{-1}), chlortetracycline (75 mg L^{-1}) and streptomycin sulphate (100 mg L^{-1}) and allowed

Table 1. The essential oil constituents obtained by Gas Chromatography-Mass Spectrometry (GC-MS) from the supplier (Bett Organics, Lagos, Nigeria)

Essential oil	Major compounds (%)
Syzygium aromaticum	Eugenol (77.09), â-caryophyllene (10.22), carvacrol (4.63), α-humulene (3.08)
Eucalyptus globulus	1, 8 Creole (71.08%), p-cymene (11.34), citronellol (6.88), linalool (4.87), aromadendrene (3.41)
Zingiber officinale	α-Zingiberene (24.55), â-phelladrene (17.71), camphene (15.13), α-curmemene (14.31), â-sesquiphellandrene (13.07)
Thymus vulgaris	Thymol (84.11), p-cymol (5.89), Terpinene (4.16), Limonene (2.88),
Cymbopogon citratus	Citral (38.33), geraniol (36.96), citronellol (5.20), limonene (3.08), E-caryophyllene (1.78)
Senna siamea	Linalool (17.80), β-damascenone (14.07), 1-octanal (5.83), iso-italicene (19.05)

25 min to solidify before inoculation with the pathogen. A circular disk of an actively growing pure culture of *C. gloeosporioides* was cut from the edge of a fungal colony using 0.4 mm sterile cork borer and inoculated with EOs. The control treatment comprised inoculated PDA plates without EO amendment. The inoculated plates were incubated at room temperature (27±2 °C) with alternating periods of 12 hr darkness and 12 hr light, Daily measurements of mycelial growth were made until the replicate control treatments were fully covered by fungal mycelia. This period took 10 days. Mycelial growth inhibition (MGI) was determined as a percentage of mycelial growth with EO against the control as:

$MGI = xc - yc/xc$

Where:

xc = colony diameter of control, *yc* = colony diameter of treatment

Effect of essential oils on spore germination

Bioassay of the EOs against *C. gloeosporioides* spore germination was carried out on PDA. For each EO and respective concentration, an aliquot containing 5 ml was dispensed into 10 ml capacity sterile test tubes. Seven concentrations: 50, 100, 300, 600, 1000, 1500, and 2000 $\mu L/L^{-1}$ were prepared as previously described and replicated thrice. Spore suspensions were prepared from actively growing cultures by adding 10 ml of sterile distilled water and Tween 80 detergent to dislodge spores. Spore suspensions were filtered through sterile muslin cloth. The initial spore

concentration for each suspension was determined using hemocytometer under 400× magnifcation through inversed optical microscopy and adjusted to an inoculum concentration of 1×10^5 conidia/ml. An aliquot of 10 ml of spore concentration was dispensed and spread evenly on PDA plates amended with respective EOs. Control treatments were amended with Tween 80 alone. PDA plates were incubated at 27±2 °C for 24 h. Percentage spore germination was determined using a random sample of at least 100 spores under photomicroscope (Olympus X330 photomicroscope, model CX31RTSF made in Tokyo, Japan) at 24 h after incubation (Samavat and Asl, 2019). Germinated spore count was made after 48 h for validation of results.

In vivo effects of essential oils on anthracnose fruit rot disease severity

Healthy moderately ripe fruits of a susceptible mango variety, Lipen, were obtained from mango orchard at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria and used in the *in vivo* assay. The harvested fruits were carefully selected to ensure that they were free from insect infestation, pathogens or mechanical injury. The experiment was arranged in a completely randomized design with seven concentrations: 50, 100, 300, 600, 1000, and 1500, and 2000 $\mu L/L^{-1}$ and three replicates. The fruits were washed with clean tap water and surface-sterilized with 5% sodium hypochlorite and subsequently rinsed with sterile distilled water. They were wounded in the middle using a sterile 2 cm cork borer. Thereafter, three replicate fruits were artificially inoculated with each of seven concentrations with a total of 21 fruits per treatment. They were inoculated with 100 µl of 1×10^5 conidia/ml inoculum concentration using micro pipette. Test EOs were then sprayed 24 h after inoculation using hand sprayer. Inoculated fruits were incubated at room temperature in perforated plastic trays for 10 days. Rot development was assessed visually. The diameter of the resulting lesions was measured on each fruit. Percent *in vivo* control was then calculated as follows:

$$\textit{Percent Disease Control} = (LD1 - LD2/LD2) \times 100$$

Where:

$LD1$ = lesion diameter on inoculated mango fruit but without EO treatment (negative control) and $LD2$ = the lesion diameter on mango fruit inoculated with the pathogen and treated with EO.

Statistical analysis

All numerical data obtained in this study were subjected to analysis of variance (ANOVA) and mean treatment differences compared by Tukey's Honest Significant Difference (HSD) test at 5% probability level using Statistical Analysis System (SAS) Institute Cary, NC, and USA, Ver. 9.2.

Results

In vitro assay of essential oils against mango fruit rot pathogen

The EOs evaluated in this study had dose and species-dependent inhibitory effects on mycelial growth of *C. gloeosporioides*. The EOs were least effective at the lowest concentration of 50 µL/L^{-1} with mycelial growth inhibition (MGI) varying between 10.0 and 28.7%, but significantly ($P<0.05$) higher than the control (Table 2). However, the efficacy of the synthetic metalaxyl fungicide which served as positive control was significantly higher than the EO at this concentration (50 µL/L^{-1}). *Syzygium aromaticum* had the highest MGI of 65.3% on the test pathogen at a concentration of 100 µL/L^{-1}. This was significantly ($P<0.05$) higher than other treatments including metalaxyl and the control. Overall, the efficacy of the EOs improved with increasing concentration. The EOs from *S. aromaticum* and *T. vulgaris* recorded absolute control of the pathogen at 1000 µL/L^{-1} with 100% MGI and this was significantly ($P<0.05$) higher than the other treatments including metalaxyl fungicide (positive control) which failed to achieve total control at same concentration. Thus, the minimum complete inhibition (MCI) for *S. aromaticum* and *T. vulgaris* was 1000 µL/L^{-1}. Comparatively, *S. siamea*, *E. globulus* and *C. citratus* EOs achieved MCI at the highest concentration of 2000 µL/L^{-1}. However, *Z. officinale* EO could not attain 100% antifungal activity even at the highest concentration of 2000 µL/L^{-1} with an MGI of 86.1% which was significantly ($P<0.05$) lower than the other treatments.

Effect of essential oil on spore germination

Conidia germination was significantly ($P<0.05$) inhibited by all the EOs irrespective of concentration (Table 3). The EOs were generally less effective against conidia germination at lower concentrations. Up to 100 µL/L^{-1}, percent conidia germination (PCG) was above 50% for all EOs tested. The EOs obtained from *S. siamea, Z. officinale* and *C. citratus* were less effective in inhibiting germination. At 300 µL/L^{-1}, PCG was still greater than 50% for the EOs. Treatments with *S. aromaticum* and *T. vulgaris* EOs were the most effective against the conidial growth. These two resulted into complete inhibition of spore germination at $\geq$ 1000 µL/L^{-1} concentrations (Table 3). The performance of these two treatments was also better than metalaxyl fungicide at same concentration. These were followed by *S. siamea* and *C. citratus* EOs which recorded complete inhibition of spore germination at 2000 µL/L^{-1}. However, EO treatment with *Z. officinale* and *E. globulu*s were the least effective, being unable to completely inhibit conidial germination of *C. gloeosporioides* even at the highest concentration of 2000 µL/L^{-1} with PCG of 10.9 and 8.2%, respectively.

Effects of essential oils on fruit rot in vivo and disease development

Anthracnose symptoms developed on fruits that were inoculated with *C. gloeosporioides* (Fig. 1). At very low concentrations, EOs were generally ineffective

Table 2. *In vitro* inhibitory effect of essential oils on percent mycelial growth of *Colletotrichum gloesporioides* at 10 days after inoculation and incubation at room temperature (27±2°C)

Essential oil	Concentration (µL/L^{-1})						
	50	100	300	600	1000	1500	2000
Thymus vulgaris	28.7±0.1b	53.3±3.0c	70.6±2.0b	91.1±0.7b	100.0±1.7a	100.0±0.8a	100.0±0.5a
Cymbopogon citratus	21.7±1.3d	59.2±2.1b	70.4±1.1b	78.0±0.4d	86.1±1.3c	90.8±0.1c	100.0±0.1a
Senna siamea	10.0±0.1e	22.2±3.4f	64.0±0.8d	79.1±2.4d	88.3±1.1c	93.3±2.1b	100.0±1.3a
Zingiber officinale	11.0±0.5e	31.7±4.1e	48.6±1.2e	57.0±2.2e	70.1±1.4d	77.3±0.6d	86.1±2.3b
Syzygium aromaticum	24.3±0.4c	65.3±1.8a	78.6±1.6a	94.1±1.7a	100.0±1.1a	100.0±0.1a	100.0±0.1a
Eucalyptus globulus	12.2±0.1e	50.0±1.1d	69.3±0.1c	82.3±1.2c	92.4±2.2b	96.0±0.2a	100.0±0.1a
Metalaxyl	33.2±2.1a	61.1±1.3b	77.3±2.0a	83.6±0.8c	92.1±0.5b	100.0±1.3a	100.0±0.5a
Control	0.0±0.0f	0.0±0.0g	0.0±0.0f	0.0±0.0f	0.0±0.0e	0.0±0.0e	0.0±0.0c

Means with same superscript along a column are not significantly different using Tukey's Honest Significant Difference (HSD) Test at P<0.05)

Table 3. Conidial germination of *Colletotrichum gloeosporioides* treated with different essential oil concentrations (µL/L^{-1})

Essential oil	Percent conidia germination (mean ±SE)						
	50	100	300	600	1000	1500	2000
Thymus vulgaris	81.4±1.8e	55.3±1.2d	31.9±0.5g	13.1±1.5g	0.0±0.0d	0.0±0.0d	0.0±0.0c
Senna siamea	90.0±2.3c	77.1±1.5b	63.8±4.1c	49.1±1.9c	17.3±1.4c	8.0±0.5c	0.0±0.0c
Zingiber officinale	87.0±2.1d	79.1±3.6b	73.4±2.8b	52.0±2.5b	41.7±2.0b	22.5±1.8b	10.9±1.3b
Cymbopogon citratus	93.8±4.0b	78.4±0.8b	58.9±3.3d	40.6±1.8d	19.2±1.3c	10.0±1.3c	0.0±0.0c
Syzygium aromaticum	88.8±3.0d	71.3±2.1c	46.0±1.3f	20.6±0.3f	0.0±0.0d	0.0±0.0d	0.0±0.0c
Eucalyptus globulus	80.0±1.6e	51.8±1.5e	31.0±1.3g	25.6±1.2e	18.0±0.1c	11.0±0.0c	8.2±0.2b
Metalaxyl	90.5±3.2c	77.6±3.0b	54.3±2.5e	26.4±2.1e	14.3±0.0d	0.0±0.0d	0.0±0.0c
Control	100.0±0.0a	100.0±0.0a	100.0±1.5a	97.3±4.6a	100.0±0.1a	97.0±4.0a	98.4±3.5a

Means with same superscript along a column are not significantly different using Tukey's Honest Significant Difference (HSD) Test at P<0.05)

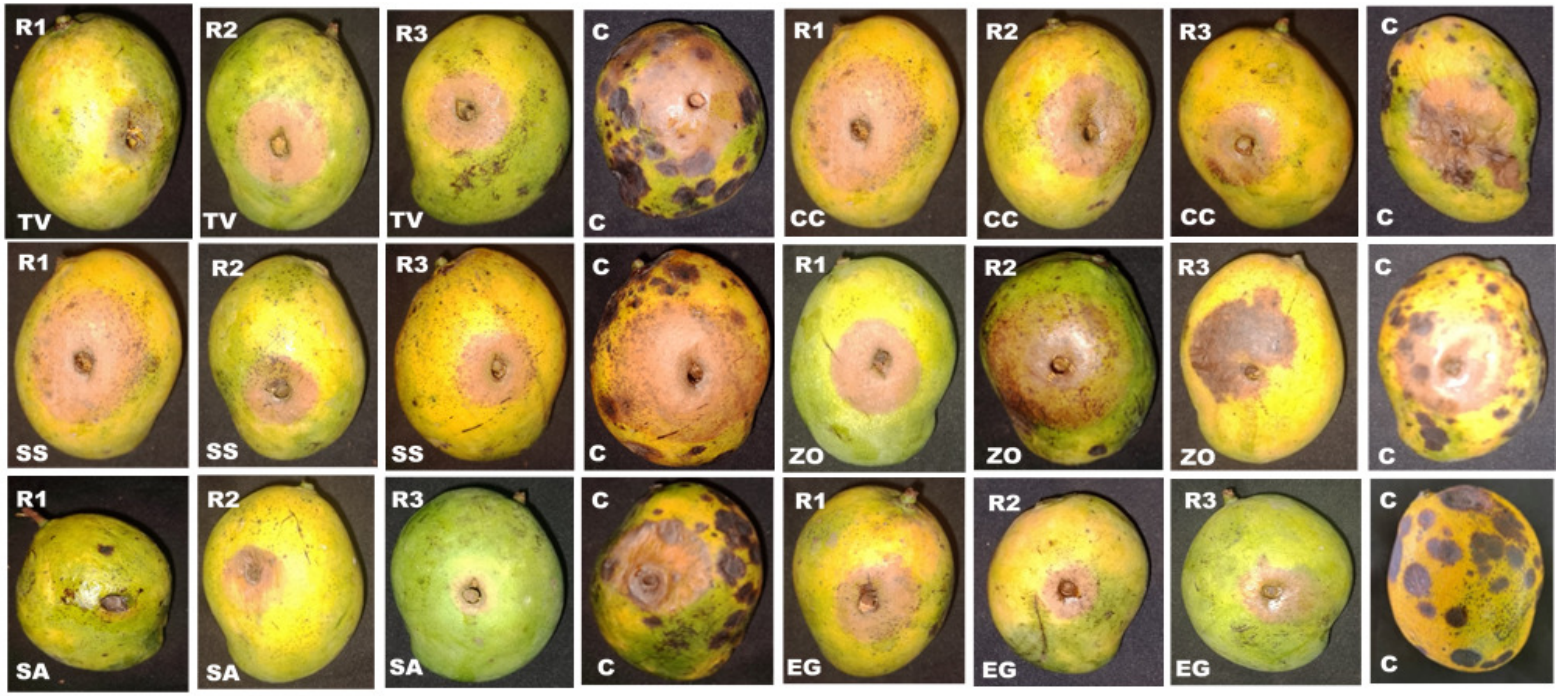

Figure 1. Representative mango fruits showing the effect of essential oils (EOs) treatment on the development of anthracnose disease after inoculation with *C. gloeosporioides*. TV = *Thymus vulgaris*, CC = *Cymbopogon citratus*, SS = *Senna siamea*, ZO = *Zingiber officinale*, SA = *Syzygium aromaticum* and EG=*Eucalyptus globulus*. C = Untreated control (R1, R2 and R3) = Replicate 1, 2 and 3, respectively.

Table 4. *In vivo* inhibitory (%) effect of essential oils on anthracnose development in mango fruits at 10 days after application and incubation at room temperature (27±2°C)

Essential oil	Concentration (µL/L^{-11})						
	50	100	300	600	1000	1500	2000
T. vulgaris	9.7±0.8b	17.4±2.2a	27.2±3.3a	43.2±3.6a	52.3±3.9a	73.5±2.6b	87.5±3.7b
Cymbopogon citratus	5.0±1.0c	11.0±1.3c	23.7±1.7b	41.0±3.0a	45.1±3.5b	47.7±3.6d	70.6±1.6e
Senna siamea	9.7±0.8b	17.4±2.2a	27.2±3.3a	43.2±3.6a	50.4±3.9a	63.5±2.6c	76.5±3.7d
Zingiber Officinale	2.8±0.4d	7.4±0.7d	15.4±2.0d	22.9±1.8c	27.2±1.1d	35.0±1.6f	62.8±2.8f
Syzygium aromaticum	7.0±1.0c	11.3±1.2c	19.2±1.0c	23.3±2.5c	53.3±3.0a	78.9±3.1a	92.5±3.1a
Eucalyptus globulus	6.0±0.9c	10.9±0.9c	14.0±1.3d	20.8±1.4c	25.8±1.2d	39.6±1.8e	66.3±2.3e
Metalaxyl	12.1±1.2a	15.9±2.4b	22.5±3.1b	30.5±3.8b	40.1±2.8c	49.7±3.4d	84.1±1.5c
Control	0.0±0.0e	0.0±0.0d	0.0±0.0 e	0.0±0.0d	0.0±0.0e	0.0±0.0f	0.0±0.0g

Means with same superscript in a column are not significantly different using Tukey's Honest Significant Difference (HSD) Test at P<0.05

in reducing anthracnose disease development in inoculated fruits (Table 4). However, rot severity reduced with increasing EO concentration. At an EO concentration of 1000 µL/L^{-1}, three of the EOs, *T. vulgaris, S. aromaticum* and *S. siamea* significantly (P<0.05) reduced fruit rot development to between 50.4 and 53.3%. Rot reduction improved as the EO concentration was increased to 1500 µL/L^{-1} with *S. aromaticum* EO having the best inhibitory effect of 78.9%, which was significantly (P<0.05) higher than the effect of other treatments. Overall, *S. aromatium* EO was the most effective in reducing anthracnose disease development among inoculated mango fruits (92.5%) at the highest concentration of 2000 µL/L^{-1}, while *Z. officinale* was least effective and only reduced disease incidence by 62.8%.

Discussion

The six EOs evaluated in this study contained volatile organic compounds reported to have antifungal properties. They were found to be effective against *C. gloeosporioides in vitro* and also significantly reduced rot development *in vivo*. EOs have been reported to contain a plethora of natural volatile compounds of plant origin with significant antifungal activity (Rabari *et al*., 2017). The efficacy of EOs could be related to the interactive effect of the major and minor bioactive constituents interacting synergistically (Singh and Pandey, 2018). For this reason, the probability of pathogen resurgence or resistance development to their effect is very low (Khan *et al*., 2021). Several authors have reported the efficacy of EOs against fungi causing post-harvest diseases, under *in vitro* conditions (Sameza *et al*., 2014; Rosato *et al*., 2018; Dania and Olaleye, 2022). Although *in vitro* assays are necessary to evaluate the inhibitory effects of EOs on fungal growth, only a few of such tests transcends the laboratories to the field under practical conditions.

Tukey's multiple comparison test (P <0.05) showed that all essential oils tested in this study had significant antifungal activity against *C. gloeosporioides* causing mango fruit rot disease. However, EOs from clove (*S. aromaticum*) and thyme (*T. vulgaris*) resulted into complete inhibition of mycelial growth at relatively low concentrations of 1000 (µL/L^{-1}) *in vitro* compared to the other treatments. Although absolute disease inhibition could not be achieved *in vivo*, their efficacy was also significantly (P<0.05) higher than other treatments including metalaxyl. The inability of the EO treatments to completely inhibit anthracnose disease could be attributed to the ability of some pathogens to degrade and absorb secondary metabolites produced by plant defence systems (Dania and Olaleye, 2022). The antimicrobial activity of EOs depends on varying indices such as their concentration, structural composition, biologically active principle, and the target pathogens (Singh and Pandey, 2018). In this study, these components were not assessed.

Clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) EOs were the most effective in inhibiting *C. gloeosporioides in vitro* and anthracnose disease development *in vivo*. Sameza *et al*. (2015) reported the potential of clove EO as a mycobiocide against *Rhizopus stolonifer* and *Fusarium solani* fungi that cause tuber rot disease in yam. Xing *et al*. (2012) reported effective inhibition of mycelial growth and conidial germination in a bioassay of clove EO against *Rhizopus, Aspergillus* and *Penicillium* genera *in vitro* and also significantly ($P<0.05$) reduced rot incidence in inoculated citrus fruits. In related research, Nana *et al*. (2015) evaluated the antifungal effect of clove EO using the food poisoning technique and found that the mycelial growth and spore germination of *Phytophthora megakarya* causing pod rot disease in cacao were significantly reduced.

Eugenol was the major constituent found in clove oil, accounting for 77.1% of the volatile compounds. Many authors have implicated this compound as the principal active component enhancing bioactivity of its EO against plant pathogens. Marei and Abdelgaleil (2018) conducted a study on the antifungal potential and biochemical effects of clove oil and reported that eugenol was the major active principle against *Fusarium solani*. Several studies have been conducted on the antimicrobial characteristics of EOs and their mechanisms of action. Prakash *et al*. (2015) and Tang *et al*. (2018) reported that bioactivity could be correlated with EOs possessing a significant proportion of volatile compounds such as monoterpenes, thymol, cinnamic aldehyde and eugenol, while Rabari *et al*. (2017) found that the main composition of EOs are phenolic compounds and terpenes which account for their fungicidal activity. The mode of action in eugenol involves the inactivation of essential fungal enzymes, reaction with the cell membrane and disruption of the genome that ultimately weaken the pathogen defence system (Cox and Markham, 2007). It could, therefore, be inferred from their study that this compound may probably be the predominant active principle in clove oil. However, it must also be emphasized that the antimicrobial activity could be attributed to volatile compounds of lower proportion or even trace constituents through additive interactions (Sameza *et al*., 2015; Tang *et al*., 2018). We observed in this study that the antifungal effect of clove oil against *C. gloeosporioides* was significantly ($P<0.05$) higher than that of the synthetic fungicide, metalaxyl, which served as the positive control. Dania and Olaleye (2022) had also reported better performance of three plant-based EOs than conventional mancozeb fungicide.

The *in vivo* assay showed that clove and thyme EOs inhibited anthracnose disease development on inoculated but treated mango fruits in a dose-dependent manner. Nana *et al*. (2015) demonstrated the ability of clove oil as a prophylactic treatment against *Phytophthora* pod rot in an *in vivo* study. In a related research, Hong *et al*. (2015) linked the effectiveness of clove oil against *C. gloeosporioides* causing pepper

anthracnose disease to its ability to inhibit conidia germination. Disease reduction *in vivo* seen in this study may have been accentuated by the lipophilic property of the EOs which enhances permeation of cell membranes into plant tissue, leading to inhibition of mycelial growth and conidial germination of plant pathogenic fungi. Seshadri *et al*. (2020) also harped on the possibility of EOs inducing resistance in plant tissue to overcome invasive tendencies of pathogens.

The antimicrobial activity of the EOs from *Thymus* species in the *in vitro* inhibition of plant pathogens had been reported previously (Rasooli and Mirmostafa, 2003; Rota *et al*., 2008). Its bioactivity has been demonstrated against *Alternaria citri* the cause of citrus fruit rot (Ramezanian *et al*., 2016) and *C. gloeosporioides* the cause of anthracnose disease (Sarkhosh *et al*., 2017. Thymol is the major constituent compound in thyme EO. Wang *et al*. (2019) demonstrated the effectiveness of thymol against *Rhizoctonia solani* causing soft rot disease in yam. Similarly, Kim (2016b) conducted an *in vitro* assay and found that the compound completely inhibited the mycelial growth of the ochratoxin-producing *Aspergillus ochraceus* in groundnut. Although the EOs used in this study, were evaluated singly, there is the likelihood of a synergistic interaction and more effective control of mycelial growth and fruit decay at lower concentrations. Thus, there is need to test these EOs in combination. Research has been carried out on safety concerns of plant EOs as antimicrobial and antioxidant substitutes. In most cases no mammalian toxicity was reported. Although cases of mild toxicity cannot be completely ruled out, eugenol and thymol identified as major active compounds in clove and thyme EOs have been registered for use as additive compounds in the food industry by the European Commission (Prakash *et al*., 2015).

Conclusion

In this study, it has been established that clove and thyme EOs possess antifungal properties which are more effective against *C. gloeosporioides in vitro* than the conventional metalaxyl fungicide. It has also been established that clove and thyme EOs significantly ($P<0.05$) reduced anthracnose disease incidence on inoculated mango fruits. These findings imply that the use of EOs from both botanicals as antimicrobial agents may practically replace the indiscriminate application of harmful chemical fungicides in mango orchards and postharvest preservation against anthracnose disease.

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Compliance with ethical standards

Conflict of interest: The Authors declare that they have no conflict of interest whatsoever in this study. This article does not contain any studies with animals performed by any of the authors.

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