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Use of Some Plant Essential Oils as Post-harvest Botanical Fungicides in the Management of Anthracnose Disease of Mango Fruits (*Mangi Feraindica L.*) Caused by *Colletotrichum Gloeosporioides* (Penz)

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Abstract Mango suffers from several diseases at all stages of its life. Anthracnose, caused by the fungus *Colletotrichum gleosporioides* is the most important post harvest disease of mango. The aim of this study was to test the possibility of the use of some plant essential oils *i.e.* Basel oil (*Ocimum basilicum*), Orange oil (*Citrus sinensis),* Lemon oil *(Citrus Medica)* and Mustard oil (*Brassica juncea* L.) to reduce postharvest losses induced by *Colletotrichum gloeosporioides* (Penz.) in mango fruits. In this study, the antifungal activity of essential oils under *in vitro* condition were assayed by tested various concentrations (0, 50,100 and 150µg/ml)) and under *in vivo* condition by used different essential oil concentrations (0, 250,500 and 1000ppm) on inoculated mango fruits. Results of the present study showed that orange oil at all tested concentrations were a significant reducing the fungal linear growth if compared with other tested essential oils. At low concentration 50 (µg/ml) orange oil caused 10.0% reduction in fungal growth, while at 100 (µg/ml) caused 72.2% and at high tested concentration 150 (µg/ml) caused a complete reduction in mycelium linear growth of pathogenic fungus. Meanwhile, at low tested concentration 50 (µg/ml), mustard oil caused a highly significantly reduction of the percentage of fungal spore germination by 70.8 % followed by basil oil by 64.7%. Results of *in vivo* studies showed that, at low concentration 250 ppm, mustard oil caused a highly reduction of anthracnose incidence of mango fruits by 79.9% followed by basil oil with 66.7% .On the other hand, orange and lemon oil at low concentration (250ppm) were showed a highly effect to reducing the percentage of rotting fruit tissue by 84.5 and 75.0%, respectively if compared with other treatments and un-treated fruits.

Keywords Plant Essential Oils, Mango Fruits, Anthracnose Disease, *Colletotrichum Gloeosporioides*

1. Introduction

Mango (*Mangifera indica* L.) is one of the top five fruit crops in the world. It is adaptable to a wide range of climates, ranging from wet tropical to dry subtropical. Among the various constraints, the most important is anthracnose caused by *Colletotrichum gloeosporioides* Penz. And Sacc. (teleomorph *Glomerellac ingulata*). Flower blight, fruit rot, and leaf spots are among the symptoms of this disease[2]. Severe infection destroys the entire inflorescence resulting in no setting of fruits. Young infected fruits develop black spots, shrivel and drop off. Fruits infected at mature stage carry the fungus into storage and cause considerable loss during storage, transit and marketing[1]. Disease control methods include the prophylactic use of fungicides such as benomyl, mancozeb, carbendazim, and thiabendazol[18]. The postharvest use of chemicals as fungicides is restricted in most countries[24]. Consumer demand for agricultural commodities without pesticide residues is high[7,24] however, pesticides may also kill various beneficial organisms and their toxic forms may persist in soil[12] and increase the incidence of resistance among pathogens towards synthetic chemicals[3,23]. Thus, a new preservation technologies are needed, which have to be considered as human-safe and environmentally friendly[9]. Among the various alternatives, natural plant products, including essential oils that are biodegradable and eco-friendly, are catching the attention of scientists worldwide. Such products from higher plants are bio-efficacious, economical, and environmentally safe and can be ideal candidates for use as agrochemicals[14]. Numerous reports showed that oils from some plant species are harmful to fungal pathogens[17]. Wilson *et al*.,[30], tested 49 essential oils from various plants and found that the oils from palmarosa (*Cymbopogon martini*), red thyme (*Thymus zygis*),cinnamon leaf (*Cinnamomum zeylanicum*), and clove buds (*Eugenia caryophyllata*) were effective in the control of *Botrytis*

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cinerea. Oils from *Eucalyptus globules* and *Ocimum canum* at 2000 ppm were effective in reducing mycelial growth and sclerotial production of *Sclerotium rolfsii*[27]. This study aimed to evaluate the effectiveness of a number of plant essential oils as alternative method against growth and spore germination of *Colletotrichum gloeosporioides* the causal agent of anthracnose disease of mango fruits under vitro conditions , and study their effect on the disease incidence under vivo conditions.

Figure 1. Anthracnose disease symptoms on mango fruits

2. Materials and Methods

2.1. Fruits

Mango (*Mangifera indica* L.) fruits Zabdia cv. were harvested at the mature stage, and sorted based on size and the absence of physical injuries or disease infection. Before treatments, fruit were surfaced disinfected with 2% sodium hypochlorite for 3 min, then rinsed with tap water, and air-dried.

2.2. Pathogen Culture

Colletotrichum gloeosporioides was cultured for 1–2 weeks on potato dextrose agar (PDA) at 25 ◦C. The isolate used was obtained from infected mango fruit in Egypt. Spores were harvested by adding 3–4 ml of sterile, de-ionized water (diH_2O) to the Petri dish. The spores were then rubbed with a sterile glass rod to free them from the PDA medium, and the spore suspension was passed through two layers of cheese cloth. The suspension was diluted with water to obtain the spore concentrations (10^6spores ml^{-1}) according to determination with a Haemocytometer slid.

2.3. Source of Tested Plant Essential Oils

Pure-grade (not containing synthetic chemicals and/or non-natural components) essential oils of Basel oil (*Ocimum basilicum*), Orange oil (*Citrus sinensis*), Lemon oil(Citrus Medica) and Mustard oil (*Brassica juncea* L.), were obtained from Cairo

Company for Oils and Aromatic Extractions CID, Egypt. These essential oils were stored in dark bottles at 4ºC for further studies.

2.3.1. In Vitro Screening of Plant Essential Oils Against *C. Gloeosporioides* Mycelium Linear Growth and Spore Germination

The antifungal tests were carried out in vitro according to the method described by Pitarokili *et al.*[22] using Petri dishes 9 cm in diameter containing potato dextrose agar (PDA). The essential oils were dispersed individually as an emulsion in sterilized water using Tween 20 (0.05%) and added to PDA immediately before it was filled into the Petri dishes at a temperature of 45-50°C. The concentrations tested were0, 50,100 and 150µg/ml. The controls included the same quantity of Tween 20 mixed with PDA. The tested fungus was inoculated immediately after preparation of the Petri dishes by placing in the centre of each plate a 5 mm diameter disk of the test fungus, cut with a sterile cork borer from the periphery of actively growing cultures on PDA plates. The Petri dishes were incubated in the dark at a temperature of 25°C. Mean growth rates were calculated from five replicates of fungus every 24 h until fungal growth in the control filled the Petri dishes completely, the percentage mycelial inhibition was calculated by the following formula:

% mycelial inhibition = dc _ dt X100

dc: where dc is mean colony diameter of control sets and dt is mean colony diameter of treatment sets conidial germination inhibition test was performed by the cavity slide technique and the results were expressed in percentage[6].

2.3.2. In Vivo Applicability of the Plant Essential Oils of Anthracnose Incidence of Mango Fruits

The Mango Fruits cv. Zabdeia were treated with different concentrations of plant essential oils by the standard techniques followed by Chandra[4] and Sharma & Yadav [25] in order to find out the efficacy of the oils against anthracnose rot disease caused by *C. gloeosporioides*. Mature healthy mango fruits of medium size were used for the experiment. The fresh mango fruits of control as well as of treatment sets were washed in running water and were surface sterilized with 0.1% sodium hypochlorite solution and were then washed with distilled water. Fruit inoculation with *C. gloeosporioides* spores obtained from a 7 day old culture, spores was suspended in sterile distilled water and 0.03% Tween- 80. Mango fruits were wounded on two sides to a depth of 1.5 mm by puncturing them with a pin. Each wound site was then inoculated with 40µl of spore suspension (106 spores/ml) of *C. gloeosporioides* , and kept at ambient temperature for drying. Air-dried fruits were dipped for 2-3 min in different concentrations of tested essential oils (250,500 and 1000ppm) individually and again kept at ambient for drying. After application of treatments mango fruits were packed in cardboard boxes and stored (13±1°C, 80± 5% RH) for 28 days. The effect of various plant essential oils on disease incidence and disease severity (percentage of fruit rotting tissue) was evaluated weekly for 28 days during cold storage. Six replicates were kept for treatment and control sets.

3. Statistical Analysis

Tukey test for multiple comparisons among means was utilized (Neler*et al*.,1985).

4. Results

4.1. In Vitro Screening of Plant Essential Oils Against *C. Gloeosporioides* Mycelium Linear Growth

Results in Table (1) showed that, orange oil at all tested concentrations was a significant reducing the fungal linear growth if compared with other tested essential oils . At low concentration 50 (µg/ml) orange oil caused 10.0% reduction in fungal growth, while at 100 (µg/ml) caused 72.2% and at high tested concentration 150 (µg/ml) caused a complete reduction in mycelium linear growth of pathogenic fungus. On the other hand, basil oil gave a moderate effect to reducing the fungal linear growth at all tested concentrations by 5.5, 20.8 and 38.5%, respectively. Lemon and mustard oil showed a lower effect at all tested concentrations if compared with the other oils and control treatments.

Table 1. Effect of various concentrations of some plant essential oils against the mycelium linear growth of *C. gloeosporioidesin vitro*

Essential oils	Conc. (µg/ml)	Fungal linear growth (mm)	Reduction %
Orange oil (*Citrus sinensis)*	50	81.0 a	**10.0**
	100	25.0 d	**72.2**
	150	00.0 e	**100.0**
Lemon oil *(Citrus Medica)*	50	90.0 a	**00.0**
	100	71.8 b	**20.2**
	150	53.0 c	**41.1**
Mustard oil (*Brassica juncea* L.)	50	90.0 a	**00.0**
	100	90.0 a	**00.0**
	150	77.1 b	**14.3**
Basil oil (*Ocimum basilicum*)	50	85.0 a	**5.5**
	100	71.2 b	**20.8**
	150	55.3 c	**38.5**
Control	0.0	90.0 a	**---**
	0.0	90.0 a	**---**
	0.0	**90.0 a**	**---**

Figures with the same letter are not significantly different (P=0.05).

4.2. In Vitro Screening of Plant Essential Oils Against *C. Gloeosporioides* Spore Germination

Results in Table (2) showed that, at low tested concentration 50 (µg/ml) ,mustard oil caused a highly significantly reduction of the percentage of fungal spore germination by 70.8 % followed by basil oil by 64.7% and orange oil by 61.2%. Meanwhile, at high tested concentration 150 (µg/ml) , orange oil caused 63.0% reduction of fungal spore germination , followed by basil oil by 39.9% and mustard oil by 22.4% . Lemon oil showed a lower effect to reducing the fungal spore germination than other tested oils at all tested concentrations.

Table 2. Effect of various concentrations of some plant essential oils against the percentage of fungal spore germination of *C. gloeosporioides in* vitro

Essential oils	Conc. (µg/ml)	Fungal spore germination %	Reduction %
Orange oil (*Citrus sinensis)*	50	31.7 d	**63.0**
	100	30.0 d	**65.0**
	150	33.3 d	**61.2**
Lemon oil *(Citrus Medica)*	50	100.0 a	**00.0**
	100	90.5 a	**6.28**
	150	51.6 c	**39.9**
Mustard oil (*Brassica juncea* L.)	50	66.6 b	**22.4**
	100	31.9 d	**62.8**
	150	25.0 d	**70.8**
Basil oil (*Ocimumbasilicum*)	50	51.6 c	**39.9**
	100	33.0 d	**61.5**
	150	30.3 d	**64.7**
Control	0.0	85.9 a	**---**
	0.0	85.9 a	**---**
	0.0	**85.9 a**	**---**

Figures with the same letter are not significantly different (P=0.05).

4.3. In Vivo Applicability of the Plant Essential Oils of Anthracnose Incidence of Mango Fruits

Results in Table (3) and Fig.2. showed that , at low concentration 250 ppm , mustard oil caused a highly reduction of anthracnose incidence of mango fruits by 79.9% followed by basil oil with 66.7% reduction of disease incidence, while orange oil and lemon oil showed a moderate effect . At high concentration 1000 ppm, basil oil caused 94.4% reduction of disease incidence followed by orange and mustard oils by 91.3 and 90.0 %, respectively. On the other hand, orange and lemon oil at low concentration (250ppm) were showed a highly effect to reducing the percentage of rotting fruit tissue by 84.5 and 75.0%, respectively if compared with other treatments and un-treated fruits. Mustard and basil oils showed moderate effect at all tested concentrations.

Table 3. Effect of fruits coating with different concentrations with plant essential oils on anthracnose disease incidence and percentage of fruit rotting tissue of mango fruits after 28 days of cold storage

Essential oils	Conc. ppm	%Anthracnose incidence	% Fruit rotting tissue
Orange oil (*Citrus sinensis)*	250	85.7 a	**15.5 c**
	500	25.3 c	**10.0 d**
	1000	8.7 e	**00.0 e**
Lemon oil *(Citrus Medica)*	250	75.6 b	**25.0 c**
	500	60.5 b	**20.0 c**
	1000	44.3 c	**10.0 d**
Mustard oil (*Brassica juncea* L.)	250	20.1 d	**52.5 c**
	500	15.0 d	**30.0 c**
	1000	10.0 d	**12.5 c**
Basil oil (*Ocimum basilicum*)	250	33.3 c	**55.1 b**
	500	13.5 d	**35.0 c**
	1000	5.6 e	**30.0 c**
Control	**00**	**100.0 a**	**75.0 a**

Figures with the same letter are not significantly different (P=0.05).

Figure 2. Effect of mango fruits coating with various concentrations of plant essential oils on anthracnose disease incidence after 15 days of storage

5. Discussion

The essential oils are reported to have some fungicidal properties against certain postharvest diseases of tropical fruits and vegetables[30,16,13] and are also safer for the environment than synthetics chemicals .The ability of four plant essential oils to inhibit the fungal growth and their effect on spore germination of *C. gloeosporioides* was evaluated. The most active oils for reducing the growth of tested fungi with significant value under vitro condition was orange oil followed by basil oil, Lemon and mustard oil showed a lower effect at all tested concentrations if compared with the other oils and control treatments. On the other hand, at low tested concentration 50 (µg/ml) ,mustard oil caused a highly significantly reduction of the percentage of fungal spore germination by 70.8 % followed by basil oil by 64.7% and orange oil by 61.2% .The literature is also silent on the mode of action of the essential oils when used as postharvest fungi toxicants[29]. A substance may inhibit the growth of fungi either temporarily (fungistatic) or permanently (fungicidal). In agreement with the findings, Thangavelu *et al.*[28] found that the extracts of *Solanumtorvum*, *Jatrophagl* and *ulifera* and *Emblica officinalis* were highly inhibitory to mycelial growth of *Colletotrichu mmusae* in banana and the inhibitory effect was directly related to the quantity of extract added to the medium. In another study by Palhano *et al.*[21] also confirmed that the inhibitory effects of citral on spore germination of *C. gloeosporioides* were higher with an increase in the concentration of essential oil. *In vivo* applicability of the plant essential oils of anthracnose incidence of mango fruits was indicated that, At high oil concentration 1000ppm, coating mango fruits by basil oil caused 94.4% reduction of disease incidence followed by orange and mustard oils by 91.3 and 90.0 %, respectively. On the other hand, mango fruits were coating with orange and lemon oil at low concentration (250ppm) were showed a highly effect to reducing the percentage of rotting fruit tissue by 84.5 and 75.0% , respectively if compared with other treatments and un-treated fruits. Maqbool *et al.,* 2010, reported that, Cinnamon oil with fungitoxic or fungistatic activity could be considered as a suitable alternative to synthetic fungicides for managing anthracnose in bananas. *In vitro* inhibition was directly related to the cinnamon oil concentrations. Dubey *et al.*[8] described that essential oil from *Eupatorium cannabinum* had an antifungal activity against *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* causing stem end rot and anthracnose diseases in mango, respectively. In addition, they found that this essential oil had an inhibitory effect on pectinase and cellulase, two important enzymes produced by phytopathog enic fungi in disease development. Farrag *et al.* [10] described that the antimicrobial activity of essential oils could be related to the presence of an aromatic nucleus and OH group that can affect hydrogen bonds of enzymes in microorganisms. Feng and Zheng , 2007, studied the effects of cassia oil on decay development in artificially inoculated and wounde tomatoes fruits. The results indicate that when wounded cherry tomatoes were treated with cassia oil, all concentrations (except 100 ppm) significantly inhibited *A. alternate*on tomatoes stored at 20 _C for 5 days ($p < 0.05$). The percentages of decayed cherry tomatoes treated by 500

ppm cassia oil was reduced by 34.2% compared to the control. Maqbool *et al.,* 2010,reported that, different concentrations of cinnamon oil not only delayed the onset of anthracnose disease in coating panama fruits but also maintained the freshness during first two weeks of storage and later on showed minimal symptoms. The highest fungicidal effect was observed in those bananas treated with 0.4% cinnamon oil (disease incidence of 8.0%) and disease severity (DS) score of 1.2 indicating fruit surface infection close to 1.0. Shelef[26] described that within several components available in essential oils, the antimicrobial activity of phenolic compounds were higher than alcoholic components, this is in agreement with the results of present study. It is known that the cell wall of pathogens is the main target of phenolic compounds and these compounds may disrupt the permeability barrier of cell membrane and inhibit respiration. Hydrophobic nature of essential oils and their components enables these compounds to penetrate lipid of fungal cell membrane and mitochondria as a result disturbing their structure[5] and these compounds accumulate in the cell membrane of pathogen causing energy deletion. In addition, in some studies, it is reported that the essential oils may affect the metabolic pathways of microorganisms. Nychas[20] found that phenolic compounds in low concentration disrupt proteins and in high concentrations damaged the enzymes outbreak in production of energy.

6. Conclusions

The results of present study showed that the possibility of the use of some plant essential oils *i.e.* Basel oil (*Ocimum basilicum*), Orange oil (*Citrus sinensis*), Lemon oil (*Citrus Medica)*and Mustard oil (*Brassica juncea* L.)to reduce postharvest losses induced by *Colletotrichum gloeosporioid es* (Penz.) in mango fruits. So essential oils can be usedas a potential source of sustainable eco-friendly botanical fungicides, after successful completion of wide range trials.

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