

# Antifungal Activity of Essential Oils Extracted from *Monodora myristica* (Gaertn), *Ocimum gratissimum* L. and *Zingiber officinalis* Roscoe on Post-harvest Anthracnose of Mango Fruit (*Mangifera indica* L.) Variety Kent in Côte d'Ivoire

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**Abstract:** This study was carried out to analyze the antifungal effect of essential oils from *Monodora myristica* (Gaertn) Dunal, *Ocimum gratissimum* L. and *Zingiber officinalis* (Roscoe) against *Colletotrichum gloeosporioides* (Penz.), the causal agent of anthracnose infecting mango fruits in Côte d'Ivoire. The inhibitory capacity of five concentrations (100, 200, 500, 1000 and 2000 ppm) of each essential oil were evaluated *in vitro* on the mycelial growth and spore germination of *C. gloeosporioides*. The efficacy of *O. gratissimum* oil on controlling postharvest anthracnose of kent mango fruit was evaluated both before and after inoculation using mycelia discs of *C. gloeosporioides*. The *O. gratissimum* oil inhibited completely (100%) the mycelial growth and spore germination of *C. gloeosporioides* at 1000 ppm *in vitro*. It is fungistatic at 1000 ppm and fungicide at 2000 ppm. The *M. myristica* oil showed the lowest inhibitory activity. The mango soaked into *O. gratissimum* oil concentration after inoculation yielded a highly protective effect against anthracnose disease in mango fruits at 1200 ppm. This treatment, presented the lowest infection rate (20%) and lesion size ( $0.22 \pm 0.04$  cm). Moreover, the four doses of *O. gratissimum* used, did not influence quality parameters of mango fruits. Therefore, essential oil of *O. gratissimum* could be an alternative to chemicals for control postharvest anthracnose of mangoes.

**Keywords:** Mango, Anthracnose, *Colletotrichum gloeosporioides*, essential oils, Côte d'Ivoire

## INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most tropical fruits produced in Côte d'Ivoire. It is the third export fruit after banana and pineapple. Côte d'Ivoire has been the top African mango exporting country and since 2008, it is the fifth most important world supplier of mangoes to the European market (Gerbaud, 2011). Mango is susceptible to postharvest diseases caused by various pathogenic fungi. *Colletotrichum gloeosporioides* (Penz) is the causal agent of anthracnose, the major postharvest disease

which leads to losses of the exportation value of Côte d'Ivoire's (FIRCA, 2008). Therefore, the control of anthracnose is essential to keep and improve mango quality. Chemical methods have been and remain the main approach used to reduce losses caused by fungal parasites on many fruits (Fend and Zheng, 2007). However, repeated use of certain synthetic fungicides on packinghouses has led to the appearance of fungicide-resistant pathogens and environmental pollution (Abd-Alla and Hagga, 2010). Moreover, the use of chemical products is increasingly restricted

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due to public concerns over toxic residues. Consequently, mango exportation is subjected to strict health standards and qualitative norms of import markets. Thus, it is necessary to research alternative, efficient and healthy solutions for controlling anthracnose of mangoes. The use of essential oils to control postharvest diseases of fruits is a promising approach; because, essential oils are volatile, biodegradable with short degradation periods (Sivakumar and Bautista-Baños, 2014). These qualities make them to be healthy phytosanitary products and environment abiding. In addition, they offer fewer possibilities for pathogens to develop resistant strains. Additionally, the antifungal and antibacterial properties of essential oils against many microorganisms have been well proven (Duamkhanmanee, 2008; Dikbas *et al.*, 2009). These properties confer the advantage upon essential oils to be preferred in order to control postharvest diseases of various fruits. This study was conducted to investigate the antifungal activity of essential oils from *Monodora myristica* (Gaertn) Dunal, *Ocimum gratissimum* L. and *Zingiber officinalis* (Roscoe) on *Colletotrichum gloeosporioides* (Penz) causal agent of anthracnose of postharvest mangoes.

## MATERIALS AND METHODS

### Essential Oils extraction

The essential oils tested were extracted from fresh leaves of *O. gratissimum* L, dried seeds of *M. myristica* (Gaertn) Dunal brought from Gouro market in Abidjan and rhizomes of *Z. officinalis* Roscoe collected in Hermankono Garo in the department of Divo (170 km Northern east of Abidjan). The essential oils were obtained by hydrodistillation of plant organs through Clevenger-type-system for 2h30min; the recovered oils were dried over anhydrous sodium sulphate and stored in darkness at -10 °C before their use for the different tests.

### Isolation of fungi

*C. gloeosporioides* used in this study was from mango (*Mangifera indica* L.) fruits of the *Kent* variety infected by anthracnose. The mango fruits were collected on a village plantation in the Department of Korhogo. The fungal strain was isolated from infected mango fruits by the tissue transplanting method. *C. gloeosporioides* was maintained on potato dextrose agar medium (PDA) and sub-cultured periodically 10 days at the pathology unit of vegetable physiology laboratory of Félix Houphouët Boigny University. The pathogens obtained were identified by microscopic examination based on the key of identification of Barnett and Hunter (1972).

### Evaluation of the antifungal activity of the essential oils *in vitro*

The antifungal activities of the essential oils were

evaluated on the radial growth of mycelia and spore germination by using agar dilution method with different concentrations.

The extracted essential oils were used at doses of 0, 100, 200, 500, 1000, and 2000 ppm. The synthetic fungicide (Azoxytrembin), used as a positive control, was dissolved in sterile distilled water to get an initial solution. From this solution, dilutions were made and mixed in PDA medium to obtain 0.01, 0.05, 0.1, 0.5, and 1 ppm concentrations.

### Effect of essential oils on mycelial growth

PDA was autoclaved at 121°C, at 1 bar for 20 min and cooled in a water bath to 40°C. An appropriate quantity of each essential oil or synthetic fungicide was added to a sterile melted medium, followed by manual rotation of Erlenmeyer to disperse the product in the medium. The mixture was poured into 90 mm Petri dishes (15ml/plate). Each Petri-dish was inoculated at the centre with a mycelia disc (5 mm diameter) taken at the periphery of *C. gloeosporioides* colony grown on PDA for 7 days. For each concentration of essential oils or synthetic fungicide, five Petri dishes were used. Negative control plates (without synthetic fungicide or essential oils) were inoculated following the same procedure. The plates were incubated at 28°C and 12/12 photoperiod for 12 days. The experiment was repeated twice. The colony diameter was measured every 24 hours following two perpendicular axes drawn at the bottom of each Petri dish which intersects at the middle of the explants. The effect of essential oil was evaluated by the percentage mycelial growth ( $I_c$ ) inhibition according to the following formula (Kwazou *et al.*, 2009):

$$I_c (\%) = [(D_o - D_c) / D_o] \times 100$$

$D_o$  = average growth of the fungus (mm) on the control Petri dish.

$D_c$  = average growth of the fungus (mm) on the Petri dish containing different concentration of each essential oil or synthetic fungicide

The explants of test plates which did not grow were transferred to dishes containing fresh PDA medium (without oil or chemical product) to assess their viability after 7 days. This procedure allowed the nature of inhibition of each product used to be determined.

### Effect of essential oils on spores germination

Spores were collected from cultures grown on PDA for 14 days by pouring 10 ml of sterile distilled water on the culture plates and scraping the plate surface with an incurved pastor pipette. The suspension of collected spores was adjusted to approximately  $1.5 \cdot 10^4$  spores/ml of distilled water. The essential oils or synthetic fungicide were directly mixed in the medium of agar culture and the mixture was poured

into 90 mm Petri plates (10 ml/plate) as above. 10 µl of spore suspension were inoculated onto Petri dishes containing agar medium following two marked parallels lines under each plate in order to ease spore counting. Three repetitions were simultaneously performed by concentration. Incubation was undertaken at 28°C, and then 200 spores per plate (100 spores per line) were examined under the microscope to determine the germination rate every 6 hours. The percentage of inhibition of spore's germination (Ig) was determined following the formula below (Bouigoumane *et al.*, 2008):

$$\text{Ig (\%)} = [(\text{No} - \text{Nc}) / \text{No}] \times 100$$

No = Number of germinated spores in the control Petri dish

Nc = number of germinated spores in the presence of a concentration of essential oil or synthetic fungicide

The effective concentration causing 50 or 90 % reduction (EC<sub>50</sub> or EC<sub>90</sub>) in the mycelial growth or spores germination were determined for each product used after 7 days of mycelia growth or after 24 hours of spore germination. The results were plotted using the linear regression equation between days (in abscissa) against the rates of reduction of the mycelial growth (in ordinate) (Serghat *et al.*, 2004).

The Minimum Inhibitory Concentration (MIC) was also determined in a parallel experiment (Remmal *et al.*, 1993; Satrani *et al.*, 2001). MIC was considered as the lowest concentration of the test product at which no spore germination or mycelia growth was observed.

#### Effect of *Ocimum gratissimum* essential oil on the control of mango fruit anthracnose

Fresh mango fruits cv. Kent health visibly undamaged and disease-free were used in this experiment. They had been harvested at commercial maturity from a plantation in the Department of Korhogo (Northern Côte d'Ivoire). Fruit surfaces were sterilized by soaking them in a solution of 1% sodium hypochlorite for 5 min, rinsed with sterile distilled water, and then air dried in the culture room. A total of 50 fruits, divided into 10 lots (5 fruits per lot) were used for the test. Nine lots were wounded with a sterile needle used to make five uniform 5 mm deep by 0.66-mm-diameter wounds on their peel at five distinct points for each fruit. Fruit was then inoculated by injecting 10 µl of *C. gloeosporioides* spore suspension (1.6x10<sup>4</sup> spores/ml) into each wounded point. The fruit was then submitted to treatment with *O. gratissimum* oil which was modified at previously determined *in vitro* EC<sub>50</sub>, EC<sub>90</sub> and MIC concentrations (150; 300; 600 and 1200 ppm). Each sample of 5 fruits was soaked in a precise concentration for 1min before or 30 min after their

inoculation (Duamkhanmanee, 2008). There were two controls: inoculated and non-inoculated mango soaked only in sterile distilled water. Treated mango fruit was put in a cardboard box and then placed in the culture room at 28 ± 2 °C. The experiment was repeated twice.

#### Disease evaluation

After 48 hours, fruits inoculated were daily observed and the number of spots that produced lesions (Ns: symptoms) was noted. The incidence of disease (ID) or the lesions rate (Lr) for each treatment was calculated as followed:

$$\text{Lr (\%)} = \frac{\text{N}_s}{\text{N}_T} \times 100$$

N<sub>T</sub>: total number of inoculated spots

The evolution of lesions was also measured following two perpendicular lines and the medium diameter of lesions (D) was calculated.

$$\text{D (mm)} = (\text{d1} + \text{d2})/2;$$

d1: lesion length following the largest diameter of the fruit

d2: lesion length following the smallest diameter of the fruit

The efficacy of each treatment (E) on the disease manifestation and evolution were then calculated according to the formula below (Mansour *et al.*, 2006):

$$\text{E (\%)} = [(\text{To} - \text{Ti}) / \text{To}] \times 100;$$

To: lesion rate (lesion size) among control fruits

Ti: lesion rate (lesion size) among treated fruits

#### Physicochemical parameters evaluation

The influence of the different doses of *O. gratissimum* oil on quality parameters of the fruit was evaluated at the 11<sup>th</sup> day after incubation. The testing parameters are described below:

**Mass loss:** the mass of each mango fruit was measured by a Sartorius balance (± 0.001g) before treatment (A) and at the 11<sup>th</sup> day of the experience (B), and mass loss was calculated as follows (Zhang *et al.*, 2009):  $\text{P}_M (\%) = [(\text{A} - \text{B}) / \text{A}] \times 100$

**Fruit firmness:** the firmness value of each fruit was determined using a crossbow type penetrometer. It evaluated the resistance inside the fruit to the penetration of the cylindrical instrument tip (length = 8mm, diameter = 3mm) (N'Ganzoua *et al.*, 2010). Firmness values were measured at 4 spots (2 along the lateral sides, one on the dorsal surface and one at the front) and the average of the measurements was

calculated for each fruit.

**Titrateable acidity:** acidity was measured by titration with sodium hydroxide solution 0.1 N in the presence of phenolphthalein in 10ml of solution obtained from 3g of each fruit pulp homogenized with 30 ml distilled water and filtered. Results are expressed as (mean  $\pm$  SE) mEq $\cdot$ 10<sup>-2</sup> g of fresh pulp,

**pH:** the pH of fruit juice was determined using a digital hand pH-meter calibrated by pH 4 and 7 buffer solutions.

**Total soluble solids (TSS):** Total soluble solids were determined by measuring the refractive index of the same juice with a digital hand refractometer (Atago pr-1) and the results were expressed as mean  $\pm$  SE °Brix.

#### Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using STATISTICA 7.1 software. Statistical significance was assessed at  $p < 0.05$  and Newman-Keuls's test was used to separate averages.

## RESULTS

### Essential oils effect on the mycelial growth

The sensitivity of *C. gloeosporioides* varied according to the concentrations used and incubation time. *Monodora myristica* oil had a low inhibitory activity on the fungal parasite. Respectively by the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day after incubation, the dose of 2000 ppm reduced the mycelia growth by 90, 50, and 40% (figure 1). At concentrations of 100, 200, 500, and 1000 ppm, the essential oil of *M. myristica* reduced the mycelial growth less than 50%. The EC<sub>50</sub> and EC<sub>90</sub> were respectively 13989 and 782268 ppm (Table 1).

All the essential oils doses of *Z. officinalis* Roscoe rhizome used, caused a pathogenic reduction of mycelial growth (Figure 2). With the concentrations of 500, 1000, and 2000 ppm, 50% of mycelial growth was obtained respectively on the 3<sup>rd</sup>, 5<sup>th</sup> and 12<sup>th</sup> day. At the concentration of 2000 ppm, 90% of reduction was observed on the 2<sup>nd</sup> day after inoculation. The EC<sub>50</sub> and EC<sub>90</sub> were respectively 1335 and 9870 ppm 7 days after incubation (Table 1).

The doses of *O. gratissimum* oil used had severely reduced mycelial growth of *C. gloeosporioides* (Figure 3). The inhibition rates varied from 34.40 to 100 % according to the concentrations and time after incubation. At 8<sup>th</sup> and 12<sup>th</sup> days after incubation, more than 50% of reduction of mycelial growth was observed respectively at 100 and 200 ppm. With the doses of 1000 and 2000 ppm mycelial growth was totally inhibited. At the 7<sup>th</sup> day, the EC<sub>50</sub> and EC<sub>90</sub> were respectively 10.30 and 447 ppm, and the MIC of mycelia growth was 1000 ppm (Table 1).

The transfer of explants at concentrations 1000 ppm and 2000 ppm showed the restart of 24.20 % for the concentration of 1000 ppm and nothing for the concentration 2000 ppm.

A high fungitoxic activity on the mycelial growth was expressed by the different doses of "Azoxystrombin" used (Figure 4). The reduction rates of mycelial growth obtained varied from 39.41 to 100% following the concentrations and the incubation time. The dose 1 ppm totally inhibited mycelial growth at 6 days after incubation. The obtained EC<sub>50</sub> and EC<sub>90</sub> at the 7<sup>th</sup> day was 0.0002 and 0.56 ppm respectively (Table 1).

### Essential oils effect on spore germination

The germination of *C. gloeosporioides* (Penz) spores was inhibited by the different essential oils and the synthetic fungicide used (Table 2). With *M. myristica* oil, the inhibition rate of spore germination varied from 22.3  $\pm$  8.8 to 69.5  $\pm$  5.9 %, 24 hours after incubation (Table 2). The EC<sub>50</sub> and EC<sub>90</sub> concentrations of the spores germination were respectively 1258.9 ppm and 12 648.10 ppm at 24 hours after incubation and no concentration had totally inhibited the germination (Table 1).

Regarding the essential oil of *O. gratissimum*, all the concentrations tested reduced the rate of spore germination more than 50%. The doses 500, 1000, and 2000 ppm reduced spore germination from 99.46  $\pm$  0.5% to 100% (Table 2). The MIC for spore germination was 1000 ppm. The EC<sub>50</sub> and EC<sub>90</sub> of that oil were determined (Table 1). Concerning the essential oil of *Z. officinalis*, it also presented a high inhibitive effect on spore germination. All the dosages have had inhibitive activities varying from 54.09  $\pm$  6.24 to 99.74  $\pm$  0.26 % (Table 2). The EC<sub>50</sub> and EC<sub>90</sub> were respectively 91.20 and 891.25ppm, and no MIC was determined at these concentrations. A high reduction in spore germination was also obtained with different concentrations of "Azoxystrombin". This activity was statistically superior to the oil of *M. myristica* and identical to those of *O. gratissimum* and *Z. officinalis* (Table 2). That product did not totally inhibit the spores germination at all of the different doses used (Table 1).

The two life stages of *C. gloeosporioides* were highly reduced by the different doses of the essential oils used. The essential oil of *O. gratissimum* was the most efficient on the two life stages. On the other side, the oil of *M. myristica* was the less efficient.

### *In vivo* activity of *Ocimum gratissimum* essential oil

#### Curative capacity of *Ocimum gratissimum* essential oil

Various doses of *Ocimum gratissimum* significantly reduced disease expression in treated fruits. Symptoms were observed on treated fruits at different dates. The average rates of the lesions obtained were spread into two statistically different groups ( $p = 0.0002$ ): the registered rates with the 150, 600, and 1200 ppm treatments were  $24 \pm 3.94\%$ ;  $24.67 \pm 4.02$  and  $20 \pm 3.12\%$  respectively; this represents the first group (Table 3). The second group is represented by the rate obtained at 300 ppm ( $38.67 \pm 4.82\%$ ).

As far as the progress of the disease is concerned, it was lower in the fruits treated during the experimental period. The mean size of the lesions produced fluctuated between  $0.22 \pm 0.04$  and  $0.46 \pm 0.07$  cm (Table 3).

All of the different doses used effectively reduced the incidence and progress of the disease on the treated fruit. The effectiveness of different doses in their ability to reduce the incidence of the disease on treated fruit varied from  $48.76 \pm 13.08$  to  $74.15 \pm 7.55\%$ . The 1200 ppm solution was shown to be the best concentration with a rate of  $74.15 \pm 7.55\%$ . That rate was significantly different from the rates of the other concentrations ( $p = 0.0004$ ). 1200 ppm also significantly reduced the disease's progression.

#### Protective capacity of *Ocimum gratissimum* essential oil

All the doses of *O. gratissimum* oil used, significantly reduced the incidence of disease on the treated fruit. In fact, the medium rates of obtained lesions in treated fruit were inferior to that of the control (Table 4). Average values of  $39.67 \pm 4.10$ ;  $39.73 \pm 5.03$  and  $32.67 \pm 4.28\%$  were respectively obtained at 300, 600, and 1200 ppm. All of these rates were statistically identical ( $p = 0.0585$ ). The average rate ( $48 \pm 4.59\%$ ) obtained at 150 ppm was statistically different from that ( $58.33 \pm 4.49\%$ ) of the control ( $P = 0.0191$ ). Disease progression was only slightly reduced. The measured lesion sizes varied from  $0.47 \pm 0.07$ cm to  $0.60 \pm 0.08$ cm. The mean sizes of the lesions measured were statistically identical with all of the treatments used ( $p = 0.1653$ ; Table 4). The concentrations 600 and 1200 ppm were slightly more effective with on the one hand, respective reduction rates by  $5.17 \pm 6.15$  and  $51.99 \pm 06.51\%$  of the incidence and on the other hand respective reduction rates of  $38.06 \pm 8.55$  and  $37.17 \pm 7.28\%$  of the anthracnose progression (Table 4). Dose of 300 ppm reduced the incidence of lesions by  $35.86 \pm 6.07\%$  and rate of disease progression by  $21.04 \pm 9.19\%$ . This same dose was more effective with the curative technique than the protective one.

#### Influences of *Ocimum gratissimum* essential oil on physicochemical parameters

➤ **Mass loss:** the rate of mass loss increased with

the oil concentration (Table 5). Thus, the highest mass loss ( $14.64 \pm 3.46\%$ ) in the treated fruit was observed at 1200 ppm. The loss of mass in fruit was similar among all the treatments used ( $P = 0.05377$ ).

➤ **Firmness:** The level of the fruit firmness was significantly reduced ( $p = 0.00004$ ) at 300 ppm. However 150; 600 and 1200 ppm dosages didn't significantly influence the firmness of the fruit (Table 5).

➤ **Soluble sugar solids:** The soluble sugar rate (TSS) was not significantly modified by the different dosages of *O. gratissimum* essential oil (Table 5). Therefore, TSS of treated fruits was statistically identical.

➤ **Titrate acidity (TA):** The titrate acidity content of the inoculated fruits showed no significant differences ( $p > 0.05$ ) between treated fruits and control one (Table 5). Fruits treated with a concentration of 150 ppm had the lowest TA value ( $2.51 \pm 0.19$  mEq. $10^{-2}$  g), while the fruits treated with the 300 ppm solution gave the highest value ( $4.25 \pm 0.41$ mEq. $10^{-2}$  g).

➤ **pH:** Results for the pH of the fruit juice (Table 5) showed that, there were no significant modifications in the fruits treated with solution concentrations of 150, 600, and 1200 ppm when compared to the control. However, the highest pH was recorded in the fruits treated with 300 ppm.

#### DISCUSSION

Among the natural compounds, essential oil offers a great potential as a biodegradable substance that has antimicrobial activity (Tripathi *et al.*, 2008). The results of the present study showed that various concentrations of essential oils extracted from *Ocimum gratissimum*, *Zingiber officinalis*, and *Monodora myristica* inhibited linear growth and spores germination of *C. gloeosporioides* but, their efficacy varied. The essential oil of *O. gratissimum* performed best against *C. gloeosporioides* causing fungicidal effects on both mycelia growth and spore germination. Results are similar to reports of previous works which described *O. gratissimum* oil as an inhibitor of mycelial growth and spore germination in several postharvest pathogens, including *C. gloeosporioides* (Souza Júnior *et al.*, 2009). They also found significant antifungal activity exhibited by *O. gratissimum* oil against others phytopathogens such as *Aspergillus flavus*, *A. fumigatus*, *Fusarium moniliforme* and *Deightonella torulosa* (N'guefack *et al.*, 2004; Camara *et al.*, 2007). On the contrary, Anaruma *et al.* (2010) revealed that the *O. gratissimum* oil from Brazil had a low antifungal activity against *C. gloeosporioides* (Penz.) & Sacc. They found MIC of the oil at 2000 ppm, while that of this study was 1000 ppm. This difference of efficacy could be due to a difference in their chemical composition, which varies with the geographical location, time of harvest and the plant

part collected (Matasyoh *et al.*, 2007). In fact, the antifungal properties of essential oils are in relationship with their content of antimicrobial compounds (Dikbas *et al.*, 2009). Farag *et al.* (1989) found that the inhibitory effect of the essential oils was mainly due to the chemical components within them. Seri-Kouassi (2004) reported a higher content of thymol (43.1%) in the essential oil from *O. gratissimum* in Côte d'Ivoire compared to the 9.80% of thymol found in Brazil (Janine de Aquino *et al.*, 2005). Many other studies reported that thymol is equipped with antifungal properties (Farag *et al.*, 1989). In 1964, thymol was documented in the United States of America as a pesticide (Tripathi and Dubey, 2004). In fact, due to its dual nature, part alcohol and part lipid, it promoted the breakdown of fungal cell membranes (Kwazou *et al.*, 2009). This may explain the higher inhibitory activity of *O. gratissimum* essential oils. Because of this, this essential oil could be recommended for a large-scale use within the scope of a biological control. Essential oils of *O. gratissimum* were found to be as effective as the synthetic fungicide Azoxystrombin. This synthetic fungicide product did not present any MIC on the two stages of life of *C. gloeosporioides* with the concentrations tested. This shows that the oil of *O. gratissimum* can be used instead of the synthetic fungicide in pathology control. The essential oils of *Z. officinale* and *M. myristica* were less effective than the oil of *O. gratissimum* and neither of those two oils presented MIC on *C. gloeosporioides*. Concerning a comparison between the two, the essential oil of *Z. officinale*, following the EC<sub>50</sub> and EC<sub>90</sub> detected, had an antifungal activity superior to that of *M. myristica*. The efficacy of *Z. officinale* oil had been previously proven by work done by El-Baroty *et al.* (2010) where they found that, the oil of *Z. officinale* highly inhibited the mycelial growth of four pathogenic fungi. The MIC they detected is at variance with the results found in this study. This difference in results may have been due to inherent differences in the parasites used. In their case, the pathogenic agents might have been more sensitive than ours. As far as the essential oils of *M. myristica*, were found to be less effective. These results were confirmed by Djeugap *et al.* (2009), who noticed that *M. myristica* oil had a lower antifungal activity against *C. gloeosporioides*.

In the present study, *O. gratissimum* essential oils effectively reduced the incidence and the evolution of disease on mango fruit as evidenced by the reduced infection rate and smaller lesion size seen on treated fruit compared to those of the control fruit. This result confirms the *in vitro* antifungal activity of *O. gratissimum* essential oils. The treatments after inoculation presented the lowest rates of infection, so it can be assumed that the essential oil of *O. gratissimum* was more effective as curative agent

than as a preventive one. Because of its volatile nature, this essential oil would best be used as an eradication agent rather than a protection agent (Duamkhanmanee, 2008). The rates of infection obtained with the two highest doses are significantly very low. However, the inhibitive effect of *O. gratissimum* essential oil is much higher *in vitro* than *in vivo*. Similar findings were previously made by Hadizadeh *et al.* (2009). The quantity of oil and its necessary components to inhibit the microbic agents' growth is also higher on food than in a medium culture. That fact could be imputed to the interaction between the essential oil phenolic compounds and the food matrix. Otherwise, this study also showed that the different doses of the essential oil did not significantly influence the firmness of the fruits. This suggests that they did not act on the activity or did not favor the synthesis of the hydrolyzing enzymes. Similar results were reported by Maqbool *et al.* (2010), who has evaluated *Cinnamom* spp essential oil effect on the post-harvest incidence of the anthracnose and the quality of bananas during the storage. These authors discovered that the different concentrations of the tested oils did not impact the firmness of the treated bananas. On the contrary, Raafat *et al.* (2012) reported that essential oils of *Cuminum cyminum* L., *Cymbopogon citratus* L. have caused an increase of treated strawberries firmness. This difference could be explained by the vegetable material. Moreover, the different doses of the oil of *O. gratissimum* also did not significantly influence the rate of TSS in the treated fruits and this result was similar to those reported by Maqbool *et al.* (2010). Concerning the mass loss, the dose of 1200 ppm of *O. gratissimum* brought out a significant loss of the treated fruits mass. This loss could be due to an increase in transpiration. On the contrary, Espitia *et al.* (2012) reported that essential oil of *Origanum vulgare*, *Cinnamomum zeylanicum* and of *Cymbopogon flexuosus* caused no significant loss of mass between treated pawpaw and controls. The essential oils of *O. gratissimum* also provoked a slight variation in the acidic titration. A dose 300 ppm favored a significant increase of the pH of the treated fruit, but with the other treatments and control there was not significant acidity change in fruit. In similar study, Tzortzakis (2007) reported that the vapor of *Eucalyptus globulus* L. and *Cinnamomum zeylanicum*, Blume, essential oils did not have a significant effect on the acidic quality in treated tomatoes and strawberries. These results contrast with the ones reported by Mohammadi and Aminifard (2012). They noticed that the pH value of peach fruit treated with essential oils was inferior to that of control fruit.

## CONCLUSION

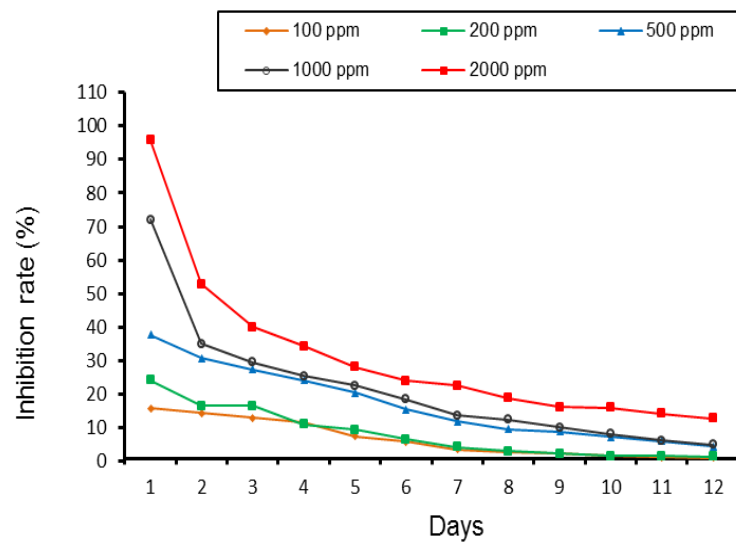
The present study demonstrated a high antifungal activity of *O. gratissimum* essential oils against *C.*

*gloeosporioides* (penz.) Penz & Sacc. *M. myristica* essential oils were also shown to have very brief activity at high concentrations. Finally, the dose 1200 ppm of *O. gratissimum* essential oils in emulsion assured a high inhibition of development of the anthracnose symptoms and their progress in artificially inoculated mangoes. Moreover, it did not alter the quality parameters of treated mangoes. So the essential oil of *O. gratissimum* can be used as an alternative way to control the postharvest anthracnose of mango fruit. Further studies on its stability as food preservative and on their use in combination with modified atmosphere packaging are recommended.

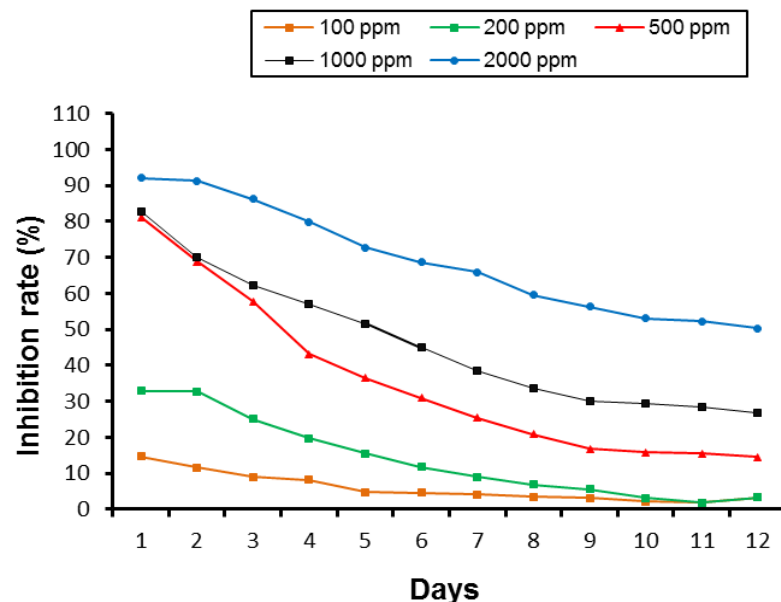
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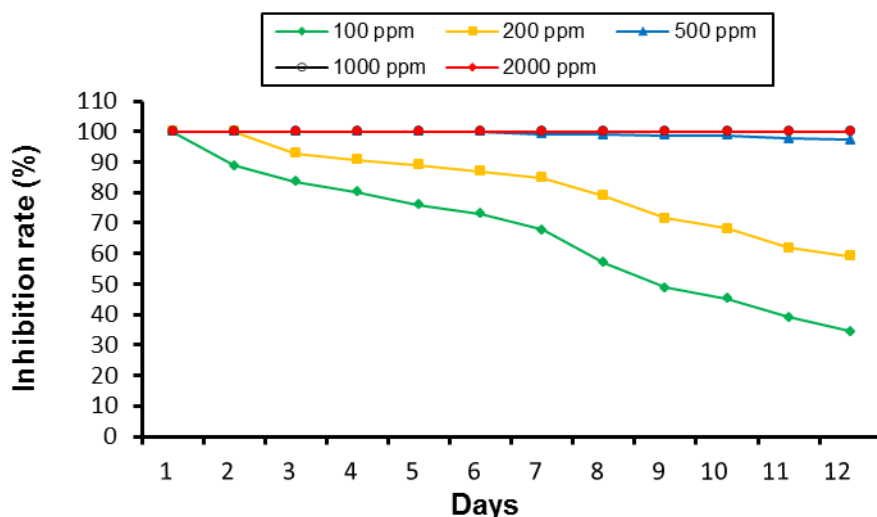


**Figure 1.** Inhibitory Effect of *Monodora myristica* essential oil on mycelia growth of *Colletotrichum gloeosporioides*

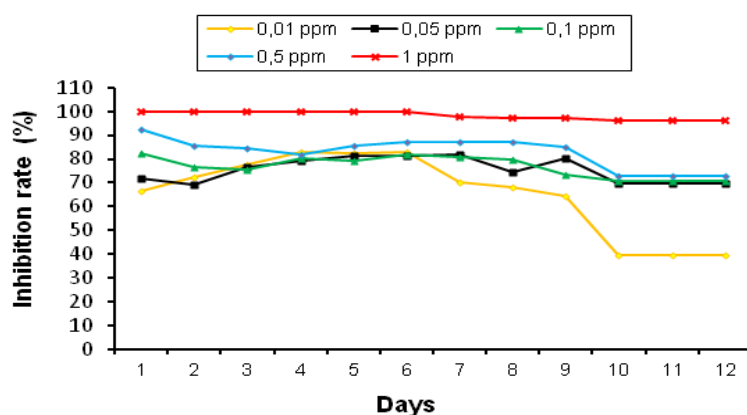


**Figure 2.** Inhibitory Effect of *Zingiber officinalis* essential oil on mycelia growth of *Colletotrichum gloeosporioides*





**Figure 3.** Inhibitory effect of *Ocimum gratissimum* essential oil on mycelial growth of *Colletotrichum gloeosporioides*



**Figure 4.** Inhibitory effect of synthetic fungicide «Azoxystrombin» on mycelial growth of *Colletotrichum gloeosporioides*

**Table 1:** Effective dose for 50 and 90% inhibition (EC<sub>50</sub>, EC<sub>90</sub>) and Minimum inhibitory concentration (MIC) of 3 essential oils and synthetic fungicide against *Colletotrichum gloeosporioides* respectively after 7 days and 24 hours of incubation

Antifungal parameters (ppm)	Mycelial growth			Spore germination		
	EC <sub>50</sub>	EC <sub>90</sub>	MIC	EC <sub>50</sub>	EC <sub>90</sub>	MIC
<i>Monodora myristica</i>	13989 a	782267 a	-	1258.9 a	12 648 a	-
<i>Ocimum gratissimum</i>	10.30 c	447 c	1000 a	81.13 b	446.7 b	1000 a
<i>Zingiber officinalis</i>	1335 b	9870 b	-	91.20 b	891.3 c	-
<i>Azoxystrombin</i>	0.0002 d	0.41 d	-	0.0003 c	0.25 d	-

Averages followed by the same letter within each column did not differ significantly at  $P \leq 0.05$  according to Newman Keuls Test. (- No concentration)

**Table 2.** Effect of different concentration of essential oils and synthetic fungicide on spore germination of *Colletotrichum gloeosporioides* *in vitro* after 24 hours of incubation.

Product Doses (ppm)	Essential oils			Synthetic fungicide
	<i>Monodora myristica</i>	<i>Ocimum gratissimum</i>	<i>Zingiber officinalis</i>	Azoxistrombin
100 (0.01)	22.3 ± 8.8 d	68.7 ± 4.1 b	54.1 ± 6.2 bc	<b>71.7 ± 6.8 ab</b>
200 (0.05)	33.4 ± 9.6 c	91.2 ± 1.8 a	59.4 ± 6.1 b	<b>78.3 ± 4.1 ab</b>
500 (0.1)	40.6 ± 7.6 d	99.9 ± 0.1 a	71.2 ± 5.0 bc	<b>86.3 ± 2.8 ab</b>
1000 (0.5)	48.2 ± 6.1 b	100 ± 0.0 a	95.5 ± 1.4 a	<b>94.2 ± 1.5 a</b>
2000 (1)	69.5 ± 5.9 b	100 ± 0.0 a	99.7 ± 0.3 a	<b>98.1 ± 0.7 a</b>

Averages followed by the same letter within each column did not differ significantly at  $P \leq 0.05$  according to Newman Keuls Test

**Table 3:** Effectiveness of essential oil of *Ocimum gratissimum* on the development and anthracnose symptoms evolution in Kent mangoes variety in treatment after inoculation (curative)

Treatments (ppm)	Infection rate (%)	Effectiveness	Lesions size (cm)	Effectiveness
00	58.33 ± 4.49 a		0.64 ± 0.08 a	
150	24.00 ± 3.94 c	64.97 ± 9.26 b	0.31 ± 0.06 cd	43.16 ± 18.17 bc
300	38.67 ± 4.82 b	48.76 ± 13.08 c	0.46 ± 0.07 b	47.97 ± 13.45 b
600	24.67 ± 4.02 c	63.75 ± 7.38 b	0.33 ± 0.06 c	40.46 ± 17.81 c
1200	20.00 ± 3.12 c	74.15 ± 7.55 a	0.22 ± 0.04 d	76.63 ± 6.93 a

Averages followed by the same letter within each column did not differ significantly at  $P \leq 0.05$  according to Newman Keuls Test

**Table 4:** Effectiveness of the essential oil of *Ocimum gratissimum* on the development and anthracnose symptoms evolution in Kent mangoes variety in treatment before inoculation (preventive).

Treatments (ppm)	Infection rate (%)	Effectiveness	Lesions size (cm)	Effectiveness
00	58.33 ± 4.49 a		0.64 ± 0.08 a	
150	48 ± 4.59 b	37.78 ± 8.91 a	0.60 ± 0.07 ab	23.20 ± 9.12 a
300	39.67 ± 4.10 bc	35.86 ± 6.07 a	0.55 ± 0.06 ab	21.04 ± 9.19 a
600	39.73 ± 5.03 bc	50.17 ± 6.15 a	0.50 ± 0.06 b	38.16 ± 8.55 a
1200	32.67 ± 4.28 c	51.99 ± 6.51 a	0.47 ± 0.07 b	37.17 ± 7.23 a

Averages followed by the same letter within each column did not differ significantly at  $P \leq 0.05$  according to Newman Keuls Test

**Table 5.** Effect of *Ocimum gratissimum* essential oil on some quality parameters of Kent mangoes variety

Treatment (ppm)	Mass loss (%)	Firmness (Kg)	Soluble sugar rate (°Brix)	Acidic Titration mEq.10 <sup>-2</sup> g	pH
Too	9.63 ± 1.04 b	2.46 ± 0.38 a	1.32 ± 0.12 b	5.24 ± 0.92 a	4.44 ± 0.10 b
00	7.98 ± 0.15 b	2.35 ± 0.28 a	1.69 ± 0.12 a	2.73 ± 0.23 b	4.43 ± 0.12 b
150	7.75 ± 0.73 b	2.44 ± 0.18 a	1.70 ± 0.12 a	2.51 ± 0.19 b	5.02 ± 0.31 ab
300	9.60 ± 1.33 b	1.09 ± 0.22 b	1.84 ± 0.07 a	4.25 ± 0.41 ab	5.55 ± 0.14 a
600	12.27 ± 1.75 ab	2.45 ± 0.24 a	1.65 ± 0.08 a	3.80 ± 0.45 ab	4.50 ± 0.13 b
1200	14.64 ± 3.46 a	2.33 ± 0.16 a	1.65 ± 0.07 a	3.54 ± 0.54 b	4.30 ± 0.15 b

Averages followed by the same letter within each column did not differ significantly at  $P \leq 0.05$  according to Newman Keuls Test. (Too: No inoculated and no treated fruits)