Biological Activity of *Syzygium aromaticum* and *Ravensara aromatica* Essential Oils from Madagascar and Their Possible Use against Postharvest Mango Anthracnose

Soloniony Navalonamanitra Andrianjafinandrasana[1,2], Marc Chillet[3], Isabelle Ratsimiala Ramonta[1], Jean-Michel Leong Pock Tsy[2,4], Jerôme Minier[3], Pascal Danthu[5]

[1]Université d'Antananarivo, Sciences et Technologies, Sciences de la Vie et de l'Environnement, Biotechnologie, Antananarivo, Madagascar
[2]DP Forêts & Biodiversité, Antananarivo, Madagascar
[3]Cirad Persyst, UMR Qualisud, 7 Chemin de l'IRAT, 97410 SAINT PIERRE, La Réunion, UMR Qualisud, Université de Montpellier, CIRAD, Université de la Réunion, Montpellier SupAgro, Université d'Avignon et des Pays de Vaucluse, Montpellier, France
[4]Département des Recherches Forestières et Gestion Ressources Naturelles-FOFIFA, Antananarivo, Madagascar
[5]Cirad, UPR HortSys, Université de Montpellier, Montpellier, France
Email: ritinaval@gmail.com

Abstract

The fungitoxicity of five Malagasy essential oils (Eos) against *Colletotrichum asianum* was assessed in terms of conidial germination and mycelial growth. Their effect on defense-related compounds content, physicochemical properties and anthracnose lesions in mango fruits was also determined. Four of the tested Eos were from *Ravensara aromatica* leaves, and the last Eo was extracted from clove leaves. Their chemical compositions were then determined through GC-MS analysis and the active compound of the most fungitoxic Eo was determined by testing the toxicity of its major component to *C. asianum*s spore germination, mycelial growth and its ability to inhibit anthracnose development on mango fruits. The *R. aromatica* Eos tested were fungistatic to *C. asianum*, whereas clove Eo was fungitoxic and the 4 chemotypes of *R. aromatica* Eo exhibited variable inhibiting capabilities: 1) all tested doses of all Eos (112.5 and 225 µL/L of air) were effective against *C. asianum* mycelial growth (10% - 100% inhibition) but doses of 225 µL/L were more inhibitory than those of 112.5 µL/L, 2) Conidial germination was more resistant to Eos toxicity since only 225 µL/L of methyl eugenol chemotype of *R. aromatica* Eo, all tested doses of the sabinene chemotype of *R. aromatica* Eo and clove Eo were found inhibitory toward conidial germination of *C. asianum*. 30 µL/L of

sprayed clove Eoweretested on inoculated mangoes and were found to be effective against anthracnose development without affecting the resorcinol content in mango peel and the physicochemical properties of mango pulp. Tests on the major components of clove Eo showed fungitoxic activities against mycelial growth and conidial germination of *C. asianum* similar to those of clove Eo.

Keywords

Biological Activity, *Syzygium aromaticum*, *Ravensara aromatic*, Fungitoxicity, Anthracnose, Essential Oils

1. Introduction

Mango fruits are commercialized worldwide for their sensorial and nutritive qualities, antioxidant and dietary properties [1] [2]. Top producers are Asian countries such as India, China, Thailand, Indonesia and Pakistan. These countries produced 15,188,000 to 1,888,449 MT of mango in 2014 (FAOSTAT Database), compared to Reunion Island that produces around 3500T of mango a year, both for exportation and local market use [3]. At preharvest stage, mango fruits suffer attack from fruit fly (*Bactrocera dorsalis*, *Tephritidae*), bacterial (*Xanthomonas*, *Xanthomonadae*; *Ralstonia*, *Rasltoniaceae*) and fungal pathogens (*Penicillium*, *Trichocomaceae*; *Alternaria*, *Pleosporaceae*; *Fusarium*, *Nectriaceae and Colletotrichum*, *Glomerellaceae*) that induce visual damages like rots and lesions at postharvest stage and cause tremendous loss during storage [4] [5]. One of the biggest challenges for the mango market is to protect stored fruit against anthracnose development. Postharvest anthracnose is a disease caused by phytopathogenic fungi included in the *Colletotrichum* genera that reduces mango fruit marketability, storability and nutritional value [6]. Current treatments combine one to multiple methods to overcome postharvest loss caused by phytopathogenic fungi: copper sprays can be applied at the preharvest stage, hot water treatment that is for use before storage; carbendazim treatment is used during storage, whereas prochloraz and benomyl are used as postharvest treatments [7]. Most of these treatments are of a chemical nature. There is thus a need for an eco-friendly and biological alternative product that can be used during mango storage to prevent anthracnose incidence and postharvest loss.

Essential oils (Eos) are natural products known through ancient time to have protective abilities against food spoilage and therefore were incorporated in stored food for their antibacterial [8] and antifungal [9] activities against a broad range of animal, human and plant pathogens, thus extending their shelf life of fruits without any negative effects on their physiochemical and sensorial qualities [10]. Eos such as cinnamon Eo [11], thyme Eo [12] [13] [14] and clove oil [15] are among the most investigated and successful against fruit postharvest pathogens. Their effects against *Colletotrichum* genera are generally focused on their *in vitro* ability to inhibit fungal growth and spore germination. Clearly,

their effectiveness against the anthracnose disease pathogen and its usability as a mango preservative would benefit from further investigation.

Many papers link the ability of Eos to induce fruit resistance against *Colletotrichum* infection to their capacity to elicit an effect on defense-related compounds such as resorcinol, chitinase enzyme synthesis in tropical fruits [16] [17]. In particular, researches on mangoes linked fruit maturity, resistance to phytopathogenic fungi to a decrease in resorcinol synthesis in mango peel [17] [18]. Such findings support the hypothesis that resorcinol compounds are involved in mango resistance to phytopathogenic fungi-caused disease.

Malagasy essential oils have recently been reported to have antifungal properties, present paper focused on two of them: clove and ravensara Eos. Clove trees were introduced into Madagascar just before the colonial period. These Eo-producing trees are actually well domesticated and play a predominant role in the economy of eastern Madagascar [19], whereas *Ravensara aromatica* Sonnerat (also known as *Cryptocarya agathophylla* Van der Werff) is an endemic tree located in the central and eastern parts of the island [20]. Clove Eo ability to inhibit phytopathogenic fungal germination and growth is already established worldwide [21]. In addition to its antifungal properties, clove Eo was also found to have antioxidant [22] and protective abilities against oxidative/nitrosative stress [23]. Similarly, clove Eo and Ravensara Eo from Madagascar are both reported to have a growth inhibition effect on human and plant pathogens such as *Aspergillus niger* and *Saccharomyces cerevisiae* [24], in addition to their phytotoxic [25] and antioxydant properties [26], and studies on the chemical composition and physical properties of both Eos [20] [27] [28] reported comparable results with previous investigations on the subject [29] [30].

This study is therefore based on the hypothesis that native Malagasy Eo also has antifungal abilities that can be used to prevent anthracnose development on stored mangoes from Reunion Island. The high variability and volatility of Ravensara Eo [31], on which its anti-germinative intensity depends [25], and the microvariability and high density of clove Eo [32] are important characteristics on which investigations were focused. Experiments were then conducted on the biological activities of these Eos against *Colletotrichum* and their effect on mango fruit anthracnose development and resorcinol production in view of their eventual use as a potential mango preservative during postharvest storage.

2. Materials and methods

2.1. The Collection of Essential Eos and Their Analysis

Five Eos extracted by hydrodistillation from fresh leaves in Madagascar were used in this study: one clove (*Syzygium aromaticum* L.) Eo and four chemotypes of *Ravensara aromatica* Sonnerat (also named *Cryptocarya agatophylla* Van der Werf) Eos: methyl chavicol (Type MC), methyl eugenol (Type ME), limonene (Type L) and sabinene chemotypes (Type S).

Eos compositions were determined by GC-MS analysis. The GC/MS analysis was conducted on a CLARUS 480+ gas chromatograph and an Elite-5MS col-

umn (length: 60 mm; I.D.: 0.25 mm). Compounds were identified by comparing the collected mass spectra with NIST08 (National Institute of Standards and Technology) database and their proportion in the Eo was established from each component's pic area in the chromatogram.

2.2. Fungal Pathogens and *in Vitro* Toxicity Assessment

Mango fruit anthracnose-isolated *Colletotrichum asianum* strains (MUC43868) were obtained from a Belgian collection (Université catholique de Louvain) and used for *in vitro* studies and to induce anthracnosis on mango fruits produced in Reunion Island.

2.2.1. Toxicity against Conidial Germination

Thirty conidia from a 15-day old culture of *C. asianum* were deposited on solidified Potato Dextrose Agar (PDA) Petri plates. The plates were reversed and 0, 10, and 20 µL of Eos were added to the plate lid (making 0, 112.5 and 225 µL/L of air concentration) prior to sealing each of them with a parafilm so as to to prevent any Eo flux between plates or any contact between the Eo and the conidia during incubation. All plates were incubated at 27°C (optimal temperature for *C. asianum* growth *in vitro*). Ten replicates were used for each treatment. Germinated conidia were counted daily (8 a.m.) for 7 days and final germination was recorded and expressed as:

$$\text{Conidial germination}(\%) = (\text{Mean of GC in each treatment} \times 100) / \text{Mean of GC in control}$$

In order to determine if germination was prevented or stopped at its early stages, optical studies were conducted on all treatments that induced no visible germination by daily inspection of the Petri plates under an optical microscope.

2.2.2. Toxicity against Mycelial Growth

1 mm^2 mycelial plug from a 15-day-old culture of *C. asianum* was grown on PDA plates with 0, 10, and 20 µL of Eos as in (2.2.1).

Radial growth was observed everyday (8 a.m.) for 7 days and the final mycelial diameter (MD) was recorded. Plugs with no growth were recultivated on freshly and the final mycelial diameter (MD) was recorded and expressed as:

$$\text{Mycelial growth}(\%) = (\text{Mean of MD in each treatment} \times 100) / \text{Mean of MD in control}$$

Criteria adopted by Bill *et al.* [33] were used to discriminate the effect of tested EO on *C. asianum*: treatments that induced no mycelial growth were defined as fungicidal, while treatments that showed mycelial growth inhibition was fungistatic.

2.3. Bioactivity of Essential Eos on Mango Fruits Assessment

2.3.1. Effect of Eos on Anthracnose Development

12 Mango fruits (Cogshall var.) were first weighed, cleaned under running water

and sterilized with 90˚ ethanol. Half of them were then inoculated with 10 µL of *C. asianum* spore suspension (10^5 conidia per mL) and maintained at 20˚C for 48 hours: a drop of conidia suspension was deposited on each mango, a small paper circle was placed over it, and each piece of paper was covered with a water-soaked cotton pad (two inoculating sites were determined for each fruit). Prior to incubation with Eos, the piece of paper and the cotton were removed.

Two closed plastic boxes (15 L contenance) were lined with aluminum foil (to prevent the Eo from permeating the boxes). In one of them, 500 µL of clove Eo were sprayed on the inner surface using 50 µL droplets of Eo (making 30 µL/L of air concentration). Ten of the above mangoes were transferred to each box and were incubated at 20˚C (storage temperature adopted by local producers and wholesalers). After 1 day of incubation, the aluminum foil coating was removed from the container with the Eo. Each box was kept open, as was the incubation chamber until all of the Eo scents had evaporated. The incubation chamber was then closed.

Lesion area (*LA*) is expressed as the mean of lesions observed in the two inoculated zones which are measured by their length (*L*1 and *L*2) and their width (*l*1 and *l*2) at the ripened stage of all mangoes:

$$LA = \left[(L1 * l1) + (L2 * l2) \right] / 2$$

2.3.2. Effect of Eos on Active Defense Response-Related Compound Content in Mango Fruit

Twenty-five mangoes were cleaned as specified above (2.3.1). For sampling, mango peels were removed, wrapped in aluminum foil, immersed in liquid nitrogen, mixed to a powder using a Retsch ® Grindomix, and stored at −80˚C. The same preparation was done to square-cut mango pulp. Five out of 25 mangoes were sampled before incubation, while the remaining 20 were incubated with or without 500 µL of sprayed clove oil as in (2.3.1). Five treated mangoes and five untreated mangoes were sampled after EO-impregnated aluminium foil removal. Final samplings were done at the ripened stage (15 days after incubation) for the remaining five treated and five untreated mangoes.

Resorcinol content was measured from 0.5 g of mango peel powders lyophilized beforehand, as described in (Knödler *et al.* 2009), using an HPLC apparatus (Dionex® Ultimate 3000 apparatus-length: 250 mm; I.D.: 4.6 mm; 5 µm; 30˚C stationary phase; Symmetry Shield RP18 column equipped with a diode array involving two eluents [A: H_2O: CH_3CN (99.8: 0.2, 0.01% HCOOH) and B (CH_3CN 100%)]). The gradient program was also adapted from (Knödler *et al.* 2009) (see Table 1). The detection of the AR was at 275 nm. Each compound was quantified and identified by comparison with a commercial standard of resorcinol (Sigma Aldrich). Pulp color (L, a*, b* indices) was measured using a Minolta ® C-400 chromameter in order to calculate °hue saturation. Freshly frozen ground pulp was used to measure total titratable acidity (ATT in meqv/100g MF), pH and total soluble solid content (measured in °Bx).

Table 1. HPLC gradient program for AR quantification (%).

Flow (mL/min)	Eluent A (%)	Eluent B (%)	Duration (min)
0.6	83	17	0
0.6	91	9	20
0.6	91	9	30
0.6	100	0	35
0.6	100	0	50
0.6	83	17	51
0.6	83	17	55

2.4. Identification of Active Compounds in Clove Eo

In order to find out if clove Eo fungitoxicity was due to its major component (eugenol) or to the synergism between its components, eugenol was purchased from a local producer (CTHT: Centre de Technique Horticole de Tamatave) and submitted to fungitoxicity tests on conidial germination and mycelial growth, anthracnose lesion area. Doses were adjusted to the eugenol content in the clove Eo tested (8.1 and 16.2 μL for *in vitro* assays, 405 μL for *in vivo* assays *i.e.* 91.125, 185.25 and 24.3 μL/L of air concentration).

2.5. Statistical Analysis

Variance analysis (ANOVA), using XLSTAT software, was used to compare the effects of each treatment on conidial germination, mycelial growth, lesion area, fruit quality and resorcinol content. Tuckey post hoc test was used to enlighten significant differences amongst the effect of each treatment on measured para-meters. In Figure 1 and Figure 2 and Tables 3-5, values with the same letter belong to the same homogeneous group.

3. Results

3.1. Essential Eo Composition

GC-MS analysis provided the EO compositions represented in Table 2. Clove Eo consisted essentially of eugenol (81%) and caryophyllene. The four *Ravensara* Eos contained similar minor components and differed in the major components and their proportions: 85% of methyl chavicol (estragole) for the first Eo, 53% of D-limonene for the second Eo, 70% of methyl eugenol for the third Eo and 28% of sabinene for the last Eo. Therefore, the collected *Ravensara* Eos belong to 4 chemotypes: an MC one, an ME one, an L one and an S one.

3.2. *In Vitro* Effects of Essential Eos on Colletotrichum Asianum

Similar to most reports on Eos, including clove Eo effects on *Colletotrichum* species, our results confirmed that clove Eos from Madagascar's eastern forests
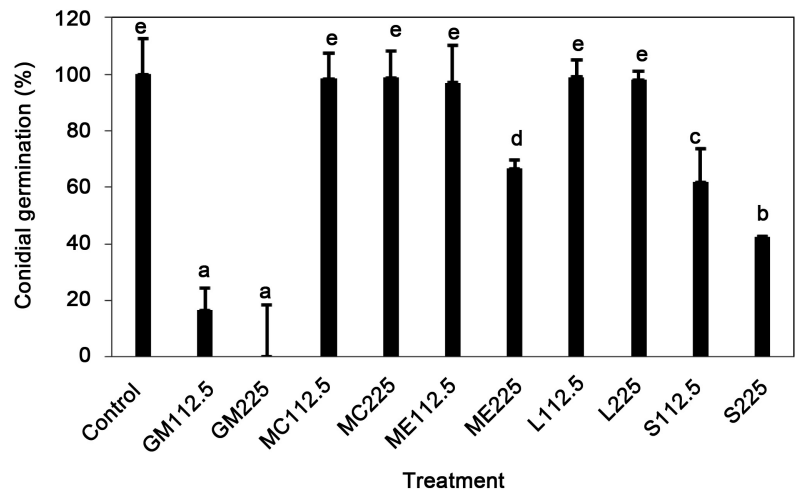
Figure 1. Effect of clove oil and *R. aromatica* oils (112.5 and 225 μL/L of air) on the percentage of conidial germination. GM stands for Malagasy clove oil, MC for methyl chavicol chemotype of *R. aromatica* oil, ME for methyl eugenol chemotype of *R. aromatica* oil, L for limonene chemotype of *R. aromatica* oil and S for sabinene chemotype of *R. aromatica* oil. Means followed by a common letter above each column are not significantly different at the 5% level.

Figure 2. Effect of clove oil and *R. aromatica* oils (112.5 and 225 μL/L of air) on the percentage of mycelial growth. GM stands for Malagasy clove oil, MC for methyl chavicol chemotype of *R. aromatica* oil, ME for methyl eugenol chemotype of *R. aromatica* oil, L for limonene chemotype of *R. aromatica* oil and S for sabinene chemotype of *R. aromatica* oil. Means followed by a common letter above each column are not significantly different at the 5% level.
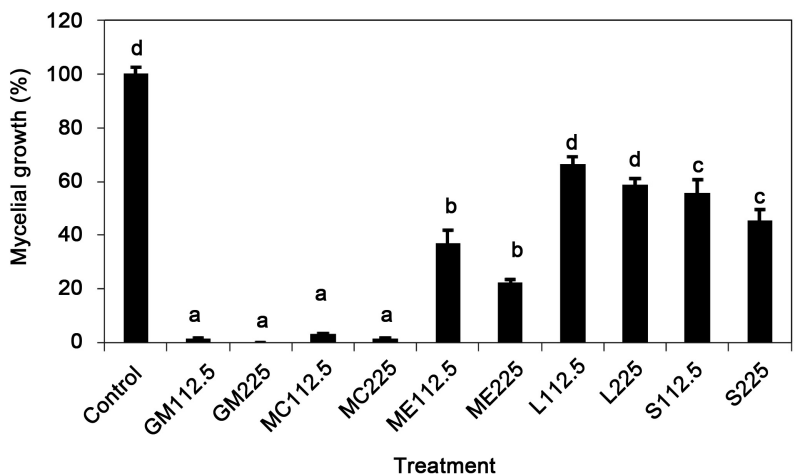
have a strong fungitoxic activity against *C. asianum* while effects of *R. aromatica* Eos were fungistatic. All tested clove Eo achieved complete inhibition of conidial germination of *C. asianum*. On the other hand, significant decreases of ($P < 0.05$) were only observed with 225 μL/L of methyl eugenol chemotypes and all tested sabinene chemotype of *R. aromatica* Eo, while limonene and methyl chavicol chemotypes of *R. aromatica* Eos showed no effect at all (see **Figure 1** and **Photo 1**).

Every tested Eo showed a significant ($P < 0.05$) decreasing effect on the mycelia
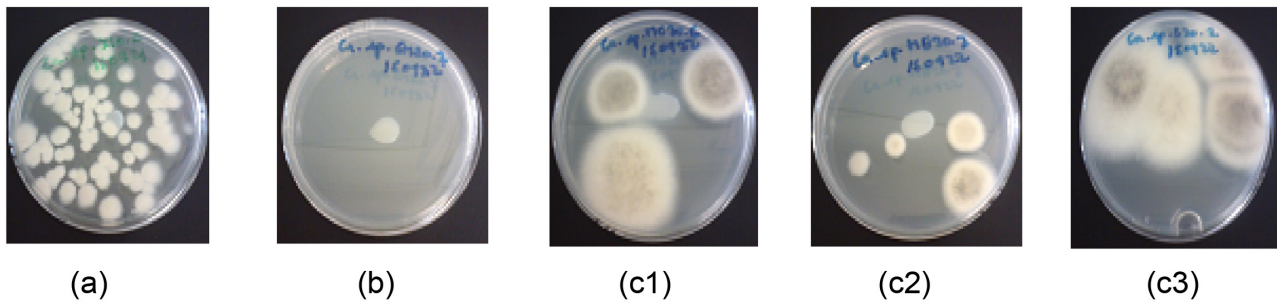
Photo 1. Conidial germination of *C. asianum* (a): without Eo; (b) With 20 μL of clove Eo; (c1) With 20 μL of Methyl chavicol chemotypes of *R. aromatica* Eo; (c2) With 20 μL of Methyl eugenol chemotypes of *R. aromatica* Eo; (c3) With 20 μL of Limonene chemotypes of *R. aromatica* Eo.
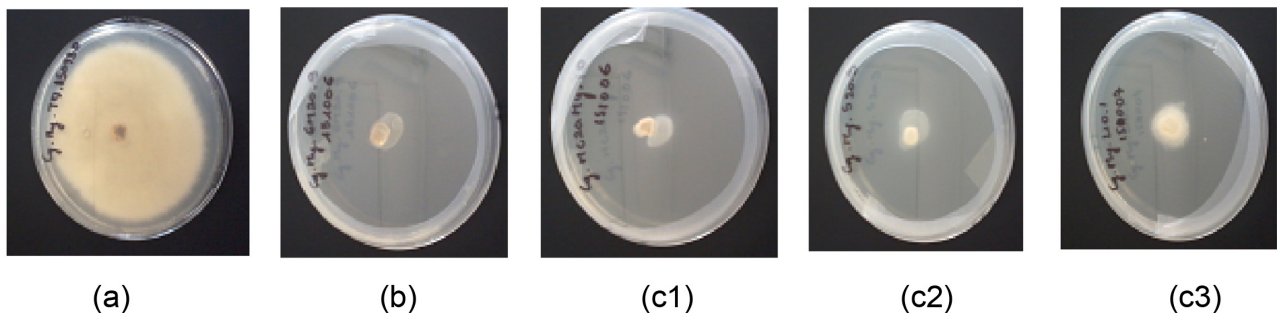

Photo 2. Mycelial growth of *C. asianum* (a): without Eo; (b) With 20 μL of clove Eo; (c1) With 20 μL of Methyl chavicol chemotypes of *R. aromatica* Eo, (c2) With 20 μL of Sabinene chemotypes of *R. aromatica* Eo, (c3) With 20 μL of Limonene chemotypes of *R. aromatica* Eo.

growth of *C. asianum*. The methyl chavicol chemotype of *R. aromatica* Eo and clove Eo totally inhibited mycelial growth of *C. asianum*. Regardless of its ineffectiveness against *C. asianum* conidial germination, the methyl chavicol chemotype of *R. aromatica* totally inhibited *C. asianum* mycelial growth (see **Figure 2** and **Photo 2**).

Therefore, the methyl chavicol chemotype of *R. aromatica* Eo also had significant fungicidal activity against mycelial growth, although its ineffectiveness against conidial germination prevents it from being the best choice for a mango anthracnose preservative. In the same range, clove Eo showed a greater inhibiting effect than the methyl eugenol chemotypes of *R. aromatica* Eo, whereas the eugenol content of tested clove Eo and the methyl eugenol content of *Ravensara aromatica* Eo are similar.

3.3. Essential Eo Treatment and Its Effect on Mangofruit Metabolism and Defense-Related Compounds

Clove Eo treatment (30 μL/L) induced a significant effect on the development of anthracnose in artificially inoculated mangoes (**Table 3**). Lesion area significantly decreased from untreated to treated mangoes at a P value < 0.05. The treatment did not alter the physical quality of mangoes pulp. No significant differences were observed on pH, total titratable acid content, total soluble solid content, weight loss and pulp color from treated to untreated mangoes at a P

Table 2. Chemical composition of the Eos tested (%).

EO components	Retention Time (min)	Clove oil	Relative area percentage			
			Ravensara oil			
			Type MC	Type ME	Type L	Type S
A-pinene	11.88	-	-	-	5	7
Camphene	12.5	-	-	-	2	-
Sabinene	13.23	-	-	-	8	28
B-pinene	13.49	-	-	-	2	-
B-myrcene	13.62	-	1	1	6	5
A-phellandrene	11.57	-	-	-	7	5
3-carene	14.53	-	1	3	-	5
D-limonene	15.28	-	5	9	33	4
B-phellandrene	13.23	-	-	-	-	6
Y-terpinene	16.24	-	-	-	-	2
Linalool	17.68	-	1	4	5	-
Terpin-4-ol	20.94	-	-	-	2	5
Methyl salicylate	21.56	-	-	-	12	-
Estragole	21.47	-	85	6	-	-
Anethole	24.67	-	4	-	-	-
A-cubebene	26.87	-	-	-	-	1
Eugenol	27.37	81	-	-	-	-
A-copaene	26.92	-	-	-	3	-
Methyl eugenol	28.39	-	2	70	-	23
Caryophyllene	29.67	14	-	2	8	3
Humulene	30.75	2	-	-	-	-
D-germacrene	31.5	-	-	-	6	5
Trans-isoeugenol or acetyl eugenol	32.17	2	-	-	-	-
Asarone	33.04	-	-	4	-	1
Caryophyllene oxide	34.76	1	-	-	-	-

value < 0.05.

5-pentadecylydroresorcinol content also showed no significant difference between treated and untreated mango peel samples ($P < 0.05$, see Table 4).

3.4. Identification of Active Compounds

Colletotrichum asianum was found to be similarly inhibited with eugenol as with clove Eo. One hundred percent inhibition was recorded on its mycelial growth and conidial germination, with all tested doses of eugenol (91.125 and 185.25 μL/L of air). Such results suggest that clove Eo fungitoxicity is not due to synergistic activities between its components but to an active compound, the

Table 3. Effect of malagasy clove Eo on anthracnose development, and physical characteristics of mango fruit 15 days after Eo treatment.

Criteria	Untreated mangoes	Treated mangoes
Lesion area (mm^2) in artificially inoculated mangoes	1067.857^c (±229.112)	530.416^a (±291.293)
Pulp color (°hue)	179.155^a (±1.34)	178.553^a (±0.03)
Total titratable acid content (ATT:meqv./100g MF)	5.96^a (±3.10)	4.38^a (±2.27)
Weight loss (g)	4.94^a (±1.28)	4.23^a (±0.53)
Total soluble solids (°Brix)	13.2^a (±0.93)	14.84^a (±2.57)
pH	4.232^a (±0.83)	4.662^a (±0.17)

Table 4. Effect of Malagasy clove Eo on resorcinol (5-pentadecylydroresorcinol) content (mg/g of fresh peel).

Sampling	Untreated mangoes	Treated mangoes
Before incubation	23.11^a (±8.15)	-
After incubation	16.42^a (±2.54)	16.68^a (±5.66)
15 days after incubation	19.30^a (±8.48)	18.52^a (±7.11)

major component of clove Eo: eugenol. The same effects were observed on anthracnose development when 405 μL of eugenol (*i.e.* 24.3 μL/L of air) induced *a* significant inhibition of lesion areas in ripening mango fruits (see Table 5). The recorded inhibition was slightly less than with clove Eo, though statistical analysis indicated that the effects of clove Eo and eugenol on lesion areas of ripening mangoes belong to similar groups (a and ab).

4. Discussion and Conclusion

The essential Eo compositions established on the basis of GC-MS are in accordance with previous reports on Malagasy clove Eoand *Ravensara aromatica* Eo compositions [27] [28], as well as with reports on other clove Eo compositions [29] [30]. The clove oil was mostly constituted of Eugenol and Caryophyllene and *Ravensara* oils exhibited similar components but varying amounts of each component. Each *R. aromatica* oils collected had one major component. Therefore, the collected Eos belong to 4 chemotypes.

Similar to its phytotoxic effects [25] and to the antibacterial effect of *Pimenta racemosa* var. *racemosa* leaf Eo against tomato wilt [34], the antifungal properties of *Ravensara aromatica* Eo also vary with the Eo chemotypes. Therefore, the antifungal activity of different doses of major components should be compared in order to confirm such a hypothesis. Since most antifungal activity reported is dose-dependent [35] [36] [37], and minor components of Eos are also known to have strong toxic abilities [9], this Eo chemotype-dependence of *Ravensara aromatic Eo* cannot solely be attributed to major Eo components, especially in view of Prakash's findings on the negative effects of minor compounds on eugenol

Table 5. Effect of eugenol on mycelial growth and conidial germination of *C. asianum* and on anthracnose development in mango fruit.

Criteria	Measurement	Treatment	Data
Conidial germination (%)	7 days after treatment	Untreated	100,000[b] (±0.000)
		Eugenol 8.1 µL	0.000[a] (±0.000)
		Eugenol 16.2 µL	0.000[a] (±0.000)
Mycelial growth (%)	7 days after treatment	Untreated	100,000[b] (±0.000)
		Eugenol 8.1 µL	0.000[a] (±0.000)
		Eugenol 16.2 µL	0.000[a] (±0.000)
Lesion area (mm^2) in artificially inoculated mangoes	15 days after treatment	Untreated	1,067,857[c] (±229,112)
		Eug 405 µL	764,583[ab] (±297,953)

toxicity [38]. Difference between clove oil *in vitro* toxicity and ME-typed ravensare Eo corroborated the previous findings on the decreasing effect of methylation of eugenol on its biological activities [39].

Many authors found Eo treatment to be significantly effective against phytopathogenic fungi-caused diseases [40] without altering the physico-chemical properties of mangoes [41]. Moderate preventive effects of sprayed clove oil were reported by Santamarina *et al.* on stored rice grain [42] while complete control of *Aspergillus flavus*, *Penicillium cinitricum* caused disease on oranges and jujube fruits were reported by Xing *et al.* [43]. Bill *et al.* [33] also reported strong curative effects of thymol oil fumigated on artificially inoculated avocadoes and demonstrated that such an effect can be partially attributed to the oil's ability to elicit resistance compounds release such as chitinase, glucanase and total phenolic compounds without altering fruit marketability. Our investigation revealed similar inhibitory effects concerning the ability of clove oil to prevent anthracnose on mango fruit in storage conditions, but no ability to induce the synthesis of resorcinol compounds was found in mango peel although our results corroborate previews statement on resorcinol content's decrease with ripening process [44]. Such findings are in agreement with the *in vitro* effects of clove oil treatment where mycelial growth and conidial germination were totally inhibited with 20 µL of clove oil. The present results suggest that inhibition of anthracnose development in ripening mangoes was mainly due to the toxic effect of clove Eo on *C. asianum* growth since more research is needed to prove that clove Eo has no effect on the internal resistance of mango to anthracnose. Some researchers directly applied the Eo on the fruit using the pulverization method or by incorporating the Eo into an edible coating such as aloes vera gel and chitosan [45]. Bautista-Banos *et al.* [46] and Bill *et al.* [33] reported that these techniques led to a greater reduction in lesion area than current commercial fungicides on fruit anthracnose.

Some works found in the literature also report that essential oil treatments have strong antifungal activity *in vitro* but weak *in vivo* and thus do not induce

significant inhibition on disease severity or defense-related enzyme activity. Shao *et al.* [47] reported similar findings when using clove oil on citrus green mold. Itako *et al.* also found that cymbopogon oil strongly inhibited spore germination *in vitro*, whereas sporulation and appressorium formation was not significantly reduced on sprayed leaves [48].

The present work attributes the toxicity of clove oil against mango anthracnose and its pathogen development to its major active compound, eugenol. Most research on the identification of the active compounds of a product is in agreement with such findings [42] [49] [50] [51] even if rare synergistic effects between components of the Eo have been reported. On the contrary, Prakash *et al.* [38] found antagonistic activities between eugenol and the remaining compounds of *Piper betle* L. essential oil to combat moisture in some edible commodities. They reported that eugenol showed better antifungal activity alone than when it was incorporated into *Piper betle* L. essential oil.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Saranwong, S., Sornsrivichai, J. and Kawano, S. (2004) Prediction of Ripe-Stage Eating Quality of Mango Fruit from Its Harvest Quality Measured Nondestructively by Near Infrared Spectroscopy. *Postharvest Biology and Technology*, **31**, 137-145. https://doi.org/10.1016/j.postharvbio.2003.08.007

[2] Evans, E.A. (2008) Recent Trends in World and U.S. Mango Production, Trade, and Consumption. EDIS document FE718, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.

[3] Amouroux, P., Normand, F., Nibouche, S. and Delatte, H. (2013) Invasive Mango Blossom Gall Midge, *Procontarinia mangiferae* (Felt) (Diptera: Cecidomyiidae) in Reunion Island: Ecological Plasticity, Permanent and Structured Populations. In: Simberloff, D., Ed., *Biological Invasions*, Springer, Berlin, 1677-1693. https://doi.org/10.1007/s10530-012-0400-0

[4] Panhwar, F. (2005) Post-Harvest Technology of Mango Fruits, Its Development, Physiology, Pathology and Marketing in Pakistan. In: Chemlin, E., Ed., Digitalverlag GmbH, Germany, 37p.

[5] Shaaban, H.A.E., El-ghorab, A.H. and Shibamoto, T. (2012) Bioactivity of Essential Oils and Their Volatile Aroma Components : Review. *Journal of Essential Oil Research*, **24**, 203-212. https://doi.org/10.1080/10412905.2012.659528

[6] Rahul, S.N., Khilari, K., Sagar, S., Chaudhary, S., Vihan, N. and Tomar, A. (2015)

Challenges in Postharvest Management of Fungal Diseases in Fruits and Vegetables—A Review. *South Asian Journal of Food Technology and Environment*, **1**, 126-130. https://doi.org/10.46370/sajfte.2015.v01i02.04

[7]   Arauz, L.F. (2000) Mango anthracnose: Economic Impact and Current Options for Integrated Management. *Plant Disease*, **84**, 600-611. https://doi.org/10.1094/PDIS.2000.84.6.600

[8]   Burt, S. (2004) Essential Oils: Their Antibacterial Properties and Potential Applications in Foods—A Review. *International Journal of Food Microbiology*, **25**, 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

[9]   Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008) Biological Effects of Essential Oil—A Review. *Food and Chemical Toxicology*, **46**, 446-475. https://doi.org/10.1016/j.fct.2007.09.106

[10] Vilaplana, R., Pérez-Revelo, K. and Valencia-Chamorro, S. (2018) Essential Eos as an Alternative Postharvest Treatment to Control Fusariosis, Caused by *Fusarium verticillioides*, in Fresh Pineapples (*Ananas comosus*). *Scientia Horticulturae*, **238**, 255-263. https://doi.org/10.1016/j.scienta.2018.04.052

[11] Perumal, A.B., Sellamuthu, P.S., Nambiar, R.B. and Sadiku, E.R. (2016) Antifungal Activity of Five Different Essential Oils in Vapour Phase for the Control of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae in Vitro* and on Mango. *International Journal of Food Science and Technology*, **51**, 411-418.

[12] Chillet, M., Minier, J., Ducrocq, M. and Meile, J.C. (2018) Postharvest Treatment of Mango: Potential Use of Essential Oil with Thymol to Control Anthracnose Development. *Fruits*, **73**, 153-157. https://doi.org/10.17660/th2018/73.3.2

[13] Chillet, M., Minier, J., Hoarau, M. and Meile, J.C. (2019) Potential Use of Thymol to Control Anthracnose Development in Mango. *European Journal of Plant Pathology*, **155**, 943-952. https://doi.org/10.1007/s10658-019-01825-9

[14] Chillet, M., Minier, J., Hoarau, M. and Meile, J.C. (2020) Optimisation of the Postharvest Treatment with Thymol to Control Mango Anthracnose. *American Journal of Plant Sciences*, **11**, 1235-1246. https://doi.org/10.4236/ajps.2020.118087

[15] Kamatou, G.P., Vermaak, I. and Viljoen, A.M. (2012) Eugenol—From the Remote Maluku Islands to the International Market Place: A Review of a Remarkable and Versatile Molecule. *Molecules*, **17**, 6953-6981. https://doi.org/10.3390/molecules17066953

[16] Sivakumar, D., Jiang, Y. and Yahia, E.M. (2011) Maintaining Mango (*Mangifera indica* L.) Fruit Quality during the Export Chain. *Food Research International*, **44**, 1254-1263. https://doi.org/10.1016/j.foodres.2010.11.022

[17] Karunanayake, L.C., Adikaram, N., Kumarihamy, B.M.M., Bandara, B.M.R. and Abayasekara, C. (2011) Role of Antifungal Gallotannins, Resorcinols and Chitinases in the Constitutive Defence of Immature Mango (*Mangifera indica* L.) against *Colletotrichum gloeosporioides*. *Journal of Phytopathology*, **159**, 657-664.

[18] Prusky, D. (1996) Pathogen Quiescence in Postharvest Diseases. *Annual Review of Phytopathology*, **34**, 413-434. https://doi.org/10.1146/annurev.phyto.34.1.413

[19] Maistre, J. (1955) Le giroflier à Madagascar et à Zanzibar. *L'agronomie Tropicale*, **10**, 413-448.

[20] Andrianoelisoa, H.S. (2008) Etude de la variabilité des huiles essentielles de Ravensara aromatica Sonnerat: Prescription pour une gestion durable de cette espece endemique malgache. PhD Thesis, Doctoral school of Chemical Sciences, Université de Montpellier II-Sciences Et Techniques Du Languedoc, 216 p.

[21] Da Cruz Cabral, L., Fernandez Pinto, V. and Patriarca, A. (2013) Application of

Plant Derived Compounds to Control Fungal Spoilage and Mycotoxin Production in Foods. *International Journal of Food Microbiology*, **166**, 1-14. https://doi.org/10.1016/j.ijfoodmicro.2013.05.026

[22] Gülçin, I., Elmastaş, M. and Aboul-Enein, H.Y. (2012) Antioxidant Activity of Clove Oil—A Powerful Antioxidant Source. *Arabian Journal of Chemistry*, **5**, 489-499. https://doi.org/10.1016/j.arabjc.2010.09.016

[23] Errafiy, N., Ammar, E. and Soukri, A. (2016) Protective Effect of Some Essential Oils against Oxidative and Nitrosative Stress on *Tetrahymena thermophila* Growth. *Journal of Essential Oil Research*, **25**, 339-347. https://doi.org/10.1080/10412905.2013.775681

[24] Hossain, M.A., AL Harbi, S.R., Weli, A.M., Al-Riyami, Q. and Al-Sabahi, J.N. (2014) Comparison of Chemical Constituents and Antimicrobial Activities of Three Essential Oils from Three Different Brands' Clove Samples Collected from Gulf Region. In *Asian Pacific Journal of Tropical Disease*, **4**, 262-268. https://doi.org/10.1016/S2222-1808(14)60570-3

[25] Andrianjafinandrasana, S.N., Andrianoelisoa, H.S., Jeanson, M.L., Ratsimiala Ramonta, I. and Danthu, P. (2013) Allelopathic Effects of Volatile Compounds of Essential Oil from *Ravensara aromatica* Sonnerat Chemotypes. *Allelopathy Journal*, **31**, 333-344.

[26] Juliani., H.R., Simon, J.E., Ramboatiana, M.M.R., Flor, P., Behra, O., Garvey, A.S. and Raskin, I. (2004) Malagasy Aromatic Plants: Essential Oils, Antioxidant and Antimicrobial Activities, *Acta Horticulturae* (*ISHS*), **629**, 77-81, https://doi.org/10.17660/ActaHortic.2004.629.9

[27] Andrianoelisoa, H.S., Menut, C., Collas De Chatelperron, P., Ramanoelina, P. and Danthu, P. (2006) Intraspecific Chemical Variability and Highlighting of Chemotypes of Leaf Essential Oils from *Ravensara aromatica* Sonnerat, a Tree Endemic to Madagascar. *Flavour and Fragrance Journal*, **21**, 833-838.

[28] Razafimamonjison, G., Jahiel, M., Duclos, T., Ramanoelina, P., Fawbush, F. and Danthu, P. (2014) Bud, Leaf and Stem Essential Oil Composition of Clove (*Syzygium aromaticum* L.) from Indonesia, Madagascar and Zanzibar. *International Journal of Basic and Applied Sciences*, **3**, 224-233. https://doi.org/10.14419/ijbas.v3i3.2473

[29] Bhuiyan, M.N.I., Begum, J., Nandi, N.C. and Akter, F. (2012) Constituents of the Essential Oil from Leaves and Buds of Clove (*Syzigium caryophyllatum* (L.) Alston). *African Journal of Pharmacy and Pharmacology*, **6**, 1260-1263.

[30] Pino, J.A., Marbot, R., Agüero, J. and Fuentes, V. (2001) Essential Oil from Buds and Leaves of Clove (*Syzygium aromaticum* (L.) Merr. et Perry) Grown in Cuba. *Journal of Essential Oil Research*, **13**, 278-279. https://doi.org/10.1080/10412905.2001.9699693

[31] Andrianoelisoa, H.S., Menut, C., Ramanoelina, P., Raobelison, F., De Chatelperron, P.C. and Danthu, P. (2010) Chemical Composition of Essential Oils from Bark and Leaves of Individual Trees of *Ravensara aromatica* Sonnerat. *Journal of Essential Oil Research*, **22**, 66-70. https://doi.org/10.1080/10412905.2010.9700267

[32] Razafimamonjison, G., Jahiel, M., Ramanoelina, P., Fawbush, F. and Danthu, P. (2013) Effects of Phenological Stages on Yield and Composition of Essential Oil of *Syzygium aromaticum* Buds from Madagascar. *International Journal of Basic and Applied Sciences*, **2**, 312-318. https://doi.org/10.14419/ijbas.v2i4.1100

[33] Bill, M., Sivakumar, D., Korsten, L. and Thompson, A.K. (2014) The Efficacy of Combined Application of Edible Coatings and Thyme Oil in inducing Resistance

Components in Avocado (*Persea americana* Mill.) against Anthracnose during Post-Harvest Storage. *Crop Protection*, **64**, 159-167.
https://doi.org/10.1016/j.cropro.2014.06.015

[34] Deberdt, P., Davezies, I., Coranson-Beaudu, R. and Jestin, A. (2018) Efficacy of Leaf Oil from *Pimenta racemosa* var. *racemosa* in Controlling Bacterial Wilt of Tomato. *Plant Disease*, **102**, 124-131. https://doi.org/10.1094/PDIS-04-17-0593-RE

[35] Amini, M., Safaie, N. and Salmani M.J. (2012) Antifungal Activity of Three Medicinal Plant Essential Oils against Some Phytopathogenic Fungi. *Trakia Journal of Sciences*, 2012, **10**, 1-8.

[36] Garcia, R., Alves, E.S.S., Santos, M.P., Aquije, G.M.F.V., Fernandes, A.A.R., Dos Santos, R.B., Ventura, J.A. and Fernandes, P.M.B. (2008) Antimicrobial Activity and Potential Use of Monoterpenes as Tropical Fruits Preservatives. *Brazilian Journal of Microbiology*, **39**, 163-168. https://doi.org/10.1590/S1517-83822008000100032

[37] Kocić-Tanackov, S., Dimić, G., Lević, J., Tanackov, I. and Tuco, D. (2011) Antifungal Activities of Basil (*Ocimum basilicum* L.) Extract on *Fusarium* Species. *African Journal of Biotechnology*, **10**, 10188-10195.
https://doi.org/10.5897/AJB11.1330

[38] Prakash, B., Shukla, R., Singh, P., Kumar, A., Mishra, P.K. and Dubey, N.K. (2010) Efficacy of Chemically Characterized *Piper betle* L. Essential Oil against Fungal and Aflatoxin Contamination of Some Edible Commodities and Its Antioxidant Activity. *International Journal of Food Microbiology*, **142**, 114-119.
https://doi.org/10.1016/j.ijfoodmicro.2010.06.011

[39] Juliani, H.R., Koroch, A.R. and Simon, J.E. (2009) Chemical Diversity of Essential Oils of *Ocimum* Species and Their Associated Antioxidant and Antimicrobial Activity. In: Chemat, K., Varshney, F., Allaf, V.K., Eds., *Essential Oils and Aromas: Green Extractions and Applications*, Har Krishan Bhalla & Sons, Dehradun, India, 284-295.

[40] La Torre, A., Caradonia, F., Gianferro, M., Molinu, M.G. and Battaglia, V. (2014) Activity of Natural Products against Some Phytopathogenic Fungi. *Communications in Agricultural and Applied Biological Sciences*, **79**, 439-449.

[41] Sefu, G., Satheesh, N. and Berecha, G. (2015) Effect of Essential Oils Treatment on Anthracnose (*Colletotrichum gloeosporioides*) Disease Development, Quality and Shelf Life of Mango Fruits (*Mangifera indica* L). *American-Eurasian Journal of Agricultural & Environmental Sciences*, **15**, 2160-2169.

[42] Santamarina, M.P., Roselló, J., Giménez, S. and Blázquez, M.A. (2016) Commercial *Laurus nobilis* L. and *Syzygium aromaticum* L. Merr. & Perry Essential Oils against Post-Harvest Phytopathogenic Fungi on Rice. *LWT—Food Science and Technology*, **65**, 325-332. https://doi.org/10.1016/j.lwt.2015.08.040

[43] Xing, Y., Xu, Q., Li, X., Che, Z. and Yun, J. (2012) Antifungal Activities of Clove Oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium citrinum in Vitro* and in Wounded Fruit Test. *Journal of Food Safety*, **32**, 84-93.

[44] Kienzle, S., Carle, R., Sruamsiri, P., Tosta, C. and Neidhart, S. (2014) Occurrence of Alk(en)ylresorcinols in the Fruits of Two Mango (*Mangifera indica* L.) Cultivars during On-Tree Maturation and Postharvest Storage. *Journal of Agricultural and Food Chemistry*, **62**, 28-40. https://doi.org/10.1021/jf4028552

[45] Herna, A.N. and Herna, M. (2006) Chitosan as a Potential Natural Compound to Control Pre and Postharvest Diseases of Horticultural Commodities. *Crop Protection*, **25**, 108-118. https://doi.org/10.1016/j.cropro.2005.03.010

[46] Bautista-Banos, S., Hernandez-Lopez, M., Bosquez-Molina, E. and Wilson, C.L.

(2003) Effects of Chitosan and Plant Extracts on Growth of *Colletotrichum gloeosporioides*, Anthracnose Levels and Quality of Papaya Fruit. *Crop Protection*, **22**, 1087-1092. https://doi.org/10.1016/S0261-2194(03)00117-0

[47] Shao, X., Cao, B., Xu, F., Xie, S., Yu, D. and Wang, H. (2015) Effect of Postharvest Application of Chitosan Combined with Clove Oil against Citrus Green Mold. *Postharvest Biology and Technology*, **99**, 37-43. https://doi.org/10.1016/j.postharvbio.2014.07.014

[48] Itako, A.T., Júnior, J.B.T. and Schwan-Estrada, K.R.F. (2013) *Cymbopogon citratus* Essential Oil Bioactivity and the Induction of Enzymes Related to the Pathogenesis of *Alternaria solani* on Tomato Plants. *Idesia*, **31**, 11-18. https://doi.org/10.4067/S0718-34292013000400002

[49] Martini, H., Weidenborner, M., Adams, S. and Kunz, B. (1996) Eugenol and Carvacrol: The Main Fungicidal Compounds in Clove and Savory. *Italian Journal of Food Science*, **8**, 63-67.

[50] Rana, I.S., Rana, A.S. and Rajak, R.C. (2011) Evaluation of Antifungal Activity in Essential Oil of the *Syzygium aromaticum* (L.) By Extraction, Purification and Analysis of Its Main Component Eugenol. *Brazilian Journal of Microbiology*, **42**, 1269-1277. https://doi.org/10.1590/S1517-83822011000400004

[51] Hong, J.K., Yang, H.J., Jung, H., Yoon, D.J., Sang, M.K. and Jeun, Y.C. (2015) Application of Volatile Antifungal Plant Essential Oils for Controlling Pepper Fruit Anthracnose by *Colletotrichum gloeosporioides*. *Plant Pathology Journal*, **31**, 269-277. https://doi.org/10.5423/PPJ.OA.03.2015.0027