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Original article

Postharvest treatment of mango: Potential use of essential oil with thymol to control anthracnose development

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Summary

Introduction – **Anthracnose, a fungal disease caused by the *Colletotrichum gloeosporioides* species is the main postharvest problem concerning mango (*Mangifera indica*) production on La Reunion Island. Traditional postharvest treatments involve chemical compounds that do not comply with the expectations of consumers or importing countries. Chemical treatments are generally used against *C. gloeosporioides*. Our goal was to develop alternative postharvest treatments using the fungitoxic properties of two essential oils (EO).** *Materials and methods* – **Two commercial essential oils, X2 and X5, were used at various concentrations and compared to a no-oil control. A first batch of treatments were tested *in vitro* for studying mycelial growth and the inhibition of conidial germination. The second experiment measured the effects of the treatments on the fruit quality of inoculated mangoes var. Tommy Atkins with a solution of *C. gloeosporioides* spores.** *Results and discussion* – ***In vitro*, X5 mainly composed of thymol was very fungitoxic against *C. gloeosporioides*. The concentrations of phenolic compounds and resorcinol in the fruits were increased after the X5 treatments, expressing some positive effects of essential oil treatments on fruit resistance mechanisms. The quality of treated fruits verified the requirements to meet consumers' expectations.** *Conclusion* – **Thymol-based EO exhibited a strong fungitoxic *in vitro* activity but it had no detectable effect when applied by volatilization on mango necrosis. Alternatives ways of treatments should be tested.**

Keywords
mango, *Mangifera indica*, anthracnose, *Colletotrichum gloeosporioides*, biological control, fruit quality, phenolics

Significance of this study

What is already known on this subject?
- Thymol oil treatments can be effective in some fruit host/pathogen interactions, such as anthracnose in avocado.

What are the new findings?
- The essential oil rich in thymol increased the biosynthesis of phenolic compounds involved in fruit resistance to anthracnose.

What is the expected impact on horticulture?
- Modifications of postharvest treatments in mango and other tropical fruits will decrease the use of chemical molecules.

Résumé

Traitement post-récolte de la mangue: utilisation potentielle de l'huile essencielle de thymol pour contrôler le développement de l'anthracnose

Introduction – **L'anthracnose, une maladie fongique causée par *Colletotrichum gloeosporioides*, est le principal problème post récolte affectant la production de mangue (*Mangifera indica*) à la Réunion. Les traitements post récolte traditionnels impliquent des molécules chimiques qui ne correspondent plus aux attentes des consommateurs ou des pays importateurs. Ces traitements chimiques sont habituellement utilisés contre *C. gloeosporioides*. Notre objectif était de développer un traitement post récolte alternatif en utilisant les propriétés fongitoxiques de deux huiles essentielles (EO).** *Matériel et méthodes* – **Deux huiles essentielles du commerce X2 et X5 ont été utilisées à diverses concentrations et comparées au contrôle sans huile. Dans un premier temps, ces traitements ont été testés *in vitro* sur la croissance mycélienne et l'inhibition de la germination. Dans un second temps, les effets des traitements ont été mesurés sur la qualité des mangues var. Tommy Atkins inoculées avec une solution de spores de *C. gloeosporioides*.** *Résultats et discussion* – ***In vitro*, l'EO à base de thymol s'est montrée très fongitoxique contre *C. gloeosporioides*. Les effets sur les mécanismes de résistance du fruit ont également été étudiés, principalement la biosynthèse des composés polyphénoliques et les résorcinols. Il y a une augmentation de la production de ce type de composés après le traitement au thymol. Des données sur les effets des huiles sur les paramètres de qualité du fruit ont aussi été mesurées. La qualité du fruit a été mesurée par des analyses biochimiques (pH, sucres) afin de vérifier si les traitements n'altéraient pas ces paramètres qualitatifs.** *Conclusion* – **L'huile essentielle à base de thymol présente une activité fongitoxique très forte *in vitro* mais appliquée par volatilisation n'a pas d'effet sur les nécroses des mangues. D'autres modes de traitements doivent être testées.**

Mots-clés
mangue, *Mangifera indica*, anthracnose, *Colletotrichum gloeosporioides*, lutte biologique, qualité du fruit, composés phénoliques

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Introduction

Mango (*Mangifera indica* L.) is considered as an important fruit crop grown throughout the tropics because of its appealing flavor as well as high nutritive and marketing values. However, mango is highly prone to postharvest decay caused by various pathogens, leading to major economic losses (Swart *et al.*, 2002). The main problems are related to anthracnose disease. Anthracnose is caused by *Colletotrichum gloeosporioides* (Penz), and is well known as one of the most destructive postharvest diseases of mango fruit. The pathogen can attack immature mango fruit as a latent infection and the lesions progressively appear after storage and during ripening (Dodd *et al.*, 1991). Disease control is achieved primarily by postharvest application of fungicides such as benomyl and prochloraz, used either alone or in combination with other treatments (Johnson *et al.*, 1997). However, because of problems related to fungicide toxicity, the development of fungicide resistance, and potential adverse effects on the environment and human health, alternative strategies for controlling postharvest rot have been proposed like biocontrol, hot water treatments or use of essential oils properties (Droby *et al.*, 2009).

Previous works described the antimicrobial properties of different essential oils to control postharvest diseases (Combrinck *et al.*, 2011; Kong *et al.*, 2016; Palou *et al.*, 2016). For Citrus disease, coating treatment with the essential oil of *Mentha spicata* and *Lippia scaberrima* reduced losses due to *Penicillium digitatum* (du Plooy *et al.*, 2009). Experiments with the same oils on two pathogens of avocado have shown promising results (Regnier *et al.*, 2010). Bill *et al.* (2014) have also demonstrated the effectiveness of thyme oil and oil-based R-carvone, as well as the stimulatory effect of natural defense pathways of this type of treatment for controlling avocado diseases.

The main objective of our study was to test alternative postharvest treatment methods against anthracnose disease of mango. The chemical properties and antimicrobial activity of essential oils can elicit physiological responses of the fruit to control *C. gloeosporioides*. In this study, we tested the fungitoxic effect of two essential oils against *C. gloeosporioides*, and the effect of these treatments on fruit physiology and quality.

Materials and methods

Plant material

The anthracnose-prone mango (*Mangifera indica*) var. Tommy Atkins was cultivated at the CIRAD research station of Saint-Pierre, La Reunion Island (21°6'S, 55°32'E, tropical climate, average annual temperature = 25 °C, rainfall = 1000 mm year^{-1}, ferrallitic soils). Fruits were harvested at a commercial maturity stage, when the discoloration of the fruit peel began to be visible.

Pathogens

Nurc-MG01, one strain of *Colletotrichum gloeosporioides* from the CIRAD pathogens collection (Montpellier, France) was used and cultivated on potato dextrose agar (PDA) medium.

Essential oils (EO)

We used two different essential oils (EO): X2 and X5, from Xeda International SA (Saint Andiol, France).

Analysis of the EO composition using GC-MS

The two oils were analyzed using a Perkin Elmer Clarus 580 gas chromatograph (GC) equipped with an Elite-5MS capillary column, 60 m long and 0.25 mm ID (oven at 250 °C), an autosampler and a flame ionization detector, and a Perkin Elmer SQ8T mass spectrophotometer.

In vitro tests – Essential oils and germination of *C. gloeosporioides*

To test the effect of each essential oil on *C. gloeosporioides* spore germination *in vitro*, 10 µL of each oil were deposited on the lid of a Petri dish (oil volatilization) and the spores (10 µL at 10^5 sp mL^{-1}) were deposited on a special microscope slide (Dutscher ref. 020302) covered with PDA medium. For each experiment, five Petri dishes were treated and five were used as controls. The duration of the experiment is 3 days: 24 h in the presence of EO, and 48 h without oil. Germination rate was measured after 6, 24 and 72 h after incubation at 26 °C. The experiment was repeated three times. To search for the minimal inhibitory concentration (MIC), we repeated this experiment with 5.0, 2.0, 1.0 and 0.5 µL of X5 oil.

In vitro tests – Essential oils and mycelial growth of *C. gloeosporioides*

To test the effect of each essential oil on *C. gloeosporioides in vitro*, 1, 2, 5 and 10 µL of each oil were deposited on the lid of a Petri dish (oil volatilization). The culture medium was thus returned with the mycelium in the middle. For each experiment, ten Petri dishes were treated and ten were used as controls. After one week of incubation at 25 °C (fungistatic activity), the inoculum was placed in another Petri dish without essential oil to test the fungitoxic effect (7 days). The mycelial growth inhibition (MGI) represents the percentage of inhibitory effect of the treatment. It is calculated by the formula:

$$\text{MGI} = 100 - \left(\frac{\text{Diameter treated}}{\text{Diameter control}}\right)$$

The experiment was repeated three times. To search for the MIC, we repeated this experiment with 2 and 1 µL of X5 oil.

In vivo tests - Fruit inoculation

The *C. gloeosporioides* strain Nurc-MG01 was cultivated for 10 days on PDA medium. To inoculate fruits, we placed a drop (10 µL of a spore solution calibrated at 10^5 mL^{-1}) on the fruit peel and stored the fruit at high humidity for 48 h to promote appressoria formation. After inoculation, the mango fruits were placed in sealed boxes (30 L) to be treated as follows: T1 (10 fruits with 250 µL of X2 essential oil for 4 days), T2 (10 fruits with 250 µL of X5 essential oil for 4 days) and T3 (Control = ventilated box without oil). We put the EO in a plate inside each sealed box for T1 and T2, and a plate with 250 µL of water for T3. The boxes were stored in a climatic room at 20 °C. The developed necrosis was observed and measured 10 days after treatment.

Biochemical analyses

Mango fruits chosen for biochemical analyses were placed in sealed boxes (30 L) to be treated as follows: T1 (10 fruits with 250 µL of X2 essential oil for 4 days), T2 (10 fruits with 250 µL of X5 essential oil for 4 days) and T3 (Control = ventilated box without oil) like the previous experiment with inoculated fruits.

After treatment, fruits were sampled. Peel and pulp were separated and frozen. Biochemical analyses were performed on quality parameters (pH and total soluble solids measured

Table 1. Composition (in %) of two essential oils (X2 and X5, Xeda International, France) analyzed by gas-chromatography coupled with mass spectrometry (GC-MS).

	X2	X5
4-carene	0.18	-
Carvone	60.17	-
Caryophyllene	6.92	-
D-limonene	0.69	-
Eugenol	30.67	-
Humulene	1.26	-
Propylene glycol (Thymol adjuvant)	-	25.02
Thymol	-	74.98
Y-terpinene	0.12	-

by hand refractometer on fruit pulp) and polyphenols and resorcinol-like compounds (analyzed by High Performance Liquid Chromatography or HPLC on fruit peel). HPLC analysis was performed using a Dionex Ultimate 300 apparatus (Dionex Co., Sunnyvale, CA, USA) equipped with a diode array detector. The column used was a reverse-phase Waters Symmetry Shield C18, 250 × 4.6 mm, 5 µ.

Statistical analysis

Analyses of variance (ANOVA) and Excel Stat (2010) were used to analyze the effect of the two EO on germination of conidia and inhibition of the mycelial growth. After applying the least significant difference (Newman-Keuls test), differences at $P \leq 0.05$ were considered to be significant.

Results and discussion

Determination of oil chemical composition using GC-MS analysis

The two essential oils used in this experiment were found to be quite different (Table 1): X2 was composed of R-carvone (60.17%), eugenol (30.67%) and caryophyllene (6.92%), whereas X5 is mainly composed of thymol (74.98%) and an adjuvant (propylene glycol). Thymol is a phenolic compound present in the essential oil (EO) of thyme *(Thymus* spp.) and several other aromatic plants.

In vitro tests – Effects on spore germination and mycelium development

Six hours after the deposition of the conidia on the microscope slides (covered with PDA), more than 80% of the conidia germinated in the absence of oil (Control) and 100% germinated after 24 h incubation (Table 2). However, no germination was observed in the presence of X2 or X5 (10 µL) after 6 h. After 24 h of the X2 treatment, only about 14% of germinated conidia were observed while no germination was observed with X5 even with 1 µL volatilization. When the slides were transferred into oil-free Petri dishes, after 2 days, 33% of the X2-treated conidia showed germination, while no germination was observed with X5 whose minimal inhibitory concentration (MIC) was 0.5 µL in the Petri dish.

The X5 essential oil showed a fungitoxic effect which totally and irreversibly blocked fungal growth with 2 µL in the Petri dish (Table 3). X2 showed a fungistatic effect as after 7 days without treatment, a partial recovery (26.5 to 54.9%) was observed.

The EO rich in thymol X5 was particularly effective at several development stages of the fungus. Oil containing thymol (75%) was reported to be effective on the pathogen, and more effective than oil containing carvone-eugenol (5). X5 exhibited the most promising properties for use in mango postharvest management.

In vivo tests and fruit analyses

No significant difference was found between the three treatments 14 days after inoculation (data not shown). We did not observe any effect of EO treatments on mango artificially inoculated with *C. gloeosporioides*. These results obtained after *in vivo* inoculation and treatments with X2 and X5 did not confirm what we observed *in vitro* on *C. gloeosporioides*. The inefficacity of the *in vivo* treatments with both EO is probably due to the treatment method. Oil volatilization for 4 days does not seem to be the most relevant method. At the end of the treatment period, it is reasonable to think that the oil volatiles have left the fruit peel, leaving the possibility for the fungus to grow again.

Similarly, no significant difference between treatments was observed on the pH and the total soluble solids of the pulp of mango (data not shown).

Gallic acid is involved in pathogen resistance by forming molecular structures like gallotannins (Cojocaru *et al.*, 1986). Its content was constant between 4 days after harvest in the control and in the X2 treatment (Figure 1). However, there was a light increase of polyphenol level in the X5 treatment. While the resorcinol content decreased between harvest and treatment times, it remained (significantly) higher in X2- and X5-treated fruits (Figure 2).

These results on resorcinol content show that the mechanism of fruit resistance against anthracnose could be en-

Table 2. Germination rate of *Colletotrichum gloeosporioides* incubated in Petri dish with or without essential oil (EO) for 6, 24 and 72 h after deposit. The control treatment consists of water; X2 and X5 are the two EO treatments.

Treatments	Volumes volatilized (in µL)	Germination rates		
		0–24 h		24–72 h
		After 6 h with EO	After 24 h with EO	After 24 h with EO and 48 h without EO
Control (water)	10	86.40 ± 7.74 a	100.00 ± 0.00 a	100.00 ± 0.00 a
X2 (carvone-eugenol)	10	0.00 ± 0.00 b	14.20 ± 23.87 b	33.30 ± 50.00 b
X5 (thymol)	10	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c
	5	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
	2	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
	1	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c

a, b, and c indicate uniform groups per column defined in ANOVA according to Newman-Keuls test ($P < 5\%$).

Table 3. Mycelial growth inhibition (MGI) of *Colletotrichum gloeosporioides* grown on PDA medium with or without the X2 or X5 essential oils (EO).

Treatments	Volumes volatilized (µL)	Mycelial area after 7-day EO treatment (mm^2)	MGI (%)	Mycelial area after 7-day treatment and 7 days without treatment (mm^2)	MGI (%)
Control	10	54.27 ± 3.84 a		53.80 ± 2.26 a	
	5	49.54 ± 2.21 a		49.38 ± 7.88 a	
	2	51.48 ± 2.35 a		54.01 ± 2.51 a	
	1	50.12 ± 2.25 a		51.24 ± 2.14 a	
X2	10	0.32 ± 0.52 c	99.41	12.69 ± 11.12 c	54.91
	5	0.04 ± 0.10 c	99.92	36.29 ± 7.96 b	26.51
X5	10	0.00 ± 0.00 c	100.00	0.00 ± 0.00 d	100.00
	5	0.00 ± 0.00 c	100.00	0.00 ± 0.00 d	100.00
	2	0.00 ± 0.00 c	100.00	0.00 ± 0.00 d	100.00
	1	21.07 ± 3.66 b	42.04	51.62 ± 2.69 a	0.00

a, b, and c indicate uniform groups per column defined in ANOVA according to Newman-Keuls test ($P<5\%$).

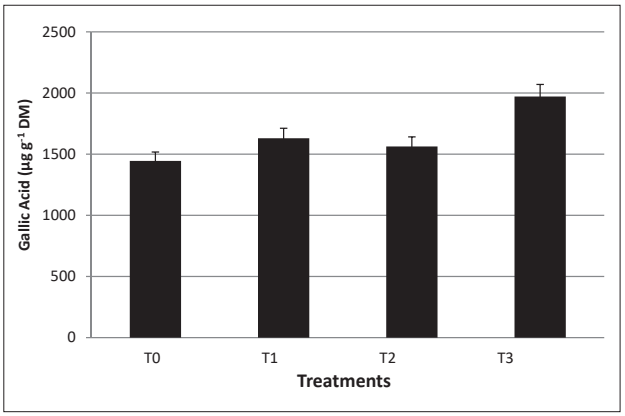

Figure 1. Gallic acid content (in µg g^{-1} dry matter – DM) in the mango fruit peel at harvesting stage (T0), and after 4 days of treatment by volatilization of 250 µL essential oil (EO) of X2 (T2) or X5 (T3). (T1) is the control treatment without EO. Mean plots and error bars from 10 fruits in triplicate.

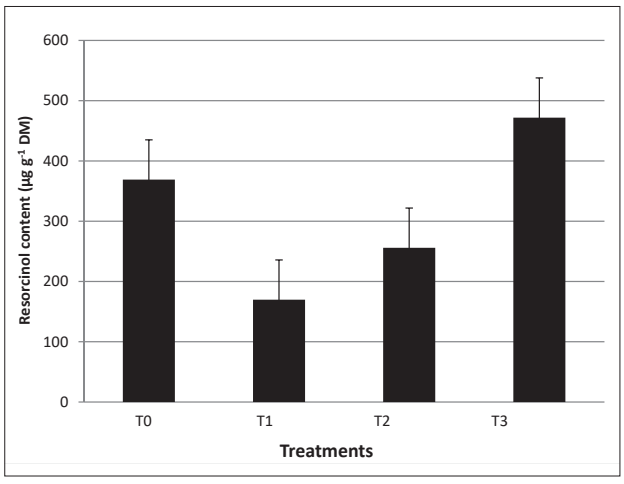

Figure 2. Resorcinol content (in µg g^{-1} dry matter – DM) in the mango fruit peel at harvesting stage (T0), and after 4 days of treatment by volatilization of 250 µL essential oil (EO) of X2 (T2) or X5 (T3). (T1) is the control treatment without EO. Mean plots and error bars from 10 fruits in triplicate.

hanced by a postharvest treatment with the volatilization of EO. The 5-pentadecyl-resorcinol is the major polyphenolic compound involved in the resistance mechanism of fruit (Karunanayake *et al.*, 2011). Sellamuthu *et al.* (2013) already reported that volatilization could enhance the defense-related enzyme activities of avocado fruits. Likewise, Bill *et al.* (2014) showed that thymol oil applied by coating on avocado fruit could elicit chitinase and PAL activities. The same EO applied by fumigation could enhance pathogenesis-related genes like ß-1,3-glucanase and chitinase gene expression (Bill *et al.*, 2016).

Conclusion

The two tested essential oils expressed a real protective potential as postharvest treatment of mango. They have a fungistatic effect on mycelial growth and spore germination, and X5 made of thymol has a fungitoxic effect. Both oils enhanced the biosynthesis of resorcinol in mango peel after 4 days of treatment, although not of other polyphenols like gallic acid.

Further studies would require a different mode of postharvest treatment of mango (*e.g.*, the use of wax or coating with thymol impregnated, or active packaging with continuous diffusion of thymol) and the related sensory analyses.

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