

Essential Oils in the Control of Anthracnose on ‘Prata Ana’ Banana

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Abstract

Alternative forms of disease control in fruits have been researched in an attempt to minimize the negative effects of chemical control on men and environment. The aim of this study was to determine the best concentration of essential oils to control the development of *Colletotrichum musae* and anthracnose intensity in ‘Prata Anã’ banana. In order to inhibit germination, mycelial growth and sporulation, four essential oils of clove (*Eugenia caryophyllus*), tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) species were used at concentrations of 2, 4, 6 and 8 µL. In fruits, the same essential oils were applied at concentrations of 80, 160, 240 and 320 µL to evaluate the incidence and severity of anthracnose. Clove and thyme oils were the most efficient in inhibiting mycelial growth, germination and sporulation at all concentrations tested. The volatile compounds present in clove and thyme oils provide complete inhibition of fungal growth. Tea tree and ginger oils have the ability to suppress anthracnose in ‘Prata Anã’ banana. Concentrations of 160 µL of ginger oil, 160 and 240 µL of tea tree oil reduced the anthracnose severity in fruits. Ginger and tea tree essential oils have the potential to control banana anthracnose, reducing the anthracnose incidence in fruits by 48% and 24%, respectively.

Keywords: alternative treatment, *Colletotrichum musae*, disease

1. Introduction

During banana production and commercialization, the volume of losses due to physical, physiological and microbiological factors is large. Infection by *Colletotrichum musae* (Berk & Curt.) von Arx. (Teleomorfo: *Glomerella musarum* Petch) is the main cause of microbiological losses. This fungus has the ability to infect green fruits, remaining in a quiescent state without the appearance of symptoms, until there are conditions for disease development (Negreiros, Salomão, Pereira, Cecon, & Siqueira, 2013). Banana anthracnose is the main post-harvest disease, causing significant losses. The infected fruit has inadequate appearance and is unsuitable for commercialization, since dark and depressed lesions are formed on the banana peel, on which, under conditions of high humidity, salmon-colored fungus fructifications appear (Garcia & Costa, 2000). In untreated fruits, anthracnose losses can reach up to 80% (Bill, Sivakumar, Korsten, & Thompson, 2014). For control of *C. musae*, management strategies such as the use of modern packing and transport systems, cultural measures and chemical control can be performed before harvest, during harvest and after harvest (Ventura & Hinz, 2002). Before harvest, chemical control is carried out by the application of thiabendazol and imazalil fungicides. However, the exclusive use of chemical control contributes to the emergence of resistant fungus species, causing damage to the environment and human health due to residues in fruit pulp (Coelho, Dias, Rodrigues, & Leal, 2010; Cruz et al., 2010). Increased consumer demand for high-quality fruits and low chemical residues resulted in market segments aimed at the marketing of differentiated products (Morandi & Bettiol, 2008). Methods to reduce the use of pesticides in the production of fruits and vegetables such as the use of alternative methods for controlling post-harvest diseases have been adopted by producers (Ferreira, São José, Bomfim, Porto, & Jesus de, 2014). Among these methods, the uses of natural substances with fungicidal action such as essential oils are commercially viable because they have low ecological and environmental impact (Werdir Gonzalez, Laumann, Silveira, Moraes, Borges, & Ferrero, 2013). Essential oils have been the subject of studies to discover

compounds present in secondary metabolites with potential for inhibiting the growth and development of microorganisms. Some essential oils are characterized by their bactericidal, insecticidal and fungicidal activities (Vilaplana, Pazmiño, & Valencia-Camorro, 2018; Khoobdel, Ahsaei, & Farzaneh, 2017). Several phytopathogens can be controlled by the application of essential oils (Knaak & Fiuza, 2010; Garcia, Juliatti, Barbosa, & Cassemiro, 2012). Results have demonstrated that essential oils can be used as biocontrol agents for the management of fungal diseases in a variety of crops infected with *Colletotrichum* species (Oliveira, Oliveira de, Vieira, Câmara, & Souza de, 2018; Rabari, Chudashama, & Thaker, 2017). In view of the above and considering the importance of banana as a food source allied to the consumer demand for pesticide-free fruits, the aim this study was to determine the best concentration of essential oils to control the development of *Colletotrichum musae* and anthracnose intensity in 'Prata Anã' banana.

2. Material and Methods

Experiments were conducted at the laboratory using clove (*Eugenia caryophyllus*), tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) essential oils. The *C. musae* isolate used in the experiment was obtained from 'Prata Anã' banana fruits with anthracnose symptoms. To determine the inhibition of mycelial growth and sporulation of *C. musae* BDA (Potato Dextrose Agar) culture medium was poured onto Petri dishes with 9 cm in diameter. Pieces of filter paper measuring 1 cm² were fixed on the lid of plates that received aliquots of essential oils at concentrations of 2, 4, 6 and 8 µL applied with the aid of an automatic pipette. In the control treatment, distilled water was added to the filter paper. Then, mycelial discs of 5 mm in diameter containing the monosporic *C. musae* culture with eight days of development were deposited on the center of each plate. The edges of plates were sealed with parafilm to avoid possible evaporation of compounds and the drying of the culture medium. Plates were placed in BOD chamber at 25 °C under 12-hour photoperiod. In order to evaluate the effect of products on the mycelial growth of the fungus, the diameter of colonies was daily measured (mean of the two diametrically opposed measurements), starting 24 hours after the experiment was set up, always at the same time and ending after mycelial growth has reached the entire plate. Subsequently, fungus sporulation was evaluated by adding 70 mL of distilled water in each plate and with the help of Drigalski spatula, colonies were scraped to release the conidia. The conidial suspension was filtered with sterile gauze. Then, a drop of each suspension was removed and placed in hemocytometer, where conidia were counted using an optical microscope and manual counter. The design was completely randomized in a 4 × 4 + 1 factorial scheme, with four essential oils and four concentrations (2, 4, 6 and 8 µL) and control. Four replicates per treatment were used; each repetition consisting of a Petri dish. To evaluate the germination of fungus conidia, essential oils were placed on each lid of Petri dishes containing the agar-water culture medium. Subsequently, 1 ml of conidial suspension at concentration of 2.5 × 10⁵ of *C. musae* was placed on water-agar medium, and spread with the aid of Drigalsk spatula. Petri dishes were taken to BOD chamber at 25 °C under 12-hour photoperiod for 24 hours. Then, they were taken to refrigerator to paralyze germination. The germination rate was evaluated by counting germinated conidia of the pathogen. Conidia that presented germination tube length greater than or equal to the conidium diameter were considered germinated. The design was completely randomized, in a 4 × 4 + 1 factorial scheme, with four essential oils and four concentrations (2, 4, 6 and 8 µL) and control.

2.1 Application of Essential Oils to Fruits

To evaluate the effect of essential oils on the anthracnose intensity of 'Prata Anã' banana fruits, commercial fruits were used in the pre-climacteric stage with peel coloration 2 according to the Von Loesecke maturation scale (PBMH & PIF, 2006). Fruits were packed in plastic boxes and transported to the Laboratory of Post-Harvest Pathology, where selection was performed according to the uniformity of color, size and absence of injury, in order to standardize fruits. Banana leaves were divided into bouquets of three fruits, washed with water and neutral detergent and placed to dry. After drying, some fruits were inoculated with *C. musae* conidia and the other part was not inoculated. Inoculated fruits were atomized to the point of drainage with the aid of a micropinture pump containing suspension of 5 × 10⁵ spores mL⁻¹. Bouquets were kept in humid chamber at 25 °C and 85% RH for 24 hours. Uninoculated fruits remained in the laboratory for 24 hours. After this period, bouquets were transferred to expanded polystyrene trays measuring 21x14 cm. Trays were packed in plastic bags with capacity of 3 kg. Each tray was added of a plastic container containing filter paper measuring 3 cm² with aliquots of the essential oils. Treatments were clove (*E. caryophyllus*), tea tree (*M. alternifolia*), thyme (*T. vulgaris*) and ginger (*Z. officinale*) essential oils at concentrations of 80, 160, 240 and 320 µL and two additional treatments. For control 1, 50 µL of distilled water was used and for control 2, Imazalil fungicide at concentration of 2 mL L⁻¹ was used, both applied in filter paper. A completely randomized design with four replications was used. Trays with treatments were closed with sealing machine and taken to the refrigeration chamber at 25 °C

and 85% of relative humidity for 12 hours. After this time, plastic bags were removed and trays remained in the refrigeration chamber. Evaluations were performed every three days for a period of 12 days. The anthracnose intensity in fruits was evaluated by incidence and severity. Incidence was obtained by the number of fruits affected by repetition, expressed in percentage by treatment. For the severity variable, the diagrammatic scale developed by (Moraes, Zambolim, & Lima, 2008) with disease severity ranging from 0.5 to 64% was used. Due to the fact that variables were discrete, the basic hypotheses were tested for the analysis of variance, additivity, error normality and variance homogeneity. Once non-compliance with these principles, non-parametric statistics were adopted using the Kruskal-Wallis test, by SAS software procedure (SAS Institute, 2000).

3. Results and Discussion

There was no significant difference ($P > 0.1$) for mycelial growth among concentrations of essential oils tested (Table 1).

Table 1. Mycelial growth (cm) of *Colletotrichum musae* submitted to different concentrations of essential oils

Concentrations ($\mu\text{L mL}^{-1}$)	Clove	Tea tree	Thyme	Ginger
2	0.0 Aa*	2.1 Ba*	0.0 Aa*	8.0 Ca
4	0.0 Aa*	2.5 Ba*	0.0 Aa*	7.0 Ca*
6	0.0 Aa*	2.1 Ba*	0.0 Aa*	6.8 Ca*
8	0.0 Aa*	2.3 Ba*	0.0 Aa*	7.7 Ca*
Control (1)	8.7	8.7	8.7	8.7

Note. Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other ($P > 0.1$) by the Kruskal-Wallis test. * Significant for control ($P < 0.1$) by the Kruskal-Wallis test.

Among species, clove and thyme oils showed greater inhibition of mycelial growth and conidia production in all tested concentrations (Table 2).

Table 2. Production of *C. musae* conidia (number of conidia. mL^{-1}) submitted to different concentrations of essential oils

Concentrations ($\mu\text{L mL}^{-1}$)	Clove	Tea tree	Thyme	Ginger
2	0.0 Aa*	63.2 Ba	0.0 Aa*	4.0 Ab*
4	0.0 Aa*	5.6 Ba*	0.0 Aa*	3.0 ABb*
6	0.0 Aa*	14.8 Ba*	0.0 Aa*	3.6 Bb*
8	0.0 Aa*	14.6 Ba*	0.0 Aa*	1.7 Ba*
Control (1)	52.8	52.8	52.8	52.8

Note. Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other ($P > 0.1$) by the Kruskal-Wallis test. * Significant for the control ($P < 0.1$) by the Kruskal-Wallis test.

These results demonstrate the efficacy of essential oils to control the development of *C. musae*, even at the lowest concentrations applied. The volatile compounds present in these oils provided 100% control of the fungus. According to (Barbosa, Vieira, & Teixeira 2015; Aquino, Sales, Soares, & Martins, 2012), the fungicide action of essential oils is able to inhibit the development of different fungi. In germination, the results follow the same trend in clove, thyme and tea tree oils, showing that from the lowest concentration applied (2 μL), there is a positive effect when compared to control. High concentration of ginger essential oil shows reduction in the conidia production and germination of *C. musae* as verified in Tables 2 and 3.

Table 3. Percentage of germinated *Colletotrichum musae* conidia submitted to different concentrations of essential oils

Concentrations ($\mu\text{L mL}^{-1}$)	Clove	Tea tree	Thyme	Ginger
2	6.3 Aa*	6.8 Aa*	0.0 Aa*	89.5 Bb
4	4.5 Aa*	0.3 Aa*	1.0 Aa*	89.0 Bb
6	1.5 Aa*	0.5 Aa*	0.3 Aa*	84.0 Cab
8	1.8 Aa*	0.0 Aa*	0.3 Aa*	78.8 Ba
Control (1)	66.5	66.5	66.5	66.5

Note. Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other ($P > 0.1$) by the Kruskal-Wallis test. * Significant for the control ($P < 0.1$) by the Kruskal-Wallis test.

The beneficial effects of higher concentrations of essential oils on reducing the development of *C. musae* are due to increased release of volatile substances capable of inhibiting fungal growth. This effect also occurs in other pathogens. Andrade and Vieira (2016) found that the conidia germination of *Colletotrichum gloeosporioides* was reduced by increasing the concentration of tree-tea essential oil applied. The fungistatic and fungicidal effect of the essential oils may be related to the presence of high concentrations of some volatile substances (Lorenzetti et al., 2011). In the floral bud of the clove plant, there is high concentration of eugenol (83.6%), an antiseptic phenolic compound that can be attributed to the control of the development of microorganisms (Costa et al., 2011). The major compounds in thyme essential oil are thymol, p-cymene and γ -terpinene, whose components have antimicrobial activities (Jakiemiu, Scheer, Oliveira, Yamamoto, & Deschamps, 2010). Several studies have demonstrated the effect of the use of essential oils in the control of post-harvest pathogens. In other pathogenic systems, it has been observed that clove essential oil causes changes in phytopathogenic hyphae. The presence of vacuoles and disorganization of cellular contents, decrease in cell wall sharpness, intense fragmentation and reduction in the turgescence of these cells are indicative of cellular degeneration (Costa et al., 2011). These factors justify the inhibition of mycelial growth, germination and sporulation of *C. musae* using this treatment (Tables 1, 2 and 3). Clove and thyme essential oils caused complete inhibition of mycelial growth and fungal sporulation. Thus, the results indicate that the essential oils are presented as a promising technique in the control of anthracnose in banana fruits.

3.1 Application of Essential Oils to Fruits

When evaluating the incidence of anthracnose, it was observed in inoculated fruits that when ginger essential oil applied at concentrations of 80 μL and 320 μL , disease control was 43.9% and 46.6%, respectively; in tea tree oil (80 μL), the fungus was controlled in 74.5%, and for the thyme oil (240 μL and 320 μL), the disease control was equal to the fungicide used, controlling for up to 42.45% and 44.56%, respectively (Table 4).

Table 4. 'Prata Anã' banana fruits inoculated with *C. musae* conidia submitted to different concentrations of essential oils

Species	Concentrations (μL)	Anthracnose intensity	
		Incidence	Severity
Clove	80	97.6 a	75.0 a
	160	97.6 a	75.0 a
	240	89.9 a	75.0 a
	320	93.5 a	75.0 a
Ginger	80	63.9 a** *	75.0 a
	160	114.0 a	75.0 a
	240	83.0 a	75.0 a
	320	60.8 a** *	75.0 a
Tea tree	80	29.1 a** *	57.0 a** *
	160	96.1 a	75.0 a
	240	75.6 a	75.0 a
	320	99.5 a	75.0 a
Thyme	80	106.9 a	75.0 a
	160	111.3 a	75.0 a
	240	65.6 a** *	75.0 a
	320	63.2 a** *	75.0 a
Control (1)		114.0 b	75.0 a
Imazalil (2)		66.1 a	75.0 a

Note. Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other ($P > 0.1$) by the Kruskal -Wallis test. * Significant for the control ($P < 0.1$) by the Kruskal-Wallis test. ** Significant for control 2 ($P < 0.1$) by the Kruskal-Wallis test.

The application of the essential oils in the fruits does not influence the ethylene production. The maturation of the fruits treated with the essential oils and the untreated oils reached the same coloration on the last day of evaluation. Ethylene is a hormone that acts in innumerable processes of the plant development, in the fruits is responsible for the biochemical and physiological changes related to the ripening of the fruits, as, for example, the changes in coloration, taste, texture, composition of reducing sugars and in the production of volatile substances (Pereira, Finger, Casali, & Brommonschenkel, 2008; Taiz & Zeiger, 2009). In the bark, yellowing occurs due to the degradation of chlorophyll and the appearance of carotenoid pigments, responsible for the yellow coloration (Medina & Pereira, 2010; Vilas Boas, Rodrigues, & de Paula, 2003). Harvesting and cleaning, sorting, packing, storage and transport processes influence the actual quality of the product, since these factors are linked to ethylene production (Spagnol, Silveira Junior, Pereira, & Guimarães Filho, 2018). When evaluating severity, only tea tree essential oil was superior to control, indicating that there was a reduction of anthracnose in treatment with inoculated fruits (Table 4). The efficiency of essential oils of other species such as neem oil and garlic oil in the control of anthracnose in banana fruits was confirmed by (Negreiros et al., 2013), who verified a reduction in the disease severity. There was no difference among essential oils tested in fruits without inoculation (Table 5).

Table 5. 'Prata Anã' banana fruits without inoculation with *C. musae* conidia submitted to different concentrations of essential oils

Species	Concentrations (μL)	Anthracnose intensity	
		Incidence	Severity
Clove	80	94.3 a	75.0 a
	160	30.1 a	75.0 a
	240	77.0 a	75.0 a
	320	67.1 a	75.0 a
Ginger	80	50.9 a	75.0 a
	160	35.6 a	39.0 a** *
	240	76.1 a	75.0 a
	320	46.0 a	75.0 a
Tea tree	80	49.6 a	75.0 a
	160	36.9 a	57.0 a** *
	240	68.4 a	57.0 a** *
	320	35.9 a	75.0 a
Thyme	80	74.8 a	75.0 a
	160	47.9 a	75.0 a
	240	72.5 a	75.0 a
	320	86.1 a	75.0 a
Control (1)		76.1 a	75.0 a
Imazalil (2)		57.0 a	75.0 a

Note. Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other ($P > 0.1$) by the Kruskal-Wallis test. * Significant for the control ($P < 0.1$) by the Kruskal-Wallis test. ** Significant for control 2 ($P < 0.1$) by the Kruskal-Wallis test.

When comparing with controls by the Kruskal Wallis test ($P = 0.10$), it was verified that the concentrations of 160 μL of ginger oil followed by tea tree oil at concentrations of 160 and 240 μL reduced the anthracnose severity by 48% and 24%, respectively (Table 5). The other treatments with essential oils presented reduction in severity equivalent to treatment with fungicide (Table 5). Corroborating the results, Maia et al. (2014) found that rosemary essential oil reduced the severity of grape downy mildew similarly to chemical treatments applied. Burt (2004) reported that essential oils are volatile secondary metabolites characterized by being complex mixtures of organic compounds, increasingly studied as antioxidants and in the control of microorganisms. Essential oils contain compounds with therapeutic and protective properties against the processes of oxidation and deterioration by microorganisms. Tea tree essential oil has as main component terpinen-4-ol, which is the component responsible for its antiseptic and biocidal effect (Thomsen, Hammer, & Riley, 2010). Probably, terpinen-4-ol showed fungicidal effect on *C. musae* as observed in the present study. Ginger essential oils contain high sesquiterpene hydrocarbons, including β -sesquiphellandrene (27.16%), caryophyllene (15.29%) and zingiberene (13.97%). These components are identified by (El-Baroty, El-Baky, Abd, Farag, & Saleh, 2010) with antioxidant and antimicrobial activity. The results obtained in the fungal development contradict the results observed in the test carried out with 'Prata Anã' banana, since clove and thyme oils controlled the development of the pathogen in Petri dishes. The inefficacy of essential oils in the control of anthracnose may be related to the inability of volatile compounds present in oils to act on the pathogen. This is because after the adhesion of the fungus to the fruit peel, germination, appressory formation and subsequent penetration into the peel, preventing the direct contact of volatile compounds of essential oils with the pathogen. As a result of this process, there is intercellular or intracellular hyphae develop and may remain quiescent for some time. The quiescence of the fungus can occur in spore germination, germ tube elongation, appressory formation and penetration or subsequent colonization (Prusky, 1996). Activation of the quiescent infection involves a series of coordinated events. This activation may occur by the breakdown of host defense and indirectly by the detoxification of antifungal agents present in the host (Prusky & Lichter, 2007). If quiescence occurs during spore germination and appressory formation, essential oils are more likely to be effective in pathogen control, since these fungal structures are on the surface of the fruit skin. Several plant species have already shown positive effect on the control of plant diseases, so other plant species have been studied in order to control phytopathogens.

4. Conclusions

In the *C. musae* development, clove and thyme oils were efficient in the inhibition of mycelial growth and in the conidia germination and production at all concentrations tested.

Ginger oil at concentrations of 2 and 4 μL controlled *C. musae* sporulation.

The anthracnose intensity was reduced by the application of tea tree and ginger oil in inoculated and not-inoculated fruits.

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