

Control of anthracnose in banana with cassava starch film associated or not with essential oils

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ABSTRACT: The objective of this work was to evaluate the *in vitro* and *in vivo* potential of cassava starch film associated or not with essential oils in the control of *Colletotrichum musae* of banana. Were tested clove oils (*Eugenia caryophyllata*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), cinnamon (*Cinnamomum zeylanicum*) and oregano (*Origanum vulgare*) at 0.1% associated or not with film 2 and 3%. Mycelial growth and conidial germination *in vitro* and anthracnose *in vivo* were evaluated. In the curative and preventive controls, the fruits were inoculated with conidia before and after coating, respectively. The major components of essential oils were: eugenol, β -caryophyllene and α -humulene in clove; thymol, p -cymene, carvacrol and α -pinene in thyme; geranial and neral in lemongrass; trans-Cinnamaldehyde and eugenol in cinnamon; carvacrol, γ -Terpinene and linalool in oregano. *In vitro*, total inhibition of mycelial growth and germination of conidia with oregano oil was observed. The films in the concentrations of 2 and 3% isolated and combined with oils of lemon grass and thyme, in addition to these isolated oils, provided a reduction in the size of anthracnose lesions.

Key words: *Colletotrichum musae*; disease; *Musa* spp.; postharvest

Controle de antracnose em banana com película de fécula de mandioca associada ou não com óleos essenciais

RESUMO: Objetivou-se com este trabalho avaliar o potencial *in vitro* e *in vivo* de película de fécula de mandioca associada ou não a óleos essenciais no controle de *Colletotrichum musae* da banana. Foram testados óleos de cravo-da-índia (*Eugenia caryophyllata*), tomilho (*Thymus vulgaris*), capim-limão (*Cymbopogon citratus*), canela (*Cinnamomum zeylanicum*) e orégano (*Origanum vulgare*) a 0,1% associados ou não com película 2 e 3%. Avaliou-se o crescimento micelial e a germinação de conídios *in vitro* e a antracnose *in vivo*. Nos controles curativo e preventivo, os frutos foram inoculados com conídios antes e depois do revestimento, respectivamente. Os principais componentes majoritários dos óleos essenciais foram: eugenol, β -cariofileno e α -humuleno em cravo-da-índia; timol, p -cimeno, carvacrol e α -pineno em tomilho; geranial e neral em capim-limão; transcinamaldeído e eugenol em canela; carvacrol, γ -terpineno e linalol em orégano. *In vitro*, foi observada inibição total do crescimento micelial e germinação de conídios com óleo de orégano. As películas nas concentrações de 2 e 3% isoladas e combinadas com óleos de capim-limão e tomilho, além desses óleos isolados, proporcionaram redução no tamanho das lesões de antracnose.

Palavras-chave: *Colletotrichum musae*; doença; *Musa* spp.; pós-colheita

Introduction

Banana plant (*Musa* spp.) is a fruit tree widely cultivated in tropical and subtropical regions (FAO, 2019). Besides being one of the most valuable agricultural products in the world, banana fruit is a nutritious, high-energy food source and is an integral part of many diets (Hapsari & Lestari, 2016). Several fungal diseases can occur in banana during the post-harvest phase, including anthracnose, whose causative agent is fungus *Colletotrichum musae* (Fernandes et al., 2019). The fungus infects green fruits in the field and remains quiescent until maturation. Symptoms manifest as dark black or brown depressed lesions that coalesce with time, forming large necrotic areas which make fruit undesirable for commerce and consumption (Maqbool et al., 2010a).

Disease control basically involves application of fungicides (Khan et al., 2001). However, misused chemicals result in greater risks to the applicator and the environment as well as in selection of fungicide-resistant populations, increases production cost and reduces producer profits (Bastos & Albuquerque, 2004).

In an attempt to reduce post-harvest losses, fruit treatment with alternative products less toxic to humans and the environment, such as biodegradable cassava starch-based films and essential oils has brought promising results (Botelho et al., 2016; Castro et al., 2017; Sarkhosh et al., 2017; Vilaplana et al., 2018).

Cassava starch is a product extracted from cassava (*Manihot esculenta*) root in the form of a white, odorless, and tasteless powder. It is used as ingredient in a range of products in various industrial sectors and is also able to form edible films applied in post-harvest fruit preservation (Oriani et al., 2014; Woggum et al., 2014).

Essential oils are lipophilic, low-molecular-weight volatile compounds extracted from various parts of medicinal plants, with characteristic smell and flavor. Application of cassava starch film and essential oils demonstrated promising effects on anthracnose control in fruits such as guava (Rozwalka et al., 2008), mango (Serpa et al., 2014), strawberry (Lorenzetti et al., 2011), papaya (Carnelossi et al., 2009; Bosquez-Molina et al., 2010; Oliveira et al., 2016), and organic banana (Vilaplana et al., 2018). Thus, knowing the action of essential oils associated with cassava starch film can contribute to the development of more promising control strategies for reducing production costs, stimulating consumption of more appealing fruits, and increasing shelf life. This study investigated the potential *in vitro* and *in vivo* of cassava starch film associated or not associated with essential oils in the control of *C. musae* in banana fruit.

Materials and Methods

Fruits, essential oils, cassava starch, and fungicide

Fruits were obtained in a plantation Banana (*Musa* sp. 'Prata-Anã AAB') at Vale dos Ventos farmstead, Lavras, state of Minas Gerais. There was no management measure at the

site, which allowed studying disease behavior under natural conditions.

Essential oils of clove (*Eugenia caryophyllata*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), cinnamon (*Cinnamomum zeylanicum*), and oregano (*Origanum vulgare*) were purchased from the Quinari[®] company. Cassava starch (Pachá[®]) and fungicide Tecto[®]SC - Syngenta were purchased in local commerce.

Fungal culture and identification

C. musae was isolated from symptomatic banana fruits. Monosporic culture was prepared by adding 10 mL of sterile water to the colony plate. A 10 µL aliquot was extracted and deposited in 9 cm diameter petri dishes containing water agar medium (20 g agar per L of distilled water) then spread with a Drigalski spatula. Dishes were incubated at 25 °C and 12 h photoperiod for six days. Then, a germinated conidium was transferred to Petri dishes containing malt agar (20 g malt extract and 20 g agar per L of distilled water). The monosporic isolate was preserved in a microtube and stored at 10 °C in the dark and cryopreserved at -80 °C in the Lavras Mycological Collection (CML), Laboratory of Systematics and Ecology of Fungi, Department of Phytopathology, Federal University of Lavras (UFLA) with access code CML 3248. The isolate was identified by morphological characters measuring 13-14 µm in length and 4.5-5.4 µm in width, typical of *C. musae* (Weir et al., 2012) and by molecular phylogeny using fragments of genes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and partial Actin (ACT). The isolate sequences generated in this study were placed in the GenBank, under accession codes KX417772 and KX417773 for GAPDH and ACT gene regions, respectively.

Chemical composition of essential oils

Chemical analyses were performed by gas chromatography coupled to mass spectrometry (GC-MS). The equipment was operated under the following experimental conditions: fused silica capillary column (30 × 0.25 mm × 0.25 µm); helium gas flow rate of 1.18 mL min⁻¹; injector and detector operating at temperatures 200 and 240 °C, respectively. Column temperature was 60 °C, with a heating ramp of 3.0 °C min⁻¹ until reaching 240 °C and 10 °C min⁻¹ until reaching 300 °C, remaining for 7 min with a cut-off time of 3.0 min. Oil samples were diluted in hexane at 1 % v/v and injected into the chromatograph with an injection volume of 1.0 µL in split mode with 1:100 ratio. The peaks obtained were compared with the Wiley 8 and FFNSC 1.2 libraries database, and the retention indices of compounds relating to co-injection of n-alkane series were calculated by the Van Den Dool & Kratz method (1963).

Compounds were quantified using flame ionization detector (FID) equipped with an RTX capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas at 1.18 mL min⁻¹ flow rate, and injector and detector temperatures were maintained at 220 and 240 °C, respectively. Column conditions and amount of injection were as described above. Results were obtained by normalizing peak areas (%).

Treatments and experimental design

Antifungal activity *in vivo* and *in vitro* of *C. musae* was evaluated in 20 treatments consisting of control with only distilled water; negative control with 0.7% Tween 20 dispersant; positive control with 0.65 mL L⁻¹ of Tecto[®]SC fungicide; cassava starch film at 2%; cassava starch film at 3%; clove essential oil (0.1%); thyme essential oil (0.1%); lemongrass essential oil (0.1%); cinnamon essential oil (0.1%); oregano essential oil (0.1%); cassava starch film at 2% associated with clove essential oil; cassava starch film at 3% associated with clove essential oil; cassava starch film at 2% associated with thyme oil; cassava starch film at 3% associated with thyme oil; cassava starch film at 2% associated with lemongrass essential oil; cassava starch film at 3% associated with lemongrass essential oil; cassava starch film at 2% associated with cinnamon essential oil; cassava starch film at 3% associated with cinnamon essential oil; 2% cassava starch film associated with oregano essential oil; and cassava starch film at 3% associated with oregano essential oil.

The experiments were conducted in a completely randomized design with four replicates per treatment. Sampling unit in experiments *in vitro* was a Petri dish while the experiment *in vivo* used a banana fruit.

Antifungal activity of essential oils and cassava starch in mycelial growth and percentage of conidial germination of *C. musae* *in vitro*

In the evaluation mycelial growth, treatments containing or not a film with essential oil, the cassava starch was autoclaved and prepared in concentrations of 2 and 3% (weight/volume), being heated in a microwave to 90 °C and stirred periodically for 10 seconds, to obtain films without granules. In the plates with already solidified malt medium, containing or not essential oils, depending on the treatment, 1 mL of films 2 and 3% were added on the surface of the medium and spread with the aid of the Drigalsky loop. For treatments containing only essential oils, 5 mL solutions containing distilled water, 0.7% Tween 20 and 5 µL of each oil were prepared. From these solutions, 1 mL was removed and added to 9 mL of the melting medium cooled to 45 °C and poured into plates.

After solidification of the medium, mycelium discs of 5 mm in diameter were transferred to the center of the plates, then they were sealed with parafilm and incubated at 25 °C and a photoperiod of 12 h. Mycelial growth was evaluated daily for eight days by measuring colony diameter in two opposite directions using a digital caliper. The mycelial growth rate index (MGRI) was calculated using the Equation 1 proposed by Maguire (1962).

$$MGRI = \frac{S(D - D_a)}{N} \quad (1)$$

where: MGRI - mycelial growth rate index; S - summation; D - current mean diameter; D_a - mean diameter of the previous day; N - number of days after inoculation.

Germination of conidia was performed by microscopic analysis of the germinated conidia in agar-water culture medium poured into 9 cm diameter Petri dishes prepared as mentioned in mycelial growth. After solidification of the culture medium in Petri dishes, 100 µL of a 2 × 10⁶ conidia mL⁻¹ suspension were added and spread with a Drigalski spatula. Petri dishes were then incubated at 25 °C, with 12 h photoperiod. Conidial germination was then stopped with two drops of lactoglycerol cotton blue. Plates were divided into four quadrants with 100 conidia each, totaling 400 conidia per treatment. Conidia with germ tubes longer or equal to conidium diameter were considered germinated.

In vivo activity of essential oils and cassava starch in anthracnose lesions

Activity of essential oils and cassava starch film was evaluated by immersion of fruits in suspension containing treatments before and after inoculation, characterizing preventive and curative control, respectively.

Cassava starch films at concentrations of 2 and 3% were prepared by diluting 16 and 24 g cassava starch in 800 mL distilled water, respectively. Films were then heated in microwave up to 90 °C and shaken every 10 seconds to obtain gels without granules. The essential oils used in the 0.1% concentration were diluted in 0.7% Tween 20.

After cooling gels in the combined treatments, the essential oils were diluted in Tween 20, added to films at 2 and 3% with automatic pipette and shaken with glass rod. Treatments with only essential oils received 800 µL of each oil. Fruits were then immersed three times in the solutions for one minute, at 3 min intervals (Oliveira et al., 2016).

In the preventive control, fruits were initially treated with cassava starch films at 2 and 3%, whether or not incorporated in essential oils, dried for 12 h, and then submitted to inoculation. In the curative control, fruits were inoculated and later submitted to the treatments with cassava starch films at 2 and 3%, whether or not incorporated in essential oils.

Inoculation was performed at four different points in the middle part of fruits, previously pierced with five holes, using multi-needles (Soares et al., 2017). For this purpose, 20 µL of the 2 × 10⁶ conidia mL⁻¹ suspension were deposited at each point with automatic pipette. Then fruits were covered with plastic bags, forming a humid chamber at 25 °C and 90% humidity during 48 h of wetting. After removal of plastic bags, fruits remained on the bench for 12 h drying at 26 °C and 60% relative humidity. Later, fruits were put on plastic supports and placed under the laboratory bench.

Evaluations were performed daily by measuring lesions in two perpendicular directions using digital caliper. Then, the mean injured area was calculated in cm² at the inoculated points, using the formula for calculating circle area: A = πr², where: A = injured area; π = constant (3.1416) and r = mean radius of the lesion.

Statistical analysis

Normality of data were confirmed using the Shapiro-Wilk test (Shapiro & Wilk 1965). Data were submitted to analysis

of variance. Significant variables in the F test ($p \leq 0.05$) were compared by the Scott-Knott test at 5% probability. Analyses were performed using the Sisvar software (Ferreira, 2011).

Results

Chemical composition of essential oils

Table 1 shows results of GC-MS analyses of the five essential oils. The main chemical components of clove oil were eugenol (83.73%), β -caryophyllene (13.40%), and α -humulene (1.51%). The most abundant compounds identified in cinnamon oil were trans-cinnamaldehyde (90.3%) and eugenol (8.45%). Geranial (54.50%) and neral (36.87%) were the main constituents of

lemongrass oil. Carvacrol (77.34%) followed by γ -terpinene (12.36%) and linalool (6.48%) were the major components of oregano oil, followed by p -cymene (24.04%), carvacrol (21.97%) and α -pinene (5.87%). Chromatogram for thyme oil showed 22 peaks. Thymol (26.84%) presented higher levels, followed by p -cymene (24.04%), carvacrol (21.97%), and α -pinene (5.87%).

Antifungal activity of essential oils and cassava starch *in vitro* in inhibition of mycelial growth and conidial germination of *Colletotrichum musae*

Treatments with oregano and cinnamon oils isolated or combined with 2 and 3% cassava starch film, likewise Tecto[®]SC fungicide, had a greater effect on reduction of mycelial growth

Table 1. Chemical composition (relative percentage) of five different essential oils.

Compounds	^a RI	<i>E. caryophyllata</i>	<i>C. zeylanicum</i>	<i>C. citratus</i>	<i>O. vulgare</i>	<i>T. vulgaris</i>
Eugenol	1289	83.73	8.45	-	-	-
β -Caryophyllene	1378	13.40	-	-	-	-
α -Humulene	1383	1.51	-	-	-	-
Caryophyllene oxide	1383	1.19	-	-	-	-
α -Copaene	1293	0.17	-	-	-	-
trans-Cinnamaldehyde	1201	-	90.3	-	-	-
Sabinene	1383	-	1.25	-	-	-
Geranial	1196	-	-	54.50	-	-
Neral	1193	-	-	36.87	-	-
Geranyl acetate	1288	-	-	1.68	-	-
Caryophyllene oxide	1392	-	-	1.26	-	-
Citronellyl formate	1190	-	-	1.13	-	-
Bergamal	1038	-	-	1.12	-	-
Citronellyl isobutyrate	1204	-	-	0.25	-	-
Lavandulol	1193	-	-	0.24	-	-
β -Caryophyllene	1293	-	-	0.23	-	-
Citronelal	1042	-	-	0.13	-	-
Germacrene D	1378	-	-	0.10	-	-
Carvacrol	1205	-	-	-	77.34	21.97
γ -Terpinene	1010	-	-	-	12.36	0.47
Linalool	1010	-	-	-	6.48	2.55
Myrcene	926	-	-	-	1.68	-
Thymol	1089	-	-	-	1.67	-
p -Cymene	1000	-	-	-	0.27	24.04
Terpinolene	998	-	-	-	0.20	-
Thymol	1279	-	-	-	-	26.84
α -Pinene	903	-	-	-	-	5.87
δ -3-Carene	907	-	-	-	-	3.54
Terpin-4-ol	1108	-	-	-	-	3.37
Limonene	1000	-	-	-	-	2.04
Isobornyl acetate	1202	-	-	-	-	1.73
Borneol	1101	-	-	-	-	1.60
Eucalyptol	1001	-	-	-	-	1.45
Bornyl acetate	1184	-	-	-	-	0.75
Camphene	1089	-	-	-	-	0.62
α -Terpineol	1110	-	-	-	-	0.58
Camphor	980	-	-	-	-	0.53
Terpinolene	1010	-	-	-	-	0.50
Myrcene	987	-	-	-	-	0.49
Sabinene	987	-	-	-	-	0.38
β -Ocimene	994	-	-	-	-	0.30
α -Phellandrene	989	-	-	-	-	0.26
α -Selinene	1280	-	-	-	-	0.12
Total		100	100	97.75	100	100

^aRI retention indices relative to the nC8-nC25 n-alkanes series on DB-5 column.

and conidial germination. Isolated clove oil showed secondary antifungal activity, inhibiting mycelial growth and germination in 5.58 mm day⁻¹ and 22.5%, respectively (Table 2).

Cassava starch films 2 and 3% combined with essential oil of thyme, as well as isolated essential oils of thyme and lemongrass had intermediate effect on mycelial growth with averages 7.10, 6.96, 6.38, and 6.62 mm day⁻¹, respectively. Intermediate effect in conidial germination was verified with the Intermediate effect in conidial germination was verified with the cinnamon essential oil (OECL) isolated, presenting germination of 22.50% (Table 2).

Cassava starch films 2 and 3% had mycelial growth of 10.56 and 10.20 mm day⁻¹ and conidial germination of 87.00 and 81.87%, respectively (Table 2). These results confirm the inefficiency of films, as they did not differ statistically from the control mycelial growth (10.63 mm day⁻¹) and negative control (10.45 mm day⁻¹), also not differing from the negative control (87.37%) in conidial germination.

Evaluation of efficiency of cassava starch film and essential oils *in vivo* in preventive and curative control of banana anthracnose

Preventively, treatments providing the greatest reduction in lesion sizes were lemongrass and thyme oils either isolated or combined with cassava starch films 2 and 3%, as well as isolated cassava starch films 2% and 3%, thyme oil associated with 2% cassava starch film and fungicide Tecto[®]SC (Figure 1A). Similarly, these same treatments presented the best results in the curative control, although with addition of clove

oil isolated or associated with 3% cassava starch film (Figure 1 B). In both types of control, preventive and curative, fruits treated with these products showed low- developed lesions.

Essential oils of cinnamon and oregano were not efficient in the control of anthracnose when not combined with cassava starch films at 2 and 3%. Lesions were larger, with sizes of 10.35 cm² and 10.68 cm² in the curative experiment (Figure 1A) and 7.88 and 9.94 cm² in the preventive experiment (Figure 1B).

Tecto[®]SC fungicide presented low efficiency in the curative control with lesion size 9.89 cm², not differing from control and Tween20 dispersant, which presented lesions with sizes 11.04 and 10.54 cm², respectively (Figure 1A).

Cassava starch films 2 and 3% either associated or not associated with essential oils were efficient in anthracnose control, being much more expressive at a concentration of 3% with average lesion size 1.69 and 1.35 cm² in preventive and curative experiments, respectively (Figure 1A and 1B).

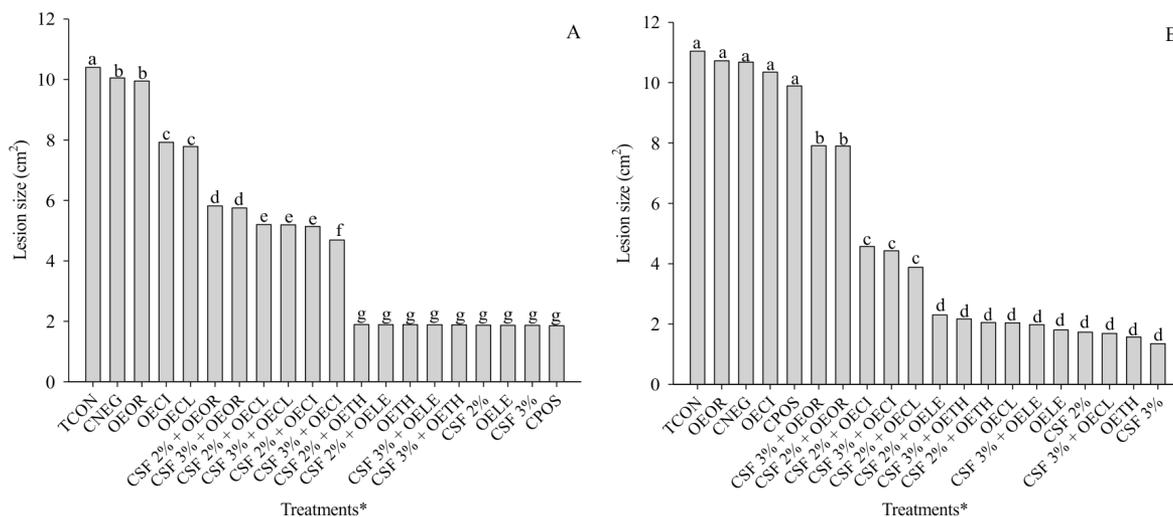
Discussion

In the present study, 11 compounds were identified in lemongrass oil, representing 97.75% of total constituents. Total constituents in clove, cinnamon, oregano, and thyme oils represented 100% of total area. Similar to our results, Scherer et al. (2009) found in clove oil the main components eugenol (83.75%) and β-caryophyllene (10.98%) followed by α-humulene (1.26%). Beraldo et al. (2013), Oliveira et al. (2009) and Snoussi et al. (2008) identified eugenol in carnation flower buds in values 77.58, 88.38, and 88.58%, respectively.

Table 2. Effect of different *in vitro* treatments on the mycelial growth rate index (MGRI) and on the percentage of germination of *Colletotrichum musae* conidia.

Treatments	*Mean of MGRI (mm day ⁻¹)	Treatments	*Germination (%)
Control	10.63 a	Control	98.12 a
CSF 2%	10.56 a	Tween20 dispersant	87.37 b
Tween20 dispersant	10.45 a	CSF 2%	87.00 b
CSF 3%	10.20 a	OELE	84.37 b
CSF 2% + OELE	10.11 a	OETH	83.75 b
CSF 3% + OELE	8.53 b	CSF 3%	81.87 b
CSF 2% + OETH	7.10 c	CSF 2% + OELE	70.50 c
CSF 3% + OETH	6.96 c	CSF 2% + OETH	62.37 d
OELE	6.62 d	CSF 3% + OETH	55.87 e
OETH	6.38 d	CSF 3% + OELE	53.37 e
OECL	5.58 e	OECL	22.50 f
OECL	2.28 f	OECL	1.75 g
CSF 2% + OECL	0.14 g	CSF 3% + OECL	1.62 g
CSF 3% + OECL	0.08 g	CSF 2% + OECL	1.50 g
CSF 2% + OECL	0.03 g	CSF 3% + OECL	1.37 g
CSF 3% + OECL	0.02 g	CSF 2% + OECL	1.55 g
OEOR	0.00 g	CSF 2% + OEOR	1.00 g
CSF 2% + OEOR	0.00 g	CSF 3% + OEOR	0.87 g
CSF 3% + OEOR	0.00 g	OEOR	0.00 g
Tecto [®] SC fungicide	0.00 g	Tecto [®] SC fungicide	0.00 g

Cassava starch film at 2% associated with clove essential oil (CSF 2% + OECL), cassava starch film at 3% associated with clove essential oil (CSF 3% + OECL), cassava starch film at 2% associated with thyme oil (CSF2% + OETH), cassava starch film at 3% associated with thyme oil (CSF 3% + OETH), cassava starch film at 2% associated with lemongrass essential oil (CSF 2% + OELE), cassava starch film at 3% associated with lemongrass essential oil (CSF 3% + OELE), cassava starch film at 2% associated with cinnamon essential oil (CSF 2% + OECL), cassava starch film at 3% associated with cinnamon essential oil (CSF 3% + OECL), 2% cassava starch film associated with oregano essential oil (CSF 2% + OEOR), cassava starch film at 3% associated with oregano essential oil (CSF 3% + OEOR). Tween20 dispersant, Tecto[®]SC fungicide and the control (only with agar-water medium without addition of any product). *Averages followed by the same letter in the columns do not differ from each other at the 5% probability level by the Scott-Knott test. CV (%) MGRI = 6.69; CV (%) conidia germination = 10.52%.



TCON: control treatment with only distilled water; CNEG: negative control with Tween20 dispersant; CPOS: positive control with Tecto[®]SC fungicide; cassava starch film at 2% (CSF 2%); cassava starch film at 3% (CSF 3%); clove essential oil (OECL), thyme essential oil (OETH), lemongrass essential oil (OELE), cinnamon essential oil (OECI), oregano essential oil (OEOER), cassava starch film at 2% associated with clove essential oil (CSF 2% + OECL), cassava starch film at 3% associated with clove essential oil (CSF 3% + OECL), cassava starch film at 2% associated with thyme oil (CSF 2% + OETH), cassava starch film at 3% associated with thyme oil (CSF 3% + OETH), cassava starch film at 2% associated with lemongrass essential oil (CSF 2% + OELE), cassava starch film at 3% associated with lemongrass essential oil (CSF 3% + OELE), cassava starch film at 2% associated with cinnamon essential oil (CSF 2% + OECI), cassava starch film at 3% associated with cinnamon essential oil (CSF 3% + OECI), 2% cassava starch film associated with oregano essential oil (CSF 2% + OEOER), cassava starch film at 3% associated with oregano essential oil (CSF 3% + OEOER). * Treatments followed by the same letter do not differ at the 5% probability level by the Scott-Knott test. CV(%) preventive = 10.28, CV(%) curative = 6.06.

Figure 1. Size of the anthracnose lesion in banana fruits treated preventive (A) and curative (B) in function on the treatments.

Andrade et al. (2012) found the presence of 14 constituents in cinnamon oil, with trans-cinnamaldehyde (77.72%) as the main component, as well as eugenol (8.5%) found by Sarkhosh et al. (2017). In lemongrass oil, Oliveira et al. (2011) identified geranial and neral components with 30.91 and 42.92%, respectively. Silva et al. (2010) identified carvacrol as the main component in oregano oil with contents ranging from 61.66 to 93.42%, which are similar to the values obtained in this study. According to Oliveira et al. (2011), phenols, such as carvacrol and thymol, can reach total chemical composition of thyme oil at 80.2 to 98.0% and promote disease control, especially in post-harvest period.

Results *in vitro* in this study suggest that the combination of clove, cinnamon, and oregano essential oil with cassava starch films at 2 and 3%, as well as with oregano oil only, had the greatest effects against anthracnose agent (*C. musae*) in banana fruits (Table 2). Several authors have reported the efficiency of essential oils of oregano, thyme, and lemongrass in experiments *in vitro*. Romero et al. (2012) found total inhibition of mycelial growth and germination of *Corynespora cassiicola*, *Fusarium sp.*, *Colletotrichum gloeosporioides* and *Rhizoctonia solani* with oregano oil. These authors attributed the results to high presence of phenolic compounds carvacrol and thymol in oregano oil, as also verified in this study (Table 1), which are able to alter constituent proteins of cell membranes and inhibit cell activities. Vilaplana et al. (2018) found that thyme oil at 500 $\mu\text{L L}^{-1}$ concentration promoted total inhibition of mycelial growth of *C. musae* in banana fruits. According to Simões & Spitzer (2000), low efficiency of some essential oils can be attributed to volatilization of chemical compounds or instability in the presence of air, heat, light, and moisture. In view of the information, it is possible to emphasize that low efficiency of clove and lemongrass oils is possibly related to variation of environmental conditions and high volatile activity

of these chemical compounds, which promoted sensitivity to the pathogen. It should also be considered that some oils may have better effects on germination but not on mycelial growth, as verified in this study.

Cinnamon oil showed antifungal activity on mycelial growth and fungus germination. These results agree with Maqbool et al. (2010b), who verified inhibition of 83.2% of growth and spore germination of *C. musae*. The authors also attributed these results to high content of cinnamaldehyde, which can react with proteins and nucleic acid and interfere with cellular processes. In view of this information, associated with the fact that trans-cinnamaldehyde and eugenol are present in high levels, it is understandable that antifungal activity is related to the action of these compounds.

Essential oils of lemongrass and thyme used *in vivo* were considered more efficient in the control of banana anthracnose. It was evidenced in other fruits that these oils are promising sources in post-harvest control of anthracnose. Smaller lesions were observed in papaya fruit treated with lemongrass, eucalyptus, mint, and tarragon oils (Carnelossi et al., 2009). Bosquez-Molina et al. (2010) reported that combination of algaroba gum 10% and thyme oil 0.1% resulted in 100% reduction of anthracnose in papaya fruits. In avocado, thyme oil 1% in combination with chitosan 1% and isolated at 0.02% reduced lesions from anthracnose (Bill et al., 2014; Sarkhosh et al., 2017). Moura et al. (2012) found in passion fruit a reduction in anthracnose severity with lemongrass oil at 0.1% concentration. Garcia et al. (2008) observed 70% reduction in development of lesions with application of citral 1.0% in papaya and banana fruits. These authors attributed the antifungal activity of lemongrass oil to the high presence of citral in its constitution, which was also identified in this study in larger quantities.

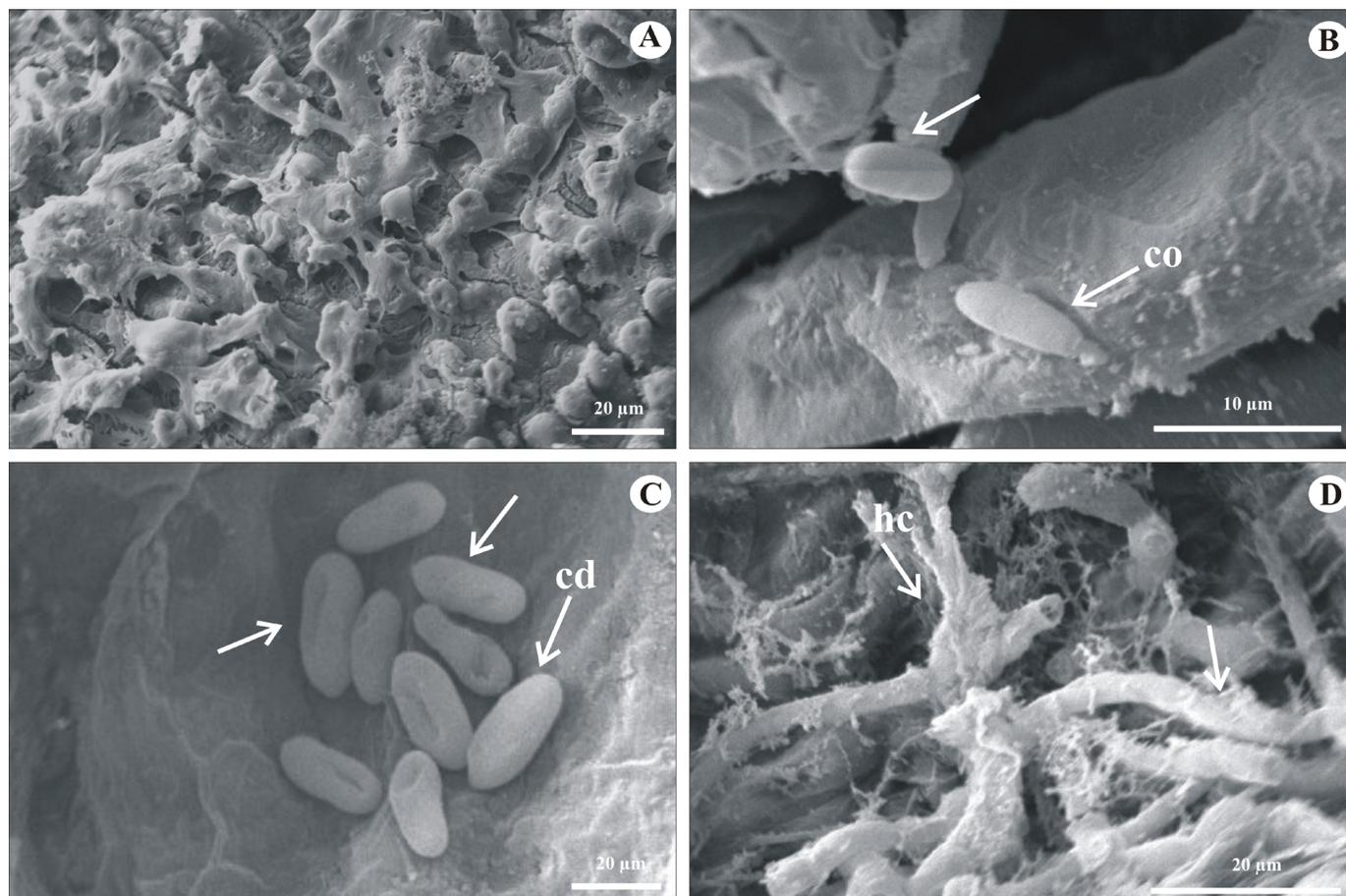


Figure 2. Scanning electromicrographs of the protective layer promoted by cassava starch film 3% (A), non-germinated and deformed *Colletotrichum musae* conidia (B, C) and collapsed hyphae (D) from the action of essential oil of lemongrass and thyme. co: conidia, cd: deformed conidia, hc: collapsed hyphae.

The efficiency of films only in analysis *in vivo* can be attributed to some property in starch composition that acts differently on the fruit, causing an obstacle to pathogen development. Oliveira et al. (2016) observed the positive effect of cassava starch films 2, 3 and 4% on the control of anthracnose in papaya fruit, attributing the efficiency of cassava starch to a protective layer that prevented the pathogen from entering fruit tissues. Images performed by scanning electron microscopy in this study showed a layer on fruit outer surface provided by films which prevented penetration of fungus *C. musae* (Figure 2A). In addition, it was possible to observe direct action of essential oils of thyme and lemongrass on the pathogen which promoted inhibition of conidia germination, leaving them in deformed appearance and causing deformations in the hyphae, thus preventing colonization in fruit tissues (Figure 2B, 2C, and 2D).

Some treatments presented both isolated and combined significant effects, which can be explained by characteristics of essential oils such as volatilization, solubility, and concentration, as well as environmental conditions of humidity, light, and temperature (Guimarães et al., 2008). However, there is great complexity in the mechanism of action of essential oils and cassava starch that is not yet known, and protein denaturation and membrane disintegration may occur during the pathogen infection process (Andrade et al., 2014).

Conclusion

The application of oregano essential oil *in vitro* completely inhibits mycelial growth and germination of *Colletotrichum musae* conidia.

Lemongrass and thyme essential oils and individual cassava starch films at 2% and 3%, as well as their combination, are effective in reducing and preventing anthracnose lesions in banana fruits and may be indicated as a viable strategy for alternative control of post-harvest anthracnose.

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