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Alternative control of *Corynespora cassiicola* in papaya seedlings and fruits by *Cinnamomum zeylanicum* essential oil

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The study aimed to evaluate the efficiency of essential oil concentrations on *Cinnamomum zeylanicum* leaves as a possible fungicide against mycelial growth, and the sporulation of *Corynespora cassiicola* in papaya seedling and fruit. The study performed three experiments, namely comparison of the anti-*C. cassiicola* activity of: 1 µL mL^{-1} *C. zeylanicum* essential oil, commercial fungicide, and control-treatment; comparison of the anti-*C. cassiicola* activity of five essential oil concentrations (0.0, 0.5, 1.0, 2.0, and 4.0 µL mL^{-1}) and a commercial fungicide (150 g ha^{-1} i.a.); and the effect of applying essential oil concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 µL mL^{-1}) before and after the application of mycelium disk fungus. It was found that the essential oil from *C. zeylanicum* inhibits mycelial growth and sporulation of the *C. cassiicola* fungus. The essential oil was able to maintain lower percentage of leaves with lesions in papaya seedling up to 14 days after the inoculation. The essential oil derived from *C. zeylanicum*, applied as a preventive treatment, is efficient in controlling the size of lesions in papaya fruits, however, the effect is not observed if the essential oil is applied after infection.

Key words: *Carica papaya*, target-spot, natural pesticides, inhibitory effect.

INTRODUCTION

The excessive use of fungicidaes to increase the fruit papaya yield gave rise to many negative effects including the appearing of diseases in all crops, including papaya crops. The presence of diseases can cause severe economic losses that reduce papaya fruit yield and marketability. The main papaya diseases are viruses such as Mosaic (Papaya ringspot virus - PRSV) and papaya sticky disease (Papaya meleira virus - PMeV),

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nematoses (*Meloidogyne incognita*), fungal diseases such as Alternaria spot (*Alternaria alternate* [Fr.] Keissl), anthracnose (*Colletotrichum gloesporioides* Penz) and the disease known as target-spot (*Corynespora cassiicola* [Berk & M. a;. Curtis] CTWei), all disease widely spread in most producing areas of world, including Brazil (Porter et al., 2014). Furthermore, in Maranhão State the presence of ideal climate and temperature conditions are response to provide the disease target-spot (*Corynespora cassiicola*) (Silva, 2011).

Target-spot is characterized by the appearance of injuries, which can affect the leaves, stem and fruits. Hundreds of injuries can occur in a single leaf leading to their premature loss as a result of foliar abscission. These injuries are pale yellow color, and vary from 1 to 2 mm in diameter, but in some cases can reach 10 mm in diameter, with necrotic centres. In some cases, the injury presents in the leaf might be superficially and easily cracked. The symptom of this disease on fruit is characterized by small and rounded injuries, approximately 1 mm in diameter. The developments of the disease on fruit occur rapidly, and can affect the fruit pulp, rendering it commercially unviable. Among the strategies to control the target-spot disease is the use of fungicides (Ventura et al., 2004; Liberato and Mctaggart, 2006).

Fungicides mainly used to control the disease are from dithiocarbamates, phthalonitrile, copper oxicloride, chlorothalonil or tebuconazole groups, which are also used for controlling other fungal diseases of papaya plants (Oliveira and Filho, 2006). It is known that the disorder use of fungicides can cause serious damage to the environment and human health. Hence, alternative sources of fungicide for disease control, especially those that could replace chemicals, cost effective and use in low or no residual power are necessary (Ootani et al., 2013).

The potential of essential oils to stop or inhibit mycelial growth has been studied as an alternative to the exclusive use of fungicides. Costa et al. (2011) reported that the eugenol is one of the main compounds found in clove essential oil (*Syzygium aromaticum* [L.] Merr. & L. M. Perry) *in vitro*. These authors showed that the utilization this essential oil was effective on the mycelial growth of *Rhizoctonia solani* Kun*, Fusarium solani* (Mart.) Sacc. and *Fusarium oxysporum Schlecht.* Studies performed by Carlos et al. (2010) reported that the essential oil of medicinal plant *Achillea millefolium* L., in the concentration of 200 µL, inhibited 63% of mycelial growth and 98 to 100% of the sporulation and the spore germination. Similarly, Carnelossi et al. (2009) using both *in vitro* and *in vivo* techniques showed a positive effect in the control of anthracnose in papaya fruit using essential oil of lemongrass leaves (*Cymbopogon citratus)*, eucalyptus (*Eucalyptus citriodora* L.), mint (*Mentha arvensis*L) and tarragon (*Artemisia dracunculus* L.).

Recent studies on the use of plant essential oil have been used as a new strategy for the control of plant disease. Studies have been performed to improve the antimicrobial activity and stability of *C. zeylanicum* (commonly known as cinnamon) essential oil with or without chitosan nanoparticles against *Phytophthora drechsleri*, the causal agent of cucumber (*Cucumis sativus* L.) fruit rot (Mohammadi et al., 2015). In addition, studies also reported the efficacy of chitosan in combination with *Zataria multiflora* or *C. zeylanicum* essential oil to inhibit *P. drechsleri in vitro* and on artificially infected cucumbers (Mohammadi et al., 2016). Those authors reported that *C. zeylanicum* combined with chitosan coatings can be an effective method for extending cucumber shelf-life and the combined treatments (chitosan with *C. zeylanicum* and chitosan with *Zataria multiflora*) were able to reduce fungal decay in the range of 77 to 85%, compared with the control at day 9.

For the development of natural products as an alternative to the use of synthetic fungicides, *in vitro* experiments are needed to verified the essential oil effectiveness on the pathogens. After the identification of essential oil efficiency on pathogen control new tests *in vivo* are needed to establish the optimum essential oil concentrations for specific inhibition of single or a group of pathogens (Combrinck et al., 2011).

The study hypothesized that essential oil have potential as environmentally safe alternative for the control of plant disease in relationship with the synthetic fungicides. Despite these natural products potentially great importance, few studies have focused specifically on the effects of *C. zeylanicum* on *C. cassiicola* in the humid tropic of Brazil. The aim of this work was to:

i) Evaluate the efficiency of the essential oil obtained from *C. zeylanicum* as a potential fungicide on mycelial growth and the sporulation of *C. cassiicola in vitro.*
ii) Evaluate the effect of various essential oil concentrations on the control of target-spot in papaya seedling *in vivo.*
iii) Evaluate the efficiency of this oil in the preventative and curative control of *C. cassiicola* in papaya fruit.

MATERIALS AND METHODS

Experimental site and obtainment of isolate

The experiments were conducted in Phytopathology Laboratory and greenhouse under constant relative humidity, temperature and irrigation conditions, at the State University of Maranhão, São Luís, Maranhão State. The predominant climate type in São Luis is mesothermal tropical and humid with pluviometrics precipitations of approximately 1900 mm, high relative humidity (±82%) and mean temperatures of 26°C. *C. cassiicola* (registry: MGSS-092) isolate was obtained from the fungus collection named “Prof. Gilson

Soares da Silva"/ UEMA. The isolate has been conserved in potato dextrose agar (PDA), which has been preserved by continuous sub-culturing.

Leaves samples and extraction of essential oil

In this study, the leaves were used only. Leaves samples used for extraction of the essential oil were obtained from *C. zeylanicum*, collected in the experimental area of Medicinal Plants of the Federal University of Maranhão, São Luís, Maranhão State, Brazil. Subsequently, the leaves were placed in paper bags and dried in an oven at 70°C until constant weight was achieved. After oven drying to a constant weight, extraction of the essential oil was performed at the laboratory of the Medicinal Plants at the Federal University of Maranhão. Distillation process was used for the extraction of essential oils.

Each distillation process used a quantity of 100 g of dry leaves of *C. zeylanicum* coarsely chopped. Distillation was performed using a modified Clevenger's glass apparatus. The distillation started after a heating time of 40 min. Subsequently, the extraction process was carried out for 3 h after the first drop of distillate until complete exhaustion of the leaves. Condensation was carried out continuously with water chilled to 10°C. The same extraction process was performed three times. The essential oil extracted was recovered, dried with anhydrous sodium sulphate and stored in a refrigerator at 4°C in tightly closed amber vials, and it was used for analysis and various functional biological tests. This process was performed in normal conditions (1.0 bar, 25°C). Chromatography analysis indicated that eugenol is the main volatile compound of extracted oil from *C. zeylanicum*. This component is present in essential oils or extracts of many other plants, and it is used as an alternative to control diseases.

Experimental design

Experiment 1

The experiment was carried out in a randomized experimental design, with 10 replicates, in a split-plot arrangement, with three treatments in the main plots and the nine periods of daily measurements in the subplots. Each Petri dish consisted of one replicate. Each Petri dish contained *C. cassiicola* fungal colonies. The experiment consisted of three treatments as follows: the essential oil from *C. zeylanicum* applied at 1 µL mL^{-1}; commercial fungicide (Tebuconazole) applied with the rates of 150 g ha^{-1} i.a., as per schedule recommendation from the manufacturer; and control treatment, comprising the fungi cultivated in PDA incubation without application of essential oil and fungicide. The subplots consisted of nine periods of daily measurements after inoculation.

The PDA was autoclaving at 121°C for 15 min. The treatments were added to melting PDA medium at 45°C. Subsequently, 20 ml of this mixture was transferred to petri dishes with 9 cm of diameter. Five-millimeter diameter discs were obtained from the fungal colonies and transferred to the center of Petri dishes with PDA medium. Petri dishes were closed with plastic film and stored in a BOD (Biological Oxygen Demand – SPLabor Model SP-225) type chambers at 25°C with a photoperiod of 12 h. The Petri dishes were closed with plastic film to avoid possible transpiration of the compounds and contamination by other microorganisms.

Evaluation of the effect of the essential oil on mycelial growth was performed by daily evaluations of colony diameter in two perpendicular axes for a period of 9 days after 48 h of initial fungus transplantation. This initial time was necessary for the control treatment in order to fill the whole Petri dish. Mycelial growth speed rate (MGSR) was measured daily as described by Oliveira (1991):

$$MGSR = \frac{\sum(D - Da)}{N}$$

Where, D = current average, Da = average diameter of the previous day, and N= number of days after inoculation. At 10 days after incubation to allow for mycelial growth of *C. cassiicola*, the percentage inhibition of mycelial growth (PIC) was evaluated as described by Bastos (1997):

$$PIC = \frac{\text{growth in the control - growth in the treatment}}{\text{growth in the control}} x100$$

Evaluation of sporulation was performed after 10 days of incubation. 10 ml distilled water was pipetted onto the Petri dishes and a Drigalski handle was used to produce conidia. After rapid manual shaking and scraping with a sterilized soft bristle brush, Petri dish contents were filtered in sterilized gauze and quantified in a Neubauer chamber.

Data obtained in the first experiment were statistically examined by analysis of variance (ANOVA) and regression. Means of qualitative factors were compared by the Tukey test at significance level up to 5% probability. The quantitative factor and models were chosen based on the significance of regression coefficients using the *t*-test at 5% probability level value of determination coefficient (R^2), and according to the biological phenomenon**.** The R^2 was obtained by relationship between regression sum and treatment sum of square.

Experiment 2

Six seeds of Sunrise Solo variety were sown in pots with 5 L capacity. The substrates used in the seeds germination comprised of soil and autoclaved manure in the proportion of 3:1 v/v. 10 days after sowing, plants were thinned to three plants per pot. Sixty days after germination, papaya seedlings were sprayed with spores suspensions of the *C. cassiicola*. After incubation, plants were kept in a humid chamber in greenhouse conditions for 48 h. Then, papaya seedlings were maintained in constant relative humidity, temperature and irrigation conditions for 28 days of evaluation period.

For inoculum prepararation, *C. cassiicola* isolates were transferred to Petri dishes containing PDA and were maintained in B.O.D. type chambers, at temperature of 25°C, with a photoperiod of 12 h, for 7 days. Then, 20 ml of distilled water was added to the Petri dishes and conidia were collected from the flooding dishes by gently scraping the colony surface with a bent glass rod. Subsequently, conidia counts were performed with the aid of a Neubauer chamber. Conidia suspensions were adjusted to approximately 1 x 10^5 conidia mL^{-1}.

The experiment was carried out in a randomized experimental design, with 6 replicates, in a split-plot arrangement, with five essential oil concentrations and commercial fungicide with active ingredient Tebuconazole (Folicur®) in the main plots and the four periods of measurements, in 7-day intervals in the subplots. Each pots contain one replicate. The five essential oil concentrations were: 0.0, 0.5, 1.0, 2.0, and 4.0 µL mL^{-1}. The commercial fungicide was applied at a rate of 150 g ha^{-1} i.a., as per schedule recommendation from the manufacturer. The subplots consisted in

7-day intervals at 7, 14, 21 and 28 days after inoculation. First disease symptoms were observed at three days after inoculation. The treatments were applied on papaya seedlings after appearing the first disease symptoms. Dimethyl sulfoxide (DMSO) (1% in distilled water) was used as a solvent to solubilize the essential oil. This solution was then applied to each sample using a 1.0 L hand sprayer.

The evaluated parameters included total number of leaves, number of plants with disease and the severity of the target-spot disease measured as the percentage of leaves with lesions and the total leaf area with lesions. The total leaf area with lesions calculation was performed at 28 days after inoculation. Then, for each pot, four leaves were collected from middle to upper level of the seedlings and were scanned in gray scale with resolution of 75 dpi (pixel/inch). The standardization of leaf area and the total leaf area with lesions were performed through the ImagemJ program, with distance adjusted in pixel for 29.52 pixel. The percentage of growth inhibition (P.I.C.) of lesions was also calculated according to the following equation:

$$\mathrm{PIC} = \frac{\text{growth of lesions in control - growth of lesions in treatment}}{\text{growth of lesions in control}} x100$$

Data obtained during second experiment were statistically examined by ANOVA and regression. The mean of each essential oil concentration was compared with that of commercial fungicide by the Dunnett's test at significance level up to 5% probability. Regression models were chosen based on the significance of regression coefficients using the *t*-test at 5% probability level, value of determination coefficient (R^2) and according to the biological phenomenon**.** The R^2 was obtained by relationship between regression sum and treatment sum of square.

Experiment 3

Healthy papaya fruit of the Sunrise Solo cultivar were acquired at intermediate maturation stage. The fruit were washed in running water, and immersed in a solution of 2% (v/v) sodium hypochlorite for 1 min. Then, the fruit were rinsed with sterile distilled water for three times and were kept in room temperature until there were completely dried. This experiment was conducted in a completely randomized design with five replicates. Five essential oil concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 µL mL^{-1}) of *C. zeylanicum* were used. Each replicate comprised of one fruit. The essential oil concentration of 0.0 µL mL^{-1} comprised of the fruits containing the mycelium disk and sterile distilled water.

For the preventative treatment, essential oil concentrations were applied 24 h before pathogen inoculation. DMSO (1% in distilled water) was used to solubilize the essential oil at various concentrations for the treatments. The application of solution containing essential oil was performed with a small handheld sprayer of 1.0 L capacity. For assessment of curative treatment with the essential oil, orifices of approximately 2 mm in depth were performed in the middle part of the fruit and later 5 mm diameter mycelium discs were inserted. The fruits were placed in transparent plastic bags and kept in plastic containers in a humid chamber for 24 h. The fruits were sprayed with essentials oils and kept in the humid chamber for 72 h. Thereafter, the fruits were kept inside the plastic containers during six days for the disease symptoms development.

The evaluation of number of infested fruits and severity of the disease (diameter of lesions) were performed six days after the inoculation. The P.I.C. of lesions was also calculated. The results obtained from the third experiment were examined by regression analysis. Regression models were chosen based on the significance of regression coefficients using the *t*-test at 5% probability level, value of determination coefficient (R^2), and according to the biological phenomenon. The R^2 was obtained by relationship between regression sum and treatment sum of square. The SAEG (version 9.1, 2007) software was used for all statistical analysis.

RESULTS AND DISCUSSION

Mycelial growth and conidium production of *C. cassiicola*

There was a significant effect of treatments on mycelial growth of *C. cassiicola* fungus through out of the experiments (Table 1). The treatment using essential oil derived from *C. zeylanicum* was found as efficient as the commercial fungicide treatment in inhibiting fungal mycelial growth. Target-spots were observed on untreated papaya leaves. However, there was no mycelial growth of *C. cassiicola* fungus in essential oil and fungicide treated papaya leaves. Thus, these results showed the efficiency of *C. zeylanicum* essential oil for the control of spot-target. Increased periods of daily measurements after inoculation resulted in a significant increase in the mycelial growth of the *C. cassiicola* fungus according to a linear model (Figure 1). The control treatment provides the mycelia growth normally. The MGSR for the control treatment was of 0.18 cm day^{-1}.

Essential oil obtained from bark and leaves of *C. zeylanicum* was reported by Ranasinghe et al. (2002) which provide inhibitory effect on mycelial growth of the fungi *Colletotrichum musae* (Berk & Curt.) Arx (causing the disease known as anthracnose), *Lasiodiplodia theobromae* and *Fusarium proliferatum* (Matsuhima) Nirenberg (responsible for rot in rhizomes of banana) *in vitro*. Combrinck et al. (2011) also performed 100% efficiency of *C. zeylanicum* essential oil on mycelial growth inhibition of the pathogens *L. theobromae*, *C. gloeosporioides*, *Alternaria citrii*, *Botrytis cinerea* and *Penicillium digitatum*. Carnelossi et al. (2009) observed total or partial inhibition of *C. gloeosporioides* sporulation with the use of essential oils of lemon grass, eucalyptus, mint and tarragon. Besides, Lorenzetti et al. (2011) verified the effect of essential oil of various species on the control of the fungus *Botrytis cinérea* in strawberry gray with concentrations of 125 and 1000 ppm. The *C. zeylanicum* oil was found to efficiently affect both mycelial growth and germination of fungi spores.

The inhibitory effects were due to the presence of eugenol, a majority compound present in the essential oil of *C. zeylanicum* (cinnamon) was reported by Carnelossi et al. (2009) and Lorenzetti et al. (2011). This substance is responsible for causing: alterations in the cytoplasmic membrane; interruption of the motive power of protons, of

Table 1. Mycelial growth (cm) *in vitro* affected by essential oil from *C. zeylanicum* (1 µL mL^{-1}).

Treatment	Days after the inoculation									PIC[1]	n^0 spores ($x10^5$) [2]
	1	2	3	4	5	6	7	8	9		
Control	1.6[b]	2.6[b]	3.2[b]	3.5[b]	4.0[b]	4.4[b]	4.7[b]	5.8[b]	6.1[b]	-	2.64 [b]*
Essential oil	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	100	0.0 [a]
Fungicide	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	100	0.0 [a]

*Means followed by the same lower case letter in the column do not differ by Tukey test at 5% probability. [1]Percentage of mycelial growth inhibition (PIC). [2]Sporulation of *C. cassiicola* evaluated at 10 days after the inoculation.

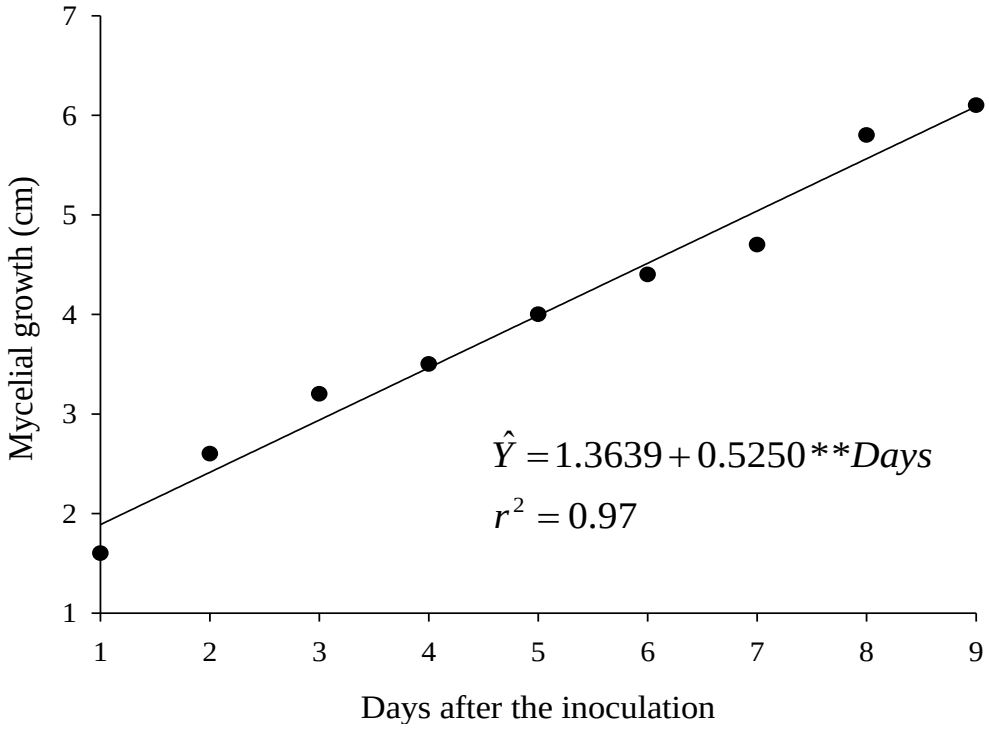

Figure 1. Mycelial growth (cm) as function of days after inoculation for control treatment. ** Significant at 1% of probability by the *t*-test (** Significant at 1% of probability by the *t*-test).

the electrons flux, of the active transport; and coagulation of the cellular content of filamentous fungi (Davidson, 1997; Abbaszadeh et al. 2014). Costa et al. (2011) reported that the essential oil obtained from cloves (*Syzygium aromaticum*) presents a huge concentration of eugenol and promotes morphological alteration in the vacuoles; disorganization of cell contents; and decrease of the cell wall distinctness beyond lower turgescence in cells of mycelia treated with this oil.

These mechanisms could explain the inhibitory action observed in the experiment, which showed the inhibition of *C. cassiicola* fungus by *C. zeylanicum in vitro* attributed, to confirm the essential oil of cinnamon as promising agent on the control of *C. cassiicola* fungus.

Control of target-spot in papaya seedling

Under greenhouse conditions, all seedlings showed symptoms of the disease three days after inoculation with fungus. The quick establishment of pathogen is probably due to the high susceptibility of the papaya plant to *C. cassiicola*, as reported by Oliveira (2007), first disease symptom occurs at 96 h after inoculation with fungus. There was no statistically significant difference between each essential oil concentration compared with fungicide treatment on measures of total leaf number for all time points after inoculation, and at 21 and 28 days after inoculation for percentage of leaves with lesions (Table 2). At 7 and 14 days after inoculation, there was a

Table 2. Total leaf number and percentage of leaves with lesions caused by *Corynespora cassiicola* on papaya seedling treated with various essential oil concentrations of *C. zeylanicum.*

Treatments	Days after inoculation			
	7	14	21	28
	Total leaf number			
Fungicide	8.0	7.6	7.7	7.0
0.0	8.8^{NS}	8.5^{NS}	7.1^{NS}	5.9^{NS}
0.5	6.7^{NS}	6.1^{NS}	6.8^{NS}	7.4^{NS}
1.0	9.1^{NS}	7.6^{NS}	8.2^{NS}	8.0^{NS}
2.0	6.2^{NS}	5.9^{NS}	5.7^{NS}	5.3^{NS}
4.0	7.4^{NS}	5.9^{NS}	5.9^{NS}	5.2^{NS}
	Percentage of leaves with lesions			
	7.8	14.7	28.6	28.1
0.0	27.5*	36.0*	51.8^{NS}	44.4^{NS}
0.5	25.9*	38.7*	43.7^{NS}	35.0^{NS}
1.0	21.8*	29.2*	31.3^{NS}	30.9^{NS}
2.0	13.2^{NS}	24.5^{NS}	25.2^{NS}	24.9^{NS}
4.0	8.2^{NS}	11.0^{NS}	24.8^{NS}	25.4^{NS}

*and ^{NS}: Means differ and do not differ from the fungicide treatment, respectively, at 5% probability by Dunnett's test.

statistically significant difference between 0.0; 0.5 and 1.0 µL mL^{-1} essential oil concentrations when compared with fungicide treatment on the measure of percentage of leaves with lesions (Table 2). However, there was no statistically significant difference of 2.0 and 4.0 µL mL^{-1} essential oil concentration when compared with fungicide treatment on percentage of leaves with lesions.

By visual analyses, at higher essential oil concentration (4.0 µL mL^{-1}), the presence of higher quantities of discolored spots on leaves were detected. This spots found on leaves of papaya seedlings are non-characteristic of the target-spot disease. These results could have potentially been caused by the phytotoxicity effect on leaves of papaya seedlings, which invalidated the concentration as promoter of the control of *C. cassiicola* in papaya seedlings. Furthermore, the discoloration spots appearing could have been the cause for the fungal not to grow on the leaves of papaya seedlings.

As described by Liberato and Mctaggart (2006), the leaf of the papaya plant is highly susceptible to target-spot, and when there is a considerable number of a lesion on the leaf, the disease causes premature leaf shedding. Martins et al. (2012) also reported that the highly aggressive fungus reduces the photosynthetic capacity of papaya leaves, and has a direct effect on foliar abscission.

The essential oil concentrations and the commercial fungicide were not able to lessen premature leaf shedding caused by the target-spot disease over the days assessed (Figure 2A). As per the *in vitro* experiment, where there was a reduction of the mycelial growth and sporulation, it was expected that in presence of *C. zeylanicum* essential oil, the *C. cassiicola* infection is suppress, thus this increased the longevity of the papaya leaves. These results were different to those reported by Médice et al. (2007) who verified that the presence of essentials oil of citronella (*C. nardus* [L.] Rendle), eucalyptus, neem (*Azadirachta indica* A. Juss.) and thyme (*Thymus vulgaris* L.) were capable of keeping the soybean plant leaves green and photosynthetically active.

The study also observed that the essential oil and commercial fungicide were able to keep the low percentage of infected leaves until the 14th day of evaluation (Figure 2B). There was a trend showing a reduction in the percentage of infected leaves between the 21st and the 28th days of evaluation (Figure 2B). The reduction in the percentage of infected leaves over the same time interval coincides with the major reduction in the number of leaves presented in this treatment (Figure 2A). This fact is related to the attack of *C. cassiicola* and it was reported by Ventura et al. (2004) and Liberato and Mctaggart (2006).

Similar effects were described by Médice et al. (2007), where a decrease was observed in the percentage of leaves with pustule of the *Phakopsora pachyrhizi* Syd. & P. Syd. after the application of essential oil from eucalyptus up to seven days after inoculation. However, the essential oil of thyme was able to reduce the incidence of disease until the 36th day of evaluation, which may be attributed to the higher or lower capacity of these oils in low active concentrations to maintain their action over time at ambient conditions. These results reinforce the idea of the necessity of new applications of *C. zeylanicum* essential oil (except at the concentration of 4 µL mL^{-1}) in controlling target-spot in papaya seedling, and in doing so, avoid continuous new fungal infections on papaya leaves. Essential oil concentrations presented no effects on leaf number for all four evaluation time points. Increased days after inoculation resulted in a significant increase in the percentage of leaves with lesions according to a linear model for all evaluation time points (Table 3).

Essential oil concentrations had no effect on leaf area and total lesioned area (Table 4). The averages were 64.8 cm^2 and 2.0 cm^2 for leaf area and total lesioned area, respectively. There was no statistically significant difference between each essential oil concentration compared with fungicide treatment on leaf area, and total lesioned area in papaya seedlings (Table 4). However, it was noted that the action of different concentrations of *C. zeylanicum* essential oil did effect the percentage of growth inhibition of lesions for the treatments of 1.0 µL mL^{-1} and 2.0 µL mL^{-1}, representing 66 and 54% of

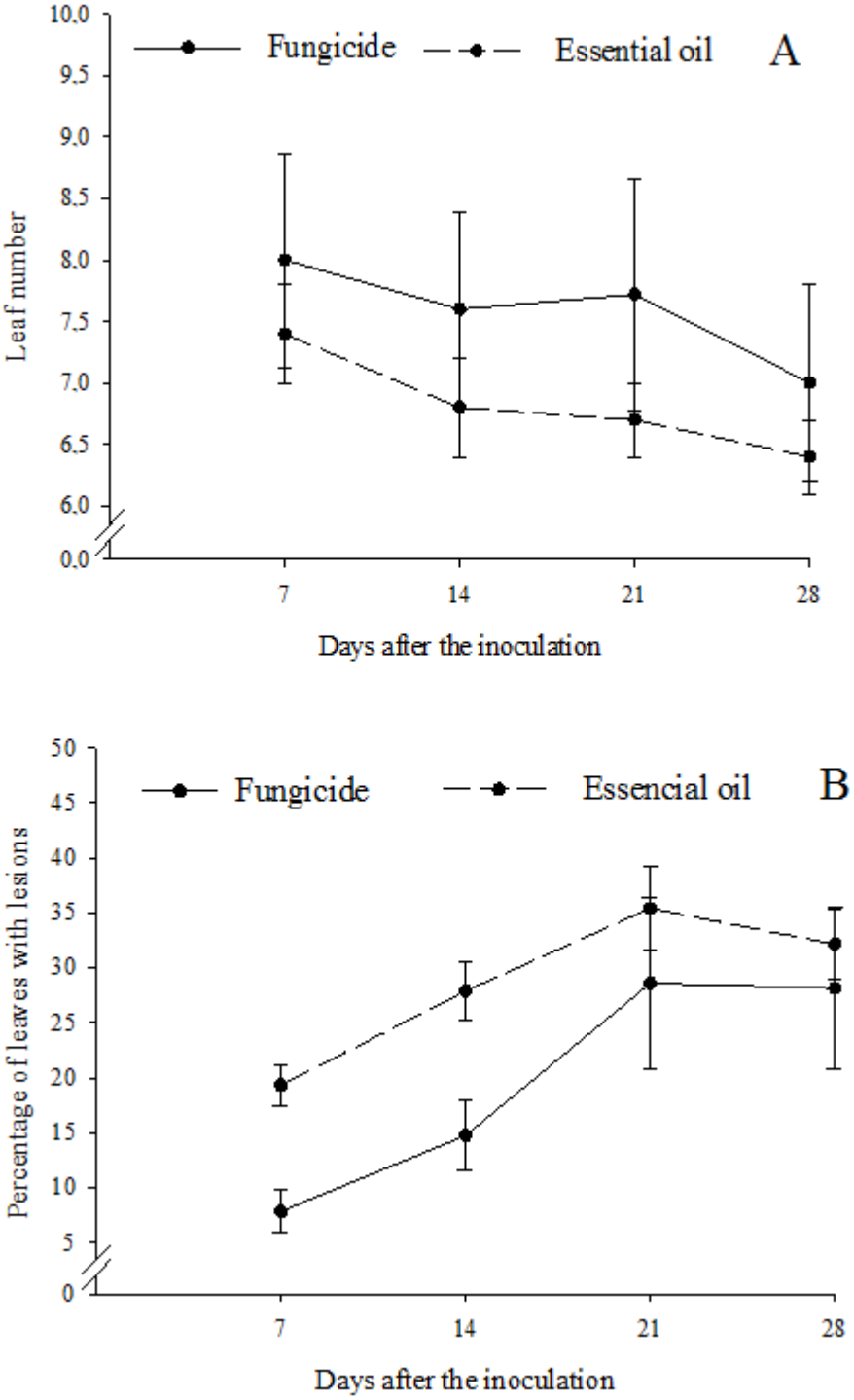

Figure 2. Leaf number (A) and percentage of leaves with lesions (B) affected x days after inoculation. B*ars denote standard error* of the mean.

inhibition, respectively, in relation to the control (Table 4).

Similar results obtained in another pathosystem were reported by Lorenzetti et al. (2012), efficiency of essential oils on reduction of severity of diseases. They found significant results with the essential oils of citronella and eucalyptus on the control of lemon grass leaf rust,

Table 3. Adjusted model of leaf number and percentage of leaves with lesions caused by *C. cassiicola* on papaya trees treated with different concentrations of *C. zeylanicum* essential oil.

Days	Adjusted model	r^2/R^2
	Leaf number	
7	$\hat{Y}$ = 7.4; SE = 0.4	-
14	$\hat{Y}$= 6.8; SE = 0.4	-
21	$\hat{Y}$ = 6.7; SE = 0.3	-
28	$\hat{Y}$ = 6.4; SE = 0.3	-
	Percentage of leaves with lesions	
7	$\hat{Y}$=26.9625 - 5.0959**X	0.93
14	$\hat{Y}$=38.0125 - 6.7550**X	0.95
21	$\hat{Y}$=44.7275 - 6.2450°X	0.68
28	$\hat{Y}$=38.2850 - 4.11°X	0.65

** and °: Significant at 1 and 10% of probability by the *t*-test, respectively. SE = standard error of the mean.

Table 4. Effect of *C. zeylanicum* essential oil on total area and total lesioned area (severity) caused by the *C. Cassiicola* fungus in papaya seedling at 28 days after inoculation with the pathogen.

Treatments	Leaf area (cm^2)	Total lesioned area (cm^2)[1]	Percentage of control
Fungicide	**42.0**	**0.42 (2,3)**	**20.7**
0.0	86.0^{NS}	0.35^{NS} (2.9)	0.0
0.5	53.5^{NS}	0.43^{NS} (2.4)	18.2
1.0	69.8^{NS}	0.23^{NS} (1.0)	66.5
2.0	54.6^{NS}	0.26^{NS} (1.3)	54.3
4.0	60.4^{NS}	0.43^{NS} (2.4)	15.6

^{NS}: Means differ and do not differ from the fungicide treatment, at 5% probability by Dunnett's test, respectively. [1]Values were transformed to Log(x+1). Values in parentheses indicate values originals.

obtaining a smaller percentage of infected leaves after 30 days of application of the essential oil when compared to the control.

The range that provides high percentage of disease control was 1.0 to 2.0 µL mL^{-1} (Table 4). These results are important in emphasizing that new research is necessary in tropical humid Brazil. This new research needs to define an optimum essential oil concentration that provides greater control of the disease in papaya crops for current conditions.

Preventative and curative control of lesions in fruits of papaya

Increased essential oil concentrations resulted in a significant decrease in the preventative ability of treatments, when considering lesion size, according to a linear plateau response (Figure 3). An essential oil concentration of 0.52 µL mL^{-1} led to the smallest estimated size lesion. After this concentration, the lesion size was constant. Essential oil concentrations presented no effects to lesion size in curative treatments. The lesion size was 11.48 cm (Table 5). These results show that after the infection of papaya fruits, the percentage of inhibition of disease is too low. Thus, it is necessary to focus efforts on the preventative treatment of fungal infection in papaya fruits.

Perhaps the presence of essential oil before inoculation with fungus (preventative treatment) was capable of providing an environment of protection in the fruit, thereby creating a barrier to the establishment and development of lesions. In the curative treatments, the time between the inoculation and the treatment should have been sufficient to fully establish the fungus in the fruit. Another consideration is the fast process of papaya

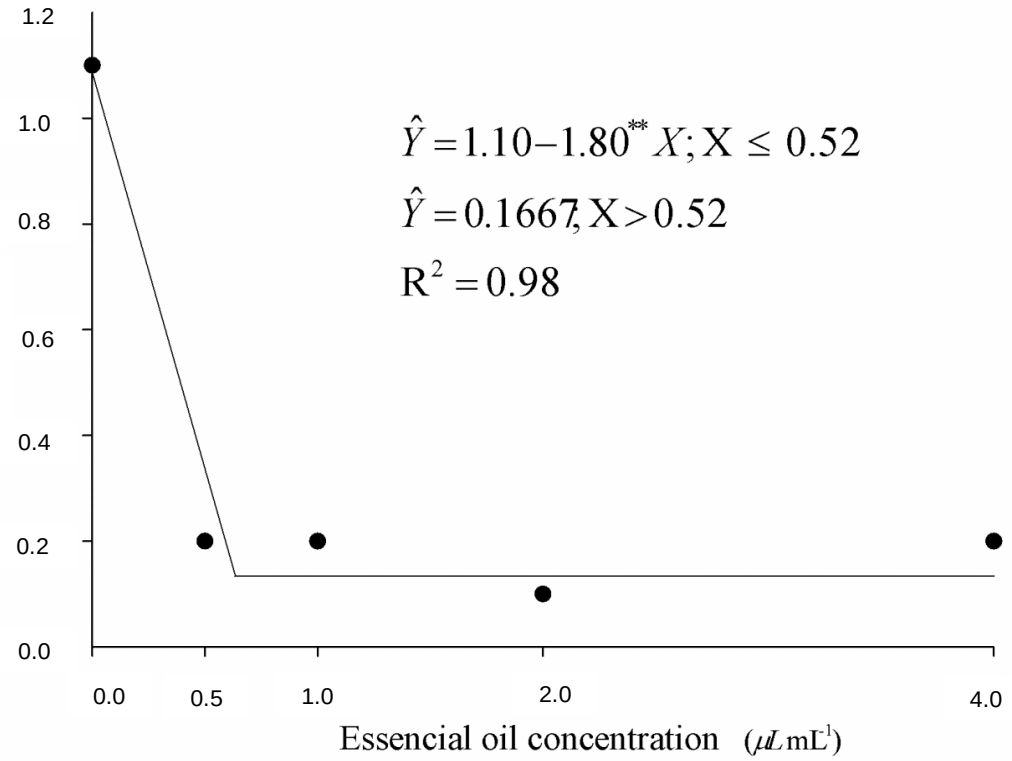

Figure 3. Lesion size (cm) in response to preventative treatment with essential oil at various concentrations **(**** Significant at 1% of probability by the *t*-test. *Values (Y) were transformed* to √x).

Table 5. Lesion size (cm) in curative treatments affected by essential oil concentration in papaya fruits at 7 days after the inoculation of the pathogen.

Treatments	Lesion size (cm)	Percentage of control
0.0	10.8	0
0.5	12.5	-15
1.0	10.9	0
2.0	9.0	17
4.0	11.7	-0.08
-	$\hat{Y} = \overline{Y} = 11.48$	-

fruits ripening, mainly in the presence of pathogen, which stimulates the production of ethylene, the hormone responsible for ripening. This could explain why curative treatments with essential oil did not result in pathogen control.

Similar results obtained by Carnelossi et al. (2009), used of the essential oil of *C. citratus* and *Eucalyptus citriodora* (at 1% concentration) in the control of *C. gloeosporioides,* the causal agent of papaya anthracnose in postharvest. The fruits were preventatively treated and subsequently inoculated after 24 h, presented higher control of disease, attesting to the potential use of essential oils as an alternative to the application of commercial fungicides in the prevention of disease. The study results showed that the application of essential oil from *C. zeylanicum* could be considered as an effective method to inhibit fungal growth, to reduce the use of synthetic fungicides in papaya seedling, and could be used as a preventative control for *C. cassiicola* in papaya fruits. In the future, more laboratory and field studies are suggested for further practical validation of *C. zeylanicum* essential oil on *C. cassiicola* control in papaya seedlings and postharvest fruits.

Conclusions

Essential oil from *C. zeylanicum* has inhibitory effect of on the mycelial growth and sporulation of the *C. cassiicola* fungus. The essential oil keeps the low percentage of leaves with lesions in papaya seedling up to 14 days after the inoculation. The essential oil from *C. zeylanicum*, applied as a preventative treatment, is effective in controlling lesion size. Essential oil has no effect in controlling lesion size after infections of *C. cassiicola* have already been established in papaya fruits. The *C. cassiicola* essential oil concentration used to

obtain the lower infection of *C. cassiicola* is 0.52 µL mL^{-1} which is applied before the infection in fruits.

Conflict of Interests

The authors have not declared any conflict of interest.

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