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Control of papaya fruits anthracnose by essential oils of medicinal plants associated to different coatings

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Chemical pesticides have been commonly used for the control of various plant diseases. Although pathogens can be controlled with the application of these products, there are hazardous impacts in human health and environment. In parallel, essential oils have been shown to be effective in plant diseases management and can be safely incorporated as suitable alternatives for synthetic fungicides. Therefore, the aim of this study was to evaluate the control of papaya fruits anthracnose by different essential oils associated with different coatings. *In vitro* evaluation of essential oils was carried out to conidial germination and mycelial growth of *Colletotrichum gloeosporioides*. Quantification of anthracnose and fruits fresh mass loss was also performed for all treatments. Rosemary pepper and Noni essential oils were the most effective in reducing conidia germination and mycelial growth of *C. gloeosporioides*. The application of essential oils associated with different coatings was as effective as the chemical control, in inhibiting the growth of the pathogen in 100%. Moreover, papaya fruits treated with the mixture of paraffin and essential oils reduced in 7% the fruits fresh mass loss infected with the fungus. These combinations are a promising alternative to chemicals to control anthracnose, a major postharvest disease on papaya during storage.

Key words: *Colletotrichum gloeosporioides*, alternative control, post-harvest, weight loss.

INTRODUCTION

Brazil is the world's second largest papaya producer (*Carica papaya* L.), with a production of 1.517.696 t year^{-1}, situated among the main exporting countries, mainly for the European market (IBGE, 2016). Papaya is one of the most susceptible fruit to phytopathogen attack, from pre-postharvest stages. Among the diseases that attack the plant, anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Is the most

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important because of the damage it can cause to fruit productivity and marketing (Dantas et al., 2003). The pathogen can infect at any stage of development, but symptoms usually appear in ripe fruits (Kimati et al., 2005).

The application of fungicides is one of the most used ways to control anthracnose (Liberato and Zambolim, 2002), however this method besides contaminating the environment and food can also affect the natural ripening of the fruits. In addition, it is known that permanent use of fungicides may lead to the emergence of resistant variations of phytopathogens (Price et al., 2015). Therefore, the search for alternative control methods is justified, including resistance induction in plants through the use of plant extracts and essential oils, among others (Stangarlin et al., 1999; Schwan-Estrada and Stangarlin, 2005).

Many studies have looked at the potential of extracts or essential oils obtained from medicinal plants in the control of pathogens (Cunico et al., 2003; Stangarlin and Schwan-Estrada, 2008; Aguiar et al., 2014; Sarmento-Brum et al., 2014a, b). These compounds should have acceptable antifungal activity with known and very low toxicological effects on mammals and minimal impact on the environment. In addition, they should be exempt from residue tolerances on agricultural commodities. Authorities confirm these characteristics by approving them as food additives or preservatives or as generally regarded as safe substances (Palou et al., 2016). Secondary metabolites with antifungal activity derived from plants can activate defense mechanisms in plants against pathogens (Stangarlin et al., 1999). Currently, there are few studies that have been developed associating essential oils with coatings to control papaya anthracnose in tropical conditions. Thus, the aim of this study was to evaluate the effectiveness of essential oils postharvest, isolated or associated with different coatings, for the control of anthracnose in papaya fruits.

MATERIALS AND METHODS

Extraction of essential oils

The medicinal plants were collected in the municipality of Gurupi, Tocantins, Brazil. They were identified by morphological characterization with the support of specialized literature. The following plants, as well as their parts for extraction, were used: Noni fruits (*Morinda citrifolia* L.) and leaves of Lemongrass (*Cymbopogon citratus* DC Stapf), Mastruz (*Chenopodium ambrosioides* L.), Citronella (*Cymbopogon nardus* L) . Rendle) and Rosemary pepper (*Lippia sidoides* Cham). Extraction of the essential oils was performed by the Clevenger hydrodistillation method (Castro et al., 2002). The supernatant was then stored at 4°C until use.

Isolation and aggressiveness test of *C. gloeosporioides* from papaya fruits

C. gloeosporioides isolates were obtained from *Formosa papaya* fruits purchased from local businesses (June/July, 2017). The identification of the fungus was performed through morphological characteristics, supported by specialized literature. Then the identification was completed through the Koch Postulates.

Fragments of fruit lesions (0.5 cm in diameter) were removed using a sterile scalpel. Subsequently, a dip in a 50% ethanol for 30 s was done before soaking in sodium hypochlorite (1%) for 40 s and washing in sterile water for three consecutive times. The fragments were transferred to Petri dishes containing PDA culture medium (Potato, Dextrose, Agar) and kept in an incubation room at 27 ± 3°C and 12 h photoperiod. After growth and sporulation of the pathogen, the plates were stored at 5°C.

For the aggressiveness test, uniform fruits in size and ripening stage acquired in local trade were sanitized before inoculation. Each isolate was inoculated separately on the surface of the fruit. All fruits were inoculated with 6 mm diameter mycelium discs for treatments with and whithout injury. Next, fruits were incubated in a 85 ± 3% RH chamber at 27 ± 3°C. Lastly, the diameter of the lesions was evaluated 7 days after inoculation.

Fungistatic action of essential oils

The fungistatic-fungicidal nature of essential oil was tested in quadruplicate by observing revival of growth of the inhibited mycelial disc following its transfer to non-treated PDA. Five essential oils were tested: Noni fruits (*M. citrifolia* L.) and leaves of Lemongrass (*C. citratus* DC Stapf), Mastruz (*C. ambrosioides* L.), Citronella (*C. nardus* L . Rendle) and Rosemary pepper (*L. sidoides* Cham.) in 7 different concentrations (0.000, 2000, 4000, 6000, 8000, and 10000 ppm). After oil distribution, a 6 mm diameter mycelium agar disc was placed in the center of the dishes. Then, the plates were sealed with PVC plastic film, identified and incubated at 27°C and photoperiod of 12 h. The effect of essential oils on phytopathogen mycelial growth was performed with four measurements at 2, 4, 6, and 8 days of incubation, from two diametrically opposite measurements of colony diameter, using a digital caliper. From these data, the mycelial growth rate index was calculated according to the following formula, described by Maia et al. (2011):

$$\mathrm{MGRI} = \frac{\sum(\mathrm{D}-\mathrm{Da})}{\mathrm{N}},$$

Where MGRI = mycelial growth rate index; D = current average diameter of the colony; Da = average diameter of the previous day colony; N = number of days after inoculation.

In vitro analysis of the effect of the five essential oils in anthracnose conidia inhibition was performed as described. Briefly, after oil extraction, dilutions were made at concentrations of 0 (control), 500, 1000, 2000, 3000, 4000 and 5000 ppm. The conidia were immersed in small flasks containing the doses described, then placed on slides and kept in a humid chamber in a gerbox for 48 h. Then, it was considered germinated when it reached about 1.5 times the size of the body length and with the formation of appressorium. A total of 200 conidia were counted for each treatment and values expressed as a percentage.

Chromatographic analysis of pepper Rosemary and Noni essential oils

Qualitative analyses were performed by gas chromatography coupled to CG-MS mass spectrometry (Shimadzu GC-2010) under the following conditions: RTX-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness); with the following column temperature setting: 60 - 240°C (3°C min^{-1}); injector temperature: 220°C; helium carrier gas; splitrate injection (1:100) with 1 µL injected volume of a 1: 1000 hexane solution. For the mass spectrometer (MS), the following conditions were used: 70 eV impact energy; ion source temperature and interface: 200°C. The constituents were identified by comparing their mass spectra with those from the Nist and Wiley 229 library databases, and also by comparing their calculated retention rates with those in the literature (Adams, 2007). The contents of the compound were expressed as a percentage based on area normalization.

Efficacy of essential oils associated with different coatings in the anthracnose preventive control and loss of fresh mass of papaya

Extract oil solutions (30 mL) of each plant was prepared and used to fruit pulverization. A completely randomized experimental design was used, with four repetitions, in a 4 × 4 factorial scheme: factor *a* = four coatings and factor *b* = two essential oils and two major compounds. Four treatments were used at the following concentrations: Noni (3%), Rosemary pepper (3%), Octanoic acid (2.5%) and Thymol (7.5%). These substances were diluted in four coatings: carnauba wax; colorless flavored gelatin, paraffin, sunflower oil and water as a control. Carnauba wax and paraffin were heated to melt to promote addition and mixing of treatments. The colorless flavored gelatin was dissolved in distilled water and sterilized to a concentration of 3% (w/v). In treatment with sunflower oil, only the dissolution of the compounds was performed. The methodology used for dissolution and treatment mixtures in coatings was adapted from Junqueira et al. (2004). For each treatment three fruits were used. Briefly, the fruit was inoculated without injury which *C. gloeosporioides,* by the deposition of mycelium-agar disc at three opposite points in the equatorial region of the fruit. Subsequently, the fruits were placed in trays and in a humid chamber 27 ± 3°C (Evangelista et al., 2014; Amarante et al., 2013). Evaluations were performed every 48 h, until maturity (optimal point of consumption and end of life). The severity of anthracnose was determined every two days, totaling four evaluations. The diameter of the lesion (mm) was quantified and posteriorly calculated the area under the disease progress curve (AUDPC), according to equation presented by Campbell and Madden (1990). Fresh weight loss (FWL) was obtained by the difference between the initial weight, using a digital scale, and the final expressed as a percentage.

Statistical analysis

A completely randomized design with four replications was used, in a factorial scheme of 5×7, being five essential oils: Noni fruits (*M. citrifolia L*.), Lemongrass (*C. citratus*), Mastruz (*C. ambrosioides* L.), Citronella (*C. nardus*) and Rosemary pepper (*L. sidoides* Cham.) in 7 different concentrations (0.000, 2000, 4000, 6000, 8000, and 10000 ppm). Statistical analysis was performed by analysis of variance and Scott-Knott test (p = 0.05) using the software SISVAR (Ferreira, 2014).

RESULTS AND DISCUSSION

Aggressiveness of *C. gloeosporioides* isolates to papaya fruits

Thirty isolates of *C. gloeosporioides* (CG1 to CG30) were evaluated. This total, 56% (17/30) caused disease with lesions fruit peel. Only CG2 and CG29 were able to cause anthracnosis without injury. Thus, 44% (13/30) of the isolates were not able to cause anthracnose symptoms and were considered non-pathogenic (Table 1).

Fungistatic dilution test

Isolate CG2 was used to evaluate the inhibitory potential of *in vitro* growth of essential oils. Among the oils tested, Rosemary pepper and Noni had the greatest inhibitory effect on mycelial growth of the pathogen (Figure 1). Rosemary pepper oil stood out in relation to the others and caused considerable inhibition of fungal mycelium from the 4000 ppm concentration. Noni essential oil had a differentiated effect from the others only from the 6000 ppm concentration (Figure 1).

Concentration of 1,000 ppm showed lower fungus inhibition capacity for all essential oils tested (MGRI values greater than 22.9). The essential oils of Lemongrass, Citronella and Mastruz had the lowest inhibitory effect (10,000 ppm and MGRI range from 21.9 to 22.6). Thus, all concentrations of essential oils inhibited the growth of fungal hyphae.

Rosemary pepper and Noni essential oils showed lower activity in this study. Sahoo et al. (2012) showed fungitoxic potential in Noni leaf extract on some phytopathogens fungal species, such as, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus oryzae*, *Helminthosporium* species, *Curvularia* species and *Sclerotium* species.

All essential oils also reduced the *C. gloeosporioides* conidia germination for all concentrations tested (Figure 2). Noni essential oils totally inhibited conidial germination at 3000 ppm. Rosemary pepper and Lemongrass inhibited conidial germination at 5000 ppm. Already, Mastruz and Citronella essential oils not totally inhibited the conidial germination even at high concentration (Figure 2).

Chromatographic analysis of Rosemary pepper and Noni essential oils

Chromatographic analysis of Rosemary pepper and Noni essential oils (Table 2) presented the major constituent, respectively, Thymol (92.68%) and Octanoic acid (82.24%).

Essential oils of Rosemary pepper also were evaluated for fungitoxic potential against mycelial growth of phytopathogens as *Rhizoctonia solani* and *Sclerotium rolfsii* by Gonçalves et al. (2015). Unlike this study, the major components found were Carvacrol (33.27%) and 1,8-cineol (24.41%). Costa et al. (2005) found thymol as a major chemical constituent (43.5%) of *L. sidoides* Dalcin et al. (2017) and Osorio et al. (2018) found similar results to Noni oil. In both studies, Octanoic acid is the major component (82.24 and 64.04%, respectively).

It is known that the composition of secondary

Table 1. Aggressiveness of *C. gloeosporioides* isolates to papaya fruits after seven days of inoculation.

Isolated	CF	SF	Isolated	CF	SF	Isolated	CF	SF
CG 1	+	-	CG 11	-	-	CG 21	+	-
CG 2	+++	++	CG 12	-	-	CG 22	+	-
CG 3	+ +	-	CG 13	-	-	CG 23	+	-
CG 4	+ +	-	CG 14	-	-	CG 24	+	-
CG 5	+ +	-	CG 15	-	-	CG 25	+	-
CG 6	+ +	-	CG 16	-	-	CG 26	+	-
CG 7	+ +	-	CG 17	-	-	CG 27	+	-
CG 8	-	-	CG 18	-	-	CG 28	+	-
CG 9	-	-	CG 19	-	-	CG 29	++	+
CG 10	-	-	CG 20	-	-	CG 30	+	-

CG: Isolates of *C. gloeosporioides*; CF: with injury to the fruit peel; SF: no injury; - non-pathogenic; + little aggressive; ++ aggressive and +++ very aggressive.

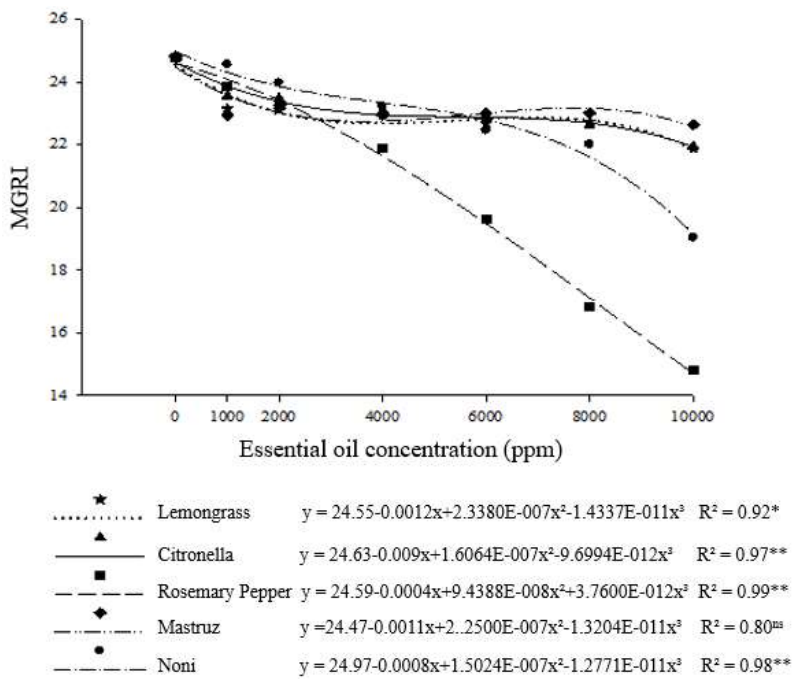

Figure 1. Mycelial growth rate index (MGRI) of *Colletotrichum gloeosporioides* as a function of increasing concentrations of essential oils.
ns = not significant, * = 5% and ** = 1% probability by Scott-Knott test.

components in plants is a result of the balance between their formation and transformation during growth, mainly due to factors genetic, environmental, and cultivation techniques (Castro et al., 2002).

Efficacy of essential oils associated with different coatings applied in preventive control of anthracnose and loss of fresh mass of papaya

A significant difference was observed for disease progression within 24 h after fruits treatment and inoculation (Figure 3). Largest area under disease progress curve (AUDPC) was observed in treatments with the following mixtures: Paraffin + Octanoic acid, Gelatin + Noni, Sunflower oil + Noni and Sunflower oil + Octanoic acid. These mixtures were less effective in anthracnose control. On the other hand, the associated with paraffin + Noni, gelatin + Octanoic acid, Sunflower oil + Thymol were more effective. This result infers that the greater anthracnose control action may be related to the combination of chemical constituents present in the

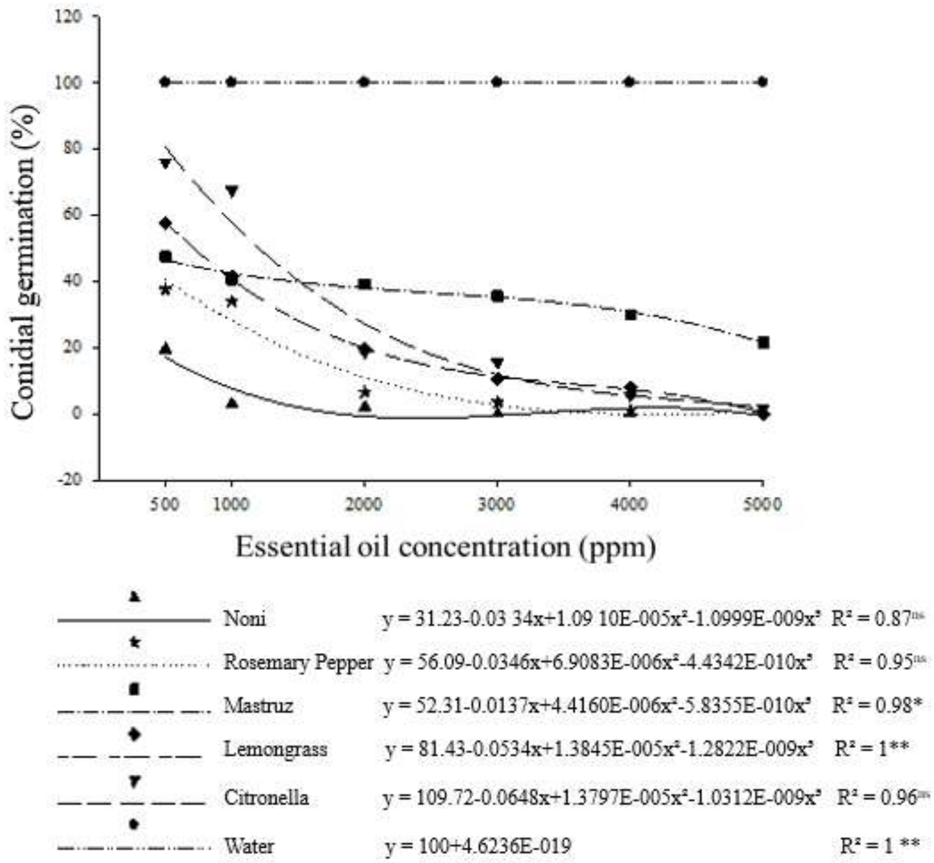

Figure 2. *In vitro* germination of *Colletotrichum gloeosporioides* conidia as a function of increasing concentrations of essential oils of Noni, Rosemary pepper, Mastruz Lemongrass and Citronella. ns = not significant, * = 5% and ** = 1% probability by Scott-Knott test.

Table 2. Chemical constituents of essential oils of Rosemary pepper *(Lippia sidoides* Cham.) and Noni (*Morinda citrifolia* L.) extracted from leaves and fruits, respectively, collected in Gurupi, Tocantins, 2017.

Compound	IR	Percentage	
		Rosemary pepper	Noni
α-tujeno	924	0.051	-
α-terpineno	1014	0.091	-
ρ-cimeno	1020	1.162	-
γ-terpineno	1054	0.250	-
hydrated cis-sabineno	1065	0.102	-
Hydrated cis-sabineno	1174	0.453	-
4-terpineol	1232	0.430	-
Thymol methyl ether	1289	92.684	-
Thymol	1417	2.235	-
(E)-caryophyllene	1452	0.134	-
α-humuleno	1582	0.617	-
Hexanoic acid	987	-	8.26
Ethyl Octanoate	999	-	2.48
Octanoic acid	1177	-	82.24
Isopentyl Octanoate	1259	-	1.60

IR: Retention Index.

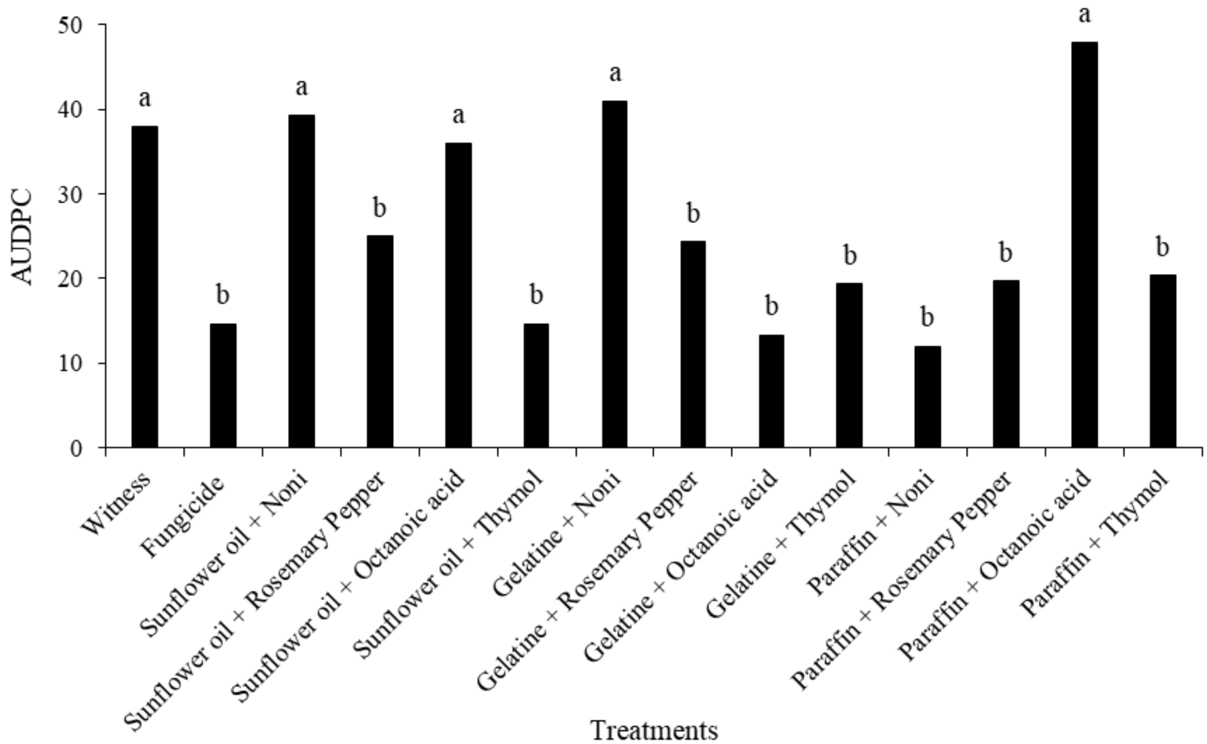

Figure 3. Area under the progress curve of anthracnose in papaya fruits subjected to the application of different coatings with incorporation of the essential oils of Noni (*Morinda citrifolia),* Rosemary pepper (*Lippia sidoides*) and their major compounds. Equal letters above the columns do not differ statistically by the Scott-Knott test (p = 0.05).

coating and/or other constituents in the oil than a simple isolated action of a major compound.

Possibly the interaction between the essential oil compounds of Noni (Octanoic acid) and Rosemary pepper (Thymol Methyl) promoted an inhibitory effect responsible for the reduction of AUDPC. Like Octanoic acid when associated with gelatin, paraffin-associated Noni oil and Sunflower oil + Thymol were skilled to disease control, demonstrating that the effect may be increased under the influence of the coating or mixture used. This effect can be observed for both essential oils and their major compounds associated with the different coatings tested.

Similar studies evaluating the potential of essential oils to control postharvest phytopathogens have been reported. Carnelossi et al. (2009), evaluating essential oils for *C. gloeosporioides* control postharvest in papaya. The authors observed smaller area with lesion under the disease progress curve (AUDPC) and lower severity for fruits treated with *C. citratus* oil. Zillo et al. (2017) showed that Rosemary pepper essential oil associated with carboxymethylcellulose film reduce severity in the *C. gloeosporioides* control. Here, the result is not similar. About Noni essential oil, there are few studies so far. Observing other postharvest alternative treatments of different fruits, Barman et al. (2017) demonstrated that chitosan applied to mango has the ability to reduce the incidence of diseases and also the loss of fruit mass. Guanhyao et al. (2019) suggested that the composite coating containing clove and cinnamon extracts can induce resistance to *Rhizopus* species in hot peppers.

All treatments result in a decrease in mass fruits steadily over the days. Moreover, all treated fruits gradually lost fresh mass as storage time increased (Table 3).

Two distinct statistical groups in relation to the percentage of mass loss were observed. The first group was composed by six treatments with higher values of papaya fruit degradation, starting from the control (13.89%) to the gelatin + Rosemary pepper mixture (10.04%). The most effective treatments for reducing papaya mass loss start with paraffin + Thymol (9.39%) to paraffin + Rosemary pepper (6.52%). The latter, together with the treatment consisting of the Sunflower oil + Noni mixture were the only ones with losses below 7%.

Carnelossi et al. (2009) also found that essential oils have the potential to increase fruit shelf life. In their study, oils from *C. citratus* (Lemongrass), *Eucalyptus citriodora* (Eucalyptus), *Mentha arvensis* (Mint) and *Artemisia dracunculus* (Tarragon), were used as treatments to mass loss. The results showed the largest losses in papaya fruits occurred with essential oil treatments of *E.*

Table 3. Loss of fresh weight of papaya fruits as a function of time (days) and application of essential oils associated with different coatings.

Treatment	Initial weight (kg)	Days				
		2	4	6	8	10
		%				
Witness	0.849	1.26[a]	6.28[a]	6.28[a]	11.46[b]	13.89[b]
Sunflower oil+ Thymol	1.064	3.07[a]	6.02[a]	6.70[a]	9.84[b]	12.16[b]
Gelatine + Thymol	0.880	1.36[a]	3.83[a]	7.16[a]	9.47[b]	11.44[b]
Gelatine+ Noni	1.043	0.42[a]	1.95[a]	5.15[a]	5.21[a]	11.22[b]
Gelatine + Octanoic acid	1.197	0.56[a]	1.25[a]	4.37[a]	7.60[a]	10.50[b]
Gelatine + Rosemary pepper	1.265	0.82[a]	1.66[a]	4.93[a]	7.51[a]	10.04[b]
Paraffin + Thymol	1.019	0.79[a]	2.13[a]	4.74[a]	7.23[a]	9.39[a]
Sunflower oil + Octanoic acid	0.927	0.65[a]	1.29[a]	4.10[a]	6.62[a]	9.06[a]
Paraffin + Noni	0.874	0.99[a]	2.55[a]	5.76[a]	7.13[a]	8.46[a]
Sunflower oil + Rosemary pepper	1.066	1.03[a]	1.84[a]	3.84[a]	5.91[a]	7.78[a]
Paraffin+ Octanoic acid	1.035	0.58[a]	1.64[a]	3.77[a]	5.28[a]	7.05[a]
Sunflower oil + Noni	1.303	0.66[a]	1.64[a]	3.27[a]	5.12[a]	6.85[a]
Paraffin + Rosemary pepper	1.038	0.84[a]	2.02[a]	3.57[a]	5.17[a]	6.52[a]

Equal letters in the column do not differ from each other by the Scott-knott test (p = 0.05).

citriodora and *C. citratus,* when compared with the other treatments. According to the results obtained in the present study, it was verified that the possible action of the coatings on fruits mass loss is more related to the decrease of water loss than the anthracnose control. Thus, treatments that were ineffective in controlling the disease, such as Sunflower oil + Noni, had good efficiency in reducing fruit water loss.

Other authors have reported that adding fruit coatings is effective in preventing weight loss. Hossain and Iqbal (2016) reported a decrease in banana weight loss with chitosan application. Jongsri et al. (2016) using the same substance also observed a reduction in weight loss in mango.

Combining essential oils with coatings to improve fruit health and shelf life is an innovative line of studies. Recent work demonstrates the effectiveness of these treatments and the ability to reduce fungicide use and have a healthier product for consumers (Muazu et al., 2018; Dunn et al., 2019; Habashi et al., 2019). Oliveira et al. (2019) also states that there is a synergism between chitosan and *Mentha piperita* essential oil, demonstrating the importance of studies with the mixture of these substances.

Conclusion

Rosemary pepper and Noni essential oils showed greater reduction in growth rate of *C. gloeosporioides,* compared to Citronella, Mastruz and Lemongrass. Low concentrations of Rosemary pepper and Noni also were effective in inhibited conidial germination in comparism to other essential oils. The interaction of these essential oils and different coatings showed similar AUDPC values for fungicide control in papaya fruits inoculated with *C. gloeosporioides*. Fruits treated with the Paraffin + Rosemary pepper and Sunflower Oil + Noni mixture were able to reduce at least than 7% the mass loss of the fruits infected with the fungus.

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