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Repellent activity of essential oils against mediterranean fly and their effects on postharvest quality in paluma guava

Atividade repelente de óleos essenciais contra mosca do mediterrâneo e suas influências na qualidade pós colheita em goiaba paluma

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ABSTRACT - Brazil is one of the world's largest guava (*Psidium guajava*) producing countries, but the economic performance of this crop can be negatively affected by the damage caused by *Ceratitis capitata* (Diptera: Tephritidae). The main methods for controlling *C. capitata* are based on broad-spectrum insecticide applications. However, the negative effects of using this control method stimulate the development of sustainable alternatives, such as the use of essential oils (EO). In this sense, the objective of the present study was to evaluate the repellent activity of EO of citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum*), and copaiba (*Copaifera officinalis*) for repellency of *C. capitata* in Paluma guava fruits and determine their effects on postharvest fruit quality, under laboratory-controlled conditions. The fruits were treated with citronella, clove, and copaiba EO and placed in covered plastic containers attached to the ends of arenas of 20 cm diameter and 15 cm height. The fruit physical and chemical analysis was carried out based on the same oils applied in the repellent test. The *C. nardus* essential oil presented a greater repellency of *C. capitata* females. The EO applications proved to be a good strategy for maintaining the fruit post-harvest quality.

Keywords: Botanical insecticides. Alternative control. Fruit flies. Postharvest fruits.

RESUMO - O Brasil está entre os maiores produtores mundiais de goiaba (*Psidium guajava*), mas o rendimento econômico dessa cultura pode ser negativamente afetado pelos danos causados por *Ceratitis capitata* (Diptera: Tephritidae). Os principais métodos de controle de *C. capitata* são baseados em aplicações de inseticidas de amplo espectro. Porém, os efeitos negativos do uso desse controle estimulam o desenvolvimento de alternativas sustentáveis, como o uso de óleos essenciais. Nesse sentido, o presente estudo teve como objetivo avaliar a atividade repelente dos óleos essenciais de citronela (*Cymbopogon nardus*), cravo (*Syzygium aromaticum*) e copaíba (*Copaifera officinalis*) em fruto de goiabeiras Paluma contra *C. capitata* e determinar suas influências na qualidade pós-colheita desses frutos trabalhados. Em condições controladas de laboratório, os frutos foram tratados com óleos essenciais de citronela, cravo e copaíba e acondicionados em recipientes de plástico com dimensões de 20 cm de diâmetro e 15 cm de altura. A análise físico-química dos frutos foi realizada com base nos mesmos óleos aplicados no teste de repelência. O óleo essencial de *C. nardus* apresentou maior repelência às fêmeas de *C. capitata*. A aplicação de óleos essenciais, mostrou-se uma estratégia para manutenção da qualidade pós-colheita.

Palavras-chave: Inseticidas botânicos. Controle alternativo. Moscas-das-frutas. Frutos pós-colheita.

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INTRODUCTION

Guava (*Psidium guajava* L.) is a fruit species from the family Myrtaceae that is widely grown in tropical and subtropical regions. Guava fruits are used for multiple purposes, due to its pleasant taste and nutritional value (WANG et al., 2018), which allowed it to enter different market niches (MOON et al., 2018). Brazil is one of the world's largest guava producing countries, with a planted area of 15,200 hectares and an average yield of 22.7 Mg ha^{-1} (GOMES et al., 2017) This fruit tree is grown mainly in the Northeast and Southeast regions of the country (IHA et al., 2018).

Guava is one of the most infested fruits by *Ceratitis capitata* (Wied., 1824) (Diptera: Tephritidae) in Brazil (MACIEL et al., 2017). Damages are caused by *C. capitata* at adults and larval stages. Females perforate the surface of fruits for oviposition, enabling the entry of pathogenic microorganisms; the larvae then feed on the fruit pulp, compromising the commercial quality (BOULAHIA-KHEDER, 2021a).

The main methods for controlling *C. capitata* are based on broad-spectrum synthetic insecticide applications, focusing mainly on adult forms (BOULAHIA-KHEDER, 2021b). The indiscriminate use of insecticides can often cause problems to the fauna and environment, such as death of natural enemies, since most of these products are not selective (YADAV et al., 2017); in addition, they lead to the development of resistant pest populations (ELFEKIH et al., 2014).

Essential oils (EO), or their chemical compounds, can attract beneficial insects or repel unwanted ones (LIU et al., 2019). In addition, these materials are biodegradable, leaving no toxic waste, denoting that they are potentially suitable for use in integrated pest management programs (LÓPEZ et al., 2011).

Some studies on the insecticidal properties of EO have reported the repellent activity of EO of *Allium tuberosum* Rottler ex Sprengel (Amaryllidaceae) (GAO et al., 2019), *Cinnamomum zeylanicum* Blume (Lauraceae) and *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae) (PLATA-RUEDA et al., 2018), and lethal action of *Artemisia absinthium* L. (Asteraceae) (RIZVI et al., 2018) against many agricultural pests. López et al. (2011) found repellent effect of *Tagetes minuta* L. (Asteraceae) and *Tagetes filifolia* L. (Asteraceae) EO against *C. capitata*.

EO are recognized for their fruit quality maintenance properties (SIVAKUMAR; BAUTISTA-BAÑOS, 2014). Botelho et al. (2016) found that the combination of cassava starch and cinnamon EO were efficient in maintaining the quality of guava fruits at room temperature and under modified atmosphere, prolonging their shelf-life. However, studies evaluating the quality of fruits treated with EO with insecticidal effect are scarce. In this sense, the objective of the present study was to evaluate the repellent activity of citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum*), and copaiba (*Copaifera officinalis*) EO against *C. capitata* in Paluma guava fruits and determine their effects on postharvest fruit quality.

MATERIAL AND METHODS

The Mediterranean fruit flies (*Ceratitis capitata*) used in the experiment were from culture stocks maintained by Invertebrate Zoology Laboratory of the Department of Biological Sciences of the Federal University of Paraiba (LABZOO/CCA/UFPB), at an average temperature of 25±2 °C, 70±10% relative humidity, and photophase of 12 hours.

Insects at larval stage were fed an artificial diet consisting of 400 g of carrot, 4 g of nipagin, and 80 g of brewer's yeast (BRITO et al., 2009); and with a 10% honey solution during the adult stage. The oils used in the study were obtained from local stores.

The repellency of the essential oils (EO) against *C. capitata* was evaluated through EO application on guava fruits and the effect of the treatments on fruit quality was evaluated through physical-chemical analysis.

The guava (*Psidium guajava* L. cv. Paluma) fruits used were at early physiological maturation stage (green to yellow fruits); they were acquired in a local market, cleaned with water and 0.1% sodium hypochlorite for 10 minutes, and dried on absorbent paper at room temperature (25 °C). The EO used were: citronella, *Cymbopogon nardus* (L.) Rendle (1899), (Poaceae); clove, *Syzygium aromaticum* (L.) Merrill and Perry (1939) (Myrtaceae); and copaiba, *Copaifera officinalis* L. (1762) (Fabaceae). They were diluted in distilled water at concentrations of 0 (control), 100, 200, 300, 400, and 500 mg mL^{-1}, composing the treatments. The guava fruits were immersed in the treatments for one minute and left to dried in plastic trays for one hour. The fruits were then placed in covered plastic containers, coupled at the ends of the arenas, covered with sheer fabric at the top and with plastic at the bottom, similar to the procedures described by Botelho et al. (1973) (Figure 1). Forty newly mated five-day-old females were released in the central region of the arenas at one day after the starting of oviposition.

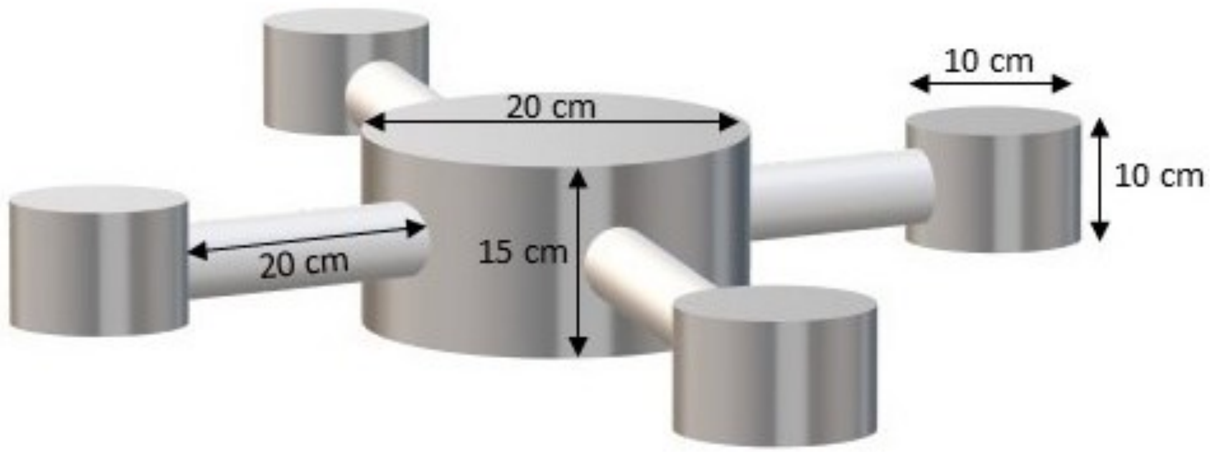

Figure 1. Dimensions of the arena used for confinement of adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae).

EO repellency test

The number of individuals (females) with the treated fruits in the containers was counted at each evaluation. The insects were counted every hour after their release for first six hours and then at 12, 24, 48, and 72 hours after the release of the females inside the arenas.

The repellency of each EO against *C. capitata* was determined by the percentage of females that visited fruits treated with one of the oils (citronella, clove, and copaiba) at different concentrations (treatments), during the exposure time. Five replications were used for each treatment. Each replication consisted of four fruits, one for each treatment (oil-concentration), and a control (untreated group). Thus, 100 fruits were used, distributed in 25 arenas.

A complete randomized experimental design was used, in a 3×6×4 factorial arrangement, with five replications. The treatments consisted of 3 oils, 6 oil concentrations, and 4 evaluation periods.

Fruit physical and chemical analysis

The physical and chemical tests were carried out using eight guava fruits (*P. guajava* L. cv. Paluma) at initial maturation stage per treatment. The highest oil concentration of 500 mg mL^{-1} was used to represent the repellency index for the citronella, clove, and copaiba essential oils, diluted in distilled water, to cover the fruits, consisted of concentration treatments and untreated group (control). After the preparation of the products in plastic containers at temperature of 25 °C, a complete randomized experimental design with four replications was used, evaluating fruit skin and pulp, in a 4×5×2 factorial arrangement, consisting of four treatments and five storage periods, which were evaluated every three days for 12 days of storage at room temperature 22±2 °C.

The analyzed parameters were:

Weight loss (%): determined by weighing all experimental units at the beginning of storage and then daily for 12 days. These data were used to calculated the weight loss percentage as a function of the difference in weight over the 12-day period (storage).

Skin color: measured through an objective, using a Minolta® Color Reader CR-10 digital colorimeter, expressing the color as the parameters: L* = luminosity; a* = green (-a*) to red (+a*); and b* = blue (-b*) to yellow (+b*) colors, in which the farther from the center (0), the more saturated the color (CALBO; CALBO, 1989).

pH: Determined using a digital pH meter, the electrode was inserted into a solution containing 5±0.5 g of the material, diluted in 50 mL of distilled water.

Total soluble solids: determined by direct reading, using a digital refractometer (Abbe, ATAGO N1) according to the analytical standards of the Instituto Adolfo Lutz - IAL (2008).

Titratable acidity (g citric acid 100 g^{-1} of pulp): determined by titration with 0.1 M NaOH solution with phenolphthalein indicator until a permanent pinkish color was obtained, using five g of the sample in 50 mL of distilled water according to the method of the Adolfo Lutz Institute - IAL (2008).

Ascorbic acid: determined by titration, using a DCPIP solution (2,6-dichlorophenolindophenol 0.002%) and 0.5% oxalic acid as an extracting solution, as described by Strohecker and Henning (1967).

Statistical analysis

The results found in the repellency test were subjected to analysis of variance, using the generalized linear model and considering the Beta distribution. Depending on to the significance of the F test ($p \leq 0.05$), response surface analysis was applied for the effects of the doses of each oil versus exposure time. The results found for the fruit physical and chemical analyses were subjected to analysis of variance by the F test ($p \leq 0.05$). The factor storage days and its interaction with EO was analyzed through second-order polynomial regression. Tukey test ($p \leq 0.05$) was applied for the factor EO. Both tests were carried out using the statistical software SAS® University Edition (2015).

RESULTS AND DISCUSSION

EO repellent activity against *C. capitata*

Significant effect was found for the different oils (p = <0.0001), concentrations (p = <0.0001), times of exposure (p = <0.0001), and interaction between oils and concentrations (p = <0.0001) (Figure 2). The application of citronella oil (Figure 2A) resulted in greater repellency of *C. capitata* when compared to applications of clove and copaiba oils (Figures 2B and 2C). The increase in citronella oil concentration continuously increased the repellency potential, resulting in 0% to 5% visiting flies for the concentration of 200 mg mL^{-1}. This effect was observed during the 72 hours of evaluation. The increase in clove oil concentrations also increased the repellency percentage (Figure 2B), but this effect was lost over time and the percentage of visiting flies became similar to that in the control treatment. Similarly, the treatment with copaiba oil had no or low effect on the number of visiting flies (Figure 2C).

Citronella EO has a high insecticidal activity against fruit flies mainly due to the presence of two important bioactive compounds (citronellal and geraniol) (ARANCIBIA et al., 2013). The citronellal monoterpene present in citronella oil has scientifically recognized insecticidal and repellent action, highlighting the potential of this oil for use as a pesticide in organic crops (REGNAULT-ROGER; VINCENT; ARNASON, 2012). In addition to its insecticide action, citronella oil exhibits rapid volatility (ARANCIBIA et al., 2013) which, combined with its great insect repellent action, resulted in the lowest percentage of individuals since the beginning of the releases.

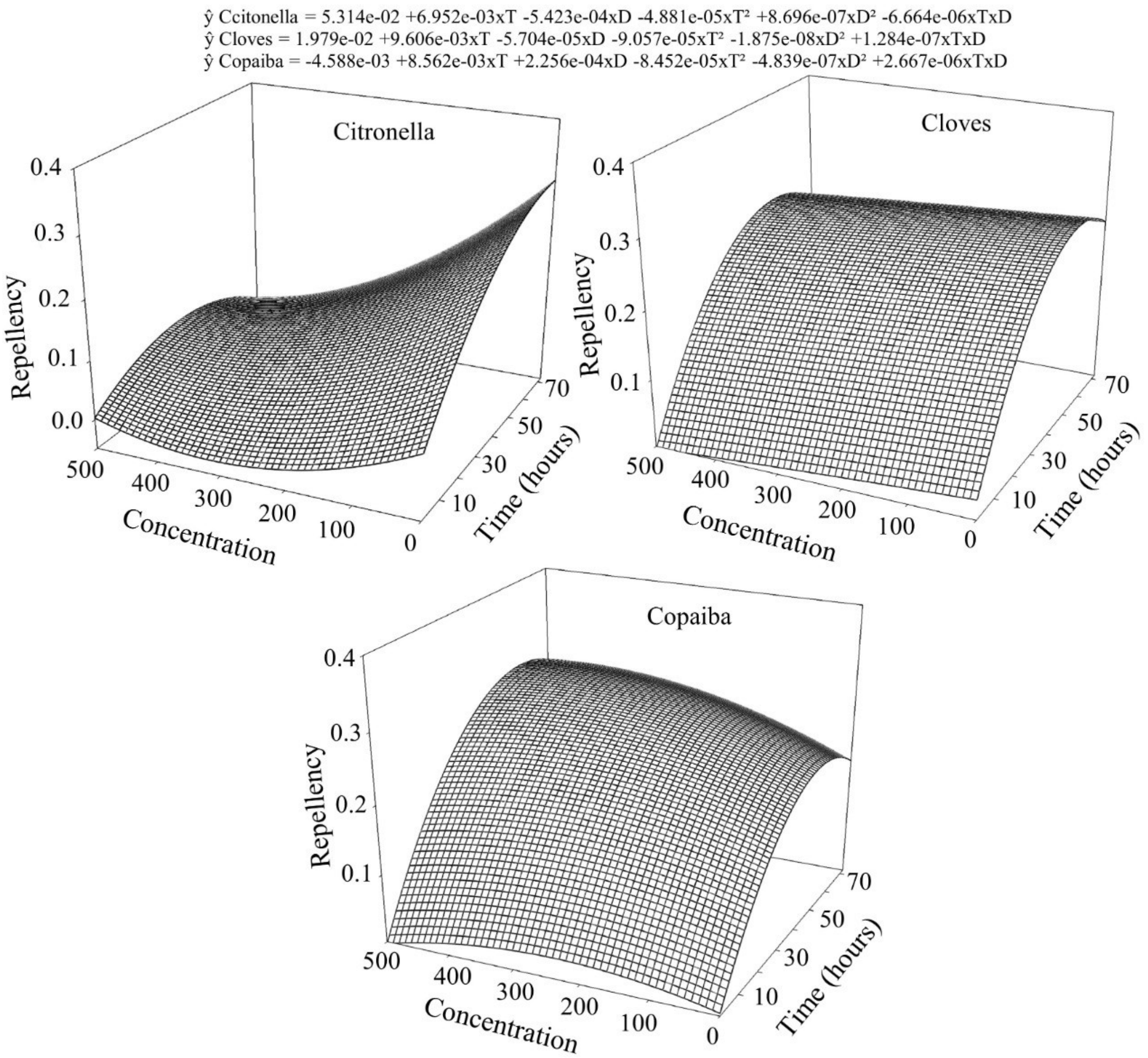

Figure 2. Probability (P) of repellency of adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae) by citronella, clove, and copaiba essential oils at concentrations of 0 (control), 100, 200, 199 300, 400, and 500 mg mL^{-1}, topically applied on guava fruits during different exposure times.

Physical and chemical analysis of guava treated with EO

The treatments and storage periods had significant effect on weight loss (Figure 3). Control fruits presented the highest weight loss percentages, with decreases of approximately 24%. This result is consistent with those found by Botelho et. al. (2016), who evaluated post-harvest responses of guava fruits of the cultivar Pedro Sato subjected to several treatments, including EO, and found similar weight losses for control fruits.

Fruits under treatments with EO presented lower weight loss. This variable is mainly connected to respiration and moisture exchanges through the fruit surface (JOSHI et al., 2017). EO are hydrophobic and compatible with the outer layer of fruit, thus, they can form a protective barrier against water loss, reducing weight loss (SANGSUWAN et al., 2016).

Fruits coated with clove and copaiba EO presented weight losses from 5% to 13%. Joshi et al. (2017) also found good results for clove oil regarding storage maintenance and postharvest quality of papaya: treatments using this oil had the lowest weight losses.

The skin brightness (L*) of guava fruits was significantly affected ($p < 0.001$) only by the storage periods (Figure 4A). The highest L* values were found after 9 days of storage (69), with a decrease after 10 days (66). These values were higher than those found by Murmu and Mishra (2018), who evaluated guava fruits of the cultivar Baruipur after 21 days under different coatings based on Arabic gum, sodium caseinate, and cinnamon and lemongrass essential oils, and found L* values between 41.23 and 60.29.

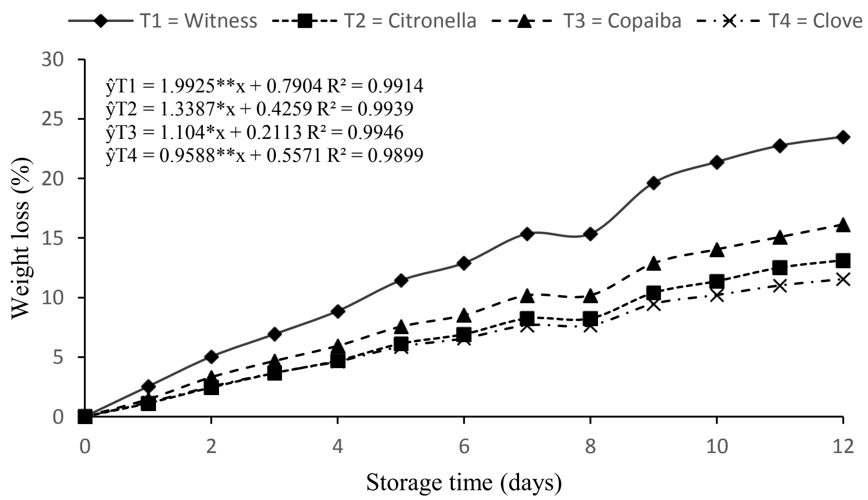

Figure 3. Weight loss of guava fruits treated with citronella, clove, and copaiba essential oils during different storage periods.

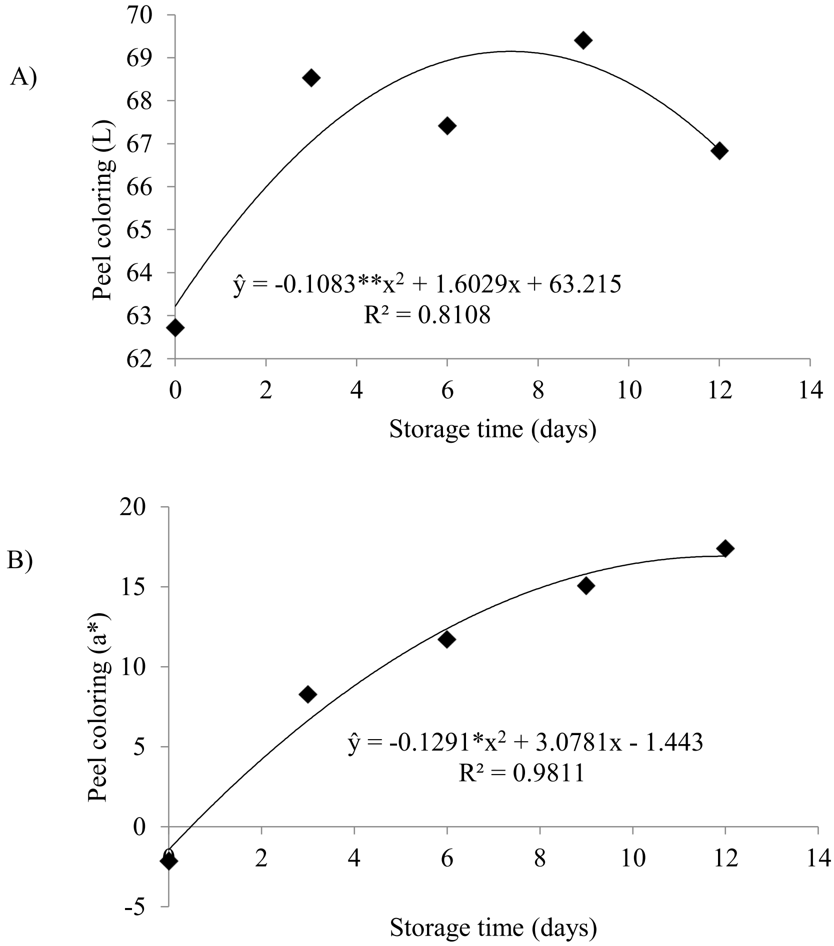

Figure 4. (A) Skin color (L*) of Paluma guava fruits treated with essential oils against adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae) as a function of storage time. (B) Skin color (a*) of guava fruits as a function of storage time.

Isolated effects of storage periods and the treatments used were found on green to red color transition (a*) (Figure 4B and 5A). The fruits showed losses in green color and yellowing from the sixth day onwards. Similar results were reported by Rodrigues et al. (2018), who observed this color transition on the fourth day in Paluma guava fruits coated with jackfruit seed starch.

The control reached the yellowish color more quickly than the other treatments, presenting the highest a* values (Figure 5A). Copaiba and clove oils were the treatment that most delayed the color transition, leading to a slower evolution of a*. These results confirm the positive effect of using EO on maintaining guava fruit quality.

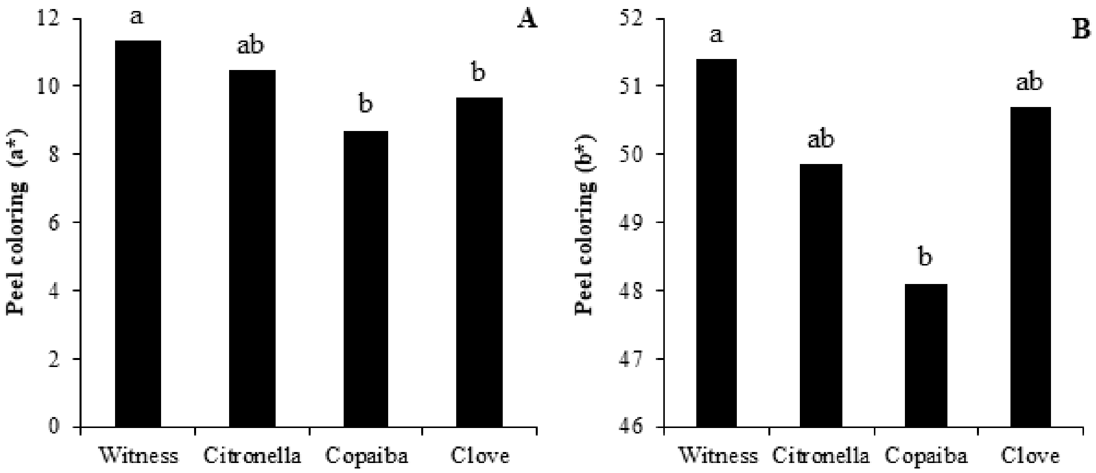

Figure 5. (A) Skin color (a*) of guava fruits as a function of storage time (5-A) and treatments with citronella, clove, and copaiba essential oils against adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae) (5-B). (B) Skin color (b*) of guava fruits treated with citronella, clove, and copaiba essential oils against adult *C. capitata*.

Similarly, Choi, Singh, and Lee (2016) found delay in color change in plums subjected to treatments with hydroxypropyl methylcellulose coating incorporated with oregano and tangerine (*Citrus reticulata*) EO, thus increasing the post-harvest quality.

The b* parameter of skin color was significantly affected by the treatments (Figure 5B). The copaiba oil resulted in the lowest values, although statistically similar to the other oils. The application of copaiba oil resulted in a longer delay in fruit ripening, with a slower yellowing.

Guava is a climacteric fruit, thus the use of coatings such as EO can delay its ripening process by changing the internal gas composition due to changes in the fruit skin permeability, leading to changes in O_2, CO_2, and ethylene productions (SIVAKUMAR; BAUTISTA-BAÑOS, 2014). Shirzadeh and Kazemi (2012) evaluated mint essential oil in apple fruits and found reduction in ethylene production rates due to the oil application, which directly affected the guava fruit shelf-life.

The skin pH varied from 4 to 5, and the highest values were found in the control (Figure 6A). The pH parameter peaked on the sixth day in all treatments. A strong reduction in pH was found on the twelfth day, mainly for citronella oil, indicating a possible effect of the components of this essential oil on the fruit metabolic activity (PERDONES et al., 2012).

According to current Brazilian legislation (BRASIL, 2018), the pH of the guava pulp used in this research was within the Brazilian Identity and Quality Standard (PIQ), which determines a minimum pH of 3.5. The pulp pH (Figure 6B) was significantly affected by the treatments and storage periods; the control presented decreasing values when compared to the other treatments. The pulp from fruits coated with citronella EO presented the the lowest skin pH values. Different results were reported by Aquino, Blank, and Santana (2015) for guava fruits coated with chitosan, cassava starch, and essential oil of *Lippia gracilis* schauer (1847), with pH significantly lower in treatments with coating application than in the control treatment, at 10 days of storage at room temperature.

In addition, pH is important for the development of some types of pests in fruit trees. Lee et al. (2016) evaluated the susceptibility of raspberry fruits to *Drosophila suzukii* Matsumura 1931 (Diptera: Drosophilidae), an important pest of small fruits, and found that the probability of oviposition increases as the analyzed fruit pH is increased. In the present study, the treatment with non-addition of EO resulted in higher pH values when compared to treatments with citronella, copaiba, and clove EO, for most of the storage periods.

The pulp pH of fruits subjected to EO application was within the limits found in other studies about guava focused on aspects of production and post-harvest improvement (AQUINO; BLANK; SANTANA, 2015).

Total soluble solids (%) of fruits were significantly affected ($p < 0.001$) only by the storage periods (Figure 7), presenting reductions at the end of the experiment.

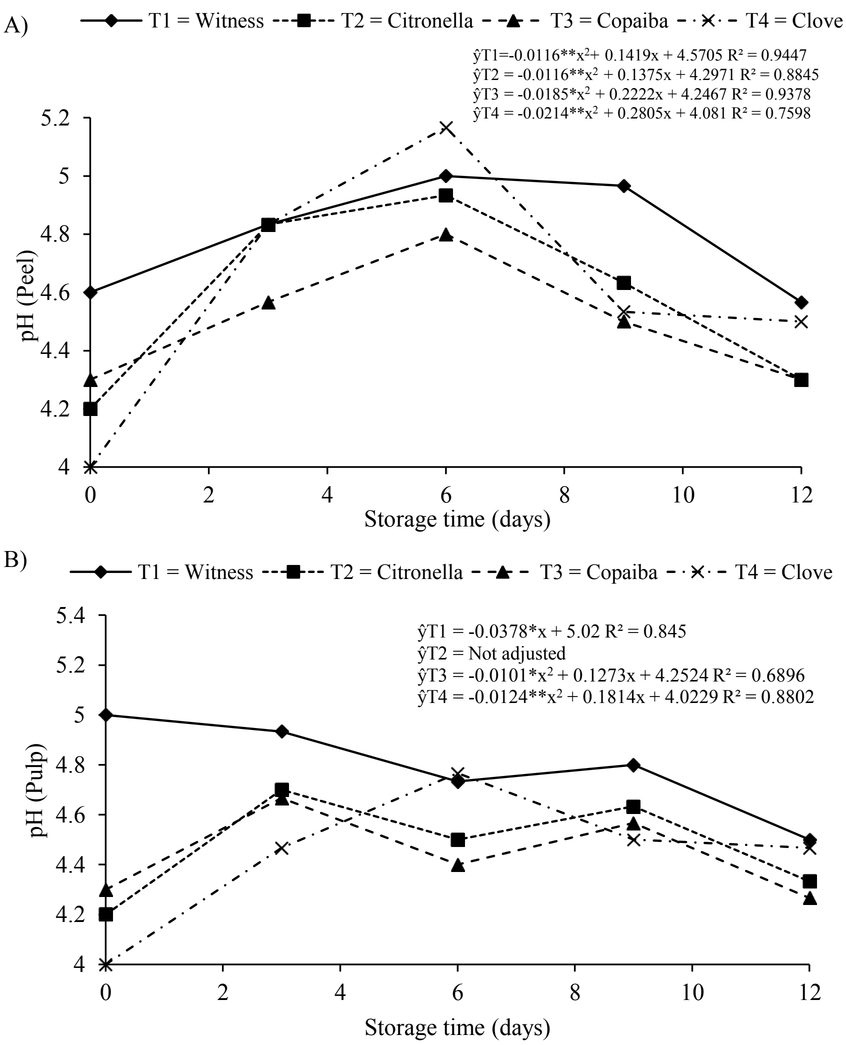

Figure 6. Skin pH (6-A) and pulp pH (6-B) of guava fruits treated with citronella, clove, and copaiba essential oils against adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae).

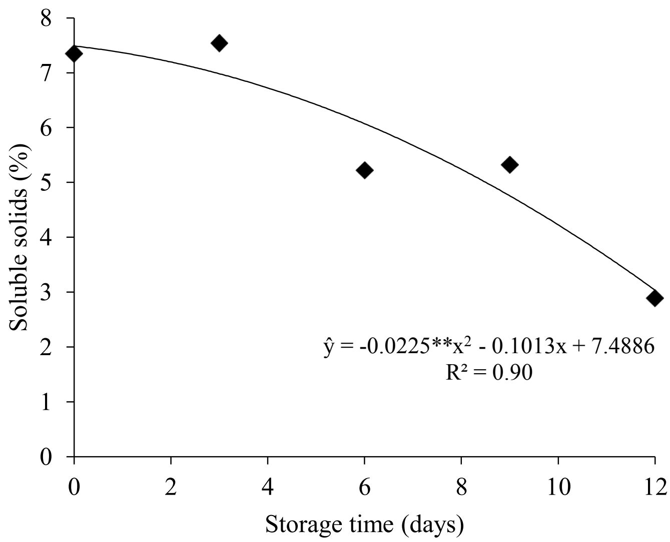

Figure 7. Total soluble solids in guava fruits treated with citronella, clove, and copaiba essential oils against adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae) as a function of storage time.

The pulp titratable acidity results found were mostly higher than the minimum value established by the Brazilian Identity and Quality Standard (PIQ) for guava pulp, which is 0.4 mg citric acid 100 g^{-1} (BRASIL, 2018). The pulp titratable acidity was affected by the storage periods (Figure 8B). The fruit titratable acidity presented a slight decrease until the sixth day, followed by an increase in the nineth and twelfth day, resulting in a similar titratable acidity to that found on the day 0 (Figure 8B). Choudhary and Jain (2018) evaluated guava fruits coated with different selenium rates and found decreases in titratable acidity as the storage period was increased.

The ascorbic acid levels in the skin were affected by the storage periods and treatments (Figure 9A). Although the control initially presented the lowest values, at the end of the twelfth day, it had higher levels of ascorbic acid than the treatments with EO.

Ascorbic acid contents, in general, decreased during the experimental period. Ascorbic acid in the skin decreased as the storage time was increased due to the instability of this compound, mainly under limiting storage conditions in terms of light, temperature, humidity, and diseases (FATEMI et al., 2011). In the present study, it was more pronounced in the treatment with clove essential oil. The ascorbic acid contents found were, in general, higher than the minimum required by the Brazilian Identity and Quality Standard (PIQ) for guava pulp, which is 24 g 100 g^{-1} (BRASIL, 2018).

The fruit pulp ascorbic acid contents were also affected by the storage time and treatments (Figure 9B). The highest pulp ascorbic acid contents on the twelfth day were found in the treatment with citronella oil, and not in the control, as found for the fruit skin. Furthermore, the ascorbic acid levels increased between the nineth and the twelfth day in all treatments, a different trend from that observed in previous days.

The treatment with citronella essential oil on day 0 presented ascorbic acid levels of 53.50 mg 100 g^{-1}, and despite the reduction on the twelfth day, it presented 51.97 mg 100 g^{-1}. Thus, it proved to be the most efficient treatment to maintain the ascorbic acid levels, which is responsible for eliminating free radicals in the fruit, thus preventing oxidative damage and prolonging post-harvest life during storage (NAIR; SAXENA; KAUR, 2018).

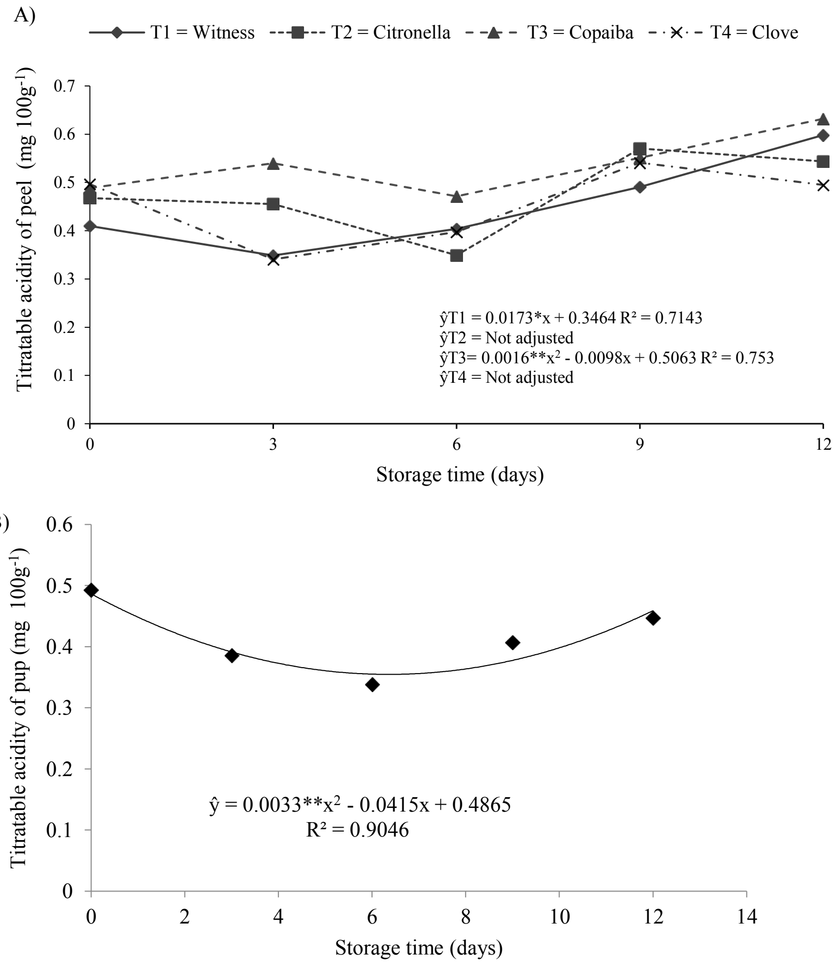

Figure 8. (A) Titratable acidity of skin (B) and pulp of guava fruits treated with citronella, clove, and copaiba essential oils against adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae), as a function of storage time.

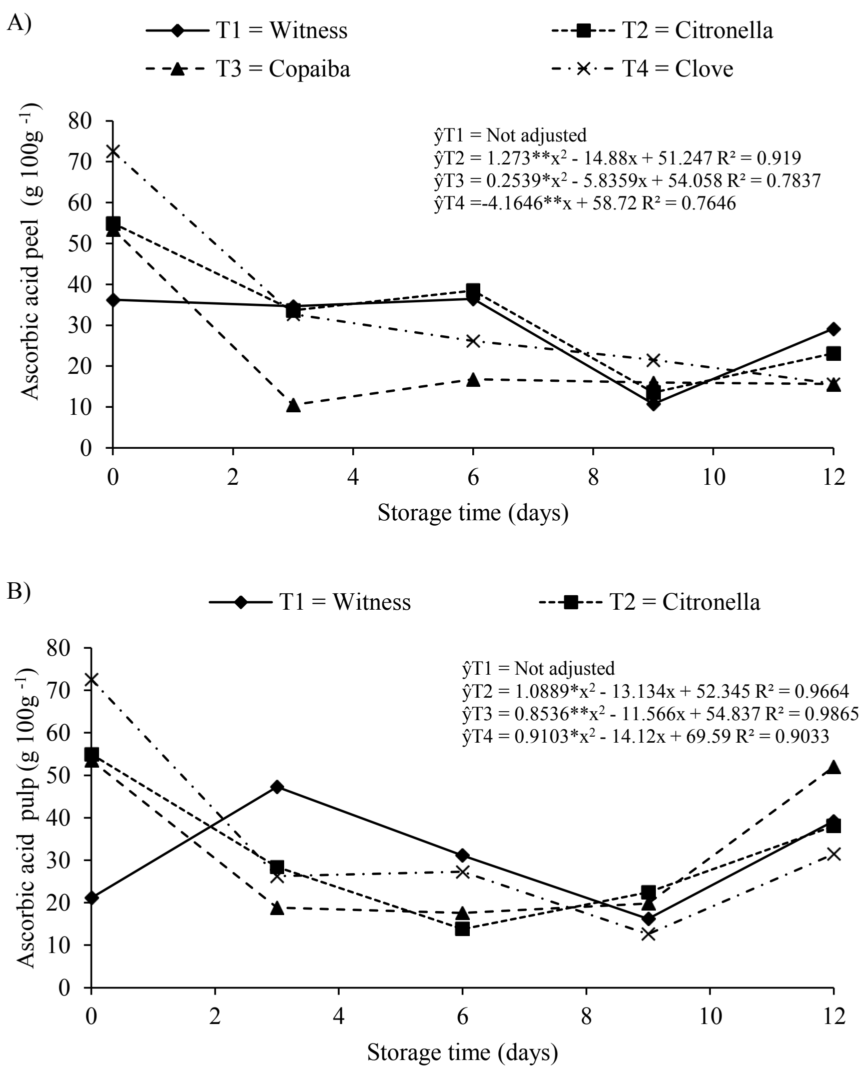

Figure 9. Ascorbic acid contents in the skin (A) and pulp (B) of guava fruits treated with citronella, clove, and copaiba essential oils against adult Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae).

CONCLUSIONS

Citronella (*Cymbopogon nardus*) essential oil presents higher repellent activity against *Ceratitis capitata* females than clove (*Syzygium aromaticum*) or copaiba (*Copaifera officinalis*) essential oils, when applied on Paluma guava fruits by immersion in water solutions at concentrations of 100, 200, 300, 400, or 500 mg mL^{-1}. The application of the essential oil solutions at concentration of 500 mg mL^{-1} had no negative effect on the analyzed post-harvest quality parameters and proved to be a promising strategy for maintaining the quality of Paluma guava fruits throughout 12 days of storage at 22±2 °C.

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