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# Insecticidal activity of bioproducts on *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

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**The aim of this study was to evaluate the insecticidal activity of the bioproducts proagrim, essential oil from fennel and orange oil, on the mortality of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). The study was divided into three parts: the first assay studied the insecticidal activity of the products on infested fruit; secondly, a bioassay was performed by ingestion of the bioproducts in an artificial diet, and the third assay assessed the activity of the products following topical application on insects. Insecticidal activity was evaluated by application of the products at concentrations of 0.0 (control), 10,000, 15,000, 20,000, 25,000 and 30,000 ppm. In general, proagrim and the essential oil of fennel induced higher mortality in larvae of *C. capitata* than orange oil. Useful information for the development of new tephritid control tools is provided by this study, because proagrim and essential oil from fennel are of potential commercial importance for the control of *C. capitata*, as they cause high mortality to the larvae of *C. capitata* in infested fruit.**

**Key words:** Bioproducts, fruit-flies, red mombin fruit, Tephritideos.

## INTRODUCTION

The red mombim fruit (*Spondias purpurea* L.) is one of the most cultivated species of the genus, due to its exotic flavor and growing market acceptance. However, there is no record of a management system for the control of insect pests that damage the crop, including fruit flies. The medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) attacks over 400 species of fruit, including red mombin (Araujo et al., 2000), and is one of the main pests affecting fruit in the world, not only by direct damage to production, but also by quarantine requirements imposed by countries that import fresh fruit. Traditionally, the control of tephritids is performed by applying toxic bait with a hydrolyzed protein associated with an insecticide. Although control is effective, this

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approach leads to problems of environmental imbalance and food safety (pesticide residues in fruits) (Silva et al., 2011).

Several papers (Benelli et al., 2013; Hidayat et al., 2013; Silva et al., 2012, 2013a; Oliveira et al., 2012, 2014) have discussed the applicability of natural products such as essential oils for pest control. The insecticidal activity of essential oils may occur in several ways, causing mortality or deformation at different stages of development (Isman, 2006). Some plants produce secondary metabolites, natural or semi-synthetic precursors in the development of a chemical product, which can be used to develop new pesticides. Several substances, including phenylpronanoids and anethole in the essential oils of fennel and orange, have been shown to have insecticidal activity (Morais, 2009).

In Brazil, the search for alternatives for the management of Tephritidae is not new in the scientific environment, in view of factors that go beyond environmental contamination and health problems related to humans. Both the demand for fruit, especially without waste chemicals, and the presence of medfly has increased in developed and developing countries (Silva et al., 2011, 2013b). In order to provide information about the potential of alternative methodologies to control fruit flies, this study evaluated the insecticidal activity of the bioproducts proagrim (based on neem oil) and the essential oils from oranges and fennel.

## MATERIALS AND METHODS

The insects used in this study were collected from an orchard of *Malpighia punicifolia* L. localized in Areia, Paraiba State, Brazil. The insects were maintained in the laboratory at 25 ± 1°C, with a 12 h photophase and relative humidity of 80 ± 10%. The colonies of *C. capitata* were maintained according to the method described by Lima et al. (2008). The essential oil from orange (Prev-Am®, sodium tetraborohydrate decahydrate) and proagrim can be freely purchased on the Brazilian market. Proagrim is a mineral compound enriched with 1% neem (Azadiratchtin from *Azadirachta indica* A. Jus). The essential oil from fennel (*Foeniculum vulgare* Mill.) was obtained from seeds and fruits by hydrodistillation for 4 h with a clevenger-type apparatus. Three assays were performed with the aim of studying the insecticidal activity in red mombin fruit and to clarify the possible modes of action (ingestion or topical).

### Bioassays

#### *Insecticidal activity of the bioproducts on C. capitata in fruit*

In this assay, the susceptibility of different larval instars was evaluated. Fully ripe fruits (fully yellow) were harvested, cleaned with water and 0.1% hypochlorite, and then dried at ambient temperature. For artificial infestation, 300 fruits were used, which were infested by *C. capitata* for a period of 72 h in rearing cages containing 1,500 sexually mature females. After this period, the fruits infested with eggs were placed in covered plastic containers and kept at room temperature. The fruits were sprayed with solutions (around 1500 µL of suspension/fruit) of proagrim, essential oil of fennel or essential oil of orange at concentrations of 10,000, 15,000, 20,000, 25,000, and 30,000 ppm. The applications were performed separately in three stadiums. Larvae reached the stadiums of first, second and third instar after 24, 72, and 120 h after infestation, respectively.

Fruits without any bioproduct application were used as a control (Oliveira et al., 2012). The concentrations were chosen based on tests of these products on the physiological quality of red mombin fruit (data not shown). A group of fruits was randomly selected and maintained as a control. The sprayed fruits were re-packaged into plastic pots containing vermiculite, which were then capped and kept at room temperature under the photoperiod and humidity conditions already mentioned for larval development. The evaluation of insect mortality was performed daily after fruit infestation by counting the number of dead larvae present in the container or inside the fruits, and survival was evaluated by counting the number of pupae present in the containers. The experimental design was completely randomized with four replicates performed for each concentration/product; each replicate consisted of ten fruits. The results of this assay are expressed as mortality rate per fruit.

#### *Application of bioproducts by ingestion*

For this assay, insects were reared on an artificial diet that contained proagrim or one of the essential oils. Larvae at different developmental stages were separated into groups of 10 per dish. Each Petri dish contained 10 g of the artificial diet of carrot, yeast, and nipagin, to which was added a suspension of 1 mL of each product (proagrim, essential oil of fennel or essential oil of orange) at a concentration of 0.0 (control), 10,000, 15,000, 20,000, 25,000, or 30,000 ppm.

The products were applied to the plates with the aid of a pipette. The mortality rate of individuals was assessed daily for a period of seven days, starting from the preparation of the plates containing the insect diet products. The experimental design was completely randomized, with three replicates consisting of 10 insects for each concentration/product. Larvae were placed in Petri dishes and subjected to the same conditions of temperature, photoperiod and humidity as for the other assays.

#### *Topical application of bioproducts*

Eggs and larvae of the first, second and third instars, and pupae of *C. capitata* were used. Individuals were placed in Petri dishes and subjected to temperatures of 25 ± 1°C, 12 h of photophase, and a relative humidity of 70 ± 10% and were provided with daily moisture according to a previously published method (Oliveira et al., 2010). Groups of larvae and pupae younger than 24 h were kept in groups of 20 per dish and were treated with proagrim or the essential oils from fennel or orange at concentrations of 0.0 (control), 10,000, 15,000, 20,000, 25,000, and 30,000 ppm (Oliveira et al., 2012). An artificial diet (10 g/dish) was provided for the larvae. Topical applications were performed with a volume of 100 µL/insect for each concentration and for each product given over the entire body of the insect. For eggs, the treatment was performed by manually spraying the whole egg chorion. Excess product that accumulated at the bottom of the Petri dish was removed with blotting paper. The mortality of individuals was evaluated daily after product application. Survival was evaluated by counting the number of hatched larvae, pupae, or adults present in the containers, to the number of applications performed on eggs, larvae, and pupae, respectively. The experimental design was completely randomized with five replicates for each concentration/product. Each replicate consisted of 20 individuals.

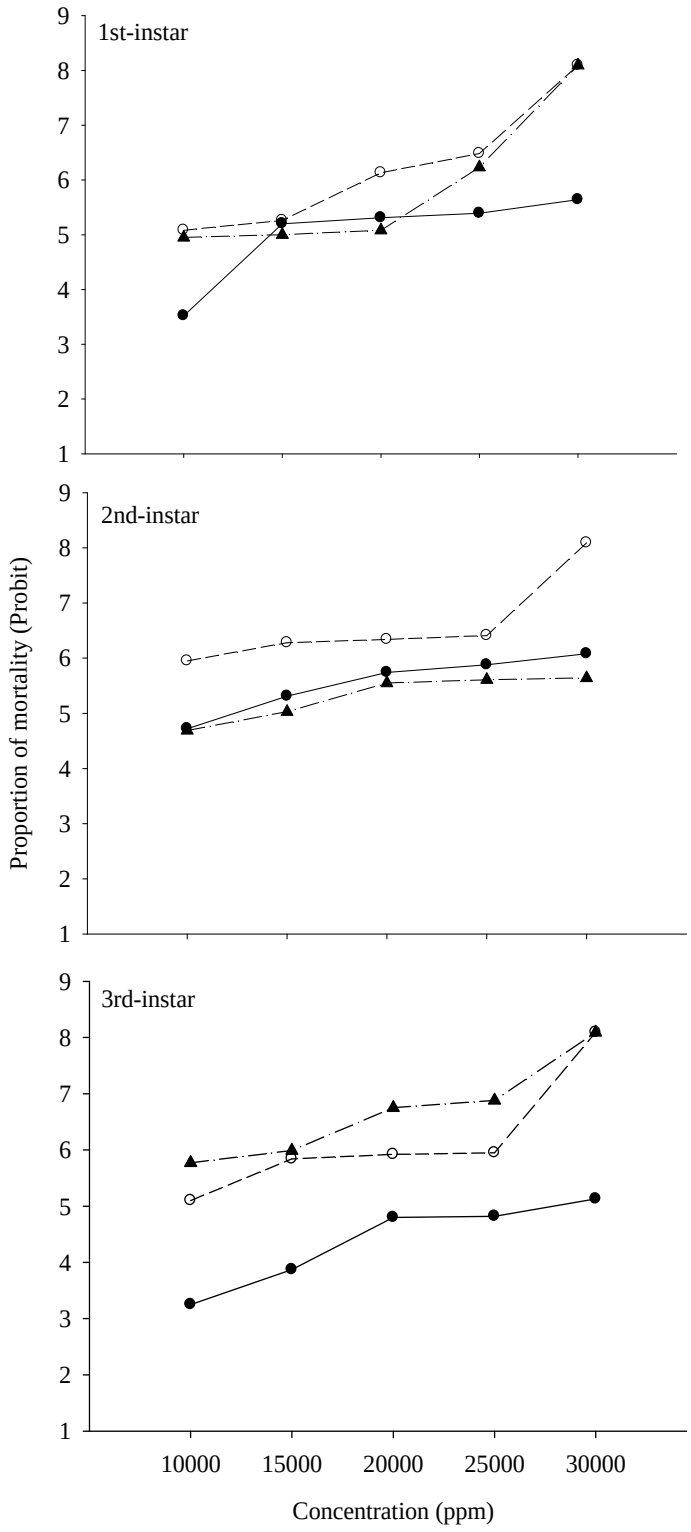


**Figure 1.** Proportion of mortality (Probit) of larvae of different stages of *Ceratitis capitata* as a function of the concentration of essential oil of orange (—●—), essential oil of fennel (– –○– –) or proagrim (—·▲·—). The bioassay was conducted in artificially-infested fruits of *Spondias purpurea*.

***Data analysis***

The mortality data (%) of all assays were adjusted based on the mortality of the control group (Abbott, 1925) and were analyzed separately. In first assay, the results are presented as percentage mortality and were converted using the Probit scale (Finney, 1971) as was performed by Brito et al. (2009). In the first assay, the original mortality data were analyzed using the Kruskal-Wallis test (α=0.05) (Sas, 2003). The mortalities data of the second and third assays were tested for normality (Kolmogorov normality test) (Massey, 1951) as well as homogeneity of variance (Bartlett's test) (Bartlett, 1937). The averages were compared using the Student-Newman-Keul's test (P= 0.05) (Begun and Gabriel, 1981; Sas, 2003). The means mortalities were subjected to a mixed-model analysis of variance (three-way ANOVA) (Proc Mixed in Sas) to allow for random effects in the model. Mortality data were transformed by the arcsine of the square root to ensure homogeneity of variance and normality of the residuals. In the second assay, the 50% lethal concentrations ($LC_{50}$) were determined for each product and for each stadium/stage of development, and these data were subjected to Probit analysis (Finney, 1971) using the Probit procedure (Sas, 2003). A binomial model was used with a complementary log-log link function.

## RESULTS

### Insecticidal activity of the bioproducts on *C. capitata* in fruit

There was no difference among instars or among products at the second and third instars. However, the maximum mortality rates were of 93.00, 65.00 and 83.00% at the first instar with 30,000 ppm of fennel oil, orange oil and proagrim, respectively (Figure 1 and Table 1). There was difference among products only at a concentration of 30,000 ppm for the first instar larvae. At this concentration, the fennel oil and proagrim were more efficient than orange oil (Figure 1 and Table 1).

### Application of bioproducts via ingestion

In this bioassay, there was no difference among the products ($F$= 1.90; $df$= 2; $P$= 0.1559). However, there was a difference among instars on the mortality of fruit flies ($F$= 4.64; $df$= 2; $P$= 0.0121). There were no interactions between product (P), concentration (C), and instar (I) ($F_{P\ by\ C\ by\ I\ (16,36)}$= 0.55; $P$= 0.9100).

Low levels of mortality were found in this assay. Nevertheless, comparing the susceptibility among instars of *C. capitata* larvae, applying fennel oil induced the highest mortality rates of third instar larvae to concentrations of 20,000 (10%; $P$= 0.012) and 30,000 ppm (16.65%; $P$= 0.001). Second instar larvae showed the greatest susceptibility to orange oil (20%; $P$= 0.013) to 30,000 ppm of this oil. The greatest susceptibility of the larvae of fruit flies to proagrim was at the second instar, with mortality rates of 10% (10,000 ppm; $P$<0.001), 23.31%

**Table 1.** Proportion of mortality (Mean ± SE) of *Ceratitis capitata* larvae as a function of the concentration of different products in bioassay with artificial infestation in red mombin fruits.

| Concentration (ppm) | Product | | | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| | Fennel oil | Orange oil | Proagrim | | |
| 1st Instar | | | | | |
| Control | 1.23 ± 1.11 | 2.22 ± 0.98 | 0.98 ± 0.45 | 0.2332 | 0.5432 |
| 10,000 | 50.00 ± 28.00 | 60.69 ± 3.88 | 37.50 ± 23.93 | 0.5789 | 0.7487 |
| 15,000 | 60.41 ± 21.34 | 43.75 ± 25.74 | 39.26 ± 21.99 | 0.2740 | 0.8720 |
| 20,000 | 65.00 ± 23.62 | 53.12 ± 27.18 | 48.01 ± 20.73 | 0.3779 | 0.8728 |
| 25,000 | 74.10 ± 17.70 | 62.14 ± 21.92 | 75.00 ± 25.00 | 2.1606 | 0.3395 |
| 30,000 | 92.85 ± 7.14 | 65.00 ± 21.79 | 83.33 ± 9.62* | 6.000 | 0.0498 |
| 2nd Instar | | | | | |
| Control | 0.96 ± 0.21 | 1.13 ± 0.38 | 1.65 ± 1.25 | 0.0980 | 0.9696 |
| 10000 | 51.11 ± 13.22 | 60.28 ± 1.38 | 47.55 ± 14.63 | 0.5990 | 0.9487 |
| 15000 | 60.41 ± 21.34 | 43.75 ± 25.74 | 41.21 ± 17.59 | 0.2740 | 0.8888 |
| 20000 | 62.12 ± 23.62 | 63.12 ± 22.18 | 58.01 ± 20.73 | 0.3779 | 0.7723 |
| 25000 | 72.140 ± 10.70 | 52.16 ± 10.29 | 75.00 ± 25.00 | 2.1606 | 0.4444 |
| 30000 | 75.23 ± 18.70 | 75.00 ± 7.70 | 64.12 ± 16.20 | 2.2600 | 0.0789 |
| 3rd Instar | | | | | |
| Control | 1.29 ± 1.14 | 1.26 ± 1.05 | 1.23 ± 0.85 | 1.2789 | 0.0987 |
| 10000 | 64.13 ± 22.22 | 69.22 ± 1.38 | 64.32 ± 12.33 | 1.8776 | 0.1076 |
| 15000 | 58.45 ± 11.45 | 56.34 ± 14.53 | 58.23 ± 12.45 | 2.0080 | 0.2345 |
| 20000 | 63.11 ± 22.52 | 71.14 ± 23.23 | 65.09 ± 14.65 | 1.4359 | 0.1456 |
| 25000 | 68.15 ± 17.20 | 71.14 ± 11.79 | 65.59 ± 23.42 | 0.1789 | 0.1234 |
| 30000 | 69.22 ± 16.40 | 69.05 ± 12.34 | 65.14 ± 12.30 | 0.1450 | 0.0898 |

SE= standard error. *Means are significantly different (within row) by non-parametric statistical Kruskal-Wallis test *(α=0.05)*.

(15,000 ppm; *P*<0.001), 10% (20,000 ppm; *P*<0.001) and 16.66% (30,000 ppm; *P*< 0.001) (Table 2).

## Topical application of bioproducts

Chi-square ($\chi^2$) values were calculated to estimate the insecticidal activity of proagrim on the eggs and larvae of *C. capitata*, and the essential oil from fennel on larvae. Values were within the preset limits and, therefore, these data conformed to the Probit model. The median lethal concentration ($LC_{50}$) of proagrim for eggs was 17,000 ppm and was higher than that for the first, second and third instars (3,400, 2,300, and 1,800 ppm, respectively). It was not possible to estimate the $LC_{50}$ for proagrim treatment of pupae, due to the weak dose-response (Table 3).

There were no significant differences among the $LC_{50}$ values estimated for essential oil of fennel for larvae of the first, second, or third instars. It was not possible to estimate the $LC_{50}$ for the egg and pupal stages of *C. capitata*, due to the weak dose-response. There was no significant difference among the $LC_{50}$ values estimated for proagrim and fennel oil under the studied conditions because there was an overlap in the confidence intervals (95% CI). However, for the first instar, there was no confidence interval overlap for the $LC_{50}$ of this product; therefore, the estimated $LC_{50}$ for proagrim treatment for first instar larvae of *C. capitata* was higher than that estimated for fennel oil treatment (Table 3).

No significant interactions between product (P), concentration (C) and stage (S) were observed ($F_{\text{P by C by S (32, 74)}} = 0.23$; $P = 1.00$). However, there was an interaction between product and stage ($F_{\text{P by S (8, 74)}} = 0.23$; $P = 1.00$). In fact, the effect of the product varied according to fruit fly developmental stage. The results show that the mortality with proagrim treatment ranged from 35 to 59, 53 to 64, 60 to 72 and 67 to 74%, for the 10,000 to 30,000 ppm treatments for eggs, first, second and third instars, respectively (Figure 2 and Table 4).

The maximum mortality caused by the application of fennel essential oil on eggs, the first, second and third instars and pupae were 27, 77, 78, 68 and 1.80% using 30,000 ppm oil, respectively. The oil from orange resulted in lower mortality at the stadiums of larvae ($L_1$, $L_2$ and $L_3$) in relation to the other tested products; but, at the egg

**Table 2.** Mortality (Mean ± SE) of different instars of *Ceratitis capitata* larvae as a function of the concentration of different products in bioassay following application by ingestion assay.

| Concentration | Fennel Oil | | | | Orange Oil | | | | Proagrim | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (ppm) | 1st | 2nd | 3rd | P>0 | 1st | 2nd | 3rd | P>0 | 1st | 2nd | 3rd | P>0 |
| Control | 0.00 ± 0.00$^{a}$ | 0.00 ± 0.00$^{a}$ | 0.10 ± 0.10$^{a}$ | *0.187* | 1.21 ± 0.34$^{a}$ | 2.08 ± 0.67$^{a}$ | 1.12 ± 0.78$^{a}$ | *0.266* | 0.00 ± 0.00$^{a}$ | 0.10 ± 0.09$^{a}$ | 0.76 ± 0.23$^{a}$ | 0.808 |
| 10000 | 0.00 ± 0.00$^{b}$ | 3.21 ± 3.20$^{b}$ | 10.00 ± 1.10$^{a}$ | *0.049* | 0.00 ± 0.00$^{a}$ | 6.70 ± 3.23$^{a}$ | 3.33 ± 0.33$^{a}$ | *0.578* | 0.00 ± 0.00$^{b}$ | 10.00 ± 0.00$^{a}$ | 0.00 ± 0.00$^{b}$ | <0.001 |
| 15000 | 0.00 ± 0.00$^{a}$ | 0.00 ± 0.00$^{a}$ | 6.67 ± 0.64$^{a}$ | *0.421* | 0.00 ± 0.00$^{a}$ | 6.66 ± 3.20$^{a}$ | 0.00 ± 0.00$^{a}$ | *0.421* | 10.00 ± 0.00$^{b}$ | 23.31 ± 9.98$^{a}$ | 3.31 ± 3.20$^{c}$ | <0.001 |
| 20000 | 0.00 ± 0.00$^{b}$ | 0.00 ± 0.00$^{b}$ | 16.65 ± 3.23$^{a}$ | *0.001* | 3.53 ± 3.33$^{a}$ | 6.79 ± 3.14$^{a}$ | 10.00 ± 0.00$^{a}$ | *0.578* | 3.33 ± 3.30$^{b}$ | 10.00 ± 0.00$^{a}$ | 0.00 ± 0.00$^{b}$ | <0.001 |
| 25000 | 3.33 ± 0.31$^{a}$ | 6.65 ± 0.68$^{a}$ | 6.64 ± 3.33$^{a}$ | *0.729* | 0.00 ± 0.00$^{a}$ | 0.00 ± 0.00$^{a}$ | 6.67 ± 0.66$^{a}$ | *0.430* | 10.00 ± 0.00$^{a}$ | 10.00 ± 0.00$^{a}$ | 13.33 ± 8.81$^{a}$ | 0.961 |
| 30000 | 0.00 ± 0.00$^{b}$ | 0.00 ± 0.00$^{b}$ | 10.00 ± 5.76$^{a}$ | *0.012* | 0.00 ± 0.00$^{b}$ | 20.00 ± 5.27$^{a}$ | 3.23 ± 0.34$^{b}$ | *0.013* | 0.00 ± 0.00$^{c}$ | 16.66 ± 8.81$^{a}$ | 6.87 ± 0.65$^{b}$ | <0.001 |

[1]Student-Newman-Keuls test: Means followed by the same lowercase letters (within the rows and the same product) are not significantly different (*P*= 0.05). SE= standard error.

**Table 3.** Median lethal concentrations ($LC_{50}$) for eggs and larvae of first, second, and third instars of *Ceratitis capitata* following topical application of proagrim and fennel oil.

| Product/Stage | Slope ± SE[1] | $LC_{50}$ (95% CI)[2] | $\chi^2$ | P > 0 |
|---|---|---|---|---|
| **Proagrim** | | | | |
| Egg | 360 ± 56 | 17000 (13700 - 24000)$^{a}$ | 6.23 | 0.013 |
| First instar | 560 ± 27 | 3400 (1900 - 3900)$^{b}$ | 4.10 | 0.040 |
| Second instar | 490 ± 24 | 2300 (2000 - 5500)$^{b}$ | 8.12 | 0.044 |
| Third instar | 180 ± 12 | 1800 (1200 - 2600)$^{b}$ | 9.28 | 0.002 |
| Pupae | | nc[3] | | |
| **Fennel oil** | | | | |
| Egg | | nc[3] | | |
| First instar | 500 ± 67 | 5400 (4299 - 10500)$^{b}$ | 5.24 | 0.0221 |
| Second instar | 440 ± 47 | 5200 (1063 - 8500)$^{b}$ | 12.96 | 0.0003 |
| Third instar | 470 ± 83 | 5500 (1777 - 5700)$^{b}$ | 4.90 | 0.0450 |
| Pupae | | nc[3] | | |

Each $\chi^2$ value refers the probability of the angular coefficient > 0; $LC_{50}$ = median lethal concentration; values and slopes are presented in % (w/v); values followed by the same letter are not significantly different; [1]SE= Standard Error. [2]CI= confidence interval. [3]nc = not calculated due to the low dose-response.

and pupal stages, there were no differences in mortality rates found after the application of this product and oil from fennel. At the egg stage, the highest mortality rates were found after topical application of proagrim. The highest mortality rates (28.00 and 20.00%) were found after the application of 25,000 and 30,000 ppm of proagrim, respectively, at the pupal stage of fruit flies, but no significant differences were found among the products (*P* > 0.05) (Table 4). Regardless of the fennel oil concentration, larvae of the first, second and third instars were more susceptible than eggs and pupae, because the highest mortality rates were observed at these stadiums. Moreover, at all the concentrations of

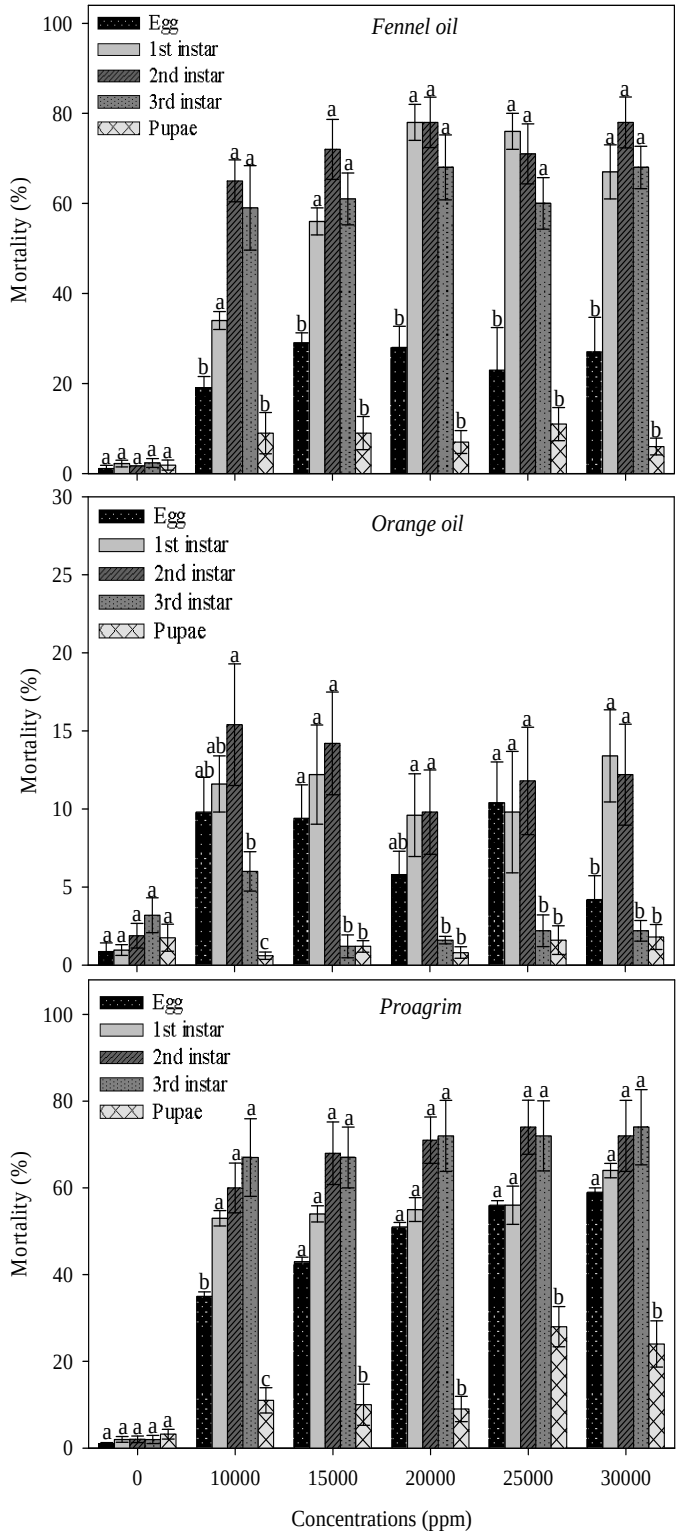


**Figure 2.** Mortality (%) of larvae at different stages of *Ceratitis capitata* after application of concentration of essential oil of orange, essential oil from fennel or proagrim. The bioassay was conducted by topical application. The vertical bars represent the standard deviation. Means followed by a common letter do not differ by SNK's test at 5% probability.

proagrim, the lowest mortality was found for pupae (Figure 2).

## DISCUSSION

The results of this study show that the insecticidal activity of the tested products is not dependent on ingestion, as low mortality was observed when the products were applied in the diet, regardless of the larval stage evaluated. Moreover, the highest mortality of the insect was caused by proagrim or the essential oil of fennel by topical application. Mortality in the topical bioassay was influenced by the insecticide tested or the developmental stage of *C. capitata*.

Proagrim and fennel oil caused high mortality in *C. capitata* in the topical application bioassay. However, mortality was also dependent on the developmental stage of the insect. Both eggs and pupae of *C. capitata* showed lower susceptibility to fennel oil, probably because of the protection that covers the eggs and pupae, which hinders the penetration of the product. Similar results to those obtained in the present study were also found by Pinto Junior et al. (2010), who demonstrated greater susceptibility of the larvae of *Alphitobius diaperinus* Panzer (1797) (Coleoptera: Tenebrionidae) to oil of *Ocotea odorifera* (Vellozo). Evaluation of the toxic effects of neem seed cake showed that this substrate acts by contact with the larvae-pupae of *C. capitata* and leads to high mortality at concentrations higher than 50%, and also prolongs the pupal period (Silva et al., 2011). The insecticidal activity of some natural products is associated with higher concentrations, as shown for anethole (Moraes et al., 2006; Figueiredo et al., 2010). It is possible that the insecticidal action of the oil of fennel is due to this substance, since the major compound in both leaves and fruits of fennel (*F. vulgare*) is trans-anethole (Mimica-Doki et al., 2003; Sousa et al., 2005). Proagrim is a mineral compound enriched with neem (Azadirachtin). Azadirachtin acts by blocking the synthesis and release of moulting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdysis in immature insects. In adult female insects, a similar mechanism of action leads to sterility.

The tolerance of the eggs and pupae of *C. capitata* to the fennel oil and the pupae to proagrim might be related to the greater protection afforded to eggs by the chorion and to the rigidity of the pupae integument provided by the puparium (Oliveira et al., 2010). In eggs, the high $LC_{50}$ found for proagrim was probably associated with the poor penetration of the product through the chorion film that covers the entire egg surface. Penetration of the insecticides might have been facilitated via alternative routes (Oliveira et al., 2011). The insecticides in this study act by contact (essential oil of fennel) and/or as a fumigant (proagrim). Pesticides and the phenylpropanoid

**Table 4.** Mortality (Mean ± SE) of larvae of different stages of *Ceratitis capitata* as a function of the concentration of different products in bioassay following topical application.

| Concentration (ppm) | Product | | | |
|---|---|---|---|---|
| | **Fennel oil** | **Orange oil** | **Proagrim** | ***Pr > F*** |
| Control | 1.12 ±0.74[a] | 0.87 ±0.55[a] | 0.96 ±0.32[a] | *0.122* |
| 10000 | 19.68 ±5.56[b] | 9.88 ±2.23[b] | 35.09 ±17.02[a] | *0.187* |
| 15000 | 29.85 ±9.27[b] | 9.46 ±2.15[c] | 43.01 ±11.02[a] | *0.003* |
| 20000 | 28.63 ±14.71[b] | 5.87 ±1.49[b] | 51.39 ±7.31[a] | *0.049* |
| 25000 | 23.42 ±9.43[b] | 10.06 ±2.61[b] | 56.01 ±14.43[a] | *<0.001* |
| 30000 | 27.19 ±7.17[b] | 4.54 ±1.52[c] | 59.00 ±12.58[a] | *<0.001* |
| **1st instar** | | | | |
| Control | 2.23 ± 0.74[a] | 0.96 ± 0.34[a] | 1.98 ± 0.66[a] | *0.234* |
| 10000 | 69.50 ± 9.66[a] | 11.68 ± 1.80[b] | 53.45 ± 11.78[a] | *0.010* |
| 15000 | 70.41 ± 13.22[a] | 12.20 ± 3.18[b] | 54.00 ± 11.87[a] | *0.018* |
| 20000 | 59.23 ± 15.60[a] | 9.60 ± 2.65[b] | 55.42 ± 12.74[a] | *0.005* |
| 25000 | 77.16 ± 18.20[a] | 9.81 ± 3.89[c] | 56.17 ± 14.43[b] | *0.002* |
| 30000 | 77.32 ± 19.33[a] | 13.41 ± 2.95[b] | 64.06 ± 11.66[a] | *0.005* |
| **2nd instar** | | | | |
| Control | 1.68 ± 0.44[a] | 1.88 ± 0.79[a] | 2.03 ± 0.77[a] | *0.559* |
| 10000 | 65.46 ± 14.66[a] | 15.44 ± 3.89[b] | 60.21 ± 15.73[a] | *0.004* |
| 15000 | 72.28 ± 9.30[a] | 14.20 ± 3.29[b] | 68.48 ± 17.21[a] | *0.017* |
| 20000 | 78.00 ± 10.19[a] | 9.80 ± 2.70[b] | 71.00 ± 15.36[a] | *<0.001* |
| 25000 | 71.22 ± 17.89[a] | 11.80 ± 3.44[b] | 74.08 ± 16.23[a] | *0.002* |
| 30000 | 78.00 ± 19.53[a] | 12.20 ± 3.23[b] | 72.16 ± 18.20[a] | *0.0031* |
| **3rd instar** | | | | |
| Control | 2.36 ± 0.96[a] | 3.19 ± 1.12[a] | 1.98 ± 0.94[a] | *0.0987* |
| 10000 | 59.12 ± 19.39[a] | 6.10 ± 1.26[b] | 67.00 ± 18.94[a] | *0.038* |
| 15000 | 61.08 ± 15.76[a] | 1.20 ± 0.73[b] | 67.00 ± 17.00[a] | *0.003* |
| 20000 | 68.24 ± 17.21[a] | 1.64 ± 0.24[b] | 72.12 ± 18.20[a] | *0.003* |
| 25000 | 60.20 ± 15.73[a] | 2.20 ± 1.01[b] | 72.14 ± 18.06[a] | *0.002* |
| 30000 | 68.46 ± 14.71[a] | 2.20 ± 0.66[b] | 74.18 ± 18.66[a] | *0.002* |
| **Pupae** | | | | |
| Control | 1.88 ± 1.12[a] | 1.75 ± 0.87[a] | 3.22 ± 1.12[a] | *0.1909* |
| 10000 | 9.00 ± 4.58[a] | 0.63 ± 0.24[a] | 11.29 ± 2.91[a] | 0.082 |
| 15000 | 9.00 ± 3.67[a] | 1.27 ± 0.37[a] | 10.00 ± 4.74[a] | 0.189 |
| 20000 | 7.00 ± 2.54[a] | 0.80 ± 0.37[a] | 9.19 ± 2.91[a] | 0.057 |
| 25000 | 11.45 ± 3.67[a] | 1.66 ± 0.92[a] | 28.26 ± 4.63[a] | 0.055 |
| 30000 | 6.33 ± 1.87[a] | 1.88 ± 0.80[a] | 24.22 ± 5.33[a] | 0.058 |

[1]Student-Newman-Keuls test: Means followed by the same lowercase letters (within the rows and the same stage/stadium) are not significantly different (P= 0.05). SE= standard error.

anethole (found in the essential oil of fennel) (Silva et al., 2009) and triterpenoids and azadirachtin (in proagrim) all act by penetrating the insect body by the respiratory system. Proagrim also acts through the cuticle (Prates and Santos, 2000).

The insecticidal activity of orange oil in this study was low for immature stages of *C. capitata*. The insecticidal activity of the essential oil from orange (sodium tetraborohydrate decahydrate) causes high mortality in the aphid *Hyadaphis foeniculi* Passerini (1860) (Hemiptera: Aphididae), ranging from 91.1 to 97.7% at concentrations of 0.3 to 0.7% (w/v), respectively (Lopes et al., 2009).

Other studies also demonstrated a potential insecticidal effect of extracts of *Citrus* species on *C. capitata* (Siskos et al., 2009).

Recent studies have shown the potential of substances from plants in the control of fruit flies (Benelli et al., 2012, 2013; Hidayat et al., 2013). In general, the $LC_{50}$ values for proagrim or the essential oil of fennel on *C. capitata* eggs and larvae were low in this study. In another study, the estimated $LC_{50}$ for adults and immature stages of *C. capitata* treated with an aqueous extract of neem kernels was 1,368 and 9,390 ppm, respectively, and 7,522 and 13,028 ppm for adults and immature adults of *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) (Silva, 2010). The essential oils of *Rosmarinus officinalis* L. and *Salvia officinalis* L., despite being rich in hydrocarbons and ketone monoterpenes, respectively, showed low insecticidal activity, whereas the oils of *Cinnamomum zeylanicum* Blume and *Thymus* sp. exhibited significant toxic effects, leading to 90% mortality after 72 h, which could be attributed to the activity of cinnamic aldehyde (Passino, 1999).

The bioproducts in this study may be used as an integral part of integrated pest management programs, since they are of low toxicity to beneficial entomofauna (Abramson et al., 2006; Silva et al., 2009). The insecticides proagrim and essential oil from fennel can be used to control *C. capitata*, since they promoted high insect mortality at low concentrations. This study shows that proagrim and essential oil of fennel are of potential commercial importance for the control of *C. capitata*, because they cause high mortality in the larvae of *C. capitata* in infested fruit, probably by penetrating inside the fruits of red mombin.

## Conclusions

The insecticidal activity of the oil from fennel was more effective for larvae of *C. capitata* than for eggs and pupae. Proagrim and the essential oil of fennel induced higher mortality in larvae of *C. capitata* compared to orange oil. The $LC_{50}$ values for these products indicate that only small application volumes are required.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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