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Full Length Research Paper

Inhibition of fruit infestation by Mediterranean fruit fly using natural products

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The inhibitory effects of some bio-products on the infestation of *Ceratitis capitata* Wied were studied. Fully ripe fruits of red mombin (*Spondias purpurea* L.) were cleaned, dried and treated with the products: Proagrim, essential oils of fennel (*Foeniculum vulgare* Mill.) and orange (*Citrus sinensis* L.), at concentrations of 0.0 (control), 1.0, 1.5, 2.0, 2.5 and 3.0% (w/v). The results obtained in the no-choice test revealed that the products significantly affected the infestation by *C. capitata* on red mombin fruit. That is, the mean of the infestation (number of larvae/female) of *C. capitata* ranged from 1.02 (3%) to 1.65 (1%) in fruits that were dressed with essential oil of orange, 0.07 (3%) to 0.25 (1%) for those treated with essential oil of fennel and 0.95 (3%) to 2.1 (1%) in the ones treated with proagrim. The free-choice test results confirmed those of the no choice test. The effective concentration to inhibit 50% infestation of *C. capitata* for fennel oil was estimated at 1.05% (w/v), which was significantly lower than the corresponding concentration for oil orange (2.11%) and proagrim (2.05%). Therefore, the essential oil of fennel is more efficient as compared to other products in reducing the infestation of this insect.

Key words: Bio-products, Mediterranean fly, *Spondias purpurea*.

INTRODUCTION

The Mediterranean fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), is a severe pest of fruit worldwide and cause major threat to this agricultural sector (Robinson and Hooper, 1989; White and Elson-Harris, 1992). Red mombin fruit (*Spondias purpurea* L.) is one of the important host of *C. capitata* (Araújo et al., 2000). It belongs to the family Anacardiaceae and is the most cultivated species of the genus in Brazil. It is probably native of Mexico and Central America (Leon and Shaw, 1990). It is a perishable tropical fruit that has a lovable flavor and a growing market acceptance (Sousa et al., 2000). Due to its excellent organoleptic quality, it is much appreciated in Northeast Brazil (Martins et al., 2003; Figueiredo et al., 2006), this is reflected by the continued increase in consumption of fresh fruit or fruits processed into various products such as ice cream, jams, squashes, usually available in the market, which has provided increasing interest for its commercial cultivation (Sacramento and Souza, 2000).

Traditionally, the medfly control is done by toxic baits, based on an attractive and stimulating food and an

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insecticide (Silva et al., 2011). The development of techniques for the control of fruit flies is of fundamental importance due to the considerable economic losses in this species (Montes and Raga, 2006). According to the study of Silva et al. (2011), 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, as the demand for fruits, especially without waste chemicals and the presence of medfly by consumers, has increased in developed and developing countries.

The interest in botanical insecticides has increased as a result of environmental concerns and insect resistance to conventional chemicals (Ayvaz et al., 2010), because female fruit flies use a variety of chemical stimuli, visual and tactile to detect and accept a proper fruit to lay their eggs (Papadopoulos et al., 2006). The biological activity of essential oils can occur in different forms, causing mortality, deformations in different stages of development as well as acting as repellent and deterrent. The repellent activity is the most common mode of action of essential oils and their major components (Isman, 2006).

The use of plant extracts, homeopathic and repellents are among the alternative tactics that meet organic production. The generation of information on the potential of alternative tactics to control fruit flies has aroused the interest of many researchers. Interesting results have been obtained in studies on the effects of substance with natural plant extracts and essential oils of different mode of action (Konstantopoulou et al., 1992; Wu et al., 1992; Jamprasert et al., 1993; Mijazawa et al., 1993; Regnault-Roger et al., 1993; Bhatnagar et al., 1993; Jembere et al., 1995; Nadarajan et al., 1996). In order to provide information of this type, this research evaluated the possible infestation inhibitory action of the following products: Proagrim (the base of neem oil and powder of stones) and essential oils of orange and fennel on *C. capitata*. The products were applied on fruits of *S. purpurea* to prevent the oviposition and damage of larvae.

MATERIALS AND METHODS

The insects used in this study were collected from an orchard and maintained in the laboratory for a generation. The insects were kept at 25 ± 2°C, 12 h of 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 products: essential oil from orange (Prev-Am®, Sodium tetraborohydrate decahydrate) and proagrim are commercial and can be purchased freely in the Brazilian market. The proagrim is a mineral compound enriched with neem (*Azadirachta indica* A. Jus) 1%. The essential oil of fennel (*Foeniculum vulgare* Mill.) was produced in the laboratory of entomology of UFPB, campus III, and obtained from the seeds and fruits using the process of hydrodistillation for 4 h with a Clevenger-type apparatus.

Bioassays

On the no choice assay, the fully-ripe *S. purpurea* were cleaned with water and 0.1% hypochlorite, and dried on absorbent paper. Four replicates of 10 fruits/treatment was used in each test. The fruits were treated with proagrim product, essential oils of fennel and orange at concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% (w/v) and fruits without application of the products as a control.

Serial dilution was performed using distilled water. Fully-ripe fruits were immersed in the solution for one minute, then placed in PVC cages with 20 cm diameter and 40 cm high, covered with organza tissue. Sixty (60) adult fruit flies, males and females (1:1), in the fertile period (ten days of age) were introduced in the cages for mating and subsequent oviposition in fruits. After 3 days, the fruits were removed and placed in polystyrene trays and covered with plastic wrap. Then, 4 days post treatment, the fruits were removed from the trays and packed in plastic containers containing sand (most of the larvae were last instar), with a capacity of 500 g, making it possible to quantify the number of insects in the fruits in each treatment.

The experiments with choice were conducted using arenas with dimensions of 20 cm in diameter and 40 cm high, covered with organza tissue type. Inside the arenas are three groups of treated fruits; each group were placed corresponding to one of the following products: proagrim, essential oil of fennel and essential oil of orange at concentrations of 1.5, 2.0 and 3.0%, respectively and an untreated group, as a corresponding control. The concentrations used in this trial were selected from the previous test results of no choice assay. The selection was based on the efficiency of the products (previous experiment) and harm reduction for some fruit quality parameters such as pH, brix degree and weight (unpublished data). Each group was composed of 4 replicates each of 5 fruits in a separate arena. A group of 80 newly mated females of *C. capitata* was released in each container.

Data analysis

The percentage of inhibition of the infestation of the no-choice test was obtained from the formula adjustments of Obeng-Ofori (1995):

$$II\% = [(NC–NT)/(NC+NT)x100]$$

Where, II%is the inhibition infestation; NC is the number of insects in control and NT is the number of insects in treated group.

The preference index (PI) was calculated for the test data-choice by adapting the formula for Kogan and Goeden (1970):

$$PI = (4T)/(T+3X)$$

Where, T is the number of insects in the test treatment; X is the sum of insects in all treatments including the control. When PI is approximately equal to 1, there is no preference in relation to treatment, when PI is >1, there is preference for the test treatment, whereas when PI < 1, there is no preference for the test treatment.

The PI was calculated for the respective treatments (viz. proagrim, essential oil of fennel and essential oil of orange, as well as the control). The number of larvae of *C. capitata* per fruit of red mombin was considered after application of each product, including the test.

The test data reflected the normality (Kolmogorov D) and

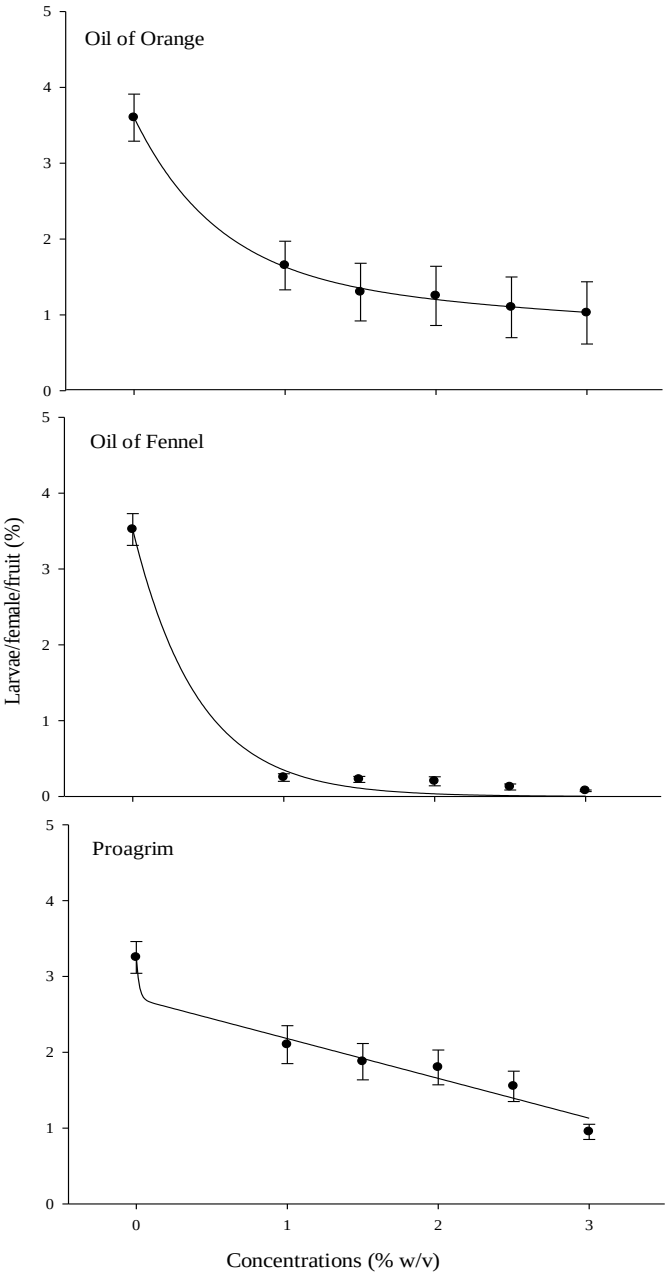

Figure 1. Effect of different concentrations of essential oil of orange, essential oil of fennel and proagrim on the average infestation (number of larvae / female) of *C. capitata* in fruits of red mombin fruit in no-choice test.

homogeneity of variances (Bartlett's test) transformed where necessary to meet the requirements of analysis of variance (ANOVA). The effective concentration values that allow the inhibition of the median infestation ($CII_{50\%}$) were calculated by PROC NLIN (Sas Institute, 2002) according to the method adopted by Camarillo et al. (2009). The results were compared by over lapping confidence interval (the 5% level of probability). The results of infestation were analyzed by polynomial regression analysis using the PROC GENMOD (Sas Institute, 2002).

RESULTS

The results obtained in no-choice test revealed that the products proagrim, essential oils of orange and fennel affected infestation significantly ($P< 0.05$) by *C. capitata* in red mombin fruit. The average infestation of *C. capitata* in red mombin fruit ranged from 1.65 (1%) to 1.025 (3%), 0.25 (1%) to 0.075 (3%) and 2.1 (1%) to 0.95 (3%), after application of the products from orange, fennel and the proagrim, respectively (Figure 1).

The effective concentration that inhibits 50% infestation of *C. capitata* was found to be 1.05, 2.11 and 2.05% (w/v) for essential oils from fennel and orange and the product proagrim, respectively. Thus, lower concentrations of essential oil from fennel can be used in preventive control of *C. capitata.* That is, the essential oil of fennel promoted infestation in a lower rate than other test products (Table 1).

In testing the free-choice, the PI was less than 1 in the treatments with applications of the products tested, while the control was 1.25, indicating no preference for oviposition when the fruits are treated with proagrim, essential oil of fennel or orange. In fruits that received applications of product from orange and the proagrim, preferably indices observed were 0.53 and 0.34, respectively (Table 2).

DISCUSSION

The results of this study revealed that the essential oil of fennel was the most efficient and should be preferred in implementing any preventive control of *C. capitata*. This action could be justified by the higher concentration of terpenes in this product over the two others, or related to differentiation of the majority of compounds in this oil. The main constituents of fennel essential oils are (E)-anethole (72.27 to 74.18%), fenchone (11.32 to 16.35%) and methyl chavicol (3.78 to 5.29%) (Mimica-Doki et al., 2003). According to the study of Sousa et al. (2005), the major compound found in both leaves and fruits of fennel (*F. vulgare*) is the trans-anethole, which is found in other plant species and has a repellent activity as in *Ocimum selloi* Benth against the mosquito *Anopheles braziliensis* (Chagas) (Diptera: Culicidae) (Paula et al., 2003).

The essential oil of fennel provided a lower preference index (0.17), thus resulting in a lower infestation of fruits (0.28 larvae/fruit), confirming previous results of the no-choice test (Table 2). The use of lower concentrations of fennel essential oil is an important factor to be considered in management programs for insect pests, given that high

Table 1. Infestation inhibition results of *C. capitata* by concentrations of bio-products in red mombin fruit.

Parameter	Product		
	Oil of Orange	**Oil of Fennel**	**Proagrim**
$CII_{50\%}$ (IC 95%)	2.11a (1.53 – 2.77)	1.05b (0.90 – 1.17)	2.05a (1.50 – 2.58)
Slope±EP	2.13±0.43	2.89±0.49	2.33±0.13
χ^2	6.84	9.13	7.03
Gl	4	4	4
P	0.0089	0.0010	0.0072

Means followed by the same letter (within line) do not differ by overlapping confidence intervals (IC 95%).

Table 2. Effects of bio-products on *C. capitata* in preference test with multiple choices.

Treatement	Larvae/ fruit[1] (no median±EP)	(PI)[z]	IC (95%)[2]
Control	1.60±0.13a	1.25a	0.98 – 1.32
Oil of orange	0.88±0.11b	0.53b	0.49 – 0.58
Proagrim	0.56±0.09c	0.34c	0.29 – 0.42
Oil of fennel	0.28±0.05d	0.17d	0.11 – 0.26

[z]Preference index; [1]Medians followed by the same letter (within column) do not differ by Tukey test (P = 0.05) or by overlapping[2] confidence intervals (95%).

concentrations of this product may adversely affect beneficial insects such as bees (Abramson et al., 2007) and natural enemies such as *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae) (Silva et al., 2009), as well as the physiological quality of fruit red mombin fruit (unpublished data).

Although, the essential oil of fennel is more efficient than proagrim and essential oil orange, these two products can be considered important substances for the control of *C. capitata*. The proagrim is a mineral compound enriched with neem. The potential of neem in controlling *C. capita* was also reported by Habibi et al. (2003), who observed toxicity on *C. capitata*. Azadirachtin has two profound effects on insects. At the physiological level, azadirachtin blocks the synthesis and release of molting 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. In addition, azadirachtin is a potent antifeedant to many insects (Isman, 2006), well as an effective insecticide, contact action expresses larval-pupal and pupal developmental delay (Silva et al., 2011). The essential oil orange can also be considered in control programs of *C. capitata*. According to Zacarin et al. (2005), terpenes present in essential oils of Valencia oranges and pears and Tahiti lemon were sufficient to promote ugliness of *Atta sexdens rubrupilosa* Forel (Hymenoptera: Formicidae). Citrus fruits essential oils have been recognised as the most critical mechanism conferring resistance to citrus species against fruit flies Papachristos et al. (2008), assessing the survival and development of immature stages of Mediterranean fruit fly (Diptera: Tephritidae) in citrus fruits, observed that the varieties of citrus have strong effects on larval stage, but with little effect on eggs and pupae. Furthermore, the area of fruit, particularly those rich in essential oils, affects the development of larvae. Other studies have confirmed the lethal effect of the extracts (Siskos et al., 2008) or essential oils (Salvatore et al., 2004; Papachristos et al., 2009) from citrus fruits as in tephritids *Bactrocera oleae (Rossi) (Diptera: Tephritidae)* and *C. capitata*, besides the deterrent effect (Levinson et al., 2003).

The substances tested in this study may be of great importance to the future pest management programs as alternative to conventional control methods. However, studies to elucidate the mechanisms responsible for reducing the infestation of larvae of *C. capitata* in terms of bio-products fennel, orange and proagrim are relevant. Some mechanisms are involved in the inhibition of infestation of fruit flies: the repellent effects (Valencia-Botín, 2004; Gomes et al., 2009), locomotor stimulation, suppressor effects, deterrent effects (Isman, 2006), ovicidal activities (Glitho et al., 1997; Tunç et al., 2000), effect on the embryogenesis (Levinson et al., 2003), larvicidal effect (Silva et al., 2011), and or in controlling the development (Silva et al., 2011; Ketoh et al., 2005) and reproduction of the insect. Further studies are

needed to understand the factors responsible for inhibition of infestation.

Conclusion

The essential oils of fennel and orange and the product proagrim promote inhibition of infestation by *C. capitata* in red mombin fruit. The essential oil of fennel is more efficient as compared to the other studied products in reducing the infestation of this insect.

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