

## THE EFFICACY OF SOME VOLATILE OIL EXTRACTS AS OLFATORY STIMULI TO THE FRUIT FLIES, *Bactrocera zonata* (Saunders) AND *Ceratitis capitata* (Wiedemann) (Diptera : Tephritidae)

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### ABSTRACT

The experiments were carried out in guava orchard at Mansoura district, Dakahlia governorate, Egypt. Eight different plant oil extracts [olive, orange, peppermint, basil, clove (Egyptian & French), parsley and black cumin] were bioassayed as attractants for the tephritids, *B. zonata* and *C. capitata* males and females. *B. zonata* males and females as well as *C. capitata* females showed no response to all the tested oils. *C. capitata* males exhibited a significantly positive response to French and Egyptian clove oils; while it had no response to olive, basil, parsley and black cumin oils. The residual effectiveness of Egyptian and French clove oils were lost after nine days of exposure.

Comparative study between prepared (local) and imported methyl eugenol was evaluated for 72 successive days. Statistical analysis indicated that there were significant differences between the local and imported methyl eugenol after 6, 12 and 18 days only. So, the local methyl eugenol traps may be used as a valuable tool in monitoring *B. zonata*.

The attractiveness of *B. zonata* males to felt and plant fibers blocks (with different thickness) saturated with methyl eugenol was evaluated. The felt blocks captured the highest number of *B. zonata* males in comparison with those of plant fibers. The efficiency of plant fiber blocks was significantly affected by block thickness; while felt blocks did not affected by thickness.

### INTRODUCTION

Fruits are considered as one of the most important crops in the whole world because of their nutritional and economical importance as well as for local consumption or exportation. *B. zonata* and *C. capitata* (Order: Diptera, Family: Tephritidae) are the most serious pests of fruits. Their females lay their eggs in the fruits and the hatching maggots devour into the pulp. Subsequently, secondary infestations with bacterial and fungal diseases mostly exist and the infested fruits drop down (White and Elson-Harris, 1994).

In Egypt, *B. zonata* became a serious pest in the last decade attacking a wide range of fruits that differ in their ripening time stage all over the year. It is a polyphagous pest attacking 40 species of fruit and vegetable crops as well as wild host plants (Syed *et al.*, 1970).

Semiochemicals are defined as "substances which transmit messages between living organisms, both plant and animals" (Law and

Regnier, 1971). The males of several economically important tephritid species are strongly attracted to particular chemical compounds, termed "male lures" or "parapheromones", that either occur naturally in plants or (presumed) synthetic analogues or plant-borne substances (Sivinski & Calkins, 1986 and Fletcher, 1987).

"Male lures" are used in the monitoring of *B. zonata* and *C. capitata* populations. According to Afia (2007), methyl eugenol could be used in male annihilation technique of *B. zonata* eradication or control programs.

So, the present work aimed to evaluate the reaction behavior of *B. zonata* and *C. capitata* (males and females) in response to some plant oil extracts. Also, comparative studies were done to compare the attractiveness of *B. zonata* males to the prepared (local) and imported methyl eugenol. Additionally, to evaluate the influence of block type on the potentiality of methyl eugenol in relation to the population of *B. zonata* males.

## **MATERIALS AND METHODS**

The experiments were carried out in an area of about two feddans cultivated with guava (*Psidium guajava*) in the experimental farm of Faculty of Agriculture, Mansoura University at Mansoura district, Dakahlia governorate, Egypt.

### **1- Evaluation the attractiveness of *B. zonata* and *C. capitata* adults to some plant oil extracts :**

To evaluate the efficacy of eight plant oil extracts [olive, orange, peppermint, basil, clove (Egyptian and French), parsley and black cumin] as attractants for males and females of *B. zonata* and *C. capitata*, bioassay was carried out in McPhail traps (McPhail *et al.*, 1963). About 100 ml of the extract were put in each trap. Each extract was replicated five times. The traps were distributed in a completely randomized design during September 2006. After three days, the traps were tested and the number of attracted males and females of each species were counted and recorded.

To estimate the residual activity of Egyptian and French clove oils in attracting *C. capitata* males, sex sticky traps (Jackson traps) were used. Every five traps (as replicates) were powered with one of the tested oil (by saturating a piece of cotton dispenser with the oil). All the prepared traps were distributed in a completely randomized design. Captured males were collected every three days throughout 21 successive days (from the 3<sup>rd</sup> till 24<sup>th</sup> of October 2006) and numbers of *C. capitata* males were counted and recorded to estimate the capture/trap/day (CTD).

### **2- Comparative efficacy of the prepared (local) and imported methyl eugenol in attracting *B. zonata* males :**

#### **2-1- Preparing the local methyl eugenol :**

Isolation and methylation of eugenol were done according to (Youssef, 1997).

**Isolation of eugenol :**

The volatile oil was extracted from clove (*Eugenia caryophyllus*) by steam distillation. This oil is the essential oil contains eugenol in 98% and caryophyllene derivatives (sesquiterpenes) non-phenolic about 2%. Eugenol was isolated by treatment of the extracted oil with an aqueous sodium hydroxid solution (3-5%). The non-phenolic constituent was extracted with chloroform. The aqueous alkaline solution was acidified with dilute hydrochloric acid to give eugenol, which was obtained by distillation.

**Methylation of eugenol by dimethyl sulphate :**

A solution of eugenol (2 gm) in acetone (8 ml) and in the presence of sodium hydroxide (20 ml, 56%) was stirred on ice bath. During the stirring, dimethyl sulphate (2 ml) was added dropwise to the reaction mixture which was stirred for 6 hours, then poured on ice cold water. Methyl eugenol ether was extracted with chloroform (3 x 20 ml), washed with water (3 x 20 ml) and dried over anhydrous sodium sulphate. The solvent (CHCl<sub>3</sub>) was evaporated under reduced pressure to give methyl eugenol ether as a pall yellow oil (1.604 gm, 80% yield).

**2-2- Field bioassay :**

Jackson traps powered with prepared (local) or imported methyl eugenol were used. Each treatment was replicated five times and distributed in a completely randomized design. Traps were tested every six days (as an interval period) and the numbers of captured males of *B. zonata* were recorded to estimate the CTD. This experiment was carried out during the period from the 3<sup>rd</sup> of October till 14<sup>th</sup> of December 2006 (72 days).

**3- Estimate the influence of block type on the potentiality of methyl eugenol as bio-control agent against *B. zonata* males :**

This experiment was conducted in the previously mentioned orchard during the period from the 13<sup>th</sup> of September till the 21<sup>st</sup> of November 2007.

**The experimental blocks :**

Two types of blocks, felt and plant fibers (25 cm<sup>2</sup>) were used as dispensers for methyl eugenol. For each block type, two thicknesses (3 & 6 mm for felt and 6 & 11 mm for plant fiber blocks) were also used for bioassay.

All of the tested blocks were dipped in a mixture of 80% methyl eugenol and 20% pure malathion for about four hours in the laboratory. After that, the blocks were transferred to the field. Each block type and thickness was replicated five times and distributed in a completely randomized design.

To collect the dead insects, a plastic bag fixed with an iron ring (40 cm in diameter) was put under the block for receiving the dead male flies.

The captured flies in the plastic bags were investigated every five days (as an interval period) and the numbers of *B. zonata* males were counted and recorded.

Statistical analysis was done as one way ANOVA and L.S.D. values were estimated at the probability of 5%. In addition, the simple regression analysis was calculated.

## RESULTS

### 1- Evaluation the attractiveness of *B. zonata* and *C. capitata* (males & females) in response to some plant oil extracts :

Eight different plant oil extracts were bioassayed as attractants for the tephritid adults, *B. zonata* and *C. capitata* (male and females). The obtained data as illustrated in Table (1) obviously indicated that females of both *B. zonata* and *C. capitata* not showed any response to any of the tested oils.

**Table (1): Capture/trap/day (CTD) of *B. zonata* (PFF) and *C. capitata* (MFF) males captured by different plant oil extracts in McPhail traps after 3 days in guava orchards at Mansoura district.**

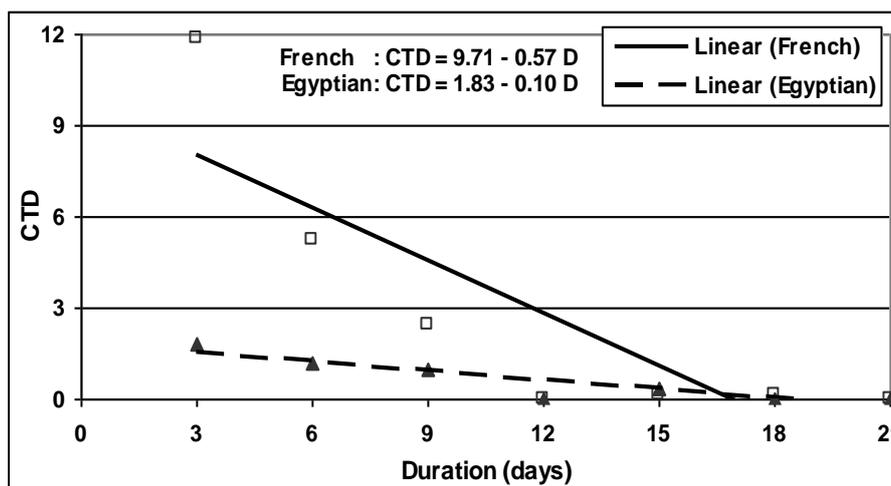
Oil extract	After 3 days			
	PFF		MFF	
	Females	Males	Females	Males
Olive	0.0	0.0	0.0	0.0
Orange	0.0	0.0	0.0	0.67
Peppermint	0.0	0.0	0.0	0.13
Basil	0.0	0.0	0.0	0.0
French clove	0.0	0.0	0.0	24.33
Egyptian clove	0.0	0.0	0.0	6.13
Parsley	0.0	0.0	0.0	0.0
Black cumin	0.0	0.0	0.0	0.0

As shown in Table (1), males of *B. zonata* showed no response to the tested plant oil extracts, while *C. capitata* males exhibited a significantly positive response to French and Egyptian clove oils (CTDs were 24.33 and 6.13). The obtained data also illustrated that *C. capitata* males had a low attractiveness to orange (CTD = 0.67) and peppermint (CTD = 0.13) oils, while *C. capitata* males showed no response to the other tested oils (olive, basil, parsley and black cumin) (Table, 1).

From the previously mentioned results, it could be concluded that clove oil can be used as a "male lure" for *C. capitata* males.

### 2- Residual activity of Egyptian and French clove oils in attracting *C. capitata* males :

Data represented in Figure (1) cleared that French and Egyptian clove oils lost their activity after nine days of exposure. The regression analysis obviously indicated that French clove oil lost its activity with an approximately higher rate of attraction than that of Egyptian one. However, the slope of regression line was -0.57 and -0.10 for French and Egyptian clove oils.



**Figure (1): Residual activity of Egyptian and French clove oils against *C. capitata* males in guava orchards at Mansoura district.**

As shown in Figure (1), at the beginning of exposure, the efficacy of French clove oil was significantly higher than the Egyptian one. The attractiveness power of them decreased gradually till the end of the experiment (21 days).

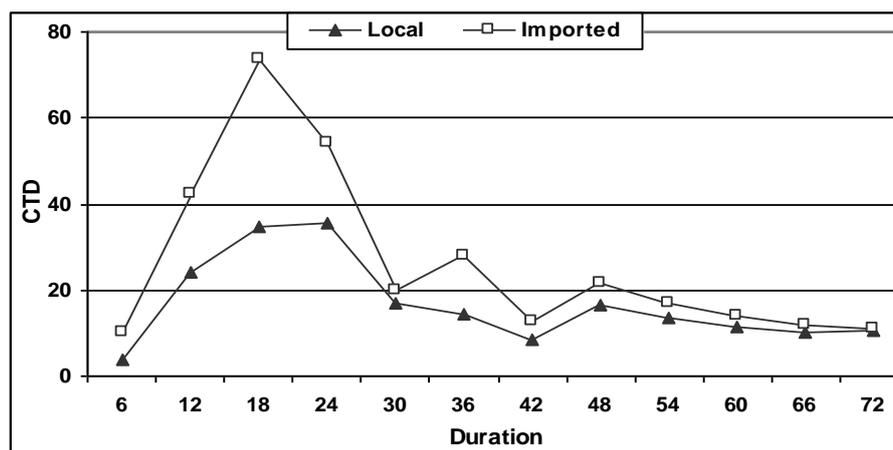
It could be concluded that, French clove oil extract could be used as male lure for *C. capitata* but with renewing it every one week.

So, these results demonstrate the potential of French eugenol oil as a cheaper alternative attractant in monitoring *C. capitata* population.

### **3- Comparative efficacy of the prepared (local) and imported methyl eugenol in attracting *B. zonata* males :**

The effectiveness of local and imported methyl eugenol was evaluated at 6 days interval by using Jackson traps under field conditions during the period from the 3<sup>rd</sup> of October till 14<sup>th</sup> of December 2006 (72 days).

The attractiveness of *B. zonata* males in response to both chemicals was illustrated in Figure (2). The CTD of local methyl eugenol after 6 days was 3.80 then increased to reach 35.50 after 24 days. After that the CTD has been decreased gradually till the end of the experiment, however the CTD reached 10.80 (after 72 days).



**Figure (2): Fluctuations in numbers of *B. zonata* males in Jackson traps baited with local and imported methyl eugenol over 72 days in guava fields at Mansoura district (without renewal the attractants).**

As shown in Figure (2), *B. zonata* males exhibited high attraction to the imported methyl eugenol than the local one. However, the CTD was 10.10 after 6 days and increased to reach 73.50 after 18 days. Then the CTD decreased gradually till reach 11.00 at the end of the experiment (after 72 days).

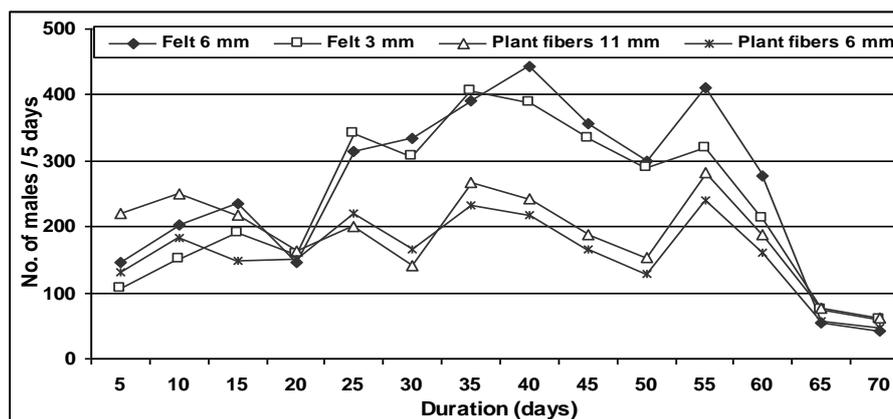
Statistical analysis indicated that there were significant differences between the local and imported methyl eugenol after 6, 12 and 18 days only.

So it could be concluded that the prepared (local) methyl eugenol may be used in monitoring *B. zonata* population.

#### **4- Influence of block type on the efficiency of methyl eugenol as bio-control agent against *B. zonata* :**

The attractiveness of *B. zonata* males to block types (felt and plant fibers) with different thickness saturated with methyl eugenol was evaluated and illustrated in Figure (3).

The number of captured males / block / five days for each block type was fluctuated over the tested period (70 days). The efficiency of the tested block types was generally higher during the second month than the first one. The highest capture of *B. zonata* males in felt (6 & 3 mm) and plant fiber blocks (11 & 6 mm) were recorded after (40 & 35) and 55 days, respectively (Figure, 3).



**Figure (3):** Fluctuation of PFF males captured by felt (6 & 3 mm) and plant fiber (11 & 6 mm thickness) blocks saturated with a mixture of 80% methyl eugenol and 20% pure malathion over 70 days in guava fields at Mansoura district (without renewal the attractant).

As shown in Figure (3), the highest capture of *B. zonata* males was recorded on felt blocks in comparison with plant fiber ones. The mean numbers for all the investigation period of captured males / block / five days were 260.9, 238.3, 189.1 and 160.6 males for felt (6 & 3 mm) and plant fibers (11 & 6 mm thickness), respectively. Statistical analysis indicated that there were significant differences between block types of felt and plant fibers (L.S.D.<sub>P=5%</sub> = 26.8).

In respect to block thickness, statistical analysis obviously indicated that the efficiency of plant fibers was significantly affected by block thickness; while, the efficiency of felt blocks did not affected by thickness.

## DISCUSSION

The present results indicated that some plant oil extracts (clove and orange) act as male lures for *C. capitata* adults. Species of Dacine basically respond more to olfactory cause than visual cause (Drew and Lioyed, 1987). The males of *Bactrocera* and *Ceratitis* may be collected in trap that has been baited with chemical attractants, which are sometimes referred to as Parapheromones (Allwood *et al.*, 1999). Some workers believe that the male lures are Kairomones, which are behavior-modifying plant chemicals.

In the present study, *C. capitata* males were attracted to clove oil extracts more than the other tested oil extracts. This may be attributed to the presence of methyl eugenol in the clove oil extracts. Also, Tan (1993 & 2000) reported that males of *B. dorsalis* (of the tephritid fruit flies) feed voraciously on methyl eugenol, a component of plant essential oil found in at least 200 species of plants from 32 families.

The methyl eugenol group consists of methyl eugenol, citronella oil and huon pine oil of citronella oil (Allwood *et al.*, 1999). Methyl eugenol attracts males of many *Bactrocera* spp. and it attracts some of subgenus

*Ceratitis*. Under recent chemical nomenclature it called methoxy eugenol and it has been known as eugenol methyl ether (Drew, 1974 & 1982).

Chemical and behavioral studies showed that methyl eugenol is converted to phenylpropanoids-DMP that functions as male sex pheromonal components (Nishida *et al.*, 1988; Tan & Nishida, 1996 & 1998 and Hee & Tan, 1998). Also, Hee & Tan (2004 & 2005) reported the presence of these pheromonal components in the hemolymph of *B. dorsalis* males fed on methyl eugenol, which suggests the hemolymph involvement in the transport of attractant-derived pheromonal components from the crop to the rectal gland organ where the compounds are released during dusk.

The present study revealed that the imported methyl eugenol had a higher efficacy in attracting *B. zonata* males than the local one. Statistically, there were significant differences between the local and imported methyl eugenol after 6, 12 and 18 days only, while there were insignificant differences between them during the rest of the tested period (72 days).

Hanafy *et al.* (1999) (in Egypt) and Gopaul & Price (1999) (in Mauritius) found locally produced food attractants as a cheaper alternative attractants for fruit flies. Also, in the present study, the local methyl eugenol may be as a cheaper alternative attractant by using some chemical operations for using in *B. zonata* population monitoring.

Methyl eugenol was successfully used in combination with several different insecticides (such as malathion or dichlorvos or naled with as poison baits) for detecting and surveying the male flies of *B. zonata* in orchards (Mitchell *et al.*, 1985; Grewal & Kapoor, 1987 and Gupta *et al.*, 1990). So, methyl eugenol can either be easily used as a standard attractant to control fruit flies.

The present study revealed that felt blocks were more suitable than the plant fiber blocks for male lures. Also, Afia (2007) found that felt dispenser was one of the highest tested dispensers for male lure (with methyl eugenol) of *B. zonata*. This may be attributed to the higher initial of release rate of male lure in plant fiber blocks than felt ones; which losses the attractiveness of plant fiber rapidly. This explanation come in the same line with that obtained by Afia (2007).

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### كفاءة بعض مستخلصات الزيوت النباتية كمنبهات شمعية لذبابة ثمار الخوخ وذبابة فاكهة البحر المتوسط

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أجريت هذه الدراسة ببستان الجوافة بالمزرعة البحثية لكلية الزراعة – جامعة المنصورة – محافظة الدقهلية . حيث تم تقييم ثمانية أنواع من الزيوت النباتية كجاذبات شمعية لكل من ذبابة ثمار الخوخ وذبابة فاكهة البحر المتوسط (وهي زيوت الزيتون ، قشر البرتقال ، النعناع ، الريحان ، القرنفل الفرنسي، القرنفل المصري ، اليقونس وحب البركة) في جذب ذكور ذبابة ثمار الخوخ وذبابة فاكهة البحر المتوسط. وقد أوضحت النتائج أن إناث كلا النوعين لم تستجيب لهذه الزيوت ، كما لم تستجيب ذكور ذبابة ثمار الخوخ لها . أما ذكور ذبابة فاكهة البحر المتوسط فقد أبدت استجابة إيجابية بدرجة معنوية تجاه كل من زيت القرنفل الفرنسي والمصري ، وأبدت استجابة منخفضة تجاه زيت قشر البرتقال ، بينما لم تبدى أى استجابة تجاه باقى الزيوت التى خضعت للدراسة . من ناحية أخرى وجد أن كل من زيت القرنفل الفرنسي والمصري فقا كفاءتهما كجاذبات بعد تسعة أيام من بداية التعليق .

وبمقارنة كفاءة الميثيل ايوجينول المصنع محلياً والمستورد فى جذب ذكور ذبابة ثمار الخوخ وجد أن الميثيل ايوجينول المستورد أعلى كفاءة من المحلى وذلك بعد ٦ ، ١٢ و ١٨ يوم فقط من بداية التعليق ؛ بينما لم يكن هناك اختلاف معنوى بينهما فى باقى فترة الدراسة (٧٢ يوم) ، ولذلك يمكن استخدام الميثيل ايوجينول المصنع محلياً بدلاً من المستورد فى تتبع تعداد ذبابة ثمار الخوخ .

كما تم دراسة كفاءة نوعين من المكعبات وهما اللباد والألياف النباتية (سمكين مختلفين لكل منها) ومشبعة بالميثيل ايوجينول تجاه ذكور ذبابة ثمار الخوخ ؛ وقد أوضحت النتائج أن المكعبات المصنعة من مادة اللباد أعلى كفاءة من تلك المصنعة من الألياف النباتية ، كما أن كفاءة المكعبات المصنعة من الألياف النباتية تأثرت بسمك المكعب بينما لم تتأثر كفاءة المكعبات المصنعة من مادة اللباد بسمك المكعب .