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CLOVE OIL AS A SOURCE OF ATTRACTANT PHEROMONES TO THE FRUIT FLIES , *Ceratits capitata* (WIEDEMANN) AND *Bactrocera zonata* (SAUNDERS) (DIPETRA :TEPHRITIDAE)

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata*(wiedemann) and the peach fruit fly, *Bactrocera zonata* (saunders) are two of the worldwide most important pests of economic importance. The clove oil (*Eugenia caryophyllata*, family Myrtaceae) was studied as a source of attractant pheromones to both the fruit flies. The clove oil was characterized by IR spectroscopy; its constituents were identified by GC/MS analysis.

Essential oil yield found to be 30.30% W/W, Major important components were found to be eugenol acetate,eugenol,iso-eugenol, *β*-Caryophyllene oxide and methyl eugenol constituting 62.50 out of 79.76, i.e. 78.63%.Clove oil at concentration of 50%, gave the highest attraction of *C. capitata* males, compared with trimedlure (as a reference attractant). The prepared (local) methyl eugenol (50,100% concentration) has approximately the same efficacy in attracting PFF males as well as imported methyl eugenol.

INTRODUCTION

The Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann) and the peach fruit fly (PFF), *Bactrocera zonata* are two of the worldwide most important pests of economic importance. These insects are known to attack 250 fruit and vegetable crops (Liquido *et al*., 1991).

The threat of Mediterranean and peach fruit flies have always been a high priority for states or countries engaged in international trade. Due to the fly's quarantine importance, an ongoing search for new and improved semi-chemical-based control and detection methods remain a high priority. The males of several economically important tephritid species are strongly attracted to particular chemical compoundes, termed" male lures" or "parapheromones" that either occur naturally in plants or (presumed) synthetic analogues or plant-born substances (Sivinski&Calkins,1986 and Fletcher,1987). The synthetic trimedlure has been used for over 25 years as the best practical attractant for survey and detection traps for MFF (Jang et al., 2005). While the PFF males show a very strong affinity to methyl eugenol which acts as very potent attractant (Keng-Hong *et al*., 2011).

Recently, researchers have searched for new and less toxic control methods. Plant extracts have been taken in consideration due to their low toxicity and low environmental impact (Verde *et al*., 2011).The whole essential oil which its main component eugenol are used in formulating insects attractants (Lee and Shibamoto, 2001)

The present study aims to evaluate the potential of the clove oil as attractants pheromone for both fruit flies (MFFand PFF).In addition, comparative of PFFmales to the prepared (local) and imported methyl eugenol.

MATERIALS AND METHODS

1-Laboratry experimentis

GC/MS: GC/MS analyses were performed, at Chemistry Department, Faculty of Science, Mansoura University, on a Varian GC interfaced to Finnegan SSQ 7000 mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m long, 0.25 mm internal diameter) coated with ploydimethylsiloxane (0.5 µm film thickness). The oven temperature was programmed from 50°C for 3 min., at isothermal, then heating by 7°C/min. to 250°C and isothermally for 10 min. at 25°C. Injector temperature was 200°C and the volume injected was 0.5µl. transition-line and ion source temperature was 250°C and 150°C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70eV.

Infrared: Infrared spectra were recorded on a Mattson 5000 FT-IR spectrophotometer at Chemistry Department, Faculty of Science, Mansoura University.

Ultraviolet: Ultraviolet spectra were determined using Unicom UV/VIS, UV spectrophotometer at Chemistry Department, Faculty of Science, and Mansoura University.

Solvents and chemicals: Methylene chloride and anhydrous sodium sulphate were obtained from Adwic Company. Methyl eugenol and trimedlure were provided by the first author.

Fixed oils: Paraffin oil was employed as a fixed oil and for obtaining a dose–response curve to different dilutions of various studied essential oils.

Plant material: Clove, Eugenia caryophyllata, as dried flower buds, was purchased from Harraz market, Bab Alkhalk, Cairo.

Processing of plant materials:

The clove dried flower buds were subjected to hydro-steam distillation. Extraction of the resulting distillate using dichloromethane, drying over anhydrous sodium sulfate and evaporation of the solvent gave the clove oil. Yield percentage was found to be 30.3%. The oil was characterized by IR spectroscopy; its constituents were identified by GC/MS analysis.

IR absorption bands of clove oil:

IR: KBr, v max, cm-1: 3250 (OH), 3077-3004 aromatic (C-H), 2975-2843 aliphatic C-H, 1638 (C=C), 1764 (CO), 1513 aromatic C=C, 1035 (C-H in plane).

U.V spectral (max, nm): eugenol acetate and eugenol at 280,282.

Preparing the synthetic methyl eugenol:

The volatile clove oil was extracted from clove by hydrosteam distillation. The obtained essential oil contains eugenol, caryophyllene derivatives (sesquiterpenes) and non phenolic compounds. Eugenol was isolated by treatment of the extracted oil with an aqueous sodium hydroxide solution (10%). A solution of eugenol (5 ml) in acetone (20 ml) and in the presence of sodium hydroxide solution (50 ml, 56%) was stirred on an ice bath. During the stirring

dimethyl sulphate (5 ml) was added drop wise to the reaction mixture which was further stirred for 6 hours then poured on ice cold water. Methyl eugenol was extracted with methylene chloride (3x20 ml) and dried over anhydrous sodium sulphate. The solvent was evaporated to give methyl eugenol as pale yellow oil. Youssef (1997).

2-Field bioassay

Experiments of the present study were carried out in the experimental farm of the Faculty of Agriculture, Mansoura University, Dakhlia government, Egypt during summer and winter 2011, seasons.The experimental farm area (about 14 feddans) cultivated with a mixture of fruit trees.

2.1-Comparative efficacy of Trimedlure and Clove oil in attracting *ceratitis capitata* males:

To evaluate the attractiveness of clove oil and trimedlure (as a check control) as attractants for MFF males, field bioassay was carried out by using white Jackson traps (Harris et al., 1971).Clove oil was investigated by using five concentrations (12.5, 25, 50, 75 and 100%), about 4 ml of different trimedlure and diluted clove oil concentrations in paraffin oil were used to saturate a piece of cotton which was placed in the trap. Each treatment was replicated three times. The traps were distributed in a completely randomized design in the shaded side of the fruit trees at 1.5-2 meters height. Each trap was far from each other by about 50 meters. The traps were inspected weekly during summer (July) and winter (December) seasons and number of attracted flies on each sticky cardboard inside traps was counted with renewal of cardboard strips.(Ghanim and Moustafa, 2009).

2.2-Comparative efficacy of the prepared and imported methyl eugenol in attracting *Bactrocera zonata* males:

To estimate the attractiveness of prepared (local) and imported methyl eugenol white Jackson traps were placed on fruit trees randomly. Prepared methyl eugenol was investigated by using three concentrations (25,50 and 100%). Each treatment had three replicates. The trapped insects were counted every week during December 2011 and the numbers of captured males of PFF were counted and recorded.

Statistical analysis was done by using one way ANOVA (CoStat, 1990).

RESULTS AND DISCUSSION

1-Analysis and characterization of clove oil volatile fraction:

Essential oil yield was found to be 30.3% w/w. The oil was characterized by IR spectroscopy. The GC/MS analytical results of clove oil volatile fraction were reported in Table (1).

Table (1): GC/MS data of clove oil volatile fraction

Compound	Moluclar Fourmla	Moluclar Weghit	Retention time	Area%
Monoterpenes				
€-Ocimene, **1**	$C_{10}H_{16}$	136.23	8.355	0.14
2-Methyl-6-methylene-1,7-octadien-3-one, **2**	$C_{10}H_{14}O$	150.22	9.73	0.07
Sesquiterpenes				
(+)-Aromadendrene,**3**	$C_{15}H_{24}$	204.35	19.92	1.44
β-Caryophyllene oxide, **4**	$C_{15}H_{24}O$	220.35	19.68	3.58
Cayrophylla-3,8(13)-dien-5β-ol, **5**	$C_{15}H_{24}O$	220.35	20.134	4.74
α-Caryophyllenol, **6**	$C_{15}H_{24}O$	220.35	20.22	5.55
Triterpene				
Trans-squalene,**7**	$C_{30}H_{50}$	410.72	31.8	0.04
Shikimates				
Benzyl alcohol, **8**	C_7H_8O	108.14	8.06	0.07
Methyl benzoate, **9**	$C_8H_8O_2$	136.15	9.34	0.03
Phenylmethyl acetate, **10**	$C_9H_{10}O_2$	150.17	10.69	0.04
Ethyl benzoate, **11**	$C_9H_{10}O_2$	150.17	10.83	0.03
Methyl salicylate, **12**	$C_8H_8O_3$	152.15	11.32	0.14
Benzyl benzoate, **13**	$C_{14}H_{12}O_2$	212.24	20.85	2.01
Benzyl salicylate, **14**	$C_{14}H_{12}O_3$	228.24	21.71	0.40
4-Benzyloxy-3-methoxybenzyl alcohol, **15**	$C_{15}H_{16}O_3$	244.29	23.83	0.03
2,3-Dihydro-2,2,5,6-tetramethyl benzofuran, **16**	$C_{12}H_{16}O$	176.25	32.32	0.07
Eugenol, **17**	$C_{10}H_{12}O_2$	164.20	14.91	15.01
€-Isoeugenol, **18**	$C_{10}H_{12}O_2$	164.20	18.55	10.70
Eugenol acetate, **19**	$C_{12}H_{14}O_3$	206.24	18.87	30.14
Dehydrodiisoeugenol, **20**	$C_{20}H_{22}O_4$	326.39	30.33	1.68
Methyleugenol, **21**	$C_{11}H_{14}O_2$	178.23	21.50	3.07
Fatty acids and esters				
Palmitic acid, **22**	$C_{16}H_{32}O_2$	256.42	22.66	0.04
α-Linoleic acid, **23**	$C_{18}H_{32}O_2$	280.45	25.06	0.02
Ethyl caproate, **24**	$C_8H_{16}O_2$	144.21	7.33	0.05
Methyl 8,11-octadecadienoate, **25**	$C_{19}H_{34}O_2$	297.47	24.30	0.03
Acetogenins				
2-Heptanone, **26**	$C_7H_{14}O$	114.19	5.03	0.07
(±)-2-Heptanol acetate, **27**	$C_9H_{18}O_2$	158.24	8.22	0.19
2-Nonanone, **28**	$C_9H_{18}O$	142.24	9.25	0.10
Heptacosane,**29**	$C_{27}H_{56}$	380.73	30.47	0.03
Nonacosane, **30**	$C_{29}H_{60}$	408.79	32.58	0.25

Fig. (1). Structures of some clove oil components

As indicated by Table 1 and Fig. 1, 30 components were identified, which constitutes 79.76% of the total components in the clove oil. As categorized in the table, the identified components were found to be two monoterpenes 1, 2; four sesquiterpenes 3 – 6; Trans squalene 7 as a triterpene, fourteen shikimate derivatives 8 – 21; four fatty acids and esters 22 – 25 and five liear acetogenins 26 – 30. Major important components were found to be eugenol acetate, eugenol, €-Isoeugenol, methyl eugenol and β-Caryophyllene oxide constituting 62.50 out of 79.76, i.e. 78.36%.This finding in agreement with those of Sivarstare *et al*. (2005) in India and Alma *et al*. (2007) in Turkey.

The presence of these components explains the attraction activity of clove oil to the MFF and PFF fruit flies.

These results seemed reasonable because Cunningham (1989) mentioned that two of the most attractive paraphermones (trimedlure) and methyl (eugenol) had been discovered as constituents of plant essential oils.

2-The attractiveness of *Ceartitas. capitata* males to clove oil:

The obtained data are summerized recorded in Table (2) and illustrated by Fig (2).

As shown in Table 2, clove oil at concentration 50% gave the highest attraction of MFF males compared with the other different concentrations of clove oil or trimedlure (as a reference attractant).However, females of the MFF don't show any response. The attractiveness of both trimedlure and clove oil were reduced with time.

The present data illustrated that MFF males showed no response to Jackson traps saturated with 12.5, 75 and 100% concentrations of clove oil. However, traps saturated with 50% clove oil lured more about one and have than those captured by trimedlure traps especially during summer season, Fig 2. The present results indicated that clove oil extract act as male lures for MFF. These results support those obtained by Ghanim (2009).

Table (2). Weekly mean number of *Ceartitas Capitata* males attracted to Jackson traps saturated with Trimedlure and different concentration of clove oil (12.5, 25, 50, 75 and 100) during summer and winter 2011, seasons.

Weeks	Summer						Winter					
	Trimedlure	Clove oil					Trimedlure	Clove oil				
		12.5%	25%	50%	75%	100%		12.5%	25%	50%	75%	100%
1st week	26	0	33	44	1	0	20	0	10	20.67	0	0
2nd week	16	0	12	18	0	0	10	0	4.67	9.33	0	0
3ed week	8	0	5	10	0	0	3.3	0	1.33	4	0	0
4th week	0	0	0	3	0	0	0	0	1.33	1	0	0
Mean	12.50	0.00	12.50	18.75	0.00	0.00	8.33	0.00	4.33	8.75	0.00	0.0

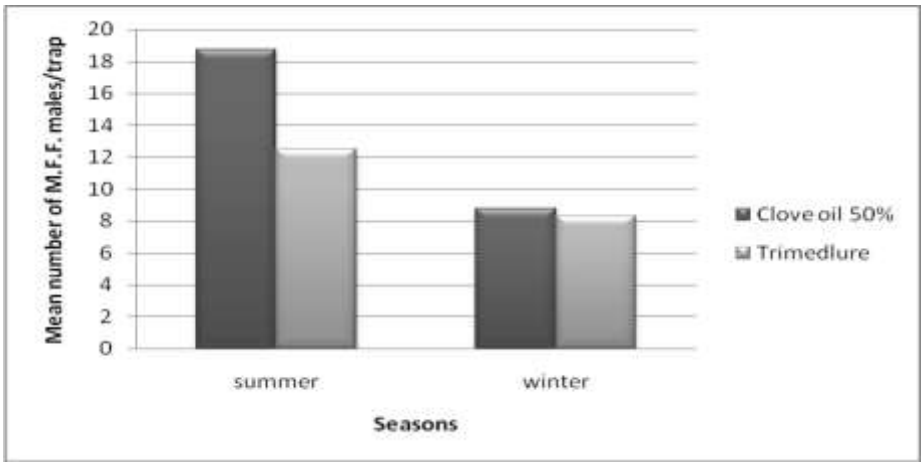

Fig. (2). The mean number of *C. capitata* males attracted to clove oil (50%) and trimedlure during summer and winter 2001, season.

3-The attractiveness of *Bactrocera. Zonata* males to the prepared and imported methyl eugenol:

From the obtained results (table3 and Fig.3) it can be stated that the prepared (local) methyl eugenol (50 and100% concentration) has approximately the same efficacy in attracting PFF males as well as imported methyl eugenol with no significant differences during the four weeks of investigation. On contrary, Ghanim (2009) revealed that the imported methyl eugenol had higher efficacy than local one.

Table (3). Weekly mean number of *Bactrocera. Zonata* males attracted to Jackson traps saturated with imported methyl eugenol and different concentration of prepared (25, 50 and 100% concentration) methyl eugenol during winter 2011, season.

weeks	ME	ME* 25%	ME* 50%	ME* 100%
1st week	10.33	7.33	11.67	14.00
2nd week	10.00	4.33	5.33	8.00
3ed week	4.33	5.67	6.00	3.00
4th week	0.00	1.33	0.33	1.00
Mean	6.17	4.67	5.83	6.50

ME*: local methyl eugenol
ME : imported methyl eugenol

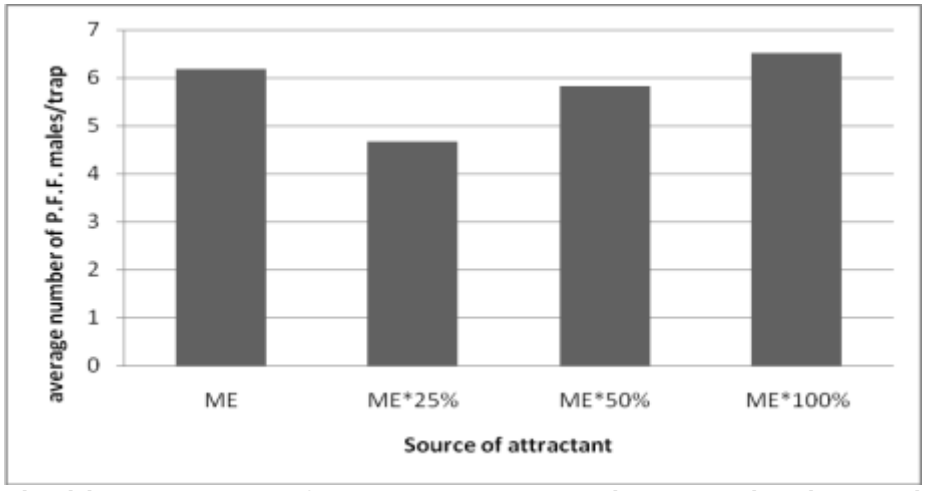

Fig. (3). Average No. of *Bactrocera Zonata* male attracted to imported and prepared (25, 50 and 100%) methyl eugenol.

So, these results demonstrate the potential of prepared (local) methyl eugenol as cheaper alternative attracting in monitoring and controlling PFF population.

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زيت القرنفل كمصدر لبعض الفرمونات الجاذبة لذبابة فاكهة البحر المتوسط وذبابة ثمار الخوخ

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تعتبر ذبابتى فاكهة البحر المتوسط وثمار الخوخ من اهم الافات الحشرية التى تصيب أشجار الفاكهة من الناحية الاقتصادية.

وفى هذة الدراسة تم تقييم كفاءة مستخلص زيت القرنفل فى جذب الحشرات الكاملة لذبابة فاكهة البحر المتوسط وذبابة ثمار الخوخ وذلك بالمزرعة البحثيه لكلية الزراعة جامعة المنصورة.

وقد أوضحت النتائج ان اناث كلا النوعين لم تستجيب لهذا الزيت- وقد ابدت ذكور ذبابة فاكهة البحر المتوسط استجابة ايجابية تجاة زيت القرنفل بتركيز(50%) بدرجة فاقت مرة ونصف استجابتها للجاذب الجنسى الترايمدلور.

وبمقارنة كفاءة الميثيل ايوجينول المصنع محليا والمستورد فى جذب ذبابة ثمار الخوخ وجد ان الميثيل ايوجينول المصنع محليا بتركيز (50, 100%) قد اعطى ذات القدرة من الكفاءة فى الجذب مقارنة بالميثيل ايوجينول المستورد, ولذلك يمكن استخدام الميثيل ايوجينول المصنع محليا بدلا من المستورد فى تتبع قدرات ومكافحة ذبابة ثمار الخوخ.

علما بانة فد تم فصل 30 مركب من زيت القرنفل حيث وجد ان مركبات Methyl eugenol , Eugenol , Eugenol acetate, Iso- eugenol, Cayrophyllene β-oxide تمثل الاغلبية فى الزيت بنسبة 62.50%.

قام بتحكيم البحث

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