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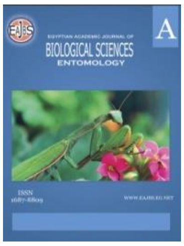

Response of Peach Fruit Fly, *Bactrocera zonata* (Saunders) to the Essential Oil of Cubeb Pepper, *Piper cubeba* Bojer

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ABSTRACT

Peach fruit fly, *Bactrocera zonata* (Fam: Tephritidae, or: Diptera), is a quarantine insect-pest that infests various commercial fruit and vegetable crops in Egypt. Almost all the monitoring and control strategies of this fly depend on the use of the para-pheromone methyl eugenol (ME) as a sex attractant of male flies. Therefore, the current study aimed to investigate the potential use of the essential oil of berries of cubeb pepper (tailed pepper) (CEO), *Piper cubeba* (Fam: Piperaceae) as an attractant for *B. zonata* flies. CEO was extracted using the hydro-distillation scavenging apparatus from cubeb berries and chemically analyzed by GC-MS. The CEO was assayed in the attraction of *B. zonata* flies compared with the recommended dose of para-pheromone ME. Results showed that ME and the CEO at the different concentrations had attracted male flies and no females. Remarkably, there were no significant differences between ME and crude CEO in attracting the male flies. The GC-MS results revealed about 29 major constituents of CEO; the eugenol was the major constituent (45.88%) of the CEO, followed by 3-methyl-pentane (15.36%), methyl-cyclopentane (9.198%), and methyl eugenol (6.093%). These findings explaine the effective role of CEO as a male attractant of *B. zonata* and it could be used as an alternative to the ME, which would conserve a huge amount of money spent on buying the chemical ME.

INTRODUCTION

The essential oils of plants perform a variety of functions in the control of insect-pests and could be biologically active for insects as attractants, repellents, and toxicants (Isman 2000). *Piper* species (as *P. nigrum* L) have been recorded as insecticidal agents or seed protectors (Bernard *et al*., 1995; Scott *et al*., 2004). Also, *P. nigrum* extracts might provide a degree of protection against the larvae of lepidoptera and European pine sawfly and can also act as a repellent and anti-feeding agent (Scott *et al*., 2004).

The attractive component of citronella oil to males of *Bactrocera dorsalis* was found to be methyl eugenol (3-4 dimethoxy-1 allylbenzene) (Howlett 1915). Sixty years later, ME was documented as the most attractant for the oriental fruit fly, *B. dorsalis*, compared with 34 chemical analogs (Metcalf *et al*. 1975). This lure was used to monitor a wide range of the Dacinae flies as well as to control and eradicate male flies via male annihilation technique (Metcalf 1990; Metcalf and Metcalf 1992; Verghese *et al*., 2012; El-Gendy and Nassar 2014). The ME interfers with the insects' pheromone communication system physiologically and/or behaviourally (Renou and Guerrero 2000), where it affects the olfactory system with a phagostimulatory action of tephritid fruit flies with a powerful attraction extended to a distance of 800 m (Roomi *et al*., 1993; Bhagat *et al*., 2013; Haq *et al*., 2014).

Some plant species of the family Piperaceae are rich in ME and eugenol compounds (Tan and Nishida 2012) of which genus of *Piper* that has varied quantities of ME from 0.1 to more than 90% of essential oils (De Vincenzi *et al*., 2000; Tan and Nishida, 2012). Cubeb pepper or tailed pepper (*Piper cubeba* Bojer) is a member of Family Piperacea, Genus *Piper*, which is mostly grown in Indonesia in Java and Sumatra islands as well as southern of India (Elfahmi *et al.*, 2007). *P. cubeba* is widely used in the Egyptian kitchen as food spice and as a main ingredient of soup in many West African countries (Juliani *et al.*, 2013). Additionally, *P. cubeba* used as a medicinal plant to treat dysentery, syphilis, gonorrhea, pain, diarrhea, enteritis and asthma, and unani (Graidistet *et al*., 2015). However, the usage of natural ME in the agricultural sector has not been spread.

In Egypt, the peach fruit fly, *B. zonata* (Diptera: Tephritidae) is a significant insect pest in horticultural crops (El-Gendy and El-Saadany 2012). That management strategies as the male annihilation technique were employed, it relies on the use of the ME as a males' lure, where males of *B. zonata* are strongly attracted to ME (Verghese *et al*., 2006; Bajaj and Singh 2018). To the best of our knowledge, no data are available about the use of essential oil of *P. cubeba* (CEO) in the attraction of *B. zonata* fly. Therefore, present study was conducted to evaluate the response of *B. zonata* flies for the OE of *P. cubeba* as a natural attractant substance under field conditions, as well as to identify the chemical constituents of the CEO using the gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant Material:

Dry cubeb berries, *P. cubeb*, were purchased from the spice market in the Damanhour city of Egypt in 2019. The cubeb samples were re-recognized in the Department of Botany, Faculty of Agriculture, Damanhour University, Damanhour, El-Beheira, Egypt.

Extraction of Essential Oil:

The CEO was extracted by the hydro-distillation method using the oil scavenger according to Elyemni *et al*. (2019) at the Laboratory of Pesticides Residue Analysis and Toxicity (PRATL), Faculty of Agriculture, Damanhour University, Damanhour, El-Beheira, Egypt. Cubeb berries were grinded to a fine powder and a quantity of 200 g of cubeb powder was added to 800 ml of distilled water in a 2-liter flask and heated for 3–4 hrs until no more oil condensed in the scavenger. By the end of the distillation, two phases were detected, the aqueous phase (aromatic water) and the organic phase (essential oil), which has less denisty than the water. The essential oil was collected and stored in dark bottles, at 4°C, until being analyzed and tested against *B. zonata*.

GC-MS Analysis Of Cubeb Essential Oil:

About 1 µl was taken from the CEO and analysed by Gas Chromatography-Mass Spectrometry (GC-MS) coupled with Varian 8200 autoinjector. The GC-MS semi-quantitative analysis was performed using the Agilent 7890A Gas Chromatography apparatus coupled with an Agilent 5975B mass selective detector (GC-MS) that equipped with an HP-5 capillary column (30 m ×0.25 mm ID, film thickness 0.25 µm, Agilent Technologies, USA). Separation of chemical components was completed at the following conditions: initial column temperature set at 50°C for 6 min. It was increased to 215°C at 15°C /min (hold for 1 min), then to 230°C at 5°C/min, and finally to 290°C at 5°C/min (hold for 2 min). The solvent delay was kept 4 min, while the injector temperature was used at 250 °C and helium gas was the carrier. Electron ionization mass spectra were recorded up to 800 *m/z* at 70 eV with the ion source temperature at 230°C. The GC-MS apparatus was controlled by a computer system, which has EI-MS libraries. The compounds in CEO were identified by the full mass spectra scans and retention time using their total ion chromatograms (TIC). The full scan mode allowed the contrast of the spectrum of obtained compounds with the EI-MS database libraries including NIST14 and Willey9.

Study Area:

The field experiments were conducted in Damanhour City, El-Beheira Governorate, Egypt. Experiments were carried out in two densely populated sites of *B. zonata* (according to preliminary tests of trapped flies). The first site has about 5 Feddans cultivated with papaz, loquat, guava, navel orange, sour orange, and palm trees, the second location has 3 Feddans cultivated with guava and navel orange. The distance between the two sites is about 1000 m.

Attraction Activity of CEO of *B. zonata* Fly:

Assay of the CEO activity in the attraction of *B. zonata* flies depended on monitoring the population of adult insects of *B. zonata*. Inspection of the presence of *B. zonata* was conducted through September 1st to December 1st, 2019 using Jackson traps (Haris *et al*., 1971). Traps were baited with CEO in four different concentrations; T1 (100%), T2 (80%), T3 (66.66%), and T4 (50%) of crude oil and the para-pheromone ME (T5) (98%, manufactured by Sinoway International, Jiangsu, Co. LTD-China) (Table 1). The CEO concentrations were prepared by mixing the crude CEO with paraffin oil (v : v). Approx., 0.4 ml of the tested treatments were injected in cylindrical cotton wicks (1 cm height × 0.5 cm diameter). The traps were hung up on the trees' canopy at 1.5-2 m above the ground at a distance of about 50 m apart (El-Gendy 2012) in a randomized block design (CBD). Traps were repeated five times per treatment. Traps were examined every seven days and the cotton wicks were renewed once a month. Trapped flies were recognized, counted, recorded by sex, and expressed as the number of capture flies/trap/day (CTD) per each site, and means were considered in the analysis.

Table 1: Description of treatments used in lure traps against *Bactrocera zonata* males

Treatment	Description	Concentration (%)
T1	cubeb oil (crude oil)	100.0
T2	1 vol (paraffin oil) : 4 vol (cubeb oil)	80.00
T3	1 vol (paraffin oil) : 2 vol (cubeb oil)	66.66
T4	1 vol (paraffin oil) : 1 vol (cubeb oil)	50.00
T5	Para-pheromone- ME (methyl eugenol)	98.00

Statistical Analysis:

Statistical analyses of the field data were achieved using CoStat (CoHort software, USA). The data were transformed to ln(x+1) to reduce the heterogeneity of variances and analyzed with ANOVA; One-way randomized blocks with repeated measures and means were compared using the LSD test. Spearman's correlation (ρ) was performed.

RESULTS AND DISCUSSION

1-Attractivness of CEO for *B. zonata* fly:

ME and CEO were assayed in the attraction of *B. zonata* flies under field conditions using Jackson traps. Results showed that the ME and CEO trapped male flies only, no females were detected in all tested treatments (lures) (Table 2). Trapped male flies differed significantly among treatments (F=18.893, p= 0.0004) and lure longevity (exposure time) (F=64.16, p= 0.0000). Additionally, highly significant interaction was reported between longevity of lures and treatments (F=4.70, p= 0.0003), with a significant impact (treatments; regreression slope = 0.09, *t*= 0.02, *p*=0.000***, lur longivety; regreression slop = - 0.17, *t*= -6.22, *p*=0.000***) of 51.88% of total variance that affecting the captured male flies according to adjusted determenation coefficient (adj R^2). As shown in Table 3, T1-treatment (crude CEO) exhibited more attraction of *B. zonata* males with 0.84-fold of that in T5-treatment (ME, reference lure), however, no significant difference was reported between them. The T2-treatment was the 2nd in the efficacy of males' attraction, followed by T4 and T3 treatments with 0.80, 0.69, and 0.61-fold of that in T5-treatment, respectively. However, no significant differences were found among T1, T4, and T2 treatments. Noticeable, the treatment T3 was the least in the attraction of *B. zonata* males and was not significantly different of T4. Previous results reported that ME attracted only male insects of *B. zonata*, *B. dorsalis*, and *B. umbrosa* (Kumar 2011; Tan 1985; Tan and Jaal 1986; Tan and Nishida 2012; Tan and Serit 1988, 1994). Besides the attraction of the males only of *B. zonata* and *B. dorsalis* to ME, *B. correcta* (Bezzi) males attracted to ME too (Verghese *et al.*, 2006, Bajaj and Singh 2018). Also, the male adults of *B. correcta* flies attracted to traps containing ME, β-caryophyllene (Wee *et al.*, 2017). However, Verghese (1998) mentioned that both males and females of *B. dorsalis* flies were attracted by ME. In addition, the *B. dorsalis* and *B. correcta* females attracted to β-caryophyllene (Jaleela *et al.*, 2019). Also, the essential oils of angelica, ginger root, manuka, orange, cubeb, and tea tree plant were found to be attracted to males of *Ceratitis capitata*, when undiluted oil (5 µl) was tested (Epsky and Niogret 2017).

Table 2: Detected flies of *Bactrocera zonata* (males and females) using Jackson traps baited with different concentrations of cubeb essential oil (CEO) and para-pheromone ME (a reference).

Treatment	Detected flies							
	Date (week)							
	1		2		3		4	
	♂	♀	♂	♀	♂	♀	♂	♀
T1	+	-	+	-	+	-	+	-
T2	+	-	+	-	+	-	+	-
T3	+	-	+	-	+	-	+	-
T4	+	-	+	-	+	-	+	-
T5	+	-	+	-	+	-	+	-

♂: male flies, ♀: female flies, +: detected, -: not detected

Table 3: Mean of captured male flies of *Bactrocera zonata* using Jackson trap baited with cubeb essential oil (CEO) and methyl eugenol (ME).

Treatment	Mean captured male flies/trap (±SE)				
	Date (week)				
	1	2	3	4	Overall mean
T1	1.67±0.02	1.56±0.04	1.08±0.13	0.84±0.21	1.29±0.12[ab]
T2	1.42±0.03	1.27±0.05	1.20±0.015	1.02±0.04	1.23±0.04[b]
T3	1.01±0.03	1.07±0.02	0.83±0.74	0.82±0.04	0.93±0.03[c]
T4	1.38±0.02	0.99±0.03	1.05±0.04	0.76±0.08	1.05±0.07[bc]
T5	1.77±0.02	1.59±0.11	1.57±0.09	1.18±0.15	1.53±0.08[a]
Overall mean	1.45±0.09[a]	1.29±0.07[ab]	1.15±0.06[bc]	0.93±0.09[c]	1.20±0.04

Means followed by the same superscript letter(s) are not significantly different according to Student's Neuman *post-hoc* multiple comparison method at $P \leq 0.05$. $LSD_{0.05}$ for overall mean treatments=0.17, $LSD_{0.05}$ for overall mean dates = 0.15.

The results revealed that responses of *B. zonata* males to CEO were concentration-dependent in a week direct relationship (ρ=0.28, *p*= 0.0045**). It was clear that the lure longivety was the most effective factor on the number of captured flies (regreression slop= -0.17, *t*=-6.22, *p*=0.000***) with 45.69% of total variance on n trapped males. Generaly, the decreased in numbers of trapped males with the decreasing of lure concentration of lure reported. These results were in agreement with the results of Dotterl *et al.* (2006), who mentioned that the responses of nursery pollinator, *Hadena bicruris* increased by increasing the concentrations of *Silene latifolia* that were diluted in paraffin oil. Ghanim (2013) reported that the weekly mean numbers of *B. zonata* males increased with an increased of ME-concentrations that were diluted in paraffin oil, where the higher mean number of males occurred significantly at the 100% concentration of ME. It is worth mentioning that paraffin oil that used as a solvent for the CEO had no effect on males' attraction.

Regarding the longevity of the tested lures (Fig. 1), the captured numbers of *B. zonata* males were inversely related to lure longevity, with a moderate negative correlation (r = -54, *p*=0.0001***). The trapped males in T3-treatment reached its highest number on the 14^{th} day then were gradually decreased. Treatments of T1, T2, and T4 had caught high number of males at the 1^{st} week similar to T5. Afterwards, the numbers of trapped males were gradually declined with elapsed time. The decreasing pattern of male flies' numbers varied not only among treatments but also among investigated times. The decreasing numbers of males occurred in the treatments of T1 by 0.07, 0.35 and 0.50-fold, T2 by 0.11, 0.15 and 0.28-fold, T4 by 0.28, 0.24 and 0.45-fold, and T5 by 0.10, 0.11 and 0.33-fold in the 2^{nd}, 3^{rd}, and 4^{th} week, respectively of captured males at the 1^{st} week of the same treatment. Whilst the decrease in the trapped males of T3- treatment was 0.18 and 0.19-fold in the 3^{rd} and 4^{th} week of males captured at the 1^{st} week. Noticeable, there were no significant differences in male catching at the 1^{st}, $2^{nd,}$ and 3^{rd} week whether in T1- or T5-treatment. These results were supported by that of Ghanim (2013), who mentioned that *B. zonata* males' numbers decreased with time along 10 weeks.

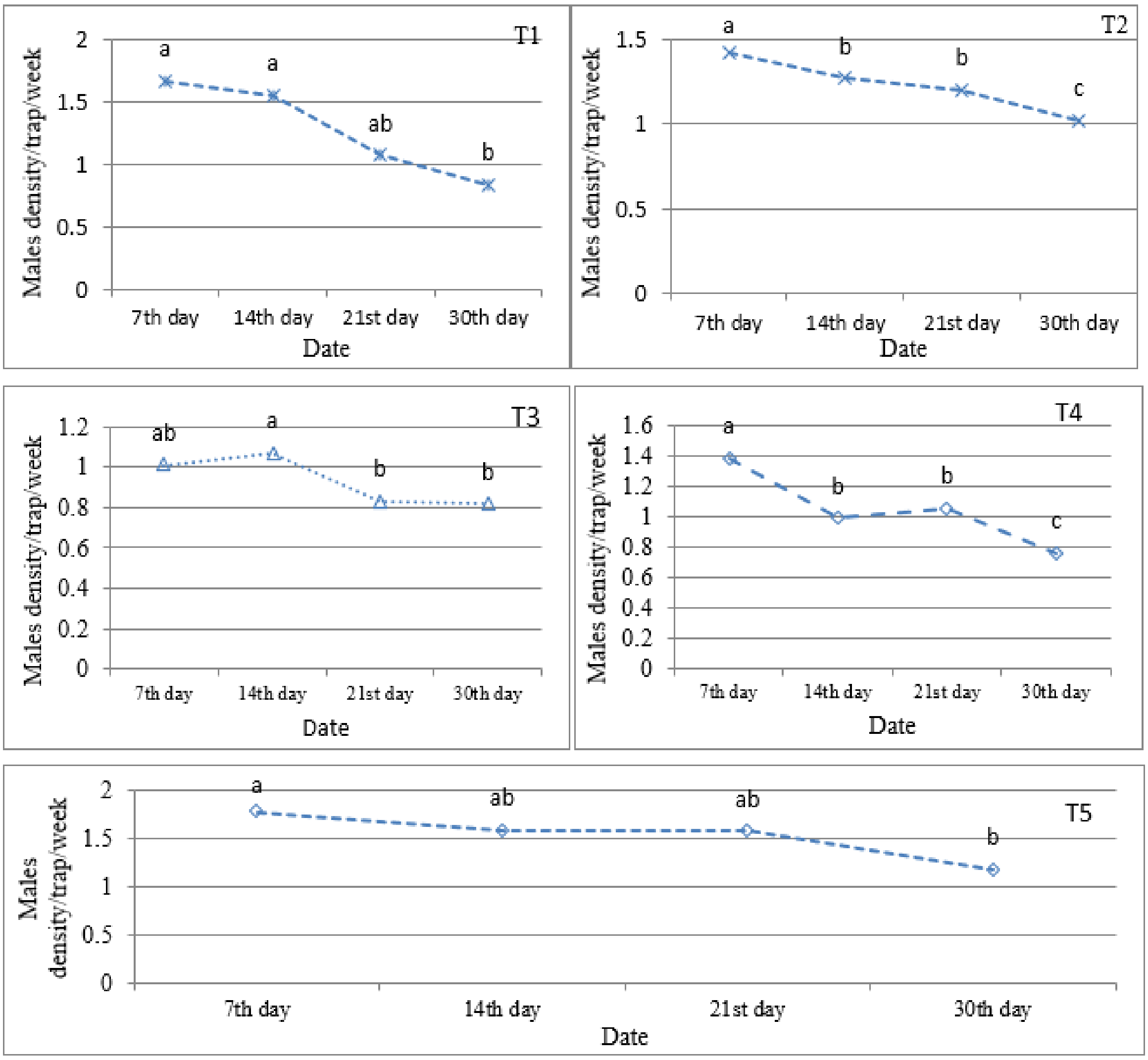

Fig.1: Mean captured numbers of male peach fruit fly, *Bactrocera zonata*, which were attracted by cubeb essential oil (CEO) concentrations (T1; 100%, T2; 80, T3; 66.6, and T4; 50% of crude oil) and methyl eugenol (ME; 98%). $LSD_{0.05}$ values were 0.55, 0.11, 0.20, 0.2, 0.54 for T1, T2, T3, T4, and T5, respectively.

2-CEO Volatile Components*:*

The GC-MS analysis of the volatile composition of CEO was presented in Table 4 and Figure 2. GC-MS results revealed the presence in 29 chemical compounds of CEO. The dominant constituents that were identified as eugenol (phenol-2-methoxy-4(2-propenyl)) (45.88%), followed by, 3-methyl-pentane (15.36%), methyl-cyclopentane (9.198%), methyl eugenol (1, 2-dimethoxy-4-(2-propenyl) benzene) (6.093%), β-myrcene (2.029%), β-caryophyllene (1.259%), and α-copaene (0.259%). The present findings revealed that the constituents of CEO were mainly eugenol and methyl eugenol. Also, the obtained results showed that the CEO is rich in eugenol compounds, which was reported as a source for o-methyl eugenol (Herrmann and Weaver 1999). In the same line with the present findings, Cheng *et al*. (2018) reported that components of the oil of berries of *P. cubeba*, that were extracted using hydro-distillation (HDE) and simultaneous distillation extraction (SDE) and identified by GC-MS were similar to the obtained finding, especially methyl eugenol with traces amount of 0.71 and 0.11% by HD and SDE, respectively. Also, terpinen-4-ol (42.41%), α-copaene (20.04%), and γ-elemene (17.68%) were the major components of *P. cubeba* EO (Andriana *et al*., 2019).

Table 4: Chemical components of cubeb essential oil (CEO), *Piper cubeba* as separated using GC-MS and identified using the chemical database libraries of NIST 14 and Willey 9.

Rt[a] (min)	Components	Area of Total (%)
2.538	3-Methyl-pentane	15.36
2.787	Methyl-cyclopentane	9.198
6.178	α-Pinene	0.038
6.980	β-Myrcene	2.029
7.257	α-Phellandrene	0.044
7.444	α-Terpinene	0.039
7.572	*p*-Cymene	0.071
7.637	dl-Limonene	0.123
7.699	Eucalyptol (1,8-Cineole)	0.474
7.868	*cis*-β-Ocimene	0.107
8.092	γ-Terpinene	0.064
8.564	α-Terpinolene	0.095
8.701	Linalool	0.110
9.976	Terpinen-4-ol	0.130
10.168	α-Terpineol	0.609
11.214	Chavicol (4-Allylphenol)	0.484
12.445	Eugenol	45.88
12.642	α-Copaene	0.259
12.854	Methyl eugenol	6.093
13.178	β-Caryophyllene	1.259
13.539	β-Selinene	0.229
13.715	γ-Muurolene	0.036
14.106	α-Cadina-4,9-diene	0.023
14.157	Δ-Cadinene	0.098
14.770	Spathulenol	0.053
14.830	Caryophyllene oxide	0.185
15.290	*t*-Cadinol	0.216
15.446	Aromandendrene	0.214
15.555	*iso*-Aromadendrene epoxide	0.072

Rt: retention time

Also, similar results were obtained in other Piperaceae plants, where ME was detected in different ratios in Betel pepper that had 4.1-6.9% of ME and 82.2-90.55% of eugenol (Sharma *et al*., 1983). EOs of *Piper divaricatum* had ME (17-93%) and eugenol (2-46%) (Maia and Andrade 2009). Generally, the present results revealed that male flies of *B. zonata* attracted to traps baited with the different concentrations of CEO, accordingly, CEO acts as a male's lure to *B. zonata*. Furthermore, the CEO analysis revealed that chemical constituents were related to the chemical ME, of which eugenol (phenol-2-methoxy-4(2-propenyl)) and methyl eugenol (1, 2-dimethoxy-4-(2-propenyl) benzene). These results might be interpreting the attraction of *B. zonata* males to CEO, but further studies are needed.

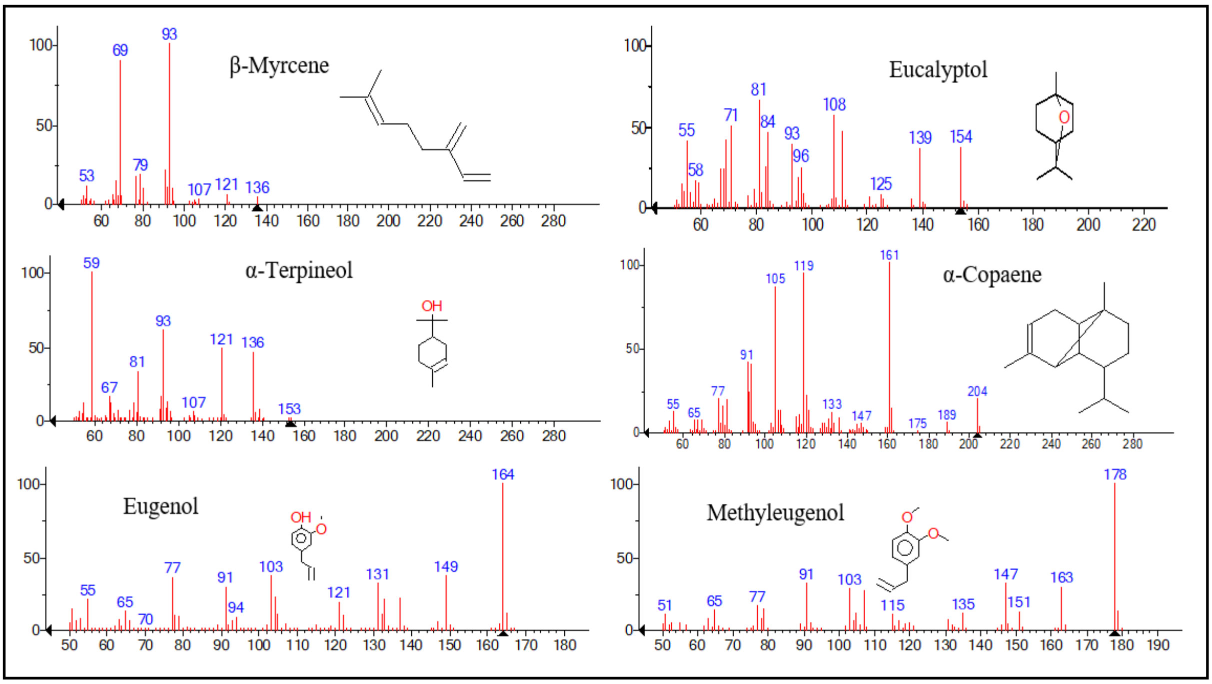

Fig. 2: Mass spectral pictograms of abundant chemical components in essential oil extract of cubeb berries (*Piper cubeba*) after analysis using GC-MS and identification using the NIST/EPA/NIH mass spectral library software version 2.2 build 2014

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ARABIC SUMMARY

استجابة ذبابة ثمار الخوخ *Bactrocera zonata* (Saunders) للمستخلص الزيتى لثمار نبات الكبيبة *Piper cubeba*

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ذبابة ثمار الخوخ *Bactrocera zonata* (Fam: Tephritidae: Diptera) هي آفة حشرية حجرية تصيب مختلف محاصيل الفاكهة التجارية في مصر. حيث تعتمد جميع استراتيجيات المراقبة والسيطرة على هذه الذبابة تقريبًا على استخدام البارا-فرمون الميثيل الأوجينول (ME) كجاذب جنسي للذباب الذكري.

وتهدف الدراسة الحالية إلى دراسة احتمال استخدام المستخلص الزيتى العطرى لثمار نبات الكبيبة Piper *cubeba* كجاذب لذبابة ثمار الخوخ *B. zonata*. حيث تم استخلاص زيت الكبيبة باستخدام جهاز المسح بالتقطير المائي لثمار نبات الكبيبة، وتم تحليله كيميائيًا بواسطة GC-MS . تم تقييم المستخلص الزيتى لثمارنبات الكبيبة في جذب ذبابة ثمار الخوخ *B. zonata* مقارنة بالجرعة الموصى بها من ME البارا-فرمون. و قد أظهرت النتائج أن مركب ME و زيت ثمار الكبيبة قد جذبوا ذكور ذبابة ثمار الخوخ، وليس الذباب الإناث. كما اوضحت النتائج عدم وجود فروق ذات دلالة إحصائية بين مركب ME وزيت ثمار الكبيبة في جذب ذكور الذبابة. كما أظهرت نتائج GC-MS أن مركب الأوجينول (eugenol) كان المكون الرئيسي (٤٥,٨٨٪) لزيت الكبيبة، يليه مركب methyl-pentane (15.36%)، ومركب methyl-cyclopentane (9.198%) ، ومركب methyl eugenol (6.093%). النتائج الحالية تبين أن زيت ثمار الكبيبة فعال فى جذب ذكورثمار الخوخ *B. zonata*، ويمكن استخدامه كبديل للمركب الكيماوى ME ، والذي سيوفر مبلغًا كبيرًا من الأموال التي يتم إنفاقها على شراء المادة الكيميائية.