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Efficiency of some Essential Oils as Attractants for *Bactrocera Zonata* Males under Field Conditions

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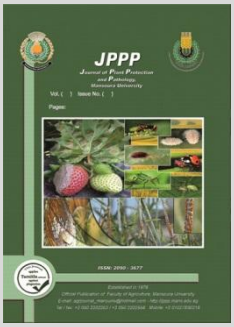

ABSTRACT

Comparative efficacy of eight plant essential oils (camphor, mint, sweet almond, thyme, basil, black seed, local clove and frankincense stones) and methyl eugenol were evaluated as attractants for PFF males, either individually, or in mixed with methyl eugenol, in a citrus orchard situated at Aga district, Dakahlia governorate, Egypt.Statistical analysis indicated that in the 2nd, 3rd and 4th weeks camphor and methyl eugenol traps lure significantly high numbers of PFF adults, they came in the superior compared with the other tested essential oils. Mint and sweet almond ranked the second in order followed by thyme. In contrast, basil, black seed, local clove, and frankincense essential oils attracted the fewest individuals. The attraction levels of camphor, mint, sweet almond, and thyme oils in relation to methyl eugenol were 84.68%, 71.95%, 47.27%, and 26.49%, respectively, in terms of the number of PFF males lured to methyl eugenol. Jackson traps baited with mixture of camphor and methyl eugenol at ratio of 1:1, showing significantly higher attraction rates without notable differences compared to traps using methyl eugenol alone. Further, the camphor-methyl eugenol mixture as well as methyl eugenol alone demonstrated similar stability over time.

***Keywords*:** Fruit flies, essential oils, attractants, methyl eugenol

INTRODUCTION

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), has become an invasive species in many countries around the world and is considered one of the most serious fruit pests (Hardy, 1973 and De Meyer *et al.*, 2007). In 1993, PFF was detected infesting guava in Kalubia governorate for the first time in Egypt (De Meyer *et al.*, 2007), then it widely dispersed across most of Egypt (Elnagar *et al.*, 2010). It infests the fruits of over 50 plant species that differ in their ripening time stage all over the year (Ghanim, 2009; and El-Afify *et al.*, 2023) and frequently causes 25%–50% yield losses in infested orchards (Siddiqui *et al.*, 2003).

Semiochemicals are defined as substances which transmit messages between living organisms, both plants and animals (Abd El-Kareim *et al.*, 2017 and 2021). Males of several economically important tephritid species are strongly attracted to male lures or parapheromones which considered as particular chemical compounds, that either occur naturally in plants or synthetic analogues or plant-borne substances (Abd El-Kareim *et al.*, 2009; Moustafa *et al.*, 2012; Singh *et al.*, 2020; Susila *et al.*, 2021; Tangpao, *et al.*, 2022; Zhang *et al.*, 2023; and Tine *et al.*, 2024). Male lures or parapheromones are used in detection, monitoring and mass trapping of many fruit fly species including PFF (Ghanim *et al.*, 2010; Ghanim, 2013; El-Adly *et al.*, 2018; El-Metwally *et al.*, 2019 and Elkelany *et al.*, 2024).

The synthetic sex attractant, methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate) is used as a male lure for many fruit flies belonging to the genus of *Bactrocera*. Manoukis *et al.* (2015) reported that methyl eugenol is a strong attractant for these insects. Inside these species, methyl eugenol converted to 2-allyl-4, 5-4-allyl-1, 2-dimethoxyphenol and (E)-coniferyl alcohol (Tan & Nishida, 1998 and Shelly *et al.*, 2004) which sequester into the male rectal glands before release during courtship (Tan & Nishida, 1998 and Hee & Tan,2005). So, methyl eugenol is recommended as an attractant for PFF males. In Egypt, the price of methyl eugenol is high and the used quantity of it for monitoring and control methods of PFF is large. Therefore, searching for less expensive alternatives to methyl eugenol should be done. Manoukis *et al.* (2015) mentioned that action programs would need to integrate information on male-targeting lures such as the ones studied with female lures and various trap types [*e.g.*, Broughton and De Lima, 2002, Heath *et al.*, 2004).

So, the current study sought to develop novel treatments that could potentially rival methyl eugenol in their ability to attract PFF males by two steps: 1) screening the attractancy of certain essential oils to PFF males, and 2) evaluating the efficiency of essential oils-methyl eugenol mixtures (by mixing the previously selected oils with methyl eugenol at different ratios) as attractants to PFF males.

MATERIALS AND METHODS

1. The used materials:

In the present study, eight plant essential oils (Table, 1) were obtained from the Pure Life Company, Cairo, Egypt. Each of Jackson traps (Harris *et al.*, 1971), cotton wicks (measured as 3 cm long and 1 cm diameter) and methyl eugenol (98% purity, Yasho Industries, India) were obtained

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from Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture.

Table 1. Essential oils used for attracting PFF males and it plant sources.

No.	Essential oil	Plant source	
		Scientific name	Family
1	Camphor	*Cinnamomum camphora* (L.)	Lauraceae
2	Mint	*Mentha spicata* L.	Lamiaceae
3	Sweet almond	*Prunus amygdalus* (Batsch.)	Rosaceae
4	Local sweet almond		
5	Thyme	*Thymus vulgaris* L.	Lamiaceae
6	Basil	*Ocimum basilicum* L.	Lamiaceae
7	Black seed	*Nigella sativa* L.	Ranunculaceae
8	Local clove	*Syzygium aromaticum* (L.)	Myrtaceae
9	Frankincense stones	*Boswellia serrata* Roxb.	Burseraceae

2. Comparative attraction of plant essential oils and the recommended attractant (methyl eugenol) to PFF males:

The eight essential oils (Table,1) and methyl eugenol were used for screening their attractancy to PFF males. From each essential oil, 3 cm^3 was injected inside a cotton wick and installed in a Jackson trap. Four traps of each essential oil were used as replicates. Additional four traps were powered with cotton wick which impregnated with methyl eugenol as a control treatment. All traps were distributed in a completely randomized design in citrus orchard located at Aga district, Dakahlia governorate, Egypt.

Traps were hung in a shady site within the canopy of the citrus trees at a height of approximately two meters from the ground. The traps were weekly inspected all over successive four weeks from the 25th of September till the 23rd of October 2022. Number of captured PFF males on each sticky cardboard inside traps was counted with renewing the sticky cardboards. The counted PFF males were recorded as flies/trap/day (FTD). Dispensers and its containing of essential oils and methyl eugenol did not renewed all over the experimental period.

3. Comparative attraction of certain essential oils mixed with methyl eugenol at different ratios compared with methyl eugenol alone.

Each essential oil (camphor, mint, thyme, black seed and sweet almond) was mixed with methyl eugenol in proportions of 3:1, 1:1, and 1:3, and assessed against each essential oil or methyl eugenol separately without any combination. From each prepared mixture, 3 cm^3 was injected inside a cotton wick and installed in a Jackson trap. All treatments were replicated four times. Jackson traps were distributed, hung and inspected in the same orchard as previously mentioned. The experiment was carried out during the period from the 1st till 29th of October 2023.

4. Statistical analysis:

The obtained data were analyzed by using analysis of variance (ANOVA) to compare treatment means. According to Steel and Torrie (1980), ANOVA was used to determine differences and significances among treatments, followed by means separation based on the Tukey HSD method and 95% confidence intervals. The means that are not related by a letter differ greatly. The regression analysis was also done. All the statistical analysis were done by MINITAB® software (version Minitab® 21.4.1).

RESULTS AND DISCUSSION

Results

1. Screening the attractancy of certain essential oils to PFF males:

The attractiveness of the eight essential oils and methyl eugenol to PFF males was estimated and is presented in Table 2 . Statistical analysis indicated that in the 2nd, 3rd and 4th weeks camphor and methyl eugenol traps lure significantly high numbers of PFF adults, they came in the superior compared with the other tested essential oils. Mint and sweet almond ranked the second in order followed by thyme. In contrast, basil, black seed, local clove, and frankincense essential oils attracted the fewest individuals.

Table 2. Attractiveness of certain essential oils to PFF males compared with methyl eugenol (recommended attractant) for four weeks under field conditions in a citrus orchard.

Essential oil	1st week	2nd week	3rd week	4th week
Camphor	3.79±0.46b	4.00±0.25a	4.03±0.12a	1.24±0.11a
Mint	4.86±0.17ab	2.65±0.11b	2.65±0.04b	0.92±0.06a
Sweet almond	2.71±0.12bc	2.14±0.33bc	2.14±0.17bc	0.28±0.09b
Thyme	1.57±0.24cd	1.12±0.19cd	1.12±0.13cd	0.28±0.13b
Basil	0.06±0.94d	0.67±0.8d	0.24±0.53d	0.28±0.12b
Black seed	1.25±0.21d	0.89±0.14d	0.67±0.15d	0.17±0.03b
Local clove	0.08±0.17d	0.60±0.17d	0.67±0.17d	0.10±0.03b
Frankincense stones	0.68±0.37d	0.24±0.34d	0.60±0.12d	0.03±0.05b
Methyl eugenol	5.76±0.44a	4.67±0.27a	3.89±0.21a	1.08±0.12a

Data illustrated in Fig. (1) showed the general mean of attracted PFF males to each tested oils all over four weeks. The average number of attracted PFF males to camphor oil (FTD = 3.26 ± 0.68 males) was statistically like methyl eugenol (FTD = 3.85 ± 1.00 males) and both treatments ranked the first position. Mint oil ranked the second (FTD = 2.77±0.81 males); while sweet almond (FTD = 1.82 ± 0.53 males) and thyme (FTD = 1.02±0.27 males) ranked the third position. With respect to the rest of the treatments, they ranked the lowest order. On another hand, Fig. (1) showed that the attraction levels of camphor, mint, sweet almond, and thyme, basil, black seed, local clove and frankincense stones oils in relation to methyl eugenol were 84.68, 71.95, 47.27, and 26.49,16.10, 8.05, 19.48, 9.35 and 10.13%, respectively, in terms of the number of PFF males lured to methyl eugenol.

The regression analysis obviously indicated that the efficiency of methyl eugenol in luring PFF males noticeably waned over time; specifically, with each passing week, there was a decrease of 1.48 PFF males/trap/day in attraction. Mint oil secured the second position, where a weekly increment in time led to a reduction of 1.18 FTDs of PFF males. In terms of camphor, sweet almond, and thyme, each weekly increase in time resulted in a decline of 0.76, 0.73, and 0.39 FTDs of PFF males, respectively (Table 3).

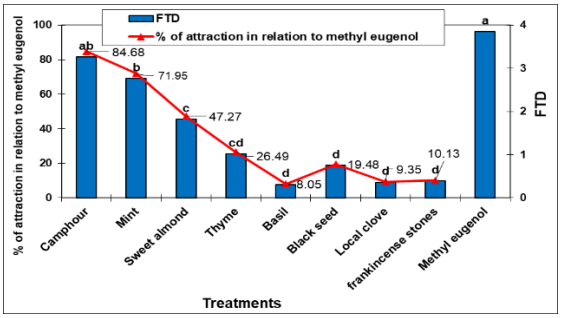

Fig. 1. General means of PFF males attracted to the tested essential oils (as FTD) and the percentage of males attracted compared to methyl eugenol over four weeks under field conditions in a citrus orchard.

Table 3. Stability of the efficiency of the tested essential oils (as the weekly attracted PFF males, FTDs) against time (as weeks, W) allover successive four weeks under field conditions of citrus orchard.

Oil	Linear relationship	R^2
Camphor	FTD = 5.17 - 0.76 W	0.528
Mint	FTD = 5.72 - 1.18 W	0.893
Sweet almond	FTD = 3.64 - 0.73 W	0.789
Thyme	FTD = 1.99 - 0.39 W	0.861
Basil	FTD = 0.25 + 0.02 W	0.013
Black seed	FTD = 1.61 - 0.35 W	0.978
Local clove	FTD = 0.33 + 0.01 W	0.003
Frankincense stones	FTD = 0.78 - 0.16 W	0.451
Methyl eugenol	FTD = 7.55 - 1.48 W	0.915

2. Comparative efficiency of certain essential oil mixed with methyl eugenol at different ratios.

Each essential oil (camphor, mint, thyme, black seed and sweet almond) was mixed with methyl eugenol in proportions of 3:1, 1:1, and 1:3, and assessed against methyl eugenol or essential oil separately without any combination. At the 1st week mixture of camphor and methyl eugenol at ratio of 1:1 proved to be the most effective, showing significantly higher attraction rates without notable differences compared to traps using methyl eugenol alone (Table,4). While camphor - methyl eugenol mixture (at ratio 3:1) ranked the second. During the 2nd, 3rd and 4th weeks, mixture of camphor or mint oils with methyl eugenol at ratio of 1:1 ranked first without notable differences compared to traps using methyl eugenol alone, while camphor or mint oils mixed with methyl eugenol at ratio (3:1) ranked second. As shown in table (4) all mixtures of sweet almond and black seed oils ranked the lowest treatments in attracting PFF males during the experimental period.

The data presented in Figure (2) demonstrated the average number of PFF males drawn to each of the essential oils (camphor, mint, thyme, black seed and sweet almond) combined with methyl eugenol in 3:1, 1:1, and 1:3 ratios, as well as to each essential oil and methyl eugenol separately over a period of four weeks. Jackson traps baited with mixture of camphor and methyl eugenol at ratio of 1:1, or 3:1 showing significantly higher attraction rates without notable differences compared to traps using methyl eugenol alone; where, mean FTDs were 11.24 ± 2.21, 10.93 ± 3.9 and 9.2 ± 6.15 males, respectively.

Table 4. Mixtures of certain essential oil-methyl eugenol (at different ratios) as attractants to PFF males allover successive four weeks under field conditions of citrus orchard.

Oil	Mixing ratio (Oil :ME.)	1st week	2nd week	3rd week	4th week
Sweet almond	Alone	2.19±0.05f	1.96±0.03g	1.64±0.14ij	0.89±0.03fgh
	3:1	2.28±0.15f	2.03±0.14g	1.64±0.28ij	1.10±0.14efg
	1:1	2.28±0.13ef	2.46±0.06g	2.14±0.17ghi	1.17±0.13efg
	1:3	2.11±0.25f	1.74±0.25g	1.42±0.29ij	0.39±0.21i
Thyme	Alone	4.96±0.21e	4.32±0.12f	3.03±0.12fgh	1.07±0.16efg
	3:1	12.11±1.05c	4.32±0.83f	3.32±0.27fg	1.24±0.06def
	1:1	3.24±0.19ef	8.42±0.43de	4.28±0.18def	1.47±0.05bcde
	1:3	13.14±0.60c	2.28±0.24g	1.82±0.12hij	0.89±0.06fgh
Mint	Alone	13.14±0.60c	10.78±0.75bc	5.60±0.13bcd	1.99±0.13a
	3:1	10.78±0.61cd	8.35±0.58de	5.78±0.38bc	1.61±0.15abcde
	1:1	9.38±0.15d	15.49±1.35a	7.85±0.62a	1.93±0.11a
	1:3	9.28±1.51d	7.64±0.39e	4.28±0.61def	1.42±0.05cde
Camphor	Alone	12.21±0.46c	10.53±1.75c	5.10±0.15cde	1.99±0.18a
	3:1	15.78±0.13b	12.32±0.32b	6.96±0.21ab	1.75±0.12abc
	1:1	19.26±2.07a	15.24±1.05a	7.32±1.71a	1.90±0.22ab
	1:3	8.61±1.15d	5.82±0.85f	4.07±0.57ef	1.39±0.14cde
Black seed	Alone	2.03±0.26f	1.82±0.18g	1.10±0.17ij	0.60±0.12hi
	3:1	2.74±0.61ef	2.32±0.17g	1.28±0.11ij	0.32±0.22i
	1:1	3.17±0.89ef	2.49±0.21g	1.17±0.27ij	0.74±0.18ghi
	1:3	2.21±0.56f	1.85±0.24g	0.74±0.17j	0.32±0.22i
ME	Alone	19.78±0.49a	15.78±0.31a	7.49±0.41a	1.92±0.09a
LSD		2.37***	1.81***	1.37***	0.43***

Note: ME means methyl eugenol

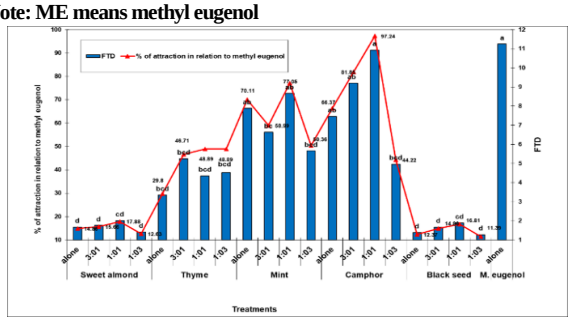

Fig. 2. General means of attracted PFF males (as FTDs) to the tested essential oil-methyl eugenol (at different ratios) in addition to their attraction as percentages of the attracted males to methyl eugenol (the recommended attractant) allover successive four weeks under field conditions of citrus orchard.

Mixture of mint-methyl eugenol at ratio of 1:1 treatment as well as mint or camphor oils alone ranked the second effective treatment as attractants for PFF males, FTDs were 8.66 ± 2.79, 7.88 ± 2.52 and 7.46 ± 2.37 males, respectively. On the other hand, mixtures of sweet almon or black seed with methyl eugenol were the lowest effective treatments. The relative attractions (in comparison with methyl eugenol) were the highest when the treatment of camphor- methyl eugenol at ratio of 1:1 was used (97.24%) followed by mint- methyl eugenol at ratio of 1:1 (77.05%), camphor- methyl eugenol at ratio of 3:1 (75.18%), mint (70.11%) and camphor (66.37%).

The relationship between the efficiency of the tested essential oil-methyl eugenol mixtures at different ratios (as FTDs) and elapsed time (as weeks) is mathematically

illustrated in Table (5). Mixtures of camphor- methyl eugenol (at 1:1 and 3:1 ratios) were relatively stable over time compared to methyl eugenol alone. Where, each elapsed time increased by one week, the captured PFF males in traps baited with methyl eugenol alone, or with mixtures of camphor-methyl eugenol (at 1:1 and 3:1 ratios) decreased by 6.19, 6.00 and 5.04 males/trap/day, respectively.

Table 5. Stability of the efficiency of the tested essential oils-methyl eugenol mixtures at different ratios (as the weekly attracted PFF males, FTDs) against time (as weeks, W) allover successive four weeks under field conditions of citrus orchard.

Treatments	Mixing ratio (Oil :ME.)	Linear relationship	R^2
Sweet almond	Alone	FTD = 2.72 - 0.42 W	0.924
	3:1	FTD = 2.74 - 0.39 W	0.973
	1:1	FTD = 2.92 - 0.36 W	0.667
	1:3	FTD = 2.78 - 0.55 W	0.916
Thyme	Alone	FTD = 6.58 - 1.30 W	0.951
	3:1	FTD = 13.65 - 3.36 W	0.834
	1:1	FTD = 6.71 - 0.94 W	0.171
	1:3	FTD = 13.83 - 3.72 W	0.694
Mint	Alone	FTD = 17.53 - 3.86 W	0.982
	3:1	FTD = 14.15 - 3.01 W	0.981
	1:1	FTD = 16.16 - 3.00 W	0.483
	1:3	FTD = 12.39 - 2.69 W	0.983
Camphor	Alone	FTD = 16.48 - 3.61 W	0.965
	3:1	FTD = 21.06 - 5.04 W	0.951
	1:1	FTD = 25.93 - 6.00 W	0.986
	1:3	FTD = 10.82 - 2.34 W	0.993
Black seed	Alone	FTD = 2.64 - 0.50 W	0.963
	3:1	FTD = 3.74 - 0.83 W	0.972
	1:1	FTD = 4.04 - 0.86 W	0.965
	1:3	FTD = 2.97 - 0.68 W	0.956
Methyl eugenol (ME)	Alone	FTD = 26.71 - 6.19 W	0.984

Discussion

The present results indicated that essential oils of camphor, mint, thyme sweet almond and black seed act as male lures for PFF adults. Camphor and methyl eugenol traps lure significantly high numbers of PFF adults, they came in the superior compared with the other tested essential oils. Mint oil ranked second in order. The attraction levels of camphor and mint oils in relation to methyl eugenol were 84.68 and 71.95%, respectively. Moreover, their attractiveness decreased gradually with time in comparison with methyl eugenol.

According to Tan (2000) males of *Bactrocera* spp. feed voraciously on methyl eugenol, a component of plant essential oil found in at least 200 species of plants. For example, methyl eugenol had been reported in clove (Moustafa *et al*., 2012), camphor (Zhang *et al.*, 2023), nutmeg (Myristrica fragrans Houtt), (Susila *et al*. (2021), thyme (Pilozo *et al.*, 2024), and basil plants (Singh *et al.*, 2020, Tangpao *et al.*, 2021 and Tine *et al.*, 2024).

Both camphor and mint essential oils exerted relatively high levels of attraction to PFF males, possibly because they contain other components that may attract PFF males. This assumption could be supported by Hernández-Sánchez *et al*. (2001), who reported that both limonene and p-cymene (isolated from the fruits of *Mangifera indica* L.) have a strong attractant effect on adults of *Ceratitis capitatia* (Wied.), which is due to their attraction to Trimedlure. Also, Nishida *et al.* (2000), Shelly *et al.* (2004) and Segura *et al.* (2018) reported that scents from plants play a role in the aggregation of male tephritid flies. Segura *et al*. (2018) added that the secondary plant metabolites may affect the sexual behavior and communication of tephritid flies, enhancing male signaling behavior, the attraction of females, and their chances to mate. So, further studies on the effects of the components of the tested essential oils as attractants for PFF (especially camphor and mint) should be done. In the present study, local clove oil was somewhat attractive to PFF males, in contrast, clove oil did not attract PFF males, but at lower concentrations it did attract *C. capitata* males (Abd El-Kareim *et al*., 2009 and Moustafa *et al*., 2012). These differences may be attributed to the differences in oil extraction resources. Moreover, the extracted essential oils are greatly affected by growing conditions (soil type, amount of water, season), the plant's genetics (Mostafa *et al*., 2020) and the environmental stresses of the plants used (Sharifi-Rad *et al*., 2017, Asadzadeh *et al*., 2023; and Zhang *et al.* 2023).

Methyl eugenol-camphor or methyl eugenol-mint mixtures (1:1 ratio) were not significantly different from methyl eugenol alone. Furthermore, these mixtures were relatively stable over time compared to methyl eugenol alone. Therefore, one might think that the amount of methyl eugenol used could be significantly reduced without significantly decreasing the number of attractive PFF males over a long period of time. These results are supported by the first experiment of this study, which stated that camphor and mint were the most attractive essential oils for PFF males and there was no difference between them. The present results are in agreements with Metwaa *et al.* (2023) and Ismail *et al.* (2024); that *Eucalyptus*, clove or lavender-methyl eugenol mixtures are higher or nearly equal to methyl eugenol alone in attracting PFF males, and their mixtures showed a relatively high stability against time in attracting PFF males (Ismail *et al*., 2024).

As a conclusion, camphor and mint essential oils could be used as attractants for PFF males alone or mixed with methyl eugenol (at a ratio of 50:50%) with no significant differences between their efficiencies and the recommended methyl eugenol as attractants for PFF males under field conditions.

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كفاءة بعض الزيوت العطرية كمواد جاذبه لذكور حشرة *Bactrocera zonata* تحت ظروف الحقل

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الملخص

أجريت هذه الدراسة ببستان موالح بمنطقة اجا محافظة الدقهلية. حيث تم تقييم ثمانية انواع من الزيوت النباتية (الكافور والنعناع واللوز الحلو والزعتر والريحان والحبة السوداء والقرنفل المحلي وحصى لبان) وميثيل يوجينول كجاذبات شمية في جذب ذكور ذبابة ثمار الخوخ إما بشكل فردي أو مخلوطًا بميثيل يوجينول. أشار التحليل الإحصائي إلى أنه في الاسبوع الثاني والثالث والرابع، أبدت ذكور الحشرة استجابة إيجابية بدرجة معنوية تجاه كل من مصائد الكافور وميثيل يوجينول ، حيث جاءت في المرتبة الأولى مقارنة بالزيوت الأساسية الأخرى التي تم اختبارها. احتل النعناع واللوز الحلو المرتبة الثانية في الترتيب يليهما الزعتر. في المقابل أبدت استجابة منخفضة تجاه زيت الريحان والحبة السوداء والقرنفل المحلي وحصى لبان بأقل عدد من الأفراد. كانت مستويات الجذب لزيوت الكافور والنعناع واللوز الحلو والزعتر مقارنة بميثيل يوجينول 84.68٪ و 71.95٪ و 47.27٪ و 26.49٪ على التوالي من حيث عدد ذكور الحشرة المنجذبة. أظهرت مصائد جاكسون المطعمة بمزيج من الكافور وميثيل يوجينول بنسبة 1: 1 معدلات جذب أعلى بشكل ملحوظ دون اختلافات معنوية مقارنة بمصائد الميثيل يوجينول فقط. علاوة على ذلك، أظهر كلا من خليط الكافور مع الميثيل يوجينول و الميثيل يوجينول وحده استقرارًا مماثلًا بمرور الوقت..