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Attraction potential of methyl eugenol-essential oils combinations for *Bactrocera zonata*
(Diptera: Tephritidae)

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Abstract

To minimize the used amounts of methyl eugenol and/or to enhance its attraction ability; the sex attractant of the peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), for detection, monitoring and controlling (Via male annihilation technique) of this deleterious insect species; combinations of methyl eugenol and clove (*Syzygium aromaticum*, Myrtaceae), *Eucalyptus* (*Eucalyptus longifolia*, Myrtaceae) and lavender (*Lavendula angustifolia*, Lamiaceae) at ratios of 1:1, 1:2 and 2:1 of each were tested for attraction potential of these combinations for males of *Bactrocera zonata* in a heavily infested citrus orchard at Giza district, Egypt using Jackson sticky traps which at random alternatively replaced. The treatment was twice repeated where traps were inspected after 2 and 7 days of the first application and after 5 days, 1, 2 and 3 weeks of the second treatment. For all treatments, clove+methyl eugenol and *Eucalyptus*+methyl eugenol attracted the highest average numbers of *B. zonata* males as FTD (Flies/Trap/Day) compared to the other treatments, whereas lavender+methyl eugenol recorded the lowest averages of FTD values. On the other hand, in the combination ratio of 1:1 and 2:1 of essential oils: methyl eugenol showed the highest averages of captured males. It could be concluded that the combinations of methyl eugenol with either clove or *Eucalyptus* at ratios of 1:1 or 1:2 of each increased the attraction potential of methyl eugenol for attracting males of *B. zonata*.

Introduction

The peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a serious insect pest for vegetables and fruits of many horticultural host plants such as apricot, peach, mango, guava, citrus and others in several countries worldwide (White and Elson-Harris, 1992). It is distributed in the

Subcontinent, Egypt (El-Minshawy *et al.*, 1999), Libya, Oman, Saudi Arabia, the United Arab Emirates, Yemen, Sudan, and the Sub-Saharan region (White and Elson-Harris, 1992).

Methyl eugenol is used as a sex attractant for males of certain species of fruit flies such as *B. zonata*. Also, food attractants

(Protein hydrolysate compounds) and olfactory stimuli (Ammonium salts) are used for attracting, especially females, of a wide range of fruit flies. Trials by several authors all over the world were conducted to minimize the used amounts of attractants or synergize attraction activity of sex or food attractants via combining or mixing them with others or with essential oils (Abd El-Kareim *et al.*, 2008; Moustafa *et al.*, 2012; Ghanim, 2013; Kardinan and Hidayat, 2013; Pinero *et al.*, 2015; Hemeida *et al.*, 2017; El-Metwally *et al.*, 2019; El-Metwally and Ragab, 2020; Singh *et al.*, 2020; GabAlla, 2021; Ghanim *et al.*, 2021 and Ragab and Elsherbeni, 2021).

This work aimed to assess or evaluate the attraction potential of methyl eugenol combined with certain essential oils (Clove, *Eucalyptus* and lavender) at different ratios to males of the peach fruit fly, *B. zonata* for minimizing the used amounts or synergizing attraction efficiency of methyl eugenol in a citrus orchard.

Materials and methods

To assess the attraction potential of methyl eugenol-certain essential oils combinations for the peach fruit fly *B. zonata* aiming to minimize the used quantities of methyl eugenol (The sex attractant of *B. zonata*) or synergize the sex attractant for controlling via Male Annihilation Technique (MAT) and monitoring of the peach fruit fly, field experiments were conducted, in a citrus orchard of about 3 feddans, on different species and varieties of citrus trees of more than 15 years, that were heavily infested with the peach fruit fly, *B. zonata*, in the Experimental Farm of Horticulture Research Institute at Giza district. The trees of about 2-2.5 meter in heights were cultivated in clay soil and irrigated by a flood system. Methyl eugenol was combined with essential oils of clove (*Syzygium aromaticum*, Myrtaceae), *Eucalyptus* (*Eucalyptus longifolia*, Myrtaceae) and Lavender (*Lavendula*

angustifolia, Lamiaceae) at the rate of 1:1, 1:2 and 2:1 for each that was carefully stirred. Sticky Jackson traps were used in three replicates for each treatment and were hung at about 2 meters of ground in the western north direction. The distance between every two adjacent traps is 15- 20 meters. The cotton wick (1.5 cm × 1 cm diameter) of each trap was saturated with 1.5 ml of each mixture. The traps were randomly hung on 18/10/2022 and inspected after two days and then a week. After that the cotton wicks were changed with new mixtures on 25/10/2022 and inspected for three weeks post treatment (after 5 days, two and three weeks on 30/10/2022, 6/11/2022 and 13/11/2022, respectively) without changing the cotton wicks to determine the persistence of the tested mixtures. The traps were alternatively replaced with their positions every inspection. Males of the peach fruit fly captured in traps were counted. The values of FTD (Flies/Trap/Day) were statistically analyzed using SPSS Programme for analysis of variance.

Results and discussion

1. First treatment:

The attraction ability of methyl eugenol combined with three essential oils (Clove, *Eucalyptus* and Lavender) at ratios of 1:1, 1:2 and 2:1 of each in citrus orchard was determined. Data compiled in Table (1) reveal that clove-methyl eugenol combination enhanced the attraction ability of methyl eugenol after two days of the first treatment and significantly showed the highest average value of FTD (124.94) that insignificantly varied with those of *Eucalyptus*-methyl eugenol combination (FTD = 106.05) and methyl eugenol alone (FTD = 105.50). The attraction ability of both *Eucalyptus*-methyl eugenol mixture and methyl eugenol alone was nearly the same and insignificantly differed from that investigated by lavender-methyl eugenol combination which recorded the lowest average value of FTD of 88.78.

Table (1): Attraction ability of certain essential oils-methyl eugenol combinations for males of *Bactrocera zonata* in a *Citrus* orchard after 2 days of the 1st treatment at Giza district.

Treatments	Combination ratio						Average
	1:1		1:2		2:1		
	FTD*	Fold	FTD	Fold	FTD	Fold	
Clove+Methyl eugenol	148.00a	1.40	127.33ab	1.21	99.50bc	0.94	124.94A
<i>Eucalyptus</i> +Methyl eugenol	86.33c	0.82	105.50bc	1.00	126.33ab	1.20	106.05AB
Lavender+Methyl eugenol	137.00a	1.30	83.00c	0.79	46.33d	0.44	88.78B
Methyl eugenol	105.50bc						105.50AB
Average	119.21A		105.33AB		94.42B		-
"F" value of compounds (LSD)					9.8479** (26.31)		
"F" value of combination ratio (LSD)					7.3543** (22.46)		
"F" value of compounds × combination ratio (LSD)					11.1230** (59.84)		

* FTD (Flies/ Trap/ Day) = Total no. of captured flies/ Traps × Exposure time.

On the other hand, in the used concentrations of 1:1 and 1:2 of essential oils: methyl eugenol insignificantly showed the highest values of FTD of 119.21 and 105.33, respectively. But the concentration of 2 essential oils: 1 methyl eugenol significantly recorded the lowest FTD value of 94.42 which insignificantly varied with the concentration of 1 essential oils: 2 methyl eugenol. Clove+methyl eugenol at 1:1 and 1:2, *Eucalyptus*+methyl eugenol at 2:1 as well as lavender+methyl eugenol at 2:1 recorded the highest FTD values showing an enhancement in attraction ability by 1.40, 1.21, 1.20 and 1.30 fold of the sex attractant of males of *B. zonata* (Methyl eugenol).

The attraction ability of the tested combinations of essential oils+methyl eugenol for males of the peach fruit fly after a week of the first treatment was tabulated in Table (2). Statistical analysis of variance of data proved that the differences between the

used treatments were highly significant, whereas the differences between the tested combination ratios were insignificant. Irrespective of the combined ratio, clove+methyl eugenol insignificantly differed from *Eucalyptus*+methyl eugenol which recorded FTD averages of 48.29 and 45.84, respectively. The attraction ability of mixture of lavender plus methyl eugenol (FTD value = 33.78) insignificantly differed from that of methyl eugenol alone (FTD = 37.40). The average number of captured males of *B. zonata* for the used ratios of combination ranged between 40.78- 41.83. The significant differences in mixtures × combination ratio indicate that clove+methyl eugenol at 1:1 as well *Eucalyptus*+methyl eugenol at 1:2 and 2:1 increased methyl eugenol-attraction capability by 1.55, 1.3 and 1.45 fold of methyl eugenol alone (As a reference), respectively.

Table (2): Attraction ability of certain essential oils-methyl eugenol combinations for males of *Bactrocera zonata* in a *Citrus* orchard after a week of the 1st treatment at Giza district.

Treatments	Combination ratio						Average
	1:1		1:2		2:1		
	FTD	Fold	FTD	Fold	FTD	Fold	
Clove+Methyl eugenol	57.93a	1.55	41.93cde	1.12	45.00bcd	1.20	48.29A
<i>Eucalyptus</i> +Methyl eugenol	34.80def	0.93	48.60abc	1.30	54.13ab	1.45	45.84AB
Lavender+Methyl eugenol	33.00ef	0.88	39.40cdef	1.05	28.93f	0.77	33.78C
Methyl eugenol	37.40cdef						37.40BC
Average	40.78		41.83		41.37		-
"F" value of compounds (LSD)					11.6287** (10.09)		
"F" value of combination ratio					1.8456 ns		
"F" value of compounds × combination ratio (LSD)					6.9325* (10.31)		

FTD (Flies/ Trap/ Day) = Total no. of captured flies/ Traps × Exposure time.

2. Second treatment:

As shown in Table (3) the differences in attraction ability after 5 days of the second treatment between both tested combinations as well used combination ratios significantly varied at 0.05 and 0.01% probability, respectively. The three tested mixtures (Clove+methyl eugenol, *Eucalyptus*+methyl eugenol and lavender+methyl eugenol) insignificantly increased the attraction ability of methyl eugenol showing FTD values of 42.84, 52.02 and 37.60, respectively. The attraction ability of the latest mixture (lavender+methyl eugenol) insignificantly

differed from that of methyl eugenol alone (FTD value = 30.60). Respecting the used combination ratios, the ratio of 1:1 and 2:1 of essential oils: methyl eugenol insignificantly recorded the highest averages of FTD values of 42.78 and 48.33, respectively. Clove+methyl eugenol at 2:1 and *Eucalyptus*+methyl eugenol at 1:1 insignificantly showed the highest values of FTD of 68.60 and 78.13 increasing the attraction capability of methyl eugenol by 2.24 and 2.55 fold of methyl eugenol alone, respectively.

Table (3): Attraction ability of certain essential oils-methyl eugenol combinations for males of *Bactrocera zonata* in a citrus orchard after 5 days of the 2nd treatment at Giza district.

Treatments	Combination ratio						Average
	1:1		1:2		2:1		
	FTD	Fold	FTD	Fold	FTD	Fold	
Clove+Methyl eugenol	33.60cde	1.10	26.33e	0.86	68.60a	2.24	42.84A
<i>Eucalyptus</i> +Methyl eugenol	78.13a	2.55	27.20e	0.89	50.73b	1.66	52.02A
Lavender+Methyl eugenol	28.80de	0.94	40.60bcd	1.33	43.40bc	1.42	37.60AB
Methyl eugenol	30.60cde						30.60B
Average	42.78A		31.18B		48.33A		-
"F" value of compounds (LSD)					8.5352* (11.42)		
"F" value of combination ratio (LSD)					29.1934** (9.07)		
"F" value of compounds × combination ratio (LSD)					20.2188** (10.79)		

FTD (Flies/ Trap/ Day) = Total no. of captured flies/ Traps × Exposure time.

Statistical analysis of data proved that the differences between clove+methyl eugenol and *Eucalyptus*+methyl eugenol, on side, and between clove+methyl eugenol, lavender+methyl eugenol and methyl eugenol alone, on another side, were insignificant (Table 4). The combination of *Eucalyptus*+methyl eugenol recorded the highest average number of capture flies an FTD value of 29.86 after two weeks of the second treatment, whereas lavender+methyl eugenol mixture showed the lowest FTD value (18.02). On the other hand, the tested combination ratios statistically showed

significant differences. In the combination ratio of 1:1 and 2:1 of essential oils: methyl eugenol insignificantly recorded the highest FTD averages of 23.30 and 28.92, respectively. The ratio of 1:1 of clove+methyl eugenol and *Eucalyptus*+methyl eugenol as well the ratio 1:2 of *Eucalyptus*+methyl eugenol and the ratio of 2:1 of the three used compounds insignificantly synergized attraction ability of methyl eugenol to 1.08, 1.23, 1.20, 1.52, 1.47 and 1.05 fold of methyl eugenol alone (The reference).

Table (4): Attraction ability of certain essential oils-methyl eugenol combinations for males of *Bactrocera zonata* in a *Citrus* orchard after 2 weeks of the 2nd treatment at Giza district.

Treatments	Combination ratio						Average
	1:1		1:2		2:1		
	FTD	Fold	FTD	Fold	FTD	Fold	
Clove+Methyl eugenol	24.81	1.08	15.95	0.69	34.86	1.52	25.21AB
<i>Eucalyptus</i> +Methyl eugenol	28.29	1.23	27.57	1.20	33.71	1.47	29.86A
Lavender+Methyl eugenol	17.10	0.74	12.86	0.56	24.10	1.05	18.02B
Methyl eugenol	23.00						23.00B
Average	23.30AB		19.85B		28.92A		-
"F" value of compounds (LSD)					12.0767** (5.83)		
"F" value of combination ratio (LSD)					11.4500** (6.69)		
"F" value of compounds × combination ratio					2.4052 ns		

FTD (Flies/ Trap/ Day) = Total no. of captured flies/ Traps × Exposure time.

Data compiled in Table (5) indicate that the attraction capability of the used combinations insignificantly reduced after 3 weeks post treatment showing FTD averages of 22.00, 24.03 and 19.86 that insignificantly lower than that recorded with methyl eugenol alone (25.14). On the other hand, and

respecting the combined ratio, the increase of essential oils, the increment of FTD value. The ratios of 1:1 and 2:1 of essential oils : methyl eugenol insignificantly showed the highest FTD averages of 25.32 and 24.88 that significantly differed from that recorded with a combination ratio of 1:2 (FTD = 18.07).

Table (5): Attraction ability of certain essential oils-methyl eugenol combinations for males of *Bactrocera zonata* in a *Citrus* orchard after 3 weeks of the 2nd treatment at Giza district.

Treatments	Combination ratio						Average
	1:1		1:2		2:1		
	FTD	Fold	FTD	Fold	FTD	Fold	
Clove+Methyl eugenol	25.14	1.00	17.29	0.69	23.57	0.94	22.00
<i>Eucalyptus</i> +Methyl eugenol	22.71	0.90	15.67	0.62	33.71	1.34	24.03
Lavender+Methyl eugenol	28.29	1.13	14.19	0.56	17.10	0.68	19.86
Methyl eugenol	25.14						25.14
Average	25.32A		18.07B		24.88AB		-
"F" value of compounds					0.3982 ns		
"F" value of combination ratio (LSD)					9.3187** (6.64)		
"F" value of compounds × combination ratio					1.2716 ns		

FTD (Flies/ Trap/ Day) = Total no. of captured flies/ Traps × Exposure time.

As shown in Figure (1) both mixtures of *Eucalyptus*+methyl eugenol and clove+methyl eugenol at ratios 1:1 and 2:1 for each as well as lavender+methyl eugenol at 1:1 recorded the highest averages of FTD values comparing to the other treatments showing an enhancement of attraction potential of methyl eugenol. The ratio of 1:2

of both clove and *Eucalyptus* combined with methyl eugenol showed nearly the same values of FTD which are approximately equal to that of methyl eugenol alone, whereas the same ratio of lavender+methyl eugenol as well as the ratio of 2:1 of the latest combination decreased methyl eugenol activity for attraction.

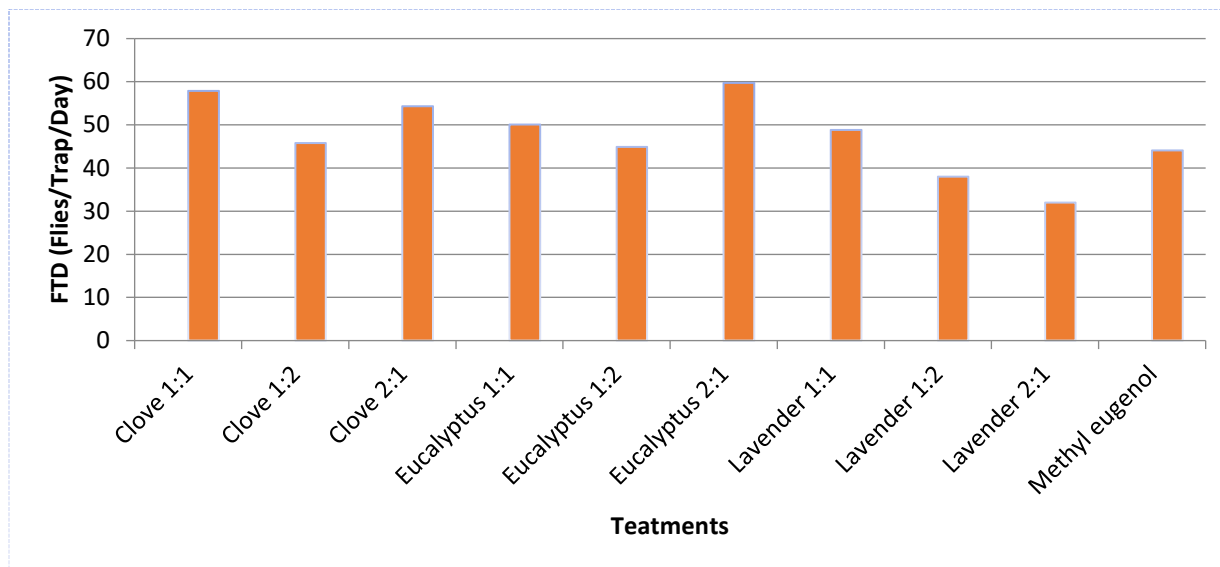


Figure (1): Averages of FTD values of essential oils-methyl eugenol at different ratios during the period of investigation.

The attraction ability of essential oils alone or essential oils mixed with sex or food attractants of fruit flies aiming to minimize the used amounts of attractants and/or to synergize attraction activity of the attractants was assessed by several authors all over the world. The previous data confirmed the obtained results which indicated that the tested combinations of essential oils+methyl eugenol enhanced the attraction potential of *B. zonata* males. Several authors tried to evaluate botanical extracts as attractants for fruit flies such as Abd El-Kareim *et al.* (2008) who bioassayed eight different plant oil extracts [Olive, orange, peppermint, basil, clove (Egyptian and French), parsley and black cumin] for *B. zonata* and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) males and females and found that *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.

Moustafa *et al.* (2012) mentioned that clove oil at a concentration of 50%, gave the highest attraction of *C. capitata* males, compared to trimedlure (As a reference attractant). The prepared (Local) methyl

eugenol (50,100% concentration) has approximately the same efficacy in attracting *B. zonata* males as well as imported methyl eugenol. Kardinan and Hidayat (2013) determined the potency of leaf extracts of *Melaleuca bracteata* and *Ocimum* sp. as fruit fly attractants against the *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) complex in guava and star fruit orchard and reported that both essential oils showed high potential as fruit fly attractants. For more than 2 weeks they attracted male fruit flies in guava and star fruit orchards as effectively as commercial methyl eugenol.

Results of El-Gendy *et al.* (2020) showed the potentiality of the essential oil of berries of cubeb pepper, *Piper cubeba* as an attractant for *B. zonata* flies and reported that methyl eugenol and the cubeb pepper essential oils insignificantly attracted males of *B. zonata* only. Singh *et al.* (2020) tested the attraction ability of the different parts (leaf, stem, inflorescence and root extracts) of basil plants (*Ocimum sanctum* L., Lamiaceae) for fruit flies compared to methyl eugenol and found that *Bactrocera cucurbitae* (Coquillett)

(*Diptera: Tephritidae*) attracted to all the basil plant parts in maximum numbers.

GabAlla (2021) conducted a laboratory evaluation on the attractiveness of some plant extracts (Guava, melon, mango, banana, apricot and orange) and plant oils (Anise, cinnamon, cloves, camphor and flaxseed) against *C. capitata* and stated that guava, mango and banana extracts attracted the highest adults of medfly. Cloves, cinnamon and anise oils were the best for attracting medfly adults.

On the other side, many researchers combined the food attractants (Protein hydrolysate compounds) and/or olfactory stimuli (Ammonium salts) for each as baits for fruit flies. Ghanim *et al.* (2014) evaluated the efficacy of six ammonium compounds (Tri-ammonium phosphate, ammonium carbonate, ammonium acetate, ammonium chloride, ammonium thiocyanate and ammonium dihydrogen phosphate at 1, 2, 3, 4 and 5% for each) as lures for the adults of zizyphus fruit fly, *Carpomya incompleta* (Becker) (*Diptera: Tephritidae*).

C. incompleta adults showed different degrees of preference for the different tested ammonium compounds. However, tri-ammonium phosphate attracted the highest numbers of *C. incompleta*. Pinero *et al.* (2015) tested the response of male and female Mediterranean fruit fly, *C. capitata*, to seven commercially available protein baits and to beer waste, alone or in combination with either ammonium acetate or ammonium carbonate.

The authors found that the presence of ammonium acetate, not ammonium carbonate, elicited a significantly greater level of response in female *C. capitata* compared to the protein baits alone. The addition of ammonium acetate to selected baits increased bait attractiveness to a level comparable with that elicited by the most widely used spinosad-based protein bait, GF-120. Hemeida *et al.* (2017) tried to enhance

buminal, agrinal and amadene (Protein-based baits) in attracting *B. zonata* using ammonium acetate and diammonium phosphate under the field conditions at ratios of 1:1, 2:1 and 1:2, respectively. Results showed that each of agrinal or amadene can be enhanced by mixing any of them with diammonium phosphate or ammonium acetate at a ratio of 1:1 which resulting significantly higher attraction ability of *B. zonata* especially females; while, buminal can be enhanced by mixing it with di-ammonium phosphate at the same ratio.

Ghanim *et al.* (2021) evaluated ammonium acetate and di-ammonium phosphate as attractants for the peach fruit fly *B. zonata* at ratios of 1, 2 and 3% of each ammonium compound lonely or mixed between them interchangeably, concluding that it may be useful in applying for integrated pest management control programs by using mixtures of ammonium acetate and di-ammonium phosphate (Ammonium acetate 3% mixed with di-ammonium phosphate 3 or 1%) because of its good attraction for *B. zonata* adults.

The obtained data revealed that the tested essential oils, in general, enhanced the attraction potential of methyl eugenol throughout 2 weeks post treatment. Thus, these results are in harmony with the previous trials which used sex attractants-essential oils mixtures such as Ghanim (2013) who diluted methyl eugenol with paraffin oil to reduce the quantity of methyl eugenol at five concentrations of methyl eugenol (10, 25, 50, 75 and 100%) and mixed it with fentrithion, spinosad and a mixture of thiamethoxam+abamactin and found that dilution of methyleugenol in paraffin oil till 50% in male annihilation technique by using spinosad (As an insecticide) did not significantly affect the captured males and had a high effect against *B. zonata* population.

El-Metwally *et al.* (2019) used a dilution of trimedlure with different oils (Paraffin, soya bean, sunflower and castor) and found that adding paraffin or castor oils to trimedlure with a concentration of 25% attracted the highest numbers of *C. capitata* males all over eight weeks; while, the lowest numbers were shown with those of adding 50% of soya bean or castor oils to trimedlure. Also, efficiency of methyl eugenol diluted with oleic acid was evaluated by El-Metwally and Ragab (2020) against the peach fruit fly, *B. zonata* at concentrations of 98, 75, 50 and 25 %. Results indicated that *B. zonata* males showed different degrees of attraction for the different tested concentrations.

Efficiency of methyl eugenol attraction ability at 75% slightly decreased by time. But, the results of Akter *et al.* (2021) contradicted the obtained data who investigated the application of plant essential oils (Basil, clove, citronella, and eucalyptus) with methyl eugenol and cuelure for attraction of *B. dorsalis* and *B. cucurbitae* and they found that 15.7- 20.3% of attraction for *B. dorsalis* using methyl eugenol, separately or mixed with basil oil. The separate cuelure gave 20.3%, while methyl eugenol+cuelure mixture provided 21.5 % attraction for *Z. cucurbitae*. In field tests, a greater number of *B. dorsalis* were trapped with methyl eugenol alone, whereas the attraction of the methyl eugenol and basil oil mixture was relatively low. For *Z. cucurbitae*, methyl eugenol+cuelure and cuelure alone trapped more flies than mixtures combining basil oil.

Ragab and Elsherbeni (2021) estimated the efficacy of the sex attractant, trimedlure diluted with oleic acid against the Mediterranean fruit fly, *C. capitata* in guava and mandarin at four concentrations (25, 50, 75 and 98 %). Results illustrated that the highest general mean of *C. capitata* males was attracted by trimedlure at a concentration of 75% followed by 50, 98 then 25%,

represented by 5.48, 3.74, 3.48 and 1.8 respectively as FTD in the first 5 weeks. Also, in the second 5 weeks of the study concentration 75% recorded the highest FTD (5.62) compared to concentrations of 50%, 98% and 25%.

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