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Stability of Methyl Eugenol-Essential Oils Combinations as Attractants for Bactrocera Zonata (Saunders) (Diptera: Tphritidae) Males Under Field Conditions

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ABSTRACT

The sex attractant (methyl eugenol) of Bactrocera zonata (Saunders) (Diptera: Tephritidae) males combined with botanical essential oils to enhance attraction ability, increase stability and/or reduce the used quantity of attractant. The present study aimed to determine stability and attractiveness of methyl eugenol mixed with lavender (Lavendula angustifolia) at 1:1, Eucalyptus (Eucalyptus longifolia) with 1:2 and clove (Syzygium aromaticum) at 2:1. Attraction potential and stability of the used combinations differently varied. Clove and lavender-methyl eugenol combinations (1:1) as well clovemethyl eugenol (2:1) nearly equal methyl eugenol alone in attraction of *B. zonata* males. But, *Eucalyptus*-methyl eugenol (1:1) and (2:1) slightly and highly more attractable for the peach fruit fly than methyl eugenol alone, respectively. In general *Eucalyptus*-methyl eugenol combination (2:1) enhanced attraction potential and stability of methyl eugenol as attractant for males of *B. zonata* for about 8 weeks.

KEYWORDS: Bactrocera zonata, methyl eugenol, Syzygium aromaticum, Eucalyptus longifolia, Lavendula angustifolia.

1. INTRODUCTION

The sex attractant, methyl eugenol was used for detection and monitoring a lot of species of the tephritid fruit flies specially belonging to *Bactrocera* genus such as peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) which attack several horticultural crops causing severe quantitative and qualitative damage reducing both local and national

income. Also, methyl eugenol was applied as mixture with pesticides for controlling fruit flies via Male Annihilation Technique (MAT) at ratio of 4 methyl eugenol : 1 pesticide at least (Ghanim et al., 2010; Khan et al., 2015; Nishida&Tan, 2016; Jang etal., 2017; Gargiulo et al., 2021 and Sim et al., 2022). The peach fruit fly was firstly recorded in Egypt in the latest decade of the twentieth century (El-Minshawy et al., 1999). The much used amounts of methyl eugenol in either monitoring or control application, necessarily require the applied quantities of the sex attractant to be minimized via combining methyl eugenol with other compounds such as botanical oils (El-Metwally et al., 2019 and El-Metwally&Ragab, 2020). The attraction potential of methyl eugenol in traps for monitoring or in controlling application reduced or declined with time (Abd El-Kareim et al., 2008; El-Adlyet al., 2018; El-Metwally et al., 2019; El-Metwally&Ragab, 2020).

This work aimed to determine stability or rate of decline and attraction activity of methyl eugenol-essential oils combinations to attract males of the peach fruit fly in citrus orchard throughout two months.

2. MATERIALS AND METHODS

Determination decline rate of methyl eugenol combined with certain essential oils for attracting B. zonata males for the purpose of determining stability of the used combinations and the effective longest period of the used combinations for attracting males of B. zonata (as monitoring method or in Male Annihilation Technique (MAT)). Experiments on different species and varieties of citrus trees (aged more than 15 years) in citrus orchard of about three feddans in the Experimental Farm of Horticulture Research Institute, Giza district took place. The farm was heavily infested with the peach fruit fly and surrounded by several horticultural crops or fruit trees (i.e. mango, olive and others). Flood system was used to irrigate trees heighted about 2-2.5 meter that cultivated in clay soil. Methyl eugenol was individually combined with essential oils of Lavender (Lavendula angustifolia, Lamiaceae), Eucalyptus (Eucalyptus longifolia, Myrtaceae) and clove (Syzygium aromaticum, Myrtaceae). All of the tested oils were mixed with methyl eugenol at ratios of 1:1, 1:2 and 2:1 and compared with methyl eugenol alone without mixing. Three replicates of Jackson traps were hung for each treatment at about two meters height in the northern-west direction of trees. The distances between traps were 15-20 meters. Each trap cotton wick was completely dipped in 1.5 ml of each mixture till saturation. The traps were hung at completely random design on and inspected 25/10/2022 every week throughout eight successive weeks without changing the tested compounds. Positions of traps were weekly changed. Captured males counted to assess FTD values were (Flies/Trap/Day). The sticky cardboards inside traps were renewed weekly.

3. RESULTS

Data in Table (1) and Figures (1-3) indicate stability or decline rates of the used combinations of the selected botanical oils with methyl eugenol throughout successive eight weeks. Clove+methyl eugenol at 1:1 persisted through the first five weeks post-treatment and recording gradual percentage of decline related to the first week in attraction ability (represented as FTD values for males of *B. zonata*) of 26.16, 25.18, 50.89and 50.89 for the 2nd, 3rd, 4th and 5th respectively. weeks post-treatment. Clove+methyl eugenol at 1:2 nearly showed the same trend to record percentage of decline in attraction potential of 39.42, 34.33 and 46.52 after 2, 3 and 4 weeks of treatment, respectively. Whereas the attraction potential of clove+methyl eugenol at 2:1 rapidly and sharply declined after a week of treatment showing 49.18% during the second week post-treatment.

Respecting Eucalyptus+methyl eugenol combinations, the attraction potential of the combination at 1:1 sharply and rapidly declined by 63.79% of the first week after two weeks post-treatment, but that recorded for the combination rate of 1:2 (Eucalyptus+methyl eugenol) slightly prolonged to a month showing 44.85% reduction in attraction potential on the 4th week post-treatment. In the conterary, the combination rate of 2:1 was more and long stable showing slight and gradual decrement in FTD values to record 49.32, 43.60 and 51.57% reduction in attraction ability after 4, 5 and 6 weeks post-treatment, respectively (Table, 1 and Figures, 4-6).

		FTD and decline rate after successive periods (week)														
Treatment		1 2		3		4		5		6		7		8		
		FTD	FTD	% Decl.												
Clove + Methyleugenol	1:1	33.60	24.81	26.16	25.14	25.18	16.50	50.89	16.50	50.89	14.86	55.77	8.24	75.48	14.00	58.33
	1:2	26.33	15.95	39.42	17.29	34.33	14.08	46.52	10.83	58.87	7.57	71.25	8.57	67.45	4.10	84.43
	2:1	68.60	34.86	49.18	23.57	65.64	22.63	67.01	22.11	67.77	16.10	76.53	4.43	93.54	11.19	83.69
Eucalyptus + Methyl eugenol	1:1	78.13	28.29	63.79	22.71	70.93	26.38	66.24	20.11	74.26	19.29	75.31	14.71	81.17	17.10	78.11
	1:2	27.20	27.57	- 1.36	15.67	42.39	15.00	44.85	9.33	65.70	4.29	84.23	3.20	88.24	3.14	88.46
	2:1	50.73	33.71	33.55	33.71	33.55	25.71	49.32	28.61	43.60	24.57	51.57	20.95	58.70	36.00	29.04
Lavender + Methyleugenol	1:1	28.80	17.10	40.63	28.29	1.77	14.71	48.92	25.78	10.49	13.95	51.56	11.57	59.83	12.24	57.50
	1:2	40.60	12.86	68.33	14.19	65.05	13.88	65.81	21.17	47.86	9.67	76.18	3.87	90.47	6.52	83.94
	2:1	43.40	24.10	44.47	17.10	60.60	19.71	54.59	18.78	56.73	10.00	76.96	4.71	89.15	4.86	88.80
Methyl eugenol		30.60	23.00	24.84	23.86	22.03	19.33	36.83	24.61	19.58	18.57	39.31	6.52	78.69	16.81	45.07

 Table 1. Decline rate of certain botanical essential oils-methyl eugenol combinations throughout eight successive weeks of exposure under field conditions of citrus orchard.

% Decline (% Decl.) = [(FTD of the first week – FTD of the other week)/ FTD of the first week] $\times 100$

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Fig. 1. Decline rates of Clove+methyl eugenol combinations at ratio of 1:1 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 2. Decline rate of Clove+methyleugenol at 1:2 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 3. Decline rate of Clove+methyleugenol at 2:1 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 4. Decline rate of *Eucalyptus*+methyleugenolat1:1 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 5. Decline rate of *Eucalyptus*+methyleugenol at 1:2 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 6. Decline rate of *Eucalyptus*+Methyleugenol at 2:1 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.

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The three combination rates of lavender+methyl eugenol (1:1, 1: 2 and 2:1) were greatly and highly unstable, where all combination rates sharply declined by 40.63, 68.33 and 44.47% of the first week after two weeks of treatment, respectively (Table, 1 and Figures, 7-9).

Attraction potential of methyl eugenol alone gradually decreased showing percentage of decline ranges from 19.58 to 39.31% throughout six weeks post-treatment to record the highest reduction percentage of 78.69 after 7 weeks of treatment (Table, 1 and Figure, 10).

In general, the attractivness of the tested combinations throughout eight successive weeks of exposure post-treatment differently varied according to the type of essential oil and rate of mixing (Figure, 11). Mixtures of methyl eugenol with clove or lavender at 1:1 for each were nearly equal methyl eugenol alone showing grand average of FTD values of 19.21, 19.06 and 20.41 males, respectively. But, attraction ability at ratio 1:2 of the three tested mixtures as well as lavender+methyl eugenol at 2:1 were lower than that of methyl eugenol alone recording mean FTDs of 13.09, 13.18, 15.35 and 17.83 males, respectively. On the other hand, Euclyptus+methyl eugenol combination at 1:1 and 2:1 as well as clove+methyl eugenol at 2:1 obviously surpassed methyl eugenol showing FTD grand averages of 28.34, 31.75 and 25.44 males, respectively.



Fig. 7. Decline rate of Lavender+Methyleugenol at 1:1 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 8. Decline rate of Lavender+Methyleugenol at 1:2 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 9. Decline rate of Lavender+Methyleugenolat 2:1 in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 10. Decline rate of Methyl eugenol in attracting *B. zonata* males throughout successive eight weeks under field conditions of citrus orchard.



Fig. 11. Comparative grand FTD values of the tested methyl eugenol-essential oils combinations throughout eight successive weeks under field conditions of citrus orchard.

4. **DISCUSSION**

Several trials were conducted to increase attraction potential and/or stability of sex attractants of insects such as the tephritid fruit flies by adding botanical oils at different rates in order to reduce the used amounts of sex attractants. The obtained data proved that Eucalyptus mixed with methyl eugenol at ratios of 2:1 and 1:1 as well as methyl eugenol with clove at 2:1 enhanced both attraction ability and stability of methyl eugenol as attractant for B. zonata males under field conditions. Previous studies of several researchers showed variable differences between certain botanicals or mixtures of sex attractants of fruit flies with botanicals which confirmed the obtained data. Ghanim (2013) reported that dilution of methyl eugenol in paraffin oil till 50% in male annihilation technique (MAT) (using spinosad as an insecticide) did not significantly affect the captured males and prolonged till ten successive weeks with a relatively high effect against B. zonata population. El-Metwally et al. (2019) found that trimedlure mixed with sun flower oil or paraffin oil at ratios of 25 or 50% enhanced the stability of trimedlure as attractant for Ceratitis capitata (Wied.) males. Casana-Gineret al. (2001) tested 79 volatile compounds emitted by C. capitata males, fruits and food baits, for attracting females of C. capitata in field trials comparing to trimedlure. Their results showed that the mixtures of ammonium compounds with corn steep liquor were the most efficient for females. Mixture ethyl acetate1acetic acid1-ethanol exhibited attraction ability for the two sexes, whereas 1.3-diethylbenzene, borneol and p-cymene was effective for females only. Navarro-Llopis et al. (2008) recorded significant differences among used types of traps and dispensers for attracting C. capitata showing noticeable variation in lifetime between dispensers. Epsky and Niogret (2017) bioassayed short-range attraction of sterile males of C. capitata to natural oils such as cubeb, angelica, manuka, tea tree, orange and ginger root. They found that oils of raw angelica, manuka and cubeb were more attractive for males than hexane-dilutions of the same oils, however more males were attracted to orange oil and tea tree oil of $100 \mu g/\mu L$ and to ginger root oil of $10\mu g/\mu L$. Overall, the highest attraction of sterile males (53%) was to tea tree oil (500 μ g).

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El-Adlyet al. (2018) found under field conditions that composite dispensers prolonged the effectiveness of methyl eugenol for attracting males of B. zonata to18 weeks or longer compared to 8 weeks only for cotton wick dispenser. They added that attraction ability of cotton wick dispenser was insignificantly higher than that of composite throughout the first six week. But, from the 7th week till the end of the experiments, composite dispensers were significantly higher to attract males. Efficacy of composite dispenser insignificantly declined by the time; while, methyl eugenol release from cotton wick dispenser significantly decreased with time. On other side, El-Metwally et al. (2019) evaluated dilutions of trimedlure with certain oils (sun flower, castor, paraffin and soya bean) for attracting C. capitata under field conditions and recorded that paraffin- or castor oils-trimedlure mixtures at 25% attracted the highest mean numbers of males throughout eight weeks. While, the lowest mean numbers of attracted males exhibited with 50% of either sova bean or castor oils added to trimedlure. Treatments diluted with paraffin oil were the most stable through eight weeks, whereas, the most inhibited treatments were those diluted with soya bean oil. El-Metwally and Ragab (2020) evaluated efficiency of methyl eugenololeic acid combination against B. zonata males at ratios of 98, 75, 50 and 25%. The reported that B. zonata males showed different levels of the used attractancy for concentrations. Attractiveness regression of tested concentrations to over 70 days males showed variation in attraction potential regarding used concentration. However, the efficiency of methyl eugenol at 75% slightly decreased by the compared to time the other tested concentrations. Akter et al. (2021) applied botanical oils (basil, citronella, clove and eucalyptus) with methyl eugenol and cue lure as attractant for Bactrocera dorsalis and Zeugodacus cucurbitae and recorded that 15.7-20.3% of attraction for *B. dorsalis* with methyl eugenol separately or combined with basil oil. The separate cue lure exhibited 20.3% attractiveness, while methyl eugenol+cue lure mixture recorded 21.5 % attraction for Z. cucurbitae. A greater number of B. dorsalis, in field tests were captured with methyl eugenol alone (FTD =20.4), whereas the attraction of the

methyl eugenol+basil oil was relatively low. Ragab and Elsherbeni (2021) diluted trimedlure with oleic acid to estimate attraction of C. capitata males at ratios of 25, 50, 75 and 98 %. They found that the highest general mean of C. capitata males, in the first 5 weeks, was attracted by trimedlure at 75% followed by 50, 98 then 25% as FTD values of 5.48, 3.74, 3.48 and 1.8, respectively. Also, in the second five weeks, 75% recorded the highest FTD (5.62). The general FTD all over 10 weeks at 75% recorded the highest FTD (5.55) followed by 50% (2.29), 98% (2.73) and 25% (1.82) males/trap/day), respectively. Hasnain et al. (2022) investigated how B. zonata males and females responded to potassium hydroxide, guava pulp, jaggery, protein hydrolysate, papaya powder and kachri powder as well as ammonium compounds additive effect. trimethylamine and putrescine as food attractants. Tested bait combinations attracted high numbers of *B. zonata* males as compared to females. Protein hydrolysate with jaggery, KOH, papaya, and kachri powder, and guava pulp showed low attractancy to *B. zonata* adults. The mixture of ammonium acetate. trimethylamine and putrescine mixed with base baits recorded a synergistic effect for the attraction of more B. zonata adults (males and females) compared to two or single componentbased baits. Hu et al. (2022) claimed that sexually mature male of B. dorsalis were strongly attracted to clove bud essential oil. Mature males differently responded throughout the day; the strongest response was elicited during the day and decreased at sunset. Vázquezet al. (2022) conducted field testing to evaluate the effectiveness and longevity of 2C [ammonium acetate and putrescine] and 3C putrescine, and [ammonium] acetate. trimethylamine] synthetic lures for the Caribbean fruit fly, Anastrepha suspensa. The torula yeast-borax mixture attracted the highest mean number of A. suspensa, according to the data obtained. Compared to 3C lures. commercial 2C lures attracted more flies. In contrast, the 3C bait lost its attraction power after 5–6 weeks, while the 2C lure started to lose it after 6-8 weeks. Under field conditions, 2C cone lures were often more attractive to A. suspensa than 3C cone lures, and they remained attractive for up to 8 weeks.

On contrary, Abd El-Kareim*etal.* (2008) used 8 plant oil extracts [peppermint, basil, olive, orange, French & Egyptian clove, black cumin and parsley] as attractants for both sexes of *B. zonata* and *C. capitata* and recorded that the residual effectiveness of French and Egyptian clove oil was lost after 9 days of exposure. Significant differences between the imported and local methyl eugenol after 6, 12 and 18 days were obtained.

5. CONCLUSION

The synthetic sex attractants of fruit flies could be combined with certain botanical essential oils to enhance the attraction ability, increase stability (releasing time) of attractants and/or in purpose reducing the used quantity of sex attractants. So, the present study revealed that Eucalyptus-methyl eugenol at 2:1 and 1:1 enhanced attraction potential and stability of methyl eugenol for *B. zonata* males throughout eight successive weeks of exposure. So, Eucalyptus essential oil could be added to methyl eugenol for monitoring and/or male annihilation application.

List of Abbreviations

FTD (Flies/Trap/Day)

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الملخص العربى

Bactrocera zonata (Saunders) ثبات مخاليط الميثيل ايجنول والزيوت العطرية كجاذبات لذكور (Diptera: Tphritidae) تحت الظروف الحقلية

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تم خلط الجاذب الجنسي (ميثيل ايجينول) الخاص بذكور (Diptera: Tephritidae) (Eucocera zonata (Saunders) (Diptera: Tephritidae) الزيوت العطرية النباتية لتعزيز القدرة على الجذب وزيادة الثبات و/أو تقليل الكمية المستخدمة من المواد الجاذبة. تهدف الدراسة الحالية إلى تحديد مدى ثبات وجاذبية الميثيل ايجينول المخلوط مع اللافندر (Lavendula angustifolia) بنسبة ١:١، والكافور (الحالية إلى تحديد مدى ثبات وجاذبية الميثيل ايجينول المخلوط مع اللافندر (Syzygium aromaticum) بنسبة ١:١، والكافور (الحالية إلى تحديد مدى ثبات وجاذبية الميثيل ايجينول المخلوط مع اللافندر (Syzygium aromaticum) بنسبة ١:١، والكافور (١:١) على جذب الذكور واختلف كذلك مدى ثبات هذه المخاليط. كانت قدرة مخاليط القرنفل واللافندرمع الميثيل ايجينول (١:١) وكذلك مدى ثبات هذه المخاليط. كانت قدرة مخاليط القرنفل واللافندرمع الميثيل ايجينول (١:١) وكذلك محلوط الميثيل ايجينول مع القرنفل واللافندرمع الميثيل ايجينول (١:١) وكذلك محلوط الميثيل ايجينول مع المخاليط. كانت قدرة مخاليط القرنفل واللافندرمع الميثيل ايجينول (١:١) وكذلك مدى ثبات هذه المخاليط. كانت قدرة مخاليط القرنفل واللافندرمع الميثيل ايجينول (١:١) وكذلك محلوط الميثيل ايجينول مع القرنفل (١:١) على جذب الذكور تساوي تقريبًا قدرة الميثيل ايجينول وحده في جذب الذكور . في حين مخلوط الميثيل ايجينول مع القرنفل (١:١) على جذب الذكور تساوي تقريبًا قدرة الميثيل ايجينول وحده في جذب الذكور . في حين مخلوط الميثيل ايجينول مع الميثيل ايجينول بنسب (١:١) و (٢: ١) أكثر جاذبية قليلاً في الحالة الأولى وكثيراً في الحالة الثانية لذبابة الخوخ مقارنة بالميثيل ايجينول وحده . بشكل عام، أدى مزج الكافور مع الميثيل ايجينول بنسبة (٢: ١) و (٢: ١) أكثر جاذبية قليلاً في الحالة الأولى وكثيراً في الحالة الثانية لذبابة الخوخ مقارنة الحده. بشكل عام، أدى مزج الكافور مع الميثيل ايجينول بنسبة (٢: ١) إلى تعزيز قدرة الميثيل الخوخ مقارنة بالميثيل عام، أدى مزج الكافور مع الميثيل ايجينول بنسبة (٢: ١) إلى تعزيز قدرة الميثيل الحوخ مقارنة بالميثيل على أدى مزج الذكور مالي الحموم ماليدة مأسبليم تقريبًا.