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Research Article

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Influence of Methyl Eugenol Diluted with Paraffin Oil on Male Annihilation Technique of Peach Fruit Fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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Abstract

Peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tiphritidae) is considered as one of the most economically important pests for several kinds of fruits and vegetables. In order to reduce the cost of male annihilation technique (MAT) for controlling *B. zonata*, dilution of methyl eugenol with paraffin oil had been evaluated to reduce the quantity of methyl eugenol. Five concentrations of methyl eugenol (10, 25, 50, 75 and 100%) were tested with three insecticides belonging to different groups of pesticides; fentrithion, spinosad and a mixture of thiamethoxam+abamactin. Methyl eugenol-fentrithion mixture at 100% of methyl eugenol showed that the weekly mean numbers of captured males all over 10 successive weeks were significantly higher (87.3 individuals/block) than those obtained at 75% (45.9), 50% (32.4), 25% (34.2) and 10% (9.0). There were no significant differences between concentrations 50, 75 and 100% of methyl eugenol in spinosad mixture, while their effects on captured males were significantly higher than that obtained when 10% and 25% were used. Mean numbers of captured males subjected to methyl eugenol-thiamethoxam+abamactin mixture averaged 8.8, 19.0, 24.9, 24.9 and 48.0 individuals/block at 10, 25, 50, 75 and 100%, respectively. Statistically, the mean number of captured males over the tested period at 100% of methyl eugenol was significantly higher than those obtained at 10, 25, 50 and 75%. Males of *B. zonata* were more attracted to methyl eugenol-spinosad mixture compared with methyl eugenol-(fentrithion and thiamethoxam+abamactin) mixtures. As a conclusion, dilution of methyl eugenol in paraffin oil till 50% in MAT by using spinosad (as an insecticide) did not significantly affect the captured males and had a high effect against *B. zonata* population.

Keywords: Male annihilation technique; Methyl eugenol; *Bactrocera zonata*; Allelochemicals; Fentrithion; Spinosad; Thiamethoxam; Abamactin

Introduction

Pharmacology has been defined as the acquisition and accumulation of plant allelochemicals through feeding for specific purposes such as defense and sexual communication [1]. This unique phenomenon occurs between insects and plant chemicals, which include lepidopterous insects-pyrrolizidine alkaloids [2], turnip sawflies-clerodendrins (triterpenoids) [3-5], diabroticite leaf beetles-cucurbitacins (triterpenoids) [3] and tephritid fruit flies-phenylpropanoids [3,6,7].

Males of many fruit flies of *Bactrocera* species were found to enhance their mating competitiveness after consumption of the kairomone lure, methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate) [6-13]. Methyl eugenol is a component of plant essential oil found in at least 200 species of plants from 32 families [14,15].

Male annihilation technique (MAT) (lure and kill) is applied as spot treatments by using dispensers as carriers of the main component of MAT, methyl eugenol (the male attractant) and toxicant [16,17].

Male annihilation technique is one of the fruit fly control methods where it aims to remove male insects, thus reducing male population. This disturbs the male: female ratio and reduces the insects' chances of mating and these females produce few progeny. Accordingly, the insect population at the target area declines and the insects could be eradicated at the end [16,18]. MAT was used successfully to eradicate the oriental fruit fly, *Bactrocera dorsalis* (Hendel) in Rota [19], Saipan [20] and Okinawa [21]; Asian papaya fruit fly, *Bactrocera papayae* in Australia [22] and *Bactrocera* species in Nauru [23].

Current control tactics of fruit flies depended mainly on organophosphorous insecticides such as malathion, naled, dichlorvos and fenitrothionas the toxic component of attract and kill mixture [24-30]. Initial identification of spinosad as promising active insecticide for MAT purposes was reported previously [31]. Recent studies demonstrated the utility of spinosad-based MAT products for the control of some fruit fly species [32-34]. Also, spinosad was examined as a toxicant agent to fruit flies [29,35-37]. Also, several authors reported the control of tephritid insects by exposing adults to neonicotinoidsas a new class of insecticides act upon nicotinic acetylcholine receptors [38-43]. Moreover, the control of cherry fruit fly, *Rhagoletis indifferents* Curran was studied by using thiamethxam and spinosad [44]; thiamethoxam with sugar is a new option for an effective bait spray against cherry fruit fly.

Dilution of odours in paraffin oil had been studied by some researchers [45]; who diluted the vapours of certain pure chemicals, typical of ripe fruits, in paraffin oil to study the ovopositional behaviour from gravid Queensland fruit fly, *Dacus tryoni* (Froggat). While, dilution amounts of common floral scent compounds of *Silene latifolia* was done [46] to study the behavioural responses of *Hadena bicruris* (Lepidoptera,

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Noctuidae). The volatiles of the fungus, *Trametes versicolor* were diluted in paraffin oil to study the response of coleopteran fungivorous insect, *Sulcacis affinis* (Cissidae) [47]. On the other hand, methyl eugenol can be mixed with cue-lure at different concentrations in MAT to control *Bactrocera dorsalis* (Hendel) and *B. cucurbitae* (Coquillett) [10,48,49].

In order to reduce the cost of MAT for *B. zonata*, which is considered one of the most economically important pests for several kinds of fruits in temperate, tropical and subtropical countries [50,51], reduction of the used quantity of methyl eugenol and/or the toxicant should be done [10]. The variation of toxicant concentrations was previously studied [30]. So, the present work concerned to determine if dilution of methyl eugenol in paraffin oil affects on the captured *B. zonata* malesin MAT or not by using three insecticides which are known to affect on insects by different methods.

Material and Methods

Dilution of methyl eugenol with paraffin oil in male annihilation technique of *B. zonata* was evaluated under field conditions. Methyl eugenol-paraffin oil preparations were investigated by using five concentrations of methyl eugenol (i.e.10, 25, 50, 75 and 100%).

To evaluate the efficiency of methyl eugenol-paraffin oil preparations as attractant for *B. zonata* males in MAT, an experiment was carried out in an area of about four feddans (1 feddan=4200 m^2) cultivated with guava (*Psidium guajava*) at the Experimental Farm of Faculty of Agriculture, Mansoura University at Dakahlia governorate, Egypt during the period from 14^{th} of September till 23^{rd} of November 2011 (10 weeks) by using three insecticides belonging to three different groups of insecticides. These insecticides were:

a) Fentrithion 95%: as an organophosphate insecticide.

b) Spinosad: as a biocide (Biosad 22.8% SC).

c) Mixture of Thiamethoxam+Abamactin: 152.4 g/L Thiamethoxam and 33.2 g/L Abamactin (Agri-Flex 186 SC).

All of the mentioned insecticides were separately mixed with each concentration of methyl eugenol (in methyl eugenol-paraffin oil preparations) in a ratio of 1: 4 (insecticide: methyl eugenol-paraffin oil preparations).

Field bioassay was carried out by using plant fibers blocks (as dispensers), measuring 5×5×1.1 cm^3. Blocks were impregnated with the solution of tested methyl eugenol-paraffin oil preparations mixed with the tested insecticides according to the above mentioned ratio of mixture for about four hours in the laboratory. After that, the impregnated blocks were transferred to the field in plastic bags. The blocks were hanged on the trees by metallic wire on a regular distance at height of about two meters in shady and airy place. Each treatment was replicated five times and distributed in a completely randomized design.

To collect the captured *B. zonata* males, plastic containers (measuring 20 cm in diameter and 20 cm in height) were fixed under the treated blocks by metallic wire for receiving the dead male flies. The captured *B. zonata* males in the plastic containers were counted and recorded once a week (without renewal the treatments).

Statistical analysis was done as one way ANOVA test where means were compared by using L.S.D. at the probability of 5% [52].

Results

Dilution of methyl eugenol with paraffin oil in male annihilation technique was examined with respect to three insecticides belonging three different groups of insecticides. These insecticides were fentrithion (organophosphate), spinosad (biocide) and a mixture of thiamethoxam+abamactin.

Methyl eugenol-fentrithion mixture

As shown in Table 1, the weekly numbers of captured males at concentration of 100% methyl eugenol were significantly higher than those captured by 75, 50, 25 and 10% at all of the tested weeks (10 weeks). The mean weekly numbers of captured males over the tested period were 9.0, 34.2, 32.4, 45.9 and 87.3 individuals/block at 10, 25, 50, 75 and 100% of methyl eugenol, respectively. Statistical analysis indicated that the mean number of captured males at 100% of methyl eugenol was significantly higher than those of 75, 25, 50 and 10%, respectively.

Methyl eugenol-spinosad mixture

The weekly numbers of captured males at 100 and 75% concentrations of methyl eugenol were higher than those captured by 50, 25 and 10% at the first three weeks of the tested period. The mean weekly numbers of captured males over 10 weeks were 34.4, 39.5, 42.5, 53.0 and 52.6 individuals/block at 10, 25, 50, 75 and 100% of methyl eugenol, respectively. These results showed that the means of captured males at 100, 75 and 50% of methyl eugenol did not differ significantly; while they were significantly higher than those obtained by 25 and 10% (Table 2).

Weeks	Captured males/block/week at (methyl eugenol%)					L.S.D. (P=5%)
	10	25	50	75	100	
1	6.6 ± 2.6c	31.0 ± 16.3b	18.6 ± 5.4bc	36.8 ± 7.2b	57.2 ± 22.8a	17.4
2	13.4 ± 5.0c	64.8 ± 7.2b	47.4 ± 10.4b	52.4 ± 12.8b	119.8 ± 24.6a	18.2
3	20.8 ± 5.8c	61.6 ± 23.4b	54.4 ± 13.9b	64.8 ± 21.8b	122.8 ± 35.0a	29.4
4	17.0 ± 5.6c	60.0 ± 24.2b	51.0 ± 12.9b	71.6 ± 27.8b	129.2 ± 40.0a	33.1
5	11.0 ± 8.7c	49.8 ± 25.8bc	43.0 ± 13.4c	87.6 ± 34.3ab	111.6 ± 50.2a	40.1
6	3.6 ± 1.8c	16.6 ± 10.5c	18.8 ± 6.9bc	33.6 ± 15.8b	54.6 ± 15.9a	15.2
7	7.6 ± 2.9c	11.0 ± 8.3c	14.2 ± 5.4c	33.8 ± 13.0b	74.8 ± 19.5a	15.1
8	4.2 ± 2.2c	22.4 ± 9.8b	26.2 ± 11.8b	26.0 ± 16.3b	65.8 ± 11.5a	14.9
9	2.8 ± 1.3c	17.6 ± 10.7bc	31.6 ± 18.2b	26.0 ± 15.7bc	72.4 ± 27.6a	22.5
10	2.6 ± 2.4c	7.0 ± 4.0c	18.4 ± 13.8bc	26.0 ± 11.6b	64.4 ± 20.7a	16.4
Mean	9.0 ± 2.7d	34.2 ± 11.3bc	32.4 ± 6.4c	45.9 ± 10.2b	87.3 ± 12.2a	12.2

-Data are represented as Mean ± SD
-Means of similar letters in the same row are not significantly differed at P=0.05

Table 1: Effect of dilution of methyl eugenol with paraffin oil on the efficiency of methyl eugenol-fentrithion mixture in MAT of B. zonata.

Weeks	Captured males/block/week at (methyl eugenol%)					L.S.D. (P=5%)
	10	25	50	75	100	
1	27.8 ± 10.7bc	16.0 ± 11.0c	39.2 ± 14.7b	87.0 ± 11.6a	92.6 ± 20.1a	18.9
2	59.4 ± 17.0b	32.2 ± 11.9c	61.8 ± 17.5b	105.2 ± 13.5a	131.0 ± 33.3a	26.9
3	69.0 ± 23.2a	30.8 ± 9.9b	59.8 ± 22.3a	77.0 ± 16.0a	76.6 ± 19.6a	25.3
4	76.2 ± 30.2a	41.0 ± 17.9b	47.6 ± 16.2b	48.6 ± 14.4b	55.6 ± 7.3ab	25.1
5	52.6 ± 20.0ab	72.2 ± 21.5a	44.2 ± 14.2b	34.6 ± 10.9b	39.6 ± 16.2b	22.7
6	19.8 ± 6.6ab	35.2 ± 20.3a	23.6 ± 12.3ab	14.0 ± 4.7b	14.2 ± 6.0b	15.3
7	17.2 ± 7.6b	41.2 ± 15.7a	26.0 ± 10.0ab	25.4 ± 14.5ab	21.8 ± 8.5b	16.0
8	5.6 ± 3.4b	32.8 ± 12.2a	47.0 ± 14.4a	44.0 ± 19.2a	35.4 ± 7.8a	17.6
9	4.2 ± 3.8b	44.0 ± 13.9a	50.6 ± 17.9a	55.2 ± 16.0a	36.6 ± 12.5a	18.7
10	12.6 ± 6.2c	49.8 ± 17.6a	25.0 ± 11.5bc	38.8 ± 12.2ab	22.2 ± 8.2bc	15.9
Mean	34.4 ± 8.0b	39.5 ± 10.5b	42.5 ± 11.1ab	53.0 ± 4.3a	52.6 ± 6.5a	11.2

-Data are represented as Mean ± SD
-Means of similar letters in the same row are not significantly differed at P=0.05

Table 2: Effect of dilution of methyl eugenol with paraffin oil on the efficiency of methyl eugenol-spinosadmixture in MAT of *B. zonata*.

Weeks	Captured males/block/week at (methyl eugenol%)					L.S.D. (P=5%)
	10	25	50	75	100	
1	7.6 ± 4.7c	12.0 ± 6.8c	19.8 ± 7.3bc	31.8 ± 12.7b	53.4 ± 21.4a	16.0
2	13.8 ± 6.8c	14.8 ± 5.7c	33.8 ± 16.1b	47.0 ± 13.8ab	58.8 ± 20.6a	18.2
3	15.0 ± 7.2c	19.2 ± 12.2c	29.2 ± 16.5bc	40.6 ± 8.3b	86.0 ± 17.5a	17.2
4	13.8 ± 11.1c	28.0 ± 8.0bc	31.4 ± 18.0bc	47.4 ± 19.1b	96.2 ± 17.7a	20.4
5	11.4 ± 3.6b	27.8 ± 8.6ab	33.2 ± 20.6a	26.4 ± 18.5ab	43.8 ± 10.9a	18.4
6	5.6 ± 1.7c	13.2 ± 4.6ab	10.4 ± 2.6bc	7.2 ± 4.9bc	18.6 ± 5.6a	5.5
7	4.6 ± 2.4b	10.4 ± 5.8ab	15.0 ± 5.8a	11.6 ± 5.4a	14.8 ± 4.0a	6.4
8	5.4 ± 3.2b	28.0 ± 7.4a	30.8 ± 14.3a	15.0 ± 10.2ab	31.2 ± 17.7a	15.5
9	5.4 ± 3.4c	24.8 ± 10.3bc	31.6 ± 14.2ab	13.2 ± 7.2bc	49.6 ± 30.3a	21.2
10	5.4 ± 2.9c	11.6 ± 4.7bc	13.8 ± 5.1b	9.2 ± 6.1bc	28.0 ± 3.7a	6.1
Mean	8.8 ± 3.8c	19.0 ± 5.7b	24.9 ± 8.0b	24.9 ± 4.8b	48.0 ± 8.7a	8.5

-Data are represented as Mean ± SD
-Means of similar letters in the same row are not significantly differed at P=0.05

Table 3: Effect of dilution of methyl eugenol with paraffin oil on the efficiency of methyl eugenol-thiamethoxam+abamactinmixture in MAT **of** *B. zonata.*

Methyl eugenol-thiamethoxam+abamactin mixture

Table 3 shows that the weekly numbers of captured males at a concentration of 100% methyl eugenol were significantly higher than those captured at 75, 50, 25 and 10% at all the tested weeks. The general mean numbers of captured males over the tested period were 8.8, 19.0, 24.9, 24.9 and 48.0 individuals/block at 10, 25, 50, 75 and 100% of methyl eugenol, respectively. Therefore, data clearly showed that the mean number of captured males at 100% of methyl eugenol was significantly higher than those obtained at 75, 25, 50 and 10%.

Discussion

Methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate) is a kairomone lure for males of many fruit flies of *Bactrocera* species. Inside these species, methyl eugenol converted to 2-allyl-4, 5-4-allyl-1, 2-dimethoxyphenol and (E)-coniferyl alcohol [6,7,11]. These metabolites are then sequestered into the male rectal gland before release during courtship at dusk. Behavioral studies have also demonstrated that both of these phenyl-propanoids function as male sex and aggregation pheromones [7,9,12].

Responses of the tephritid fly, *B. zonata* to methyl eugenol-insecticide obviously varied according to methyl eugenol concentration. However, Figure 1 shows that, each increase of methyl eugenol concentration by 1% increased the weekly means of captured males by 0.73, 0.36 and 0.21 individuals/block at fentrithion, thiamethoxam+abamactin and spinosad mixtures, respectively. So, it can be concluded that numbers of captured *B. zonata* males increased by increasing methyl eugenol concentration especially by using fentrithion and thiamethoxam + abamactin. Similar results were obtained; however, responses of *H. bicruris* and *S. affinis* increased by the increase of odours' concentrations diluted in paraffin oil [46,47]. Also, when *C. capitata* and *Anastrepha fraterculus* (Wiedemann) were sprayed with neonicotinoid insecticides, the time required for killing them was inversely proportional to the insecticide concentrations [53].

Using the organophosphorous insecticide (malathion) in Hawaii, captures of *B. cucurbitae* for traps baited with 100, 75 and 50% cue-lure (its sex attractant) were not significantly different; while, captures of *B. dorsalis* significantly varied with methyl eugenol concentrations [10]. Results of research on mixture of methyl eugrnol and cue-lure were highly influenced by insect species, population density and locality [48,49,54-56].

Also, the present results showed that there were no significant differences between captured *B. zonata* males treated with 50, 75 and 100% of methyl eugenol in spinosad mixture. While, with fentrithion and thiamethoxam+abamactin mixtures, the captured males were significantly reduced by the reduction of methyl eugenol concentration. This may be attributed to the relatively low repellent effect of spinosad in comparison with fentrithion and thiamethoxam+abamactin.

The present results also showed that responses of *B. zonata* males to methyl eugenol-insecticide varied according to the insecticide used.

However, *B. zonata* males were more attracted to methyl eugenol-spinosad mixture (44.4 individuals/block/week) in comparison with methyl eugenol-fentrithion (41.8 individuals/block/week) and methyl eugenol-thiamethoxam+abamactin (25.1 individuals/block/week) mixtures. The present results agree with the previously findings which mentioned that spinosad was more effective than the organophosphate insecticide, naled in MAT of *B. dorsalis* in Taiwan [34].

Also, many authors mentioned that spinosad had a high effect against some fruit fly species. However, spinosad could be used in controlling *Bactrocera oleae* (Gmelin) [57]. Also, spinosad was effective on Mexican fruit fly especially to immature adults [58]. In addition, spinosad was more efficient than malathion in controlling *Ceratitis capitata* Wiedemann, *B. zonata* and *B.oleae* [29,59].

On the other hand, malathion can be added to the mixture of methyl eugenol and cue-lure for control *B. dorsalis* and *B. cucurbitae* [10]. Also, spinosad is a promising substitute for organophosphate insecticides for control of *B. dorsalis* and *B. cucurbitae* in Hawaii and California [32,60].

Spinosad was more effective on *C. capitata* and *A. fraterculus* in comparison with neonicotinoid compounds [52]. This may be related to the mode of action of spinosad. However, spinosad normally kills the insects by causing the cessation of feeding and paralysis [61]. In Washington and Utah, thiamethoxam with sugar is a new option for an effective bait spray against cherry fruit fly in comparison with spinosad in sugar and protein bait applied [44].

Additionally, organophosphate insecticides have received widespread scrutiny for their negative impacts on non-target animals and human health. Thus, the identification of reduced-risk alternatives for use in these area-wide control programs is of a high priority [32,62]. So, spinosad can be used in MAT to the male of fruit flies [32,34,60].

From the previous results it can be concluded that dilution of methyl eugenolin paraffin oil till 50% in MAT by using spinosad (as an insecticide) did not significantly affect the captured males and had a relatively high effect against *B. zonata* population. For a better understanding of such interactions and behavioral responses of *B. zonata* males to methyl eugenol concentrations in MAT, extensive investigations on the effect of dilution of methyl eugenol in different mineral oils or other materials are highly required.

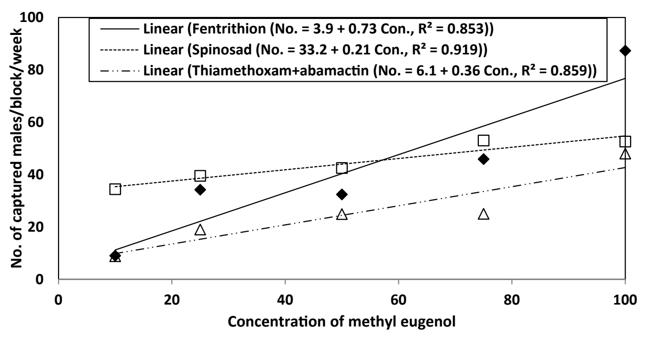

Figure 1: The relationship between methyl eugenol concentrations and the mean numbers of captured *B. zonata* males/block/week by methyl eugenol-mixtures.

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