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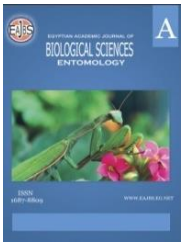

Modifications in Male Annihilation Technique for Attracting *Bactrocera zonata* Under Field Conditions

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ABSTRACT

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders, 1841) (Diptera: Tephritidae) is a destructive polyphagous pest infesting a lot of horticultural fruits and some vegetables. At Damietta governorate, Egypt, the present study aimed to maximize the efficiency of the male annihilation technique (MAT) by estimating different types of blocks (as dispensers) impregnated with different doses (3, 6, and 9 cm^3/block) of methyl eugenol to attract PFF males in guava orchards. Two different types of blocks [Hard woodpulpluff (HWF) and plant fibers] were selected for the present study. These blocks were tested as uncovered blocks and covered with aluminum polyethylene polyester. The study was carried out over eight successive weeks (from 27th September to 15th November 2023). The obtained results showed that the covered HWF blocks impregnated with the dose of 9 cm^3/block were superior in attracting PFF males all over the investigation period followed by uncovered plant fibers, uncovered HWF, and covered plant fibers' blocks. There were positive relationships between the captured PFF males and the concentration of methyl eugenol. The covered HWF blocks were relatively more stable against elapsed time in comparison with uncovered HWF or plant fibers' blocks. In conclusion, the covered HWF blocks were more effective dispensers all over eight weeks for methyl eugenol to attract PFF males in comparison with uncovered HWF blocks and plant fibers.

INTRODUCTION

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders, 1841) (Diptera: Tephritidae) was detected for the first time in Egypt in 1924 at Port-Said area as a quarantine insect pest (Efflatoun, 1924). In 1995, it was recorded again with misidentification as *Bactrocera pallidus* Perkins and May (Abuel-Ela *et al*., 1998). In 1997, this insect was recorded as *B. zonata*, and the identification was corrected (El-Minshawy *et al*., 1999). Now,

it is widely dispersed across most of Egypt even in arid desert areas (Elnagar *et al*., 2010). The peach fruit fly (PFF) is a destructive polyphagous pest infesting a lot of horticultural fruits and some vegetables (Allwood *et al*. 1999). The attack of fruit flies reduces the quantity and quality of fruit yield, where females of PFF insert their ovipositor and lay eggs beneath the skin of fruits; then the hatched larvae feed on the pulp of ripe fruits causing great damage and making fruits unfavorable for marketing or export (White & Elson-Harris, 1994; Borge & Basedow, 1997; Khan *et al.*, 2015 and Bayoumy *et al*., 2021). Losses in orchards' yield by PFF infestation reached 25%–50% (Siddiqui *et al*., 2003), and has become a significant threat to fruit production in the Mediterranean region (Duyck *et al*., 2004). In Egypt, PFF attacks a wide range of fruits which differ in ripening times all over the year including mango, guava, peach, citrus, and others (Shehata *et al*., 2008; Ghanim, 2009; Ghanim *et al*., 2015 and Bayoumy *et al*., 2021 and El-Afify *et al.*, 2023).

In managing fruit flies, using the male annihilation technique (MAT), which involves trapping males using a lure, is highly advantageous as these traps have high specificity and efficiency against numerous fruit fly species (White and Elson Harris, 1992). Among the selected lures, methyl eugenol was most effective against PFF males. Methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate) is a synthetic sex attractant for males of many fruit flies belonging to the genus of *Bactrocera*, and inside these species, methyl eugenol converted to 2-allyl-4, 5-4-allyl-1, 2-dimethoxyphenol and (E)-coniferyl alcohol (Tan & Nishida, 1996; Tan & Nishida, 1998 and Shelly & Nishida, 2004). These metabolites sequester into the male rectal glands before release during courtship (Hee & Tan, 1998; 2006, Tan & Nishida, 1998). So, methyl eugenol is recommended as an attractant for PFF males in MAT. In 2008, an application of the National Area-Wide Fruit Flies Extermination Program for PFF began in Egypt by the Ministry of Agriculture, Egypt. MAT was the main method used in addition to the bait application technique (BAT). MAT depends on attracting and killing adult males of PFF using methyl eugenol mixed with insecticide.

To protect the attractant (either pheromone or para-pheromone constituents) and its emission over a prolonged period for controlling pests, it requires formulation in suitable dispensing systems (Leonhardt *et al*., 1987; Dharanivasan *et al*., 2017 and El-Adly *et al.*, 2018). So, the present study aimed to maximize the efficiency of MAT by estimating different types of dispensers impregnated with different doses of methyl in guava orchards located in Damietta governorate (where it was noted that there was a notable scarcity of studies on PFF conducted in this governorate).

MATERIALS AND METHODS

Experiments were conducted in Kafr Saad region (Damietta governorate, Egypt), there is an area where guava (*Psidium guajava* L.) cultivation is concentrated (31◦ 26′ 12″ N) (31◦ 40′ 01″ E). About 40 feddans (1 feddan = 4200 m^2) of this area were selected for the present study. Guava trees were about four meters in height, aged about 15 years, cultivated with intervals of four meters, and had the recommended agricultural practices.

Two different types of blocks were selected for the present study: the first one was Hard Woodpulp Fluff (HWF) (obtained from the company of Golden Isles CO® Fluff Pulp, USA), and the second was the plant fibers' blocks (obtained from Plant Protection Research Institute, Agricultural Research Center, Egypt). The plant fibers' blocks had a standard measure (5x5x1.1 cm^3); while HWF blocks were cut by using a sharp cutter to obtain the same measure of the plant fibers.

Three doses of methyl eugenol (3, 6, and 9 cm^3/block) were used to impregnate HWF and plant fibers' blocks. Eight blocks of each type were impregnated with each dose and were divided into two groups (four blocks for each group as replicates). The first group

was uncovered blocks; while the second group was covered with aluminum polyethylene polyester (obtained from the company of Constantia Flexibles, a global leader in consumer and pharmaceutical flexible packaging, Austria) (Fig., 1). The net internal measurements of the aluminum polyethylene polyester cover were 8.1x6.5 cm (to make it easier to enter the block and close it). On one side of the cover, a circular hole (3.5 cm diameter) was made to allow the scent of methyl eugenol to come out.

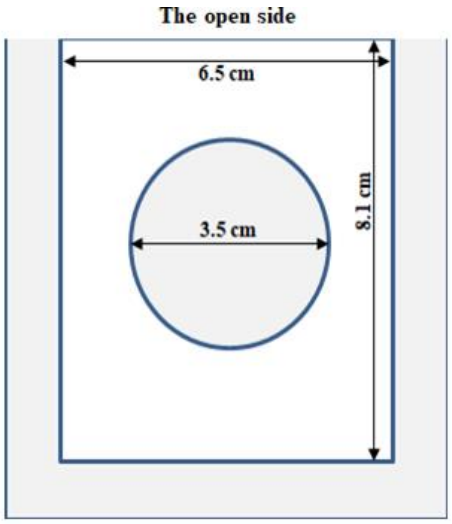

Fig. 1: Illustration showing Aluminum polyethylene polyester cover dimensions.

The impregnated blocks were put inside Makkar and El-Abbassi traps (Makkar *et al.*, 2015) as one block per trap. Traps were hung on guava trees by metallic wires at regular distances at a height of about two meters in shady and airy places. Traps were distributed in the selected area (in a completely randomized design) at a distance of 50 meters. Traps were hung from 27th September till 15th November 2023 (eight successive weeks) without renewal of the blocks or methyl eugenol.

The captured male flies were weekly collected, counted, and recorded. The number of captured flies per trap per day (FTD) was estimated for each trap according to the following formula:

FTD = F/D; where: F = Number of captured flies, and D = Length of time the trap spent in the field or period of investigation (7 days).

Data were analyzed by using one-way ANOVA followed by least significant difference (LSD) at a probability level of 0.05. Regression analysis was also performed. All analyses were performed using CoHort Software (2004).

RESULTS

As shown in Table (1), the highest captured PFF males by uncovered Hard Woodpulp Fluff (HWF) blocks injected by a dose of 3 cm^3/block (FTD = 22.67±1.25) was recorded at the first week of hanging (with significant differences), then its efficiency gradually decreased till the end of the eight weeks of the experiment. Respecting the doses of 6 and 9 cm^3/block, the highest efficiencies were recorded for each at the third and fourth weeks (FTD = 67.72±1.55 and 54.18±1.65, respectively), then their efficiency decreased by the elapsed time. On the other hand, with regard to covered HWF, the highest efficiency of the doses 3 or 6 cm^3/block was recorded at the fifth week of handling application (FTD = 14.18±0.34 and 15.96±0.17, respectively); while that recorded with the dose of 9 cm^3/block, the highest efficiency was recorded at the first week of FTD = 70.54±2.49 with significant difference.

Table 1: The efficiency of HWF and plant fibers' blocks injected by different doses of methyl eugenol in attracting PFF adult males under field conditions of guava orchard.

Block type	Dose (cm/block)	First four weeks					Second four weeks				
		1	2	3	4	LSD	5	6	7	8	LSD
Uncovered HWF	3	22.67 ±1.25	20.86 ±0.77	18.18 ±0.99	14.57 ±0.34	2.77 ***	15.25 ±0.31	15.61 ±0.24	11.82 ±0.42	7.68 ±0.42	1.09 ***
	6	44.11 ±0.56	31.89 ±0.12	67.72 ±1.55	46.54 ± 1.89	3.87 ***	30.25 ±0.78	14.22 ±0.47	10.14 ± 0.43	6.82 ± 0.19	1.58 ***
	9	28.21 ±0.38	38.46 ±1.97	51.43 ± 1.26	54.18 ± 1.65	4.44 ***	35.72 ±1.84	32.39 ±0.38	25.00 ±0.64	19.61 ±0.46	3.14 ***
	LSD	2.63 ***	3.90 ***	4.11 ***	4.67 ***		3.74 ***	1.21 ***	1.63 ***	1.20 ***	
Covered HWF	3	8.57± 0.46	8.97 ± 0.51	12.72 ± 0.31	7.46 ±0.19	1.25 ***	14.18 ± 0.34	10.89 ±0.36	8.75 ± 0.48	5.5 ± 0.28	1.13 ***
	6	10.89 ±0.63	8.97 ± 0.60	14.07 ±0.32	7.64 ± 0.09	1.44 ***	15.96 ±0.17	9.32 ±0.59	9.04 ± 0.36	5.61 ± 0.12	5.65 ns
	9	70.54 ±2.49	56.25 ±1.75	64.96 ±1.37	43.18 ±1.27	5.49 ***	37.93 ±1.34	27.93 ±0.46	26.15 ±1.02	21.82 ±0.67	5.79 ***
	LSD	4.80 ***	3.54 ***	2.70 ***	2.37 ***		2.56 ***	1.54 ***	2.23 ***	9.05 *	
Uncovered plant fibers	3	30.39 ±0.18	38.46 ±1.19	34.25 ±0.95	22.22 ±0.45	5.20 ***	16.11 ±0.16	14.18 ±0.26	11.65 ±0.39	10.61 ±0.71	1.33 ***
	6	32.07 ±0.72	29.75±0 .78	28.17 ±0.72	21.32 ±0.62	2.19 ***	19.00 ±0.35	19.61 ±0.96	12.61 ±0.67	8.96 ±0.54	2.05 ***
	9	67.39 ±1.02	39.86 ±0.76	34.50 ±0.41	62.29 ±1.03	2.59 ***	43.86 ±0.73	34.21 ±0.63	24.79 ±0.61	19.89 ±0.80	1.99 ***
	LSD	2.32 ***	2.98 ***	5.96 *	2.37 ***		1.52 ***	1.95 ***	1.82 ***	2.21 ***	
Covered plant fibers	3	15.36±0. 50	14.54 ±0.24	14.86 ±0.36	8.36 ±0.57	1.55 ***	10.21 ±0.39	5.96±0. 36	5.43 ±0.34	2.43 ±0.21	1.02 ***
	6	32.04 ±0.42	27.54 ±0.71	27.57 ±0.84	30.97 ±0.81	2.20 ***	24.82 ±0.53	19.93 ±0.76	17.93 ±0.89	9.64 ± 1.51	2.52 ***
	9	47.61 ±0.19	50.36 ±0.53	38.36 ±0.15	38.61 ±0.61	1.30 ***	27.82 ±0.37	26.72 ±0.72	16.5 ±0.57	11.43 ±0.78	1.70 ***
	LSD	1.26 ***	1.69 ***	1.95 ***	2.14 ***		1.40 ***	2.05 ***	2.06 ***	2.10 ***	
General LSD		15.64 ns	13.35 ns	14.30 ns	14.36 *		8.55 ns	7.27 ns	5.74 ns	4.7 ns	

ns: nonsignificant difference
*significant difference $p < 0.05$
*** very highly significant difference $p < 0.001$

Concerning plant fiber blocks, the uncovered ones injected with the dose of 3 cm^3 of methyl eugenol per block recorded the highest efficiency in attracting PFF males during the second week of hanging (FTD = 38.46±1.19); while recorded its highest efficiency in the first week with (FTD = 32.07±0.72 and 67.39±1.02, respectively) (Table, 1). But in the case of plant fibers, the highest efficiency with the doses of 3 and 6 cm^3/block was recorded in the first week showing FTD values of 15.36±0.50 and 32.04±0.42, respectively. The highest FTD values of 50.36±0.53 occurred with 9cm3/ plant fibers covered block at the second week of application.

As seen in Table (1), the efficiency of uncovered blocks (especially the plant fibers) reduced during the second four weeks in comparison with the first four weeks. These reductions were relatively low in the case of covered blocks.

According to mean FTD values of the tested treatments (Fig., 2), during the first four

weeks of investigation, the covered HWF and both uncovered and covered plant fibers, impregnated with 9 cm^3/block significantly recorded the highest mean FTD values of 58.73±5.95, 51.01±8.12 and 43.73±3.08, respectively. But, in the case of HWF the highest FTD value was obtained with the uncovered HWFones at 6 cm^3/ block (FTD= = 47.56±7.44) which insignificantly differed from that of 9 cm^3/ block. During the second four weeks and the eighth weeks, the rate of 9cm^3/ block significantly recorded the highest values of FTD for all used blocks. Whereas the blocks impregnated with the dose of 3 cm^3/block, were significantly the lowest in attracting PFF males.

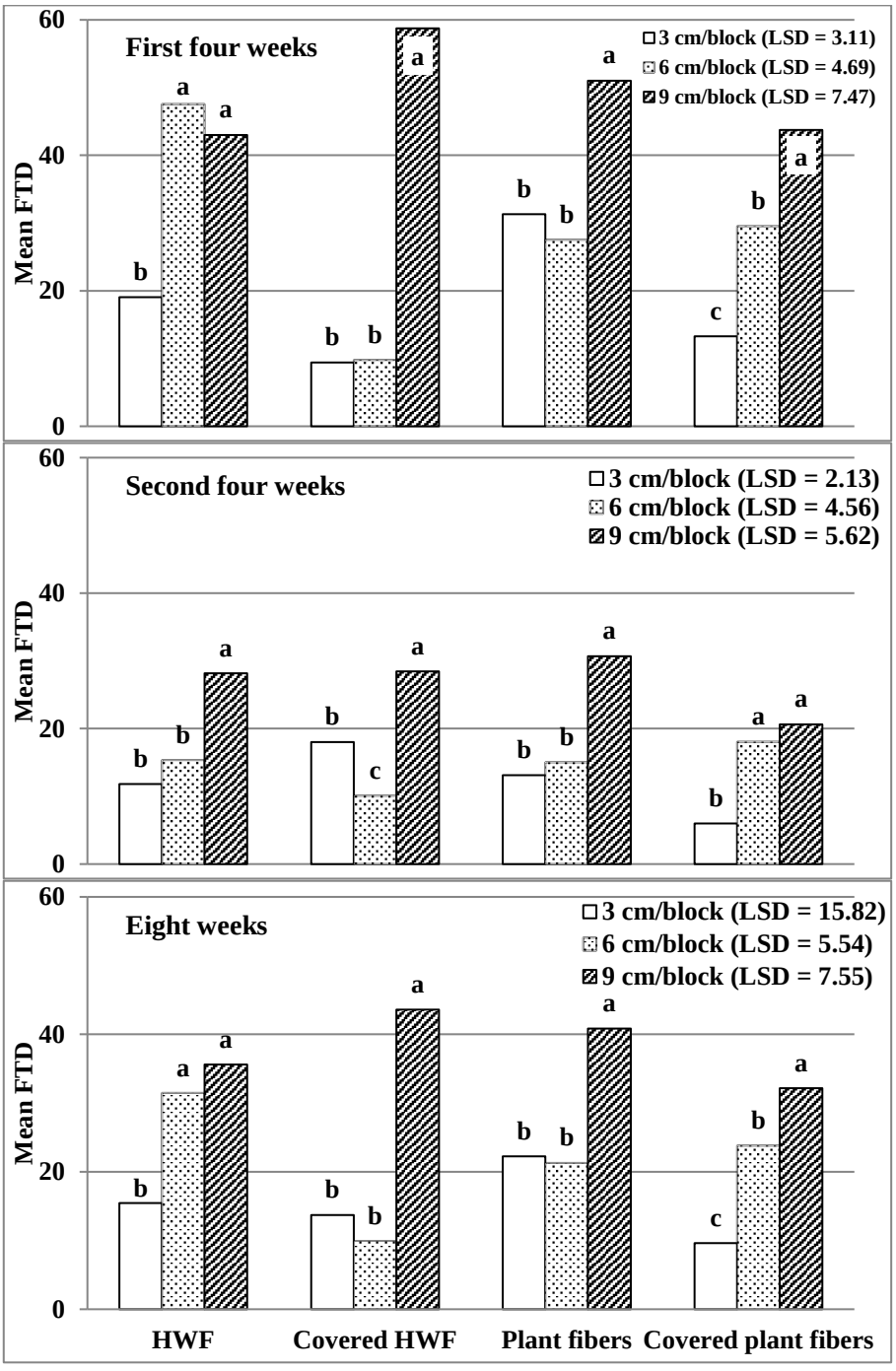

Fig. 2: Mean FTDs of captured PFF males by HWF and plant fibers' blocks impregnated by different doses of methyl eugenol all over the first four weeks, the second four weeks as well as all the tested eight weeks under field conditions of guava orchard (note: in each type of blocks, columns have the same letter are not significantly differed at a probability of 5%).

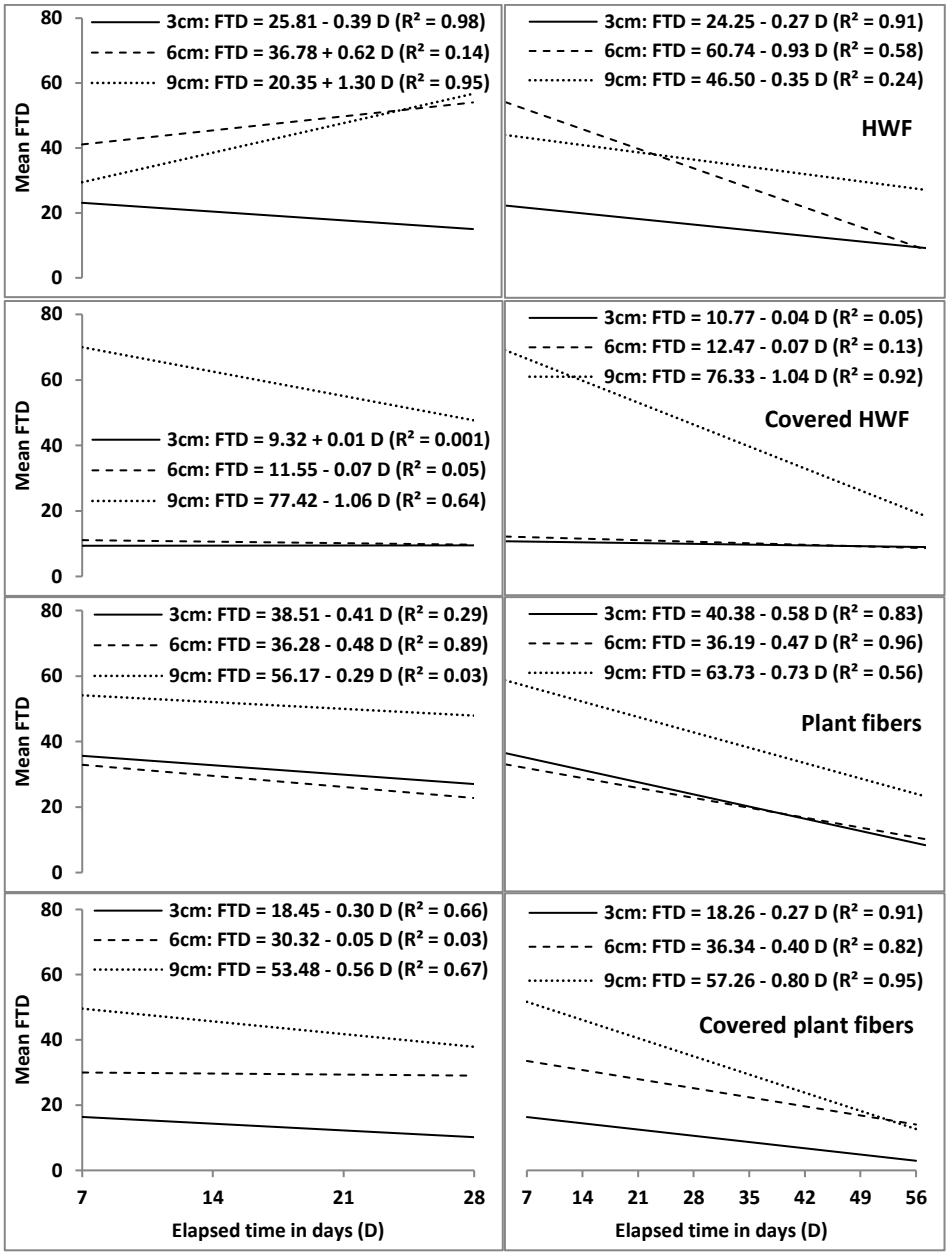

Fig. 3: Stability of uncovered and covered blocks of HWF and plant fibers impregnated with the doses of 3, 6, and 9 cm/block during the first four weeks (from 7 to 28 days), and all over the eight weeks under field conditions of guava orchard.

The stability of the tested blocks with the different amounts of methyl eugenol injected per block is mathematically illustrated in Figure (3). As shown in the figure the HWF blocks injected with the dose of 3 cm^3/block, exhibited relatively the most stable (b = -0.27) followed by the dose of 9 cm/block (b = -0.35) and 6 cm/block (b = -0.93) all over the eight weeks. For the covered HWF, the most stable doses were those of 3 and 6 cm^3/block (b = -0.04 and -0.07); while the efficiency of the dose of 9 cm/block decreased; where the mean FTD captured by block in the used traps reduced by 1.04 males every elapsed one day. Every elapsed time by one day decreased the block efficiency of the uncovered plant fibers as an attractant for PFF males by 0.58, 0.47, and 0.73 males per trap per day when it injected by the doses of 3, 6, and 9 cm^3/block, respectively. The means of FTDs of the covered blocks of plant fibers, which were injected by 3, 6, and 9 cm^3/block, decreased by 0.27, 0.40 and

0.80 for males with elapsed time by one day (Fig., 3).

In general, it can be noticed from Figure (3) that the HWF blocks were relatively more stable than plant fibers especially at rates of 3 and 6 cm^3/block when the blocks were covered with aluminum polyethylene polyester.

The relationship between the dose of methyl eugenol per block and its efficiency in attracting PFF males showed that the covered plant fibers were the most affected by the dose over the eight weeks; the calculated R^2-value reached 0.98 (Fig., 4). Uncovered HWF blocks ranked second in affecting the injected dose per block (R^2 = 0.90) followed by uncovered plant fibers (R^2 = 0.71) and covered HWF blocks (R^2 = 0.66).

On another hand, Figure (4), showed an increase in the efficiency of the blocks in attracting PFF males according to the increase of the injected dose per block which was higher during the first four weeks comparing to the second four weeks. Where, each increase of the injected dose by 1 cm^3/block increased the FTDs of HWF, covered HWF, plant fibers, and covered plant fibers' blocks during the first four weeks by 11.97, 24.65, 9.84 and 15.23 males, respectively. While, during the second four weeks, the comparison rates of increase reached 8.17, 5.23, 8.77 and 7.30 for males, respectively.

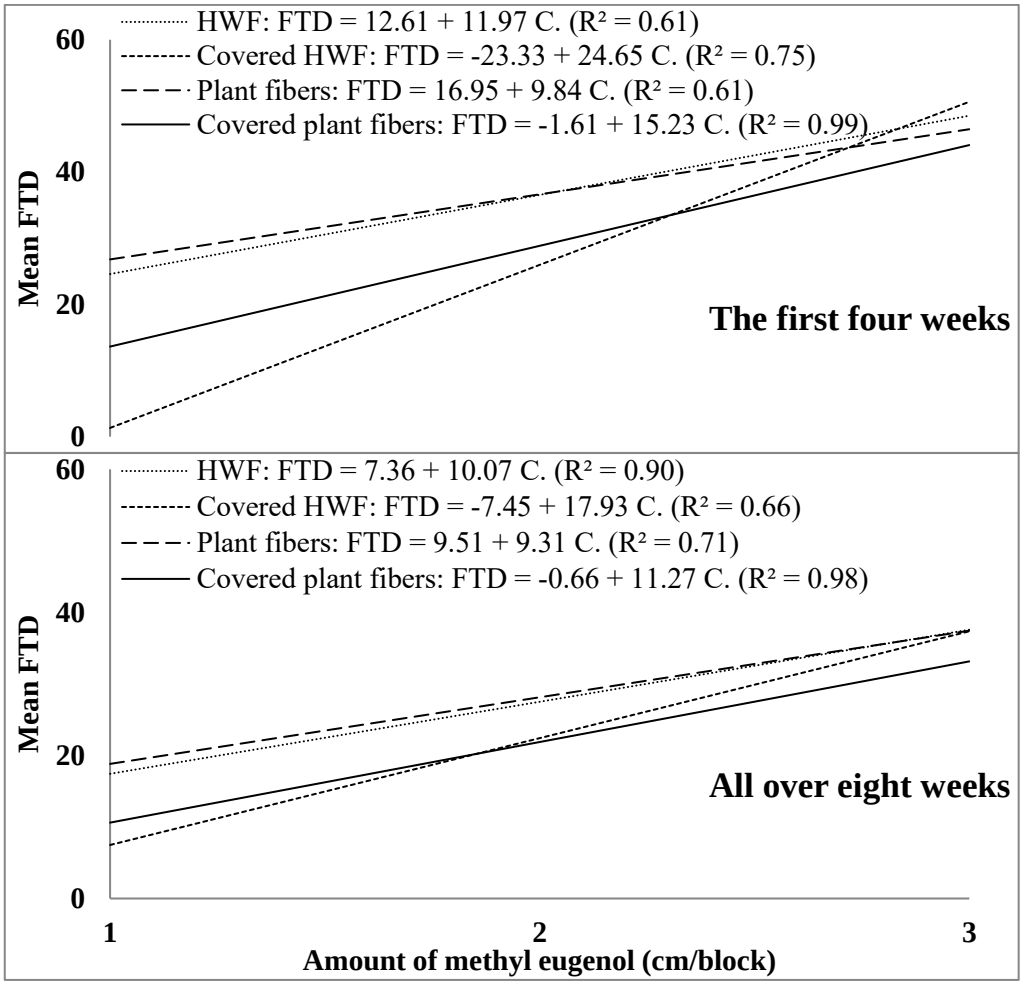

Fig. 4: Effect of the injected amount of methyl eugenol per block on their efficiency (either uncovered or covered blocks of HWF and plant fibers) during the first four weeks (from 7 to 28 days), and all over the eight weeks under field conditions of guava orchard.

DISCUSSION

The male annihilation technique (MAT); which is used for controlling several *Bactrocera* spp. is affected by the dispenser type used; this is because the dispenser controls the release rate of the attractant that impregnated with it (Bajpai and Giri, 2002, Abd El-Kareim *et al*, 2009, Sidahmed *et al*., 2014 and El-Adly *et al.*, 2018). These findings confirm what was found in the current study; where the covered HWF blocks were superior in attracting PFF males all over the tested period of eight weeks at the dose of 9 cm^3/block followed by uncovered plant fibers, uncovered HWF and covered plant fibers' blocks, respectively. Similarly, Abd El-Kareim *et al* (2009) reported that the efficiencies of plant fibers blocks (as dispensers of methyl eugenol in MAT for PFF males) were less than that of felt blocks. Sidahmed *et al*. (2014) and Mir & Ahmad (2017) reported that dispensers of plywood and white wood (impregnated with a mixture of methyl eugenol and a recommended insecticide) exhibited relatively high efficiencies as attractants for *Bactrocera invadens* Drew, Tsuruta and White and *Bactrocera dorsalis* (Hendel). On another hand, the covered HWF blocks were more effective in attracting PFF males than uncovered ones; on contrary, the uncovered plant fibers' blocks were more effective than the covered ones. According to Bajpai and Giri (2002), Leblanc *et al*. (2011), Dharanivasan *et al*. (2017) and Bayoumy *et al*. (2020), the design and chemistry of dispenser system directly affected the constant release rate of the attractant.

The present results showed that blocks of the Hard Woodpulp Fluff (HWF), especially the covered ones, were relatively more stable against elapsed time compared to plant fibers' blocks. This may be attributed to that the cover on HWF blocks helps in controlling methyl eugenol release rates and maintaining its concentration in the system within limits near the optimum. These explanations agree with those of El-Metwally *et al*. (2017); who concluded that the attracting efficiency of methyl eugenol and trimedlure for attracting both *B. zonata* and *C. capitata* males are improved when coating by using some polymers like St Acylate 8% and PVA 0.5%. Also, the present finding may be explained by Abd El-Kareim *et al*. (2009); who reported that the plant fibers' blocks (uncovered ones) proved to exhibit a relatively higher initial release rate of methyl eugenol compared to felt blocks; so, its efficiency were less stable against the elapsed time. El-Metwally and Amin (2015) stated that methyl eugenol diluted with paraffin oil was more stable against passed time in comparison with that diluted with sunflower oil. According to Patel *et al*. (2005), Rameash *et al*. (2009), Sidahmed *et al*. (2014), and Mir & Ahmad (2017), plywood dispensers impregnated with methyl eugenol mixed with insecticides were best suited for capturing *Bactrocera* spp. as it exhibited a constant release rate of methyl eugenol, longer persistence, and a comparatively moderate cost of preparation. Bayoumy *et al.* (2020) reported that trimedlure-polyethylene matrix dispensers prolonged the release of trimedlure (the attractant of *C. capitata* males) more than the cotton wick dispensers. El-Metwally *et al.* (2019) found that dilution of trimedlure with sunflower oil or paraffin oil changed it to be relatively more stable against past time under field conditions.

According to Ravikumar (2006), Ghanim (2013), and El-Metwally and Amin (2015), the number of captured PFF males increased by increasing methyl eugenol concentration impregnated dispensers. Also, the present study showed positive relationships between the captured PFF males and the concentration of methyl eugenol, where each increase of methyl eugenol concentration by 1 cm/block increased the rate of FTDs by obvious numbers. Ghanim (2013) added that dilution of methyl eugenol in paraffin oil till 50% in MAT (using spinosad as insecticide) did not significantly affect the captured males and had a relatively high effect against PFF population. According to Vargas *et al.* (2000), captures of *B. dorsalis* males were significantly correlated with methyl eugenol

concentrations. Similar results were concluded between other fruit flies (i.e. *Ceratitis capitata*) and its male attractant (trimedlure), where El-Abbassi and El-Metwally (2013), El-Metwally (2017), El-Metwally *et al*. (2019) and Bayoumy *et al*. (2020) reported that numbers of captured *C. capitata* males were higher in the treatments which had high concentrations of trimedlure.

As a conclusion, the covered HWF blocks were more effective dispensers all over eight weeks for methyl eugenol to attract PFF males in comparison with uncovered HWF blocks and plant fibers

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Authors Contributions: Prof. Dr. Nabil M. Ghanim and Prof. Dr. Mostapha M. Elmetwally designed the experiments, reviewed drafts of the article, and approved the final draft.

Prof. Dr. Mohammed M. Elbokl and Mrs. Mayada N. Elkelany conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored and reviewed drafts of the article, and approved the final draft.

Competing Interests: The authors declare that they have no competing interests.

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