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A Novel Composite as a Dispenser for Methyl Eugunol to Attract the Peach Fruit Fly, *Bactrocera zonata* (Saunders) Males under Field Conditions

El-Adly, R. A.[1]; M. M. El-Metwally[3] and N. M. Ghanim[2]
[1]Chemistry department, Faculty of Science, Taif University, Kingdom of Saudi Arabia
[2]Egyptian Petroleum Research Institute, Cairo, Egypt
[3]Plant Protection Research Institute, ARC. Dokki, Giza, Egypt

ABSTRACT

Peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is considered one of the most economic important insect pest for several kinds of fruit species. Present work seeks to explore for the preparation of a novel composite based on petroleum coke with polyethylene, oleic acid and natural rubber as a dispenser for methyl eugunol. This target aims to get efficient dispenser composite to attract PFF males under field conditions in comparison with standard liquid lure dispenser (cotton wick). In addition to favorably regulate the release rate of methyl eugenol (ME) to extend its effectiveness and stability for PFF attraction in comparison with standard lure dispenser (cotton wick) by using Jackson traps under field conditions. The obtained results showed that Composite prolonged the length of effective field ME duration to 18 weeks or longer; while, the length of effective field duration of cotton wick dispenser was 8 weeks only. The ability of cotton wick dispenser to attract PFF males was higher than that of Composite during the first six weeks (with no significant differences between the two dispensers in some of these weeks). On contrary, from the 7th week till the end of the experiments, Composite dispenser showed significantly higher ability to attract PFF males. Efficacy from Composite dispenser did not significantly affected by the time passed after hanging traps; while, ME released from cotton wick dispenser decreased significantly by the time passed. On another hand, the efficacy of Composite dispenser did not significantly affected by air temperature degrees; while, the efficacy of cotton wick dispenser was significantly affected by air temperature degrees. Therefore, it could be concluded that the prepared composite may efficiently replace the cotton wick and could be applied at large scale in integrated pest management programs for PFF.

Keywords*:* *Bactrocera zonata*, Methyl Eugenol, Petroleum coke ,dispenser composite , lure dispenser

INTRODUCTION

Peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is considered one of the most economic important pests for several kinds of fruit species in temperate, tropical and subtropical countries (Fletcher, 1987; Ghanim, 2009 and Younes *et al*., 2009). In Egypt, PFF attacking a wide range of fruits that differ in their ripening time stage all over the year (El-Minshawy *et al*., 1999; Hashem *et al*., 2001 and Ghanim, 2009). This pest can affect both fruit yield and quality (Shinwari *et al*., 2015) causing 25-50% damage to fruits, alone particularly in the summer season (Syed *et al*., 1970).

A current emerging topic in eco-friendly PFF management is the efficient deployment of its attractants in detection, monitoring and mass trapping (Afia, 2007; Abd El-Kareim *et al*., 2008&2009; Moustafa, 2009; Ghanim*et al*., 2010; Ghanim, 2013; El-Metwally and Amin, 2015 and Hemeida *et al*., 2017). Pheromone or para-pheromone constituents require formulation in suitable dispensing systems to protect them and their emission over prolonged period for controlling pests (Leonhardt *et al*., 1987 and Dharanivasan *et al*., 2017). Methyl eugenol (ME), 4-allyl-1,2-dimethoxybenzene, is a component of plant essential oil occurs naturally in more than 450 plant species from 80 families that grow mainly in the tropics and is a fundamental nutrient of some Bactrocera spp. (Tan, 1993;Aluja and Norrbom, 1999; Tan, 2000; Vayssieres *et al*., 2007 and Tan and Nishida, 2012). The use of ME as attractant in traps is known globally for the control of PFF; whereas, ME is deployed as powerful male-specific lure to PFF in Egypt (Afia, 2007; Abd El-Kareim *et al*.,2009; Ghanim *et al*., 2010; Ghanim, 2013; El-Metwally and Amin, 2015 and El-Metwally *et al*., 2017).

Several effective commercial dispenser-formulations were made by cotton rolls, polyethylene tubes, bags, or ropes baited with pheromones (Brown *et al*., 1992; Suckling, 2000; Johansson *et al*., 2001 and Sidahmed *et al*., 2014). ME has been used routinely in suitable dispenser systems to monitor and count the PFF populations in agricultural ecosystems (Afia, 2007; Abd El-Kareim *et al*.,2009 and El-Metwally *et al*., 2017). Based on its physical and chemical properties, ME is not expected to adsorb on solids and sediment (Lyman, 1990 and HSDB, 2006). ME dissipates rapidly from both soil and water at ambient temperature; whereas, dissipation half-life of ME was 6 and 34 h in water at 32 and 22°C, respectively (Shaver and Bull, 1980). The potential of temperature for ME is expected to exist almost entirely as a vapor in the ambient atmosphere (HSDB, 2006). ME vapor-phase is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 h at an atmospheric concentration (Meylan and Howard, 1993). Also, other factors that could influence release of ME in traps, including relative humidity, precipitation, topography, and landscape vegetation cover affected (Suckling *et al*., 2008).

According to Isiklan (2006), controlled release technology is a recent technology, which has considerable potential in the fields of medicine, pharmacy, and agriculture. The aims of controlled release formulations are to protect the supply of the agent, to allow the automatic release of the agent to the target at a controlled rate, and to maintain its concentration in the system within the optimum limits, over a specified period of time, thereby providing great specificity and persistence (Bajpai and Giri, 2002). Ideses and Shani (1988), Knutson *et al*. (1988), Millar (1995) and Cork *et al*. (2008) added that the dispenser-formulation should protect the pheromone constituents form oxidation and degradation by UV light which can be accomplished by trapping the pheromone constituents in a protective matrix and using antioxidants and UV inhibitors. Therefore, release rate of attractants and stability against photochemical reaction are important factors in determining the cost effectiveness of the lure dispenser system. Wilkins *et al*. (1984), Zdarek*et al*. (1988) and Chamberlain *et al*. (2000) mentioned that a constant release rate in the monitoring or mating disruption systems are affected directly by the design and chemistry of dispenser system. So, to reduce the cost of the dispenser system and increase the stability of attractants, development of a new approach to be considered as important (Dharanivasan *et al*, 2017). Bhattacharyya *et al*. (2010) have hypothesized the scope for extending the duration of attractants in insect lures through the use of nanotechnology. Nano-gel was used by Tiboni *et al*. (2008) and Bhagat *et al*. (2013) to develop nano-dispensers to slow down the release rate of pheromones for cost effective insect catches. Also, Dharanivasan *et al* (2017) hypothesized that using mesoporous metal oxide nanoparticles (SiO_2, ZnO and TiO_2 NPs) as physical adsorbents to adsorb ME through weaker interactions which may regulate the releasing rate of ME and increase their half-life and stability. Different dispensers of polymers including chitosan, sodium alginate, styrene acrylate and polyvinyl alcohol were used by El-Metwally *et al*. (2017) to coat ME for attracting PFF.

There has been a trend toward replacement of liquid lures with solid formulations (which lave no insecticides); so, the development of these less toxic and more user-friendly alternatives also has important applications to detect, monitor and control of fruit flies where species of Bactrocera and Dacus are serious economic pests (Leblanc *et al*., 2011).

Petroleum coke is a granular coal-like product that is produced by coking of feedstock obtained from residue of primary and secondary oil refining processes. It is have porous, amorphous structure and crystalline broken needle structure. It has chemically produced through cross-linking of condensed aromatic hydrocarbons during coking reactions. Approximately 75% of the worldwide production of petroleum coke is utilized as a source of combustible fuel, whereas higher grades of calcined coke are used in steel and titanium dioxide production and the manufacture of graphite electrodes used in aluminum smelting. Additional details regarding the coking processes have been described by Maxim *et al*. 2006

Based on the above-mentioned characterization of petroleum coke the present work seeks to explore for the preparation of a novel composite based on petroleum coke as carrier for methyl eugunol to attract B. zonata males under field conditions in comparison with standard liquid lure dispenser (cotton wick). In addition, studying the stability of Composite and cotton wick against time and determining the effect of air temperature degrees on the activity of the two dispensers.

MATERIALS AND METHODS

Materials: The dispenser used in this study was called Composite using novel compounds and technique in this field. The prepared composite based on petroleum coke which produced in Suez Oil Petroleum Company during cooking for heavy petroleum crude. The physicochemical properties of petroleum coke used in this investigation are presented in Table (1). In addition, polyethylene, oleic acid and natural rubber were associated with petroleum coke as additives and modifier for the surface property of prepared composite.

Procedure:

The obtained composite under investigation was prepared using autoclave device. This process was carried out in the Andrea's Hofer high pressure autoclave which made of stainless steel. It was equipped with a magnetic stirrer and automatic regulating device with a temperature indicator. The operating conditions were at low pressure and temperature 130 °C. Accordingly, the procedure accomplished in two steps. First, petroleum coke was impregnated with oleic acid and methyl eugenol (ME) solution to obtain 3% wt of ME in the final Composite. Secondly, the autoclave was charged with suitable mass of PE and then closed and heated with stirring. Then the impregnated petroleum coke was gradually added. The temperature of the blending mixture had to be held at 130°C for approximately 30-50 minutes to ensure complete mixing. Then SBR solution was gradually pumped into autoclave. The obtained Composite then was released suddenly from the opening gate in autoclave to suddenly cooling and mold in suitable shape.

Field bioassay:

The present experiments were carried out in guava orchards (*Psidium guajava L.*) located in two governorates; Dakahlia governorate (Mansoura district) and Gharbia governorate (Samanoud district). In Dakahlia, guava orchard was about ten feddans; while, it was about eight feddans in Gharbia. Experiment in Dakahlia started from the 7^{th} of August till the 11^{th} of December 2017; while, it started from the 22^{nd} August till the 12^{th} of December 2017 in Gharbia.

A composite dispenser mounted with 0.75 ml of commercial methyl eugenol (98% purity, Yasho Industries, India) was compared with cotton wick dispenser (measured as 2 cm long and 1 cm diameter) injected with the same amount of ME. Dispensers were install in Jackson traps (Harris et al., 1971). Six traps (as replicates) of each dispenser were distributed in a completely randomized design in guava orchard of each governorate. Traps were hung in a shady site within the canopy of the guava trees at a height of 1.5 – 2 meters from the ground. The distance between every two adjacent traps was not less than 50 meters to avoid the interaction between lures. The traps were weekly inspected and number of captured PFF males on each sticky cardboard inside traps was counted and recorded with renewing the sticky cardboards. Dispensers and its containing of ME did not renewed all over the experiment.

Data were analyzed by using analysis of variance (ANOVA) followed by least significant difference (LSD) at the probability of 0.05(as considered significant). In addition, the regression analysis was done to the obtained data. All statistical analysis was done with Co Hort Software (2004).

RESULTS

The physicochemical properties of Petroleum coke Table 1. show that the petroleum coke under investigation is a carbonaceous hydrophobic black solid material with fixed carbon ranging 82-88wt%. It contain a considerable amount from volatile compounds ranged between 11 to 17 %. This range is one of the most important factors to interact with methyl eugunol. The ash content indicates the presence of inorganic admixtures in coke. The analyzed sample contains 0.5 % of ash, in which the content of metals vanadium and nickel as ppm – components of coke.

Table 1. Physicochemical characterization of petroleum coke.

Test	Method	Result
True Specific gravity	ASTM-D 167	1.2-1.4
Apparent specific gravity	ASTM-D 167	1.0-1.2
Ash content, mass%	ASTM-D 3174	0.5 max
Volatile matter, mass%	ASTM-D 3175	11-17
Total moisture, mass%	ASTM-D 346	5 max
Moisture in analytical sample, mass%	ASTM-D 3173	0.4 max
Total Sulphur, mass%	ASTM-D 3177	5.3 max
Hard grove index	ASTM-D 409	60-80
Vanadium content, ppm	ASTM-D 3682	650 max
Nickel content, ppm	ASTM-D 3682	500max
Fixed carbon, mass%	ASTM-D 3172	82-88

Data illustrated in Fig. (1) shows that the length of effective field life of Composite dispenser (as releasing ME) reached 18 and 16 weeks in Dakahlia and Gharbia governorates; while, the length of effective field life of cotton wick dispenser was 8 weeks only. Whereas, PFF males was attracted to traps powered with Composite all over the experimental periods; while, it was attracted to traps powered with cotton wick for the first 8 weeks only; and after that there were no or neglected numbers attracted to the traps in the two governorates.

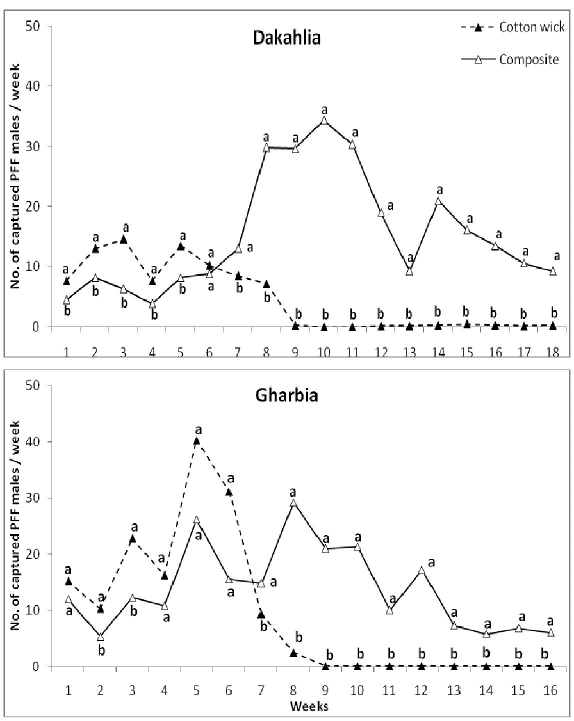

Fig. 1. Number of attracted PFF males to traps powered with cotton wick and Composite dispensers impregnated with ME all over 18 and 16 weeks in Dakahlia and Gharbia governorates (Note: in each week of each govenorate, values have the same letter did not differ signficantly at propability of 0.05).

On another hand, the ability of cotton wick dispenser to attract PFF males was higher than that of Composite during the first six weeks (with no significant differences between the two dispensers in some of these weeks). On contrary, from the 7th week till the end of the experiments, Composite dispenser showed significantly higher ability to attract PFF males (Fig., 1).

The general mean of attracted PFF males to traps powered with ME impregnated in Composite dispenser (15.32 males) was significantly higher than those of cotton wick dispenser (4.69) in Dakahlia governorate (Fig., 2). Also, in Gharbia governorate, there was significant difference between the general means of attracted PFF males to traps powered with ME impregnated in Composite (13.84 males) and cotton wicks (9.24) dispensers.

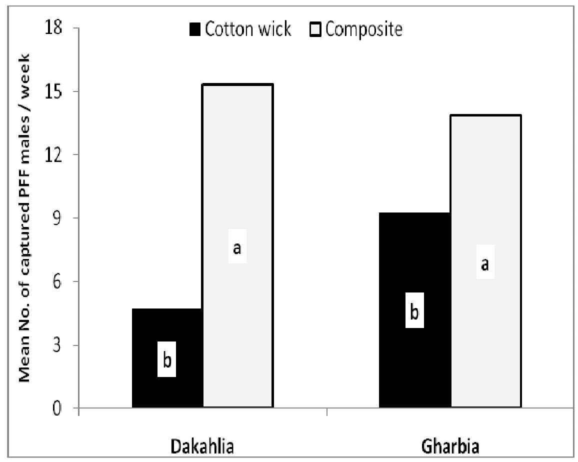

Fig. 2. Mean number of attracted PFF males to traps powered with cotton wick and Composite dispensers impregnated with ME all over the experimental period in Dakahlia and Gharbia governorates (Note: in each govenorate, colomns have the same letter did not differ signficantly at propability of 0.05).

The obtained results arranged in (Fig. 3) showed that, methyl eugenol released from Composite dispenser did not significantly affected by the time passed; while, ME released from cotton wick dispenser decreased significantly by the time passed. Whereas, the correlation coefficient value between passed time in weeks and the attracted PFF males in traps powered with ME impregnated in cotton wick and Composite dispensers were -0.84** and 0.29^{ns} in Dakahlia governorate and were -0.67** and -0.24^{ns} in Gharbia.

On another hand, each increase in the passed time by one week insignificantly increased the attracted PFF males to traps powered with ME impregnated in Composite dispenser by 0.52 males/week (R^2 = 0.08, P = 0.252) in Dakahlia governorate and insignificantly decreased them by 0.37 (R^2 = 0.06, P = 0.367) in Gharbia governorate (Fig., 3). With respect to cotton wick, each increase in the passed time by one week significantly decreased the attracted PFF males to traps by 0.85 and 1.80 males/week in Dakahlia and Gharbia governorates (R^2 = 0.70 and 0.45, P = 0.000 and 0.004, respectively).

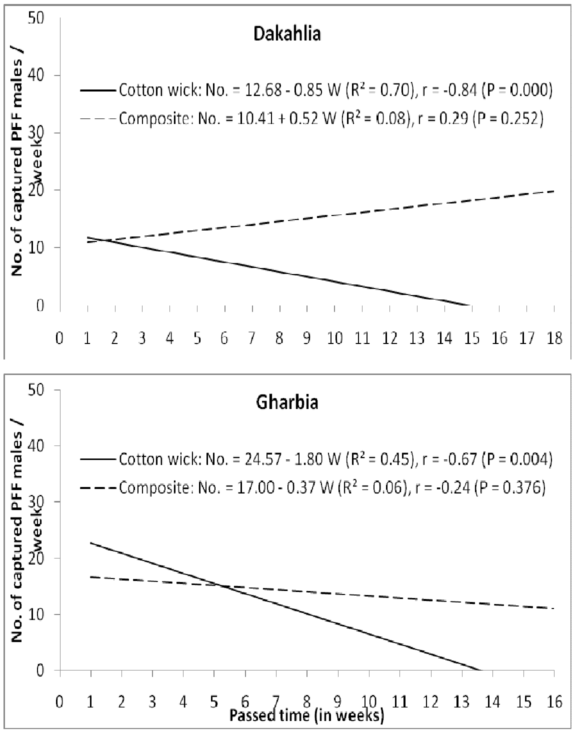

Fig. 3. Stability of the released ME (determined as attracted PFF males) from cotton wick and Composite dispensers inside traps over the time in Dakahlia and Gharbia governorates.

Temperature degrees (maximum, minimum and mean) had no significant effects on the ability of ME impregnated in Composite dispenser to attract PFF males (Table,2). Whereas, correlation coefficient values between captured PFF males and each of maximum, minimum and mean temperature degrees were insignificant in Dakahlia (r = -0.27^{ns}, -0.24^{ns} and -0.26^{ns}, respectively) and Gharbia (r = 0.26^{ns}, 0.27^{ns} and 0.27^{ns}, respectively) governorates.Also, the determination coefficient values of maximum, minimum and mean temperature degrees were relatively low in Dakahlia (R^2 = 0.07, 0.06 and 0.07, respectively) and (R^2 = 0.07, 0.08 and 0.07, respectively) governorates.

Table 2. Effect of temperature degrees on the released ME (measured as captured PFF males) from cotton wicks and Composite dispensers in Dakahlia and Gharbia governorates.

Treatment	Factor	r	b	P	R^2
		Dakahlia			
	Max. T.	0.85	1.27	0.000	0.73
Cotton wick	Min. T.	0.86	1.11	0.000	0.74
	Mean T.	0.86	1.20	0.000	0.74
	Max. T.	-0.27	-0.74	0.272	0.07
Composite	Min. T.	-0.24	-0.56	0.340	0.06
	Mean T.	-0.26	-0.64	0.305	0.07
		Gharbia			
	Max. T.	0.72	2.66	0.002	0.52
Cotton wick	Min. T.	0.71	2.33	0.002	0.51
	Mean T.	0.72	2.49	0.002	0.52
	Max. T.	0.26	0.56	0.335	0.07
Composite	Min. T.	0.27	0.52	0.302	0.08
	Mean T.	0.27	0.54	0.316	0.07

On contrary, maximum, minimum and mean temperature degrees showed highly significant extrusive effects on released ME from cotton wick dispenser (measured as attracted PFF males). Where the calculated r-values between captured PFF males and each of maximum, minimum and mean temperature degrees were 0.85**, 0.86** and 0.86**, respectively in Dakahlia governorate, and were 0.72**, 0.71** and 0.72**, respectively in Gharbia governorate. The determination coefficient values (R^2) of maximum, minimum and mean temperature degrees were 0.73, 0.74 and 0.74 in Dakahlia and were 0.52, 0.51 and 0.52, respectively in Gharbia governorates.

DISCUSSION

Controlled release technology protect the supply of an attractant to allow automatic release of it to the target at a controlled rate, and to maintain its concentration in the system within the optimum limits, over a specified period of time, thereby providing great specificity and persistence (Bajpai and Giri, 2002). The obtained results showed that the length of effective field life of Composite dispenser (as solid dispenser for ME) reached 18 and 16 weeks in Dakahlia and Gharbia governorates; while, the length of effective field life of cotton wick dispenser (as standard dispenser) was 8 weeks only. So, Composite prolonged the effective duration of releasing ME for about 3 times of that with cotton wick. This could be attributed to the ability of methyl eugunol to maintain contact with composite surface through adhesive forces or the bonding of the composite ingredients, resulting from intermolecular interactions when the ME and composite are brought together. Statistical analysis revealed that the activity of Composite dispenser showed significant stability against passed time; while, the activity of cotton wick dispenser significantly decreased as the time passed. These results are in agreement with those obtained by Leblanc *et al.* (2011) in French Polynesia (Tahiti Island), they mentioned that the solid Mallet dispensers with raspberry ketone including ME (Mallet ME wafer) outperformed other dispensers including the liquid ME standard in attracting Bactrocera dorsalis (Hendel). Also, a nanogelled ME (prepared from ME using a low-molecular mass gelator) was very stable at open ambient conditions and slowed down the evaporation of ME significantly (Bhagat *et al.*, 2013). In Sudan, Sidahmed *et al.* (2014) showed that the application of plywood dispenser impregnated with ME remained effective for more than four months in comparison only one month when used cotton wick and sponge dispensers. In Hawaii, traps baited with Mallet cuelure wafers impregnated with performed as well as standard Jackson traps that were baited with liquid cuelure in a wick against Bactrocera cucurbitae (Coquillett) were active over 8 weeks (Vargas *et al.*, 2003, 2009 and 2010).

This hypothesis is supported by Wilkins *et al.* (1984), Zdarek *et al.* (1988) and Chamberlain *et al.* (2000); they mentioned that the design and chemistry of dispenser system is directly affected the constant release rate in the monitoring or mating disruption systems. Also, Leblanc *et al.* (2011) reported that the proprietary constitution of the solid Mallet dispensers with raspberry ketone (Mallet wafer) support retard evaporation of ME against B. dorsalis. Using mesoporous metal oxide nanoparticles

(SiO2, ZnO and TiO2) as physical adsorbents to adsorb ME through weaker interactions, which may regulate the releasing rate of ME and increase their half-life and stability (Dharanivasan*et al*., 2017). The physical adsorption between ME and metal oxide nanoparticles could be held by hydrophobic interactions and stacking forces which were influenced by temperature and the more interactions slower the releasing rate (Tiboni*et al*., 2008).

According to Shaver and Bull (1980), HSDB (2006), Suckling *et al*. (2008), Tiboni*et al*. (2008) and Dharanivasan *et al*. (2017), air temperature degrees was of the highest effective factors on the duration of ME half-life as a vapor in the ambient atmosphere. With respect to the obtained data, methyl eugenol released from Composite dispenser (determined as its efficacy) did not affected significantly by temperature degrees; on contrary, ME released from cotton wick dispenser was highly significant affected by temperature degrees. The findings of cotton wick dispenser may be due to the potential of temperature on ME; which expected to exist almost entirely as a vapor in the ambient atmosphere (HSDB, 2006). Also, Shaver and Bull (1980) mentioned that ME dissipates rapidly from both soil and water at ambient temperature; whereas, dissipation half-life of ME was 6 and 34 h in water at 32 and 22°C, respectively. Dharanivasan *et al*. (2017) reported that the effect of temperature on controlled release of ME with metal oxide nanoparticles was found to be significant. While, Tiboni *et al*. (2008) mentioned that weather changes including temperature may also play a significant role in the insect catches.

Methyl eugenol released from Composite dispenser did not affected significantly by temperature degrees. Many factors, such as chemical composition, porosity, polarity, and texture of the dispenser composite must be taken into account to make sure that the methyl eugunol continues to catch peach fruit fly. Also, The physical and chemical properties of Composite (Table, 1) may be the reason of the prolonged duration of releasing ME in traps under field conditions.

CONCLUSION

From the above results, we can conclude that:

1- The petroleum coke, polyethylene, oleic acid and natural rubber are promising ingredients which can be used for the prepared of controlled release dispensers of active ingredients.
2- The prepared dispenser composite under investigation is favorably regulating the release of ME to extend its effectiveness and stability for PFF catches in comparison with standard lure dispenser.
3- The use of such prepared dispenser composite will have an economic impact.

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المتراكب الجديد (Composite) كناشر للميثيل إيوجينول لجذب ذكور ذبابة ثمار الخوخ تحت الظروف الحقليه

رفعت أحمد العادلي[١] ، مصطفى مهران المتولي[٣] و نبيل محمد غانم[٢]

[١] قسم الكيمياء ـ كلية العلوم ـ جامعة الطائف المملكة العربيه السعوديه

[٢] معهد بحوث البترول المصري ــ القاهرة ــ مصر

[٣] معهد بحوث وقاية النباتات ــ مركز البحوث الزراعية ــ الدقي ــ الجيزة ـ مصر

تعتبر ذبابة ثمار الخوخ واحده من أهم الآفات الأقتصاديه للعديد من أنواع الفاكهة. ويسعى هذا العمل الى إستكشاف متراكب جديد (Composite) مكون من فحم الكوك والبولي إيثلين وحمض الأوليك والمطاط الطبيعي كناشر للميثيل إيوجينول لجذب ذكور ذبابة ثمار الخوخ تحت الظروف الحقلية بالمقارنه بالناشر القياسي (فتيل القطن). أيضاً دراسة تنظيم معدل إطلاق الميثيل إيوجينول لكي يتم إطالة مدة ثباته وفعاليته لجذب ذكور ذبابة ثمار الخوخ وذلك باستخدام مصائد جاكسون. وقد أوضحت النتائج المتحصل عليها أن مدة فعالية الـ Composite فى الحقل بلغت ١٨ أسبوع في حين أن مدة فعالية فتيل القطن أستمرت ٨ أسابيع فقط. وخلال الستة أسابيع الأولى كانت كفاءة فتيل القطن لجذب ذكور ذبابة ثمار الخوخ أعلى من الـ Composite مع عدم وجود أختلافات معنوية بينهما فى بعض القراءات، وعلى العكس من ذلك وجد أن الـ Composite كان الأعلى من الأسبوع السابع حتى نهاية التجارب. من ناحية أخرى أظهر التحليل الإحصائي أن فعالية الـ Composite لم تتأثر معنويا بمرور الوقت بينما انخفضت فعالية الفتيل القطني بدرجة معنوية بمرور الوقت. كما أظهر التحليل الإحصائي أن فعالية الـ Composite لم تتأثر معنوياً بالتغير في درجات حرارة الجو وعلى النقيض من ذلك فان فعالية فتيل القطن تأثرت معنوياً بها. ولذلك يمكن أن نوصي باستخدام الـ Composite بدلاً من فتيل القطن وأن يطبق على نطاق واسع فى برامج المكافحه المتكامله لذبابة ثمار الخوخ.