

Oviposition Deterrent of *Bactrocera carambolae* Resulted from Eggs Deposition on Mango

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ABSTRACT

Oviposition deterrent is chemical compounds which are used for avoiding eggs deposition. The oviposition deterrent resulted from eggs deposition is valuable information that can be manipulated for managing its population. The objective of this research was to determine the presence of oviposition deterrent resulted by female *Bactrocera carambolae* on mango. Extraction of oviposition deterrent was conducted by maceration method. The preference test was performed using two arms olfactometer, meanwhile the oviposition deterrent test was conducted by exposed gravid females to fruit that already smeared with extracts. The result revealed that gravid females of *B. carambolae* were attracted to methanol extract of 1 day after egg deposition, whereas the methanol extract of 3 and 5 days after egg deposition repelled gravid females. Oviposition deterrent test indicated that methanol extract at category 3 and 5 acted as a deterrent. The preference of gravid females of *B. carambolae* to different category of infested fruit extracts was probably influenced by the chemicals modification on mango after oviposition. This result suggested that the female *B. carambolae* do not deposit oviposition deterrent. The phenomenon of deterrence was probably as a result of chemical changes in fruit as a consequence of eggs infestation.

Keywords: *Bactrocera carambolae*; fruit extract; mango; oviposition deterrent

INTRODUCTION

In some insect species, a chemical stimulus from the host plant plays an important role in recognition of its host plant. Chemical compounds produced by host plants attract herbivores to come

and alight on the host plant (Wang & Kays, 2002; Powell, Tosh, & Hardie, 2006; Pinero & Dorn, 2007; Castells & Berenbaum, 2008; Meagher & Landolt, 2008; Patt & Pfannenstiel, 2008; Riffell, Abrell, & Hildebrand, 2008). Insects come to the host plants purpose to get nutrients for themselves or their offspring in order to grow and develop properly for completing their life cycle, so that the generations can continue to grow (Averill & Prokopy, 1989; Genç, 2006). Therefore, in order to obtain sufficient nutrients particularly for offspring, insects usually avoid competition by marking areas that have been infested with the eggs by the female with certain compounds, which is called host marking pheromone (Nufio & Papaj, 2001; Adesso, McAuslane, Stansly, & Schuster, 2007). In addition to the host marking pheromone, a change of host chemical and physical after the host is attacked by insect pest may also prevent the attack or the laying of an egg from conspecific individual (Marchand & McNeil, 2004).

Fruit flies, like other insects also use semiochemicals as an important medium for communication with the host plant (Light & Jang, 1987; Jang & Light, 1991; Cossé, Todd, Millar, Martínez, & Baker, 1995; Cornelius, Nergel, Duan, & Messing, 2014; Papadopoulos, Kouloussis, & Katsoyannos, 2006; Ravikumar & Viraktamath, 2007; Manrakhan & Lux, 2008; Brévault & Quilici, 2010; Siderhurst & Jang, 2010; Niogret, Montgomery, Kendra, Heath, & Epsky, 2011) and most of fruit fly species produce host marking pheromone (Schoonhoven, Sparnaay, van Wissen, & Meerman, 1981; Sakai, Honda, Oshima, & Yamamoto, 1986; Roitberg & Mangel, 1988; Růžicka & Havelka, 1998; Nufio & Papaj, 2001; Nufio & Papaj, 2004; Arredondo & Diaz-Fleischer, 2006; Aluja & Mangan, 2008; Stelinski, Zhang, Onagbola, & Meyer, 2009).

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Bactrocera dorsalis complex is a major pest in Asia and Pacific region, where the larvae damage both fruit and vegetable. Economic losses can be caused by fruit flies due to direct damage to the fruit, the fruit falls and loss of export markets due to quarantine regulations of the importing country. Adult fruit flies are a high mobility species, wide spreading, high fecundity, and some species are very polyphag, including the invasive pest in some areas so that pests are included in the top ranking on the list of targets quarantine (Joomaye & Price, 1999; Clarke et al., 2005).

Bactrocera carambolae is a species of fruit fly belonging to *B. dorsalis* complex which has a wide host range, including mango (White & Hancock, 1997; van Sauers-Muller, 2005; Muryati, Hasyim, & Riska, 2008). Clarke et al. (2005) recorded that the host plant of *B. carambolae* includes 77 species from 50 genera belong to 27 families. The success of controlling this pest will benefit farmers, especially mango farmers to increase profits from the mango agribusiness.

Information about chemical compounds as a communication medium for *B. carambolae* with the environment, particularly host marking pheromone that is usually functioning as an oviposition deterrent for conspecific females, is useful and has a good prospect to be used to manage its population (Norin, 2007; Jeyasankar, 2009). Based on field observations on mango, *B. carambolae* in a fruit usually found in the same stadium. There was suspected that a certain compound may cause conspecific females did not lay their eggs in the same site. So far, information about the presence of certain chemicals on the fruit as a result of egg deposition by *B. carambolae* which can act as oviposition deterrent is not available yet. This information needs to be obtained in order to develop an oviposition deterrent-based technology, both as a reference for developing synthetic materials as well as looking for other materials that serve as oviposition deterrent.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Pesticide Toxicology and Laboratory of Basic Entomology Faculty of Agriculture Universitas Gadjah Mada Yogyakarta from 2012 to 2013.

Bactrocera carambolae Mass Production

Fruit fly population was initially cultured from

star fruit (*Averhoa carambolae*) and maintained in the laboratory of Basic Entomology, Department of Pests and Diseases, Faculty of Agriculture, Universitas Gadjah Mada since 2006 (Suputa, personal communication). The materials used in the fruit fly mass production were cage with screen wall and wooden floor and ceiling (30 x 30 x 30 in size); sawdust as pupa medium; plastic tray as a container of artificial food for maintaining maggot; and artificial food that was made of wheat husk (160 g), yeast extract, fermipan (8.7 g), sugar (35 g), antibacterial (0.33 g), antifungi (0.33 g), and aquadest (180 ml). The medium for female fruit fly laying eggs was plastic cup that was perforated with needle evenly and then was glued in the middle of a wall. Furthermore, eggs on the plastic cup were poured into plastic tray with artificial food for maggot, then the tray put into a box with sawdust inside. An adult fly was fed with sugar and yeast extract.

Mango Fruit for Oviposition Deterrent Extraction

Arumanis 143 cultivar used in this experiment was obtained from Cukurgondang Mango Field Experiment, Pasuruan, East Java. One hundred and twenty mango fruits were selected based on their relatively uniform maturity and size, then divided into two parts. A part (60 pieces) selected fruits were exposed to gravid females of *B. carambolae* by hanging them inside the screen cage, each containing 5 fruits, subsequently 10 gravid females were released into fruit-containing cage for 24 hours, while the remaining 60 pieces were used as control. Egg-infested fruits and egg-free fruits (control) were used as a material to be extracted in three categories.

Extraction of Suspected Oviposition Deterrent from Egg-Infested Fruit

Solvent extraction of oviposition deterrent using distilled water and methanol was conducted on three categories of day after oviposition, namely first, third and fifth category. The one day of 10 egg-infested fruits (1st category) were soaked individually in glass bowl containing 10 ml solvent for 30 minute. Fruit soaking was also done on control fruit with the same quantity. The same treatment was also conducted on third and fifth categories. Extracts from the same category were put together, hence there were 12 extracts each 100 ml. Those were methanol extract of egg-infested fruit at first category (OD-MeOH 1), third category (OD-MeOH 3), fifth category (OD-MeOH 5); water extract of

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egg-infested fruit at first category (OD-water 1), third category (OD-water 3), fifth category (OD-water 5); methanol extract of control fruit at first category (BA-MeOH 1), third category (BA-MeOH 3), fifth category (BA-MeOH 5); and water extract at first category (BA-water 1), third category (BA-water 3), fifth category (BA-water 5).

Extraction of Suspected Oviposition Deterrent from *B. carambolae* Egg

Extraction of *B. carambolae* eggs at three categories of eggs old, namely first, third, and fifth day after laying was done by maceration in methanol for 24 hours. The composition between egg and solvent was 1:20 (w/v).

Olfactometry Test of Egg-Infested Fruit Extract

A preference test followed method performed by Muryati, Trisyono, Witjaksono, & Wahyono (2012). The small piece of filter paper was dipped in OD extract and placed at an olfactometer tip, while in the other tip was placed filter paper that was dipped in control (extract BA from the same category). Hereinafter, 10 gravid females (7-10 days after emergence from cocoon) were allowed to choose which one they preferred. Each bioassay was repeated six times. The observation was carried out on the number of *B. carambolae* females chosen olfactometer tip. The data analysis was performed by using *t*-test at $\alpha = 5\%$.

Oviposition Deterrent Activity Test

Choice test was done to understand the oviposition deterrent activity of compounds extracted from egg-infested fruit (OD) compared to healthy (control) fruit against *B. carambolae*. Mango fruits that have been smeared with OD and BA were hung in the screen cage, and then 10 *B. carambolae* gravid females were released into the cage. This test was conducted through six series of experiment, namely 1) OD-water 1 vs. BA-water 1, 2) OD-water 3 vs. BA-water 3, 3) OD-water 5 vs. BA-water 5, 4) OD-MeOH 1 vs. OD-MeOH 1, 5) OD-MeOH 3 vs. OD-MeOH 3, and 6) OD-MeOH 5 vs. OD-MeOH 5. The treatments were replicated 4 times and each replication consisting of 2 sample units for water extract and 3 sample units for methanol extract. A parameter observed was the number of eggs laid. The proportion of eggs laid was calculated by:

$$\text{Proportion of eggs laid} = \frac{\text{No. ELAT}}{\text{No. ELBT}} \times 100\%$$

Remarks:

No. ELAT : Number egg laid in each treatment

NO.ELBT : Number egg laid in both treatment

The data of proportion of eggs laid in each treatment was analyzed using *t test* at $\alpha 5\%$. Oviposition activity index (OAI) of the Kramer & Mulla (1979) formula as performed by Muryati, Trisyono, Witjaksono, & Wahyono (2012) was used to determine the level of deterrence. Positive values of OAI indicate more eggs are laid in the treatment than in the control, it means that the extracts act as attractant. Conversely, negative values of OAI indicate that more eggs laid in the control than in the treatment, and that the extracts act as a deterrent/repellent.

Identification of Chemical Compounds of Extracts

Identification of the chemical compounds was performed by using GC-MS from Agilent Technologies 6890 Gas Chromatograph with auto sampler coupled to 5973 Mass Selective detectors and chemstation data system that was equipped with an HP Ultra 2 capillary column (30 m in length x 0.25 mm in diameter, film thickness 0.25 μm). The oven temperature was programmed from 70 $^{\circ}\text{C}$ (0 minute), then increased at 5 $^{\circ}\text{C}/\text{min}$ to 200 $^{\circ}\text{C}$ for 1 minute, and finally increased at 20 $^{\circ}\text{C}/\text{minutes}$ to 280 $^{\circ}\text{C}$ for 28 minutes. Injection port temperature was programed at 250 $^{\circ}\text{C}$. The carrier gas was He, with the constant flow rate of 0.8 $\mu\text{l}/\text{minutes}$. The mass spectrometer was operated at 70 eV.

RESULT AND DISCUSSION

Olfactometry Test of Egg-Infested Fruit Extract

Olfactometry test revealed that *B. carambolae* females had the same preference to water extract of egg-infested fruit for all extract categories (OD-water 1, 3 and 5) and control (BA-water 1, 3 and 5) (*t test*; $P = 0.801, 0.342, 0.976$ respectively) (Fig. 1). This presumably compounds associated with insect olfactory organs are volatile, non-polar compounds, while water is a polar solvent and unable to dissolve non-polar compounds, although Averill & Prokopy (1989) stated that the host marking pheromones of tephritid are soluble in water and methanol. Parekh, Jadeja, & Chanda (2005) and Paiva *et al.* (2010) revealed that a plant that was extracted with methanol had higher bioactive compounds against bacteria than extracted with water.

B. carambolae had different preferences over the methanol extract of eggs-infested fruits at 1st, 3rd and 5th day after oviposition (Fig. 2). *B. carambolae* females prefer extract of eggs-infested fruit 1st day after oviposition (OD-MeOH 1) to extract of healthy fruit (BA-MeOH 1) (*t test*, *P* = 0.00). The preference of *B. carambolae* females then changed in the next category of extract, i.e. the female prefer

3rd and 5th extract of healthy fruit (BA-MeOH 3 and BA-MeOH 5) to 3rd and 5th day after oviposition extract of of eggs-infested fruit [OD-MeOH 3 (*t test*, *P* = 0.00) and OD-MeOH 5 (*t test*, *P* = 0.00)]. This phenomenon indicated that there was substance/s dissolved in the methanol whether from healthy or egg-infested fruit that attract or repel the female of *B. carambolae*.

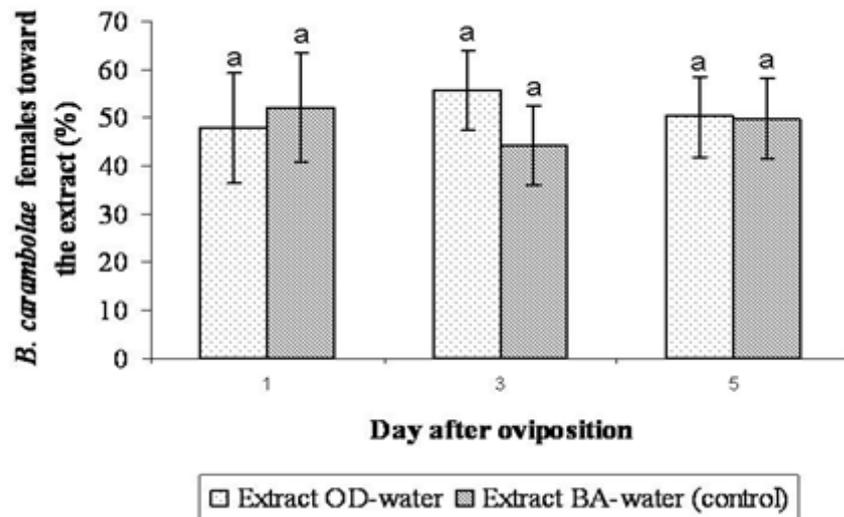


Fig. 1. The preference of *Bactrocera carambolae* females to water extract of egg-infested fruit (OD-water) and control (BA-water) base on olfactometry test

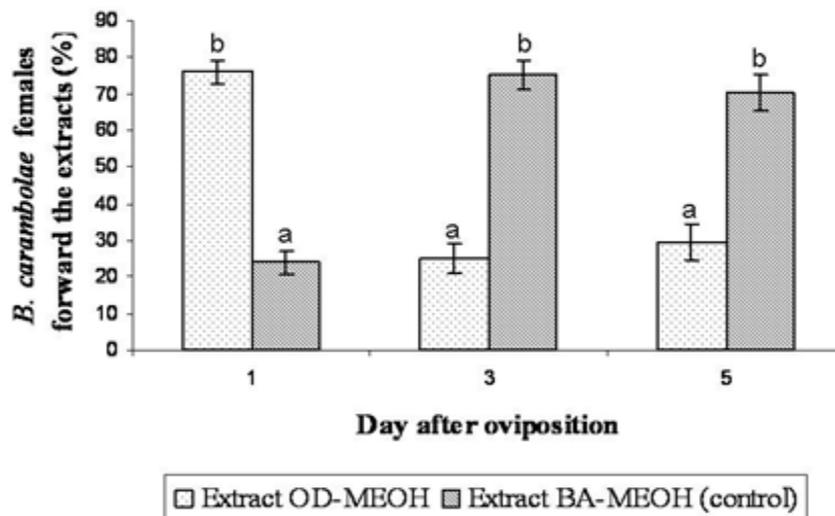


Fig. 2. Preferences of *Bactrocera carambolae* females to methanol extract of egg-infested fruit (OD-MeOH) and control (BA-MeOH) base on olfactometry test

Test of Oviposition Deterrent Activity

The proportion of eggs laid in the treated fruit with all categories of OD-water (OD-water 1, 3 and 5) was not significantly different from that in the control (base on *t* test, $P=0.562$, 0.450 , 0.398 respectively) although there was a tendency that all categories of OD-water acted as a stimulant with the OAI values of category 1, 3, and 5 (water extracts

of egg-infested fruit 1st, 3rd and 5th after oviposition) were 0.25, 0.16, and 0.11 respectively (Fig. 3). This is probably caused by some compounds or nutrients in the fruit dissolved in the water can act as oviposition stimulants. Thompson & Pellmyr (1991) stated that water-soluble compounds were the main factor that influenced host selection by *Pieris rapae*.

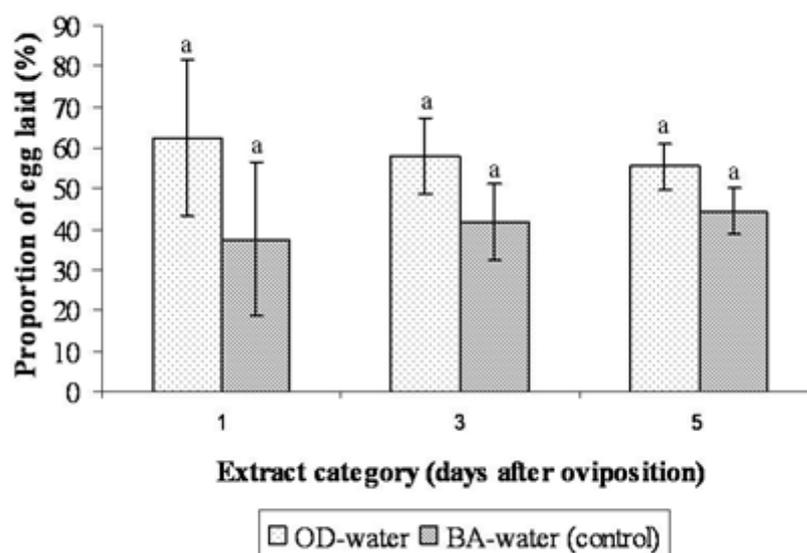


Fig. 3. Proportion of eggs laid by *Bactrocera carambolae* females in fruit treated with water extract of egg-infested fruit (OD-water) and control (BA-water)

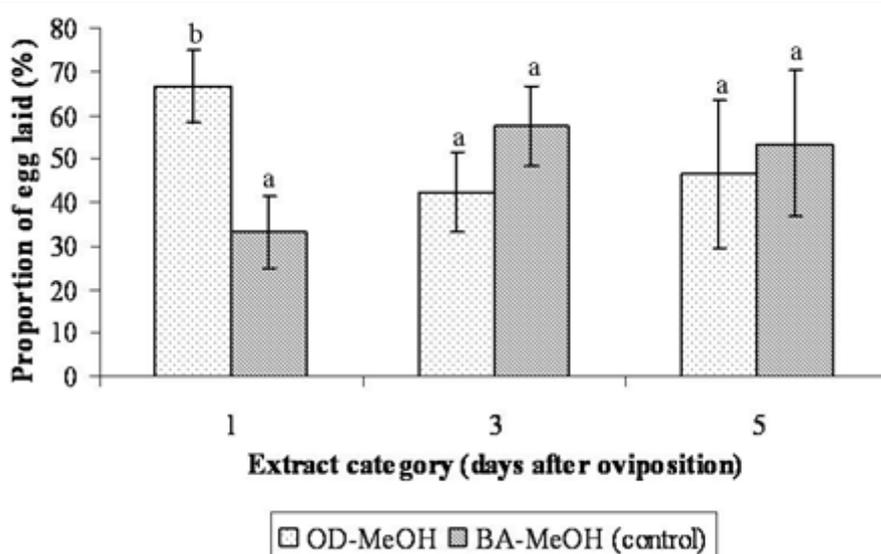


Fig. 4. Proportion of eggs laid by *Bactrocera carambolae* females in fruit treated with methanol extract of egg-infested fruit (OD-MeOH) and control (BA-MeOH)

Proportion of eggs laid in treated fruit with OD-MeOH 1 was significantly different from that in the control (BA-MeOH 1) (based on *t* test, $P=0.006$). While the proportion of eggs laid in fruits treated with OD-MeOH 3 and OD-MeOH 5 was not significantly different from that in control (BA-MeOH 3 and BA-MeOH 5) (based on *t* test, $P=0.115$ and 0.05 respectively). An extract OD-MeOH 1 acted as stimulant with the OAI of 0.36, whereas OD-MeOH 3 and OD-MeOH 5 acted as oviposition deterrent with the OAI values of -0.15 and -0.07 , respectively (Fig. 4). Oviposition deterrent activity test of OD-MeOH 1 also showed that the extract acts as a stimulant for *B. carambolae* oviposition. This is likely due to the injuries caused by the oviposition process producing chemical compounds derived from the fruit flesh, which then becomes attracted *B. carambolae* female to come and lay eggs on fruit that had already contained eggs. A similar result had been reported by Katsoyannos, Kouloussis, & Papadopoulos (1997), it stated that *Ceratitidis capitata* females prefer chemical compounds from citrus pulp to chemical compounds from the citrus skin. Data of GC-MS analysis also confirmed this result, that there were some chemical compounds just detected in the MeOH extract of OD-1 but none on BA-MeOH 1 and vice versa. Differences in the chemical content of the extracts caused differences in preference of *B. carambolae* female to both types of extracts. The fruit odor is likely to be an indicator for *B. carambola* females that there is sufficient moisture and nutrients for the larvae. Papadopoulos, Kouloussis, & Katsoyannos (2006) found that moisture or water and nutrients were the important factors for *C. capitata* in determining the suitable location of egg laying. Several previous studies also proved that female *C. capitata* and *Anastrepha ludens* were more attracted to lay eggs on egg-deposited fruits than on egg-free fruits (Díaz-Fleischer & Aluja, 2003; Rull, Prokopy, & Vargas, 2003).

Analysis of the Chemical Compounds of the Methanol Extract of Egg-Infested Fruit

Based on GC-MS analysis, OD-MeOH (1 dao) and BA-MeOH (1 dao) had the same dominant compounds, namely *1,3-benzenediol, 5-pentyl-* (the concentrations were 72.37% and 75.09% respectively) (Table 1). The eggs extract of *B. carambolae* 1st day after eggs laying (TL 1) contained *hexadecanoic acid* (20.42%) (Table 2). OD-MeOH (3 dao) and BA-MeOH (3 dao) also had the same dominant compounds, i.e. *1,3-benzenediol, 5-pentyl-* (the concentrations

were 72.71 % and 29.64 % respectively) (Table 1). Meanwhile, the eggs extract 3rd day after egg laying (TL 3) contained *hexadecanoic acid, methyl ester* as predominant compounds (the concentration was 31.39 %). OD-MeOH (5 dao) and BA-MeOH (5 dao) also contained the same predominant compounds, namely *linoleic acid* (the concentrations were 44.69 % and 40.75 % respectively) (Table 1). Whereas, the predominant compounds off eggs extract 5th day after egg laid (TL 5) was *9-octadecenoic acid* (the concentration was 11.65 %).

B. carambolae female had less preference to MeOH extract of OD-3 and OD-MeOH 5 than to controls (BA-3 and BA-MeOH MeOH 5). Oviposition deterrent test showed that both types of extracts were a weak deterrent. Mangoes infested with fruit fly eggs were initially attractive to *B. carambolae* females but later on became unattractive three days after the eggs laid. This was due to further reactions occurred in fruit wounds caused by the ovipositor injection so that the odor becomes less favored by *B. carambolae* females. Behar, Jurkevitch, & Yuval (2008), Robacker (2007) and Sood & Nath (2005) stated that fruit flies associated with bacteria that caused fruit decay after eggs deposition although visually fruit still looked good. Papadopoulos, Kouloussis, & Katsoyannos (2006) also stated that the scent produced from fermented fruit repelled the females to lay eggs. The decay process was proved by the GC-MS data which showed that the OD-MeOH 5 contained aflatoxin G1. Aflatoxin G1 is a toxin produced by fungus belong to the *Aspergillus* group that typically grows on rotting substance (Bokhari & Aly, 2009).

No compound of egg extracts (TL) was detected in the egg-infested fruit extract (OD-MeOH) for all time after the eggs deposition, this indicated that preferences of *B. carambolae* female to egg-infested fruit extract was not affected by the chemical compounds of the eggs. Some previous researches revealed that the eggs of some insects contain oviposition deterrent materials. Messina, Barmore, & Renwick (1987) examined the extract of *Callosobruchus maculatus* eggs that was identified by other females as oviposition deterrent. Thiery & Le Quere (1991) detected a compound produced by the eggs of European corn borer, *Ostrinia nubilalis*, inhibits the other females to lay eggs. Furthermore, Li & Ishikawa (2005) also stated that the eggs of *Ostrinia furnacalis* and *Ostrinia scapulalis* emit chemical compounds that prevented the other females to lay eggs in the same site.

Table 2. The predominant compounds of methanol extract of *Bactrocera carambolae* eggs 1st, 3rd, and 5th day after laid

No	Compounds	TL 1		TL 3		TL 5	
		Concentration (%)	Quality (%)	Concentration (%)	Quality (%)	Concentration (%)	Quality (%)
1	14- β -H-Pregna	2.08	96				
2	2,5-Dimethylpyrroline	2.81	49	1.43	46		
3	9-Hexadecenoic acid, methyl ester	2.79	99	24.68	99		
4	Hexadecanoic acid, methyl ester		99	31.39	99		
5	Hexadecanoic acid / Palmitic acid	20.42	99				
6	9-Hexadecenoic acid	19.22	96				
7	(+)-15-Hexadecanolide	16.94	93				
8	9-Octadecenoic acid (Z)-	14.26	99				
9	9-Octadecenoic acid, methyl ester	1.95	99	24.79	99		
10	Octadecanoic acid, methyl ester		99	7.48	99		
11	Ethylpentamethyl-disiloxane	2.04	78				
12	Taraxasterol						
13	Dimethyloctadecyl [(dimethyloctadecylsilyl) oxy]-silane	4.41	91				
14	Tetradecanoic acid, methyl ester			1.19	98		
15	1H-Pyrrole-2,5-dione, 1-(hydroxyme			1.33	46		
16	Pregn-4-ene-3,20-dione, 17- (acetyloxy)-6-methyl-, (6.alpha.)-			3.43	47		
17	d1-Limonene					3.36	60
18	1-Menthol					4.26	91
19	Terpineol-4/2-Cyclohexen-1-ol, 1-methyl-4-(1-m					7.95	96
20	9-Hexadecenoic acid, methyl ester					10.18	99
21	Hexadecanoic acid, methyl ester					11.08	99
22	9-Octadecenoic acid (Z)-					11.65	99
23	Thiosulfuric acid (H2S2O3), S-(2-a					3.78	98
24	9,17-Octadecadienal, (Z)-					8.05	99
25	Cyclopropaneoctanal, 2-octyl-					3.79	70
26	6 (Z), 9 (E) - Heptadecadiene					3.72	64

Remarks: analysis was performed by GC-MS; TL: Day after laid

The results of this study indicated that the oviposition process by *B. carambolae* female in the mango did not produce any chemical compound that could be recognized by conspecific females as a sign that the fruit had already contained eggs. It was likely caused by a relatively large size of *B. carambolae*, such as mango, so there was a sufficient space and nutrients available for a lot of larvae. Papaj & Messing (1996) who tested the oviposition behavior of *Ceratitidis capitata* in coffee revealed that more females came to the larger size of coffee infested with eggs than to a small one.

CONCLUSION

The odor of egg-infested fruit initially attracted to *B. carambolae* female to deposit more eggs, but became repellent or deterrent after three days later. Attraction or rejection mechanism was more likely as a result of processes occurring in the fruit after oviposition than the result of chemical compounds released by females coincide with oviposition. Chemical compounds of the egg-infested fruits that have oviposition deterrent activity were soluble in methanol and insoluble in water.

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