EFFECTS OF CITRONELLA GRASS EXTRACT ON THE OVIPOSITION BEHAVIOR OF CARAMBOLA FRUIT FLY (*Bactrocera carambolae*) IN MANGO

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ABSTRACT
Carambola fruit fly (*Bactrocera carambolae*) causes economic losses because the female deposit her eggs in fruits and the larvae bore into the fruits. The availability of non-preferred and safe substance in deterring the females to lays eggs will provide an effective tool to protect the fruits. This research examined three types of citronella grass extracts in regard with their effects on disturbing the oviposition behaviour. Water, methanol, and n-hexane citronella grass extracts were applied to fruits and the fruits were then exposed to gravid females. Olfactometry tests were carried out to investigate the mechanisms by which extracts modify *B. carambolae* oviposition. The methanol extract deterred the females for oviposition with OAI = -0.83, whereas the water extract and n-hexane extract had no effect on oviposition with OAI ranged -0.02 to 0.29 and -0.02 to -0.38, respectively. Furthermore, the olfactory test showed that the methanol extract had no effect on repellency. Therefore, the mechanism of reducing the oviposition was most likely through gustatory rather than olfactory processes. The major compounds in the methanol extract were silane, [[(3.beta)-lanosta-8, 24-dien-3-yl] oxy] trimethyl and elemol. The methanol extract of citronella grass has the potency to be used as a tool to protect mango from *B. carambolae* oviposition.

Keywords: mango, carambola fruit fly, citronella grass, methanol, olfactory tests.

INTRODUCTION
Fruit fly is one of the most destroying insects for fruits and vegetables in Indonesia and in some other countries (Clarke *et al*., 2005; Daini *et al*., 1983; Harris *et al*., 1986; Kalshoven, 1981; Koyama *et al*., 2004; Sookar *et al*., 2006; Vargas *et al*., 2010). There are many species of fruit fly found in Indonesia. Among those species, *Bactrocera carambolae* (Diptera: Tephritidae) has the widest host range and also the widest geographic distribution (Clarke *et al*., 2005; Muryati *et al*., 2008; Sauers-Muller, 2005). One of its hosts is mango, which has a high economic value for Indonesia. Therefore, fruit fly infestation on mango is a constraint for Indonesia to export. Furthermore, it is important to have effective, environmentally friendly and simple control technique(s) to help farmer lifting their profit from mango agribusiness.

Some control measures have been implemented for controling fruit fly. Sterile insect technique is commonly applied in some other countries (Chiu and Chu, 1991; Cladera *et al*., 2006; Kawasaki, 1991; Koyama *et al*., 2004; Linares, 1991; Orankanok *et al*., 2005; Zavala *et al*., 1991). In Indonesia, farmers usually apply fruit bagging and synthetic chemicals for fruit fly control. Some other control techniques were developed in research scale, namely: sanitation, the use of insect attractant, release of sterile males and natural enemies. There are some constrains to adopt those techniques in large scale because of Indonesian geographic, sociology and farming system. Individual approach (orchard to orchard) is seemingly more suitable for fruit fly control in Indonesia by preventing fruits from oviposition.

Fruit fly particularly use chemical as a medium for communication with their environment, including their host (Brevault and Quilici, 2010; Cornelius *et al*., 2000; Light and Jang, 1987; Manrakhan and Lux, 2008; Papadopoulos *et al*., 2006; Ravikumar and Viraktamath, 2007; Siderhurst and Jang, 2010). We can take advantage from this process to control its population by disturbing the communication using a compound that inhibits fruit fly to find their host. Such compounds were usually found in the non-host plants (Cook *et al*., 2007; Eriksson *et al*., 2008; Gokce *et al*., 2006).

Indonesia has diverse natural resources of plants for pest control. Citronella grass (*Cymbopogon nardus*) has been reported to have biological activity to different species of insects (Hasyim *et al*., 2010; Issa *et al*., 2011; Kianmatee and Ranamukhaarachchi, 2007; Koul *et al*., 2008; Pangnakorn *et al*., 2011; Zaridah *et al*., 2006). The significance of using natural product is environmentally friendly and easy to be obtained by farmers. Moreover, farmer can produce the intended materials by using a simple technology. This research objective was to determine the role of the citronella grass extracts in disturbing the oviposition behaviour of carambola fruit fly (*B. carambolae*). The raw materials were extracted using water, methanol, and n-hexane.

MATERIALS AND METHODS

Insect preparation
B. carambolae for bioassay was provided by the laboratory of Basic Entomology, Department of Pest and

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Diseases, Faculty of Agriculture, Universitas Gadjah Mada using established laboratory mass rearing procedures. This population was initially obtained from carambola fruit in 2006 (Suputa, 2010, personal communication).

Extraction

The citronella plants were obtained from Laing Research Station of Indonesian Medicinal and Aromatic Crop Research Institute, in Solok, West Sumatera. The leaves of citronella grass were cut into pieces (± 10 cm) and then dried in an oven under a temperature of 40°C and blended into powder. Dry materials (3 kg) were used for extraction process. The material was divided into three equal amounts to be macerated in three different solvents. Two parts (2 kg) of them were submerged in 2.5 l n-hexane and 3 l methanol respectively for about 24 hours. The remaining (1 kg) was dissolved in 4.5 l hot water by the infusion method for 30 minutes. The methanol and n-hexane solutions were then filtered with filter paper in vacuum flask which was then evaporated by a rotary evaporator. The water solution was first filtered with the same method using methanol and n-hexane solutions, and then evaporated using waterbath to obtain crude extracts. The results of each extract were used for bioassay. Each extract was dissolved in distillated water and added with emulsifier (0.75% tween 40) to obtain the intended concentration.

Oviposition deterrency test

Choice test with direct exposure to the fruit was conducted by using Completely Randomized Design consisting of four extracts (water, methanol, n-hexane, and control) and each treatment was repeated four times. After spraying (10 ml/fruit), the four treated fruits were then hung in the screen cage (60 x 60 x 70 cm) randomly. Ten gravid females were released in each cage. The number of eggs masses and eggs laid on each fruit was counted and the proportion of eggs laid in each treatment was calculated by dividing the number of eggs by the total number of eggs found in all four treatments multiplied by 100%. Three different concentrations for each extract (1,000; 2,500 and 5,000 ppm) were tested separately using a similar design.

The proportion of eggs laid were analyzed using analysis of variance (ANOVA) and if there was a significant difference between treatments then followed by LSD test at 95% confidence level. The oviposition activity index (OAI) was calculated by the formula proposed by Kramer and Mulla (1979):

$$OAI = \frac{N_T - N_S}{N_T + N_S}$$

Where N_T = no. of eggs in the treatment and N_S = no. of ovipositions in the standard (control). The OAI values lie within the range from +1 to -1. Positive values indicate that more ovipositions were observed in the treatment than in the control. In other words, the material functions as an attractant. In contrast, when more ovipositions were found in the control than that in the treatment, the OAI would be negative, suggesting that the material acts as a repellent. Furthermore, for determining the activity of citronella grass, we adopted classification made by Elango *et al*. (2009) which proposed that OAI of +0.3 and above are considered as attractants while that OAI of -0.3 and below are considered as repellents.

Olfactometry test

Choice test using two arms olfactometer (Y shape) was done to study the mechanisms by which extracts modify *B. carambolae* oviposition. A filter paper (1 cm^2 in width) that had been dipped in 5,000 ppm of plant extract was put on the one upper end of olfactometer, and the filter paper with the same size that had been dipped in distilled water as control was put on the other upper end (Figure-1). Subsequently, ten gravid females were released at the base of olfactometer and observed their response in an hour. The experiment was repeated six times. The number of fruit flies went toward each upper end of the olfactometer was recorded. Data was analyzed using *t* test at 95% confidence level.

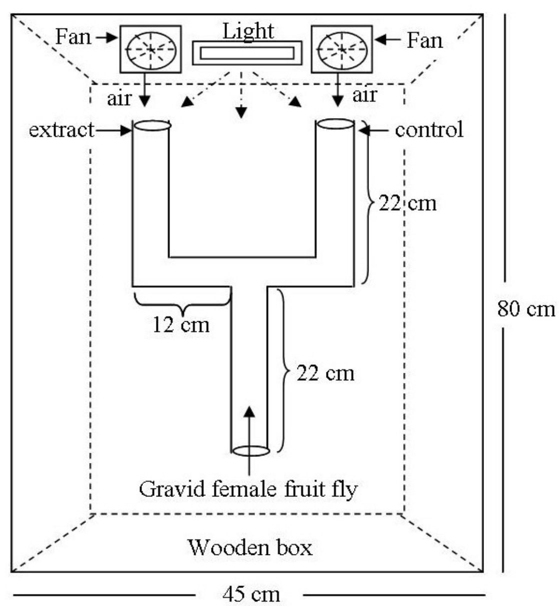

Figure-1. Scheme of olfactometry test of carambolae fruit fly (*Bactrocera carambolae*) female to citronella grass (*Cymbopogon nardus*) extract.

Identification of chemical compounds in the extracts

The compounds present in each extract were identified at the Laboratory of Local Health, Province of DKI Jakarta using Gas Chromatography Mass Spectrophotometry (GC-MS). GC-MS analyses were performed using an Agilent Technologies 6890 Gas Chromatograph with auto sampler coupled to 5973 Mass Selective detector and chemstation data system, equipped

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with an HP Ultra 2 capilary coloumn (30 m x 0.25 mm, film thickness 0.25 μm). The oven temperature was programmed from 70 hold for 0 minute, rising at 5°C/min to 200°C hold for 1 minute, and finally rising at 20°C/min to 280°C hold for 28 minutes. Injection port temperature was 250°C. Helium was used as carrier gas with the constant flow column mode at a flow rate of 0.8 μl/min. The mass spectrometer was operated at 70 eV.

RESULTS

Materials extraction

The citronella grass extracted with various solvent resulted different amount of extracts. Infusion by using water solvent yielded most (11.3% by dry matter), followed by maceration with methanol (7.0% by dry matter) and maceration with n-hexane (2.3% by dry matter) (Figure-2).

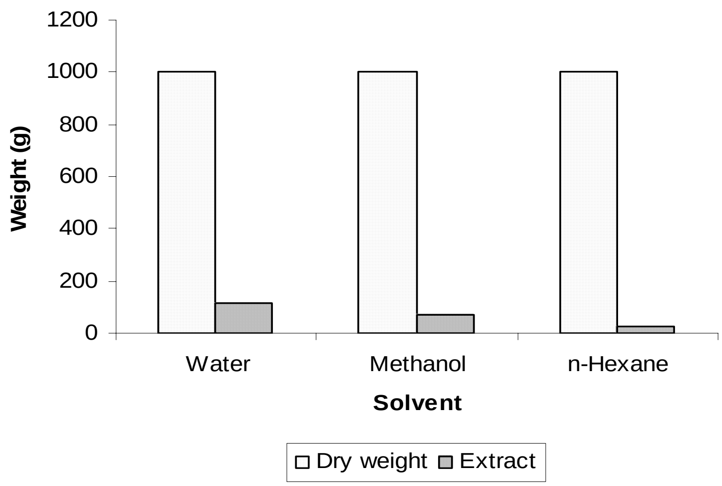

Figure-2. The amounts of extracts from citronella grass (*Cymbopogon nardus*) obtained using three different solvents.

Oviposition deterrency test

The number of eggs masses laid in the fruit treated with 1,000 ppm of methanol extract was significantly less than that of control (P = 0.035) and water extracts (P = 0.012). Moreover, the number of eggs masses laid in the fruit treated with 2,500 ppm of methanol extract was significantly less than that of water extracts (P = 0.044). The proportion of eggs laid in the fruit treated with 1,000 ppm of methanol extract was significantly less than that of control (P = 0.022), water extracts (P=0.020) and n-hexane extracts (P = 0.025), meanwhile the proportion of eggs laid in the fruit treated with 2,500 ppm of methanol extract was significantly less than that of control (P = 0.011) and water extracts (P = 0.003). Based on OAI value, the methanol extract of citronella grass acted as an oviposition deterrent against *B. carambolae*, whereas the water extract and n-hexane extracts were neutral. The deterrency activity increased when the concentration of that extract was increased from 1,000 to 2,500 ppm, but it decreased when concentration was further increased to 5,000 ppm (Table-1).

Table-1. The effects of *Cymbopogon nardus* extracts applied on mango fruits on the oviposition of *Bactrocera carambolae*.

Treatments (Extract)	n	Concentrations								
		1, 000 ppm			2, 500 ppm			5, 000 ppm		
		No. eggs masses	No. eggs laid (%)	OAI	No. eggs masses	No. eggs laid (%)	OAI	No. eggs masses	No. eggs laid (%)	OAI
Control	4	4,2 b	29.2 b		2,5 ab	32,0 b		8,0 ab	32,4 a	
Water	4	5,0 b	29,7 b	-0,02 a	3,2 b	47,9 bc	-0,29 a	11,7 b	31,5 a	0,29 a
Methanol	4	1,2 a	12,2 a	-0,50 a	0,5 a	1,4 a	-0,83 b	4,5 ab	11,2 a	-0,59 a
n-Hexane	4	3,5 ab	28,9 b	-0,02 a	1,5 ab	18,7 ab	-0,20 ab	2,7 a	24,8 a	-0,38 a

[1]Data was transformed to log10 prior to analysis. Each replicate employed 10 gravid females

[2]The numbers followed by the same letter in the same column are not significantly different based on LSD test with α = 0.05

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Olfactometry test

Based on two arms (Y) olfactometry test, the number of *B. carambolae* gravid females went to control filter paper or filter paper treated with citronella grass extracted using either water or methanol was not different (*t* test, P = 0.99 and P = 0.12, respectively). However, the number of *B. carambolae* gravid females that went toward n-hexane extract was significantly less than those went to the control (*t* test, P = 0.00) (Figure-3). Small portion of the gravid females (< 10%) did not response to either treated or control filter paper.

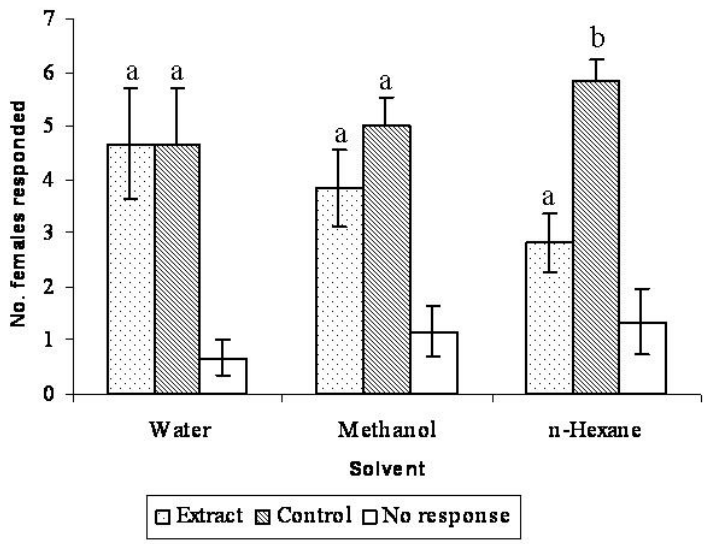

Figure-3. The preference of gravid females of *Bactrocera carambolae* to citronella grass (*Cymbopogon nardus*) extracts by using two arms (Y shape) olfactometry test. Ten females were released in each run, and each was replicated 6 times.

Identification of chemical compounds in the extracts

Based on GC-MS analysis, the chromatogram of methanol extract consisted of 31 peaks with different retention times. The three highest peaks laid on retention time of 34.78, 13.64 and 22.98 respectively (Figure-4). The three most abundant compounds on the methanol extract consecutively were silane, [[(3.beta)-lanosta-8, 24-dien-3-yl] oxy] trimethyl (18.58%), elemol (7.61%) and alpha.-trans-sesquicyclogeraniol (7.59%) (Table-2).

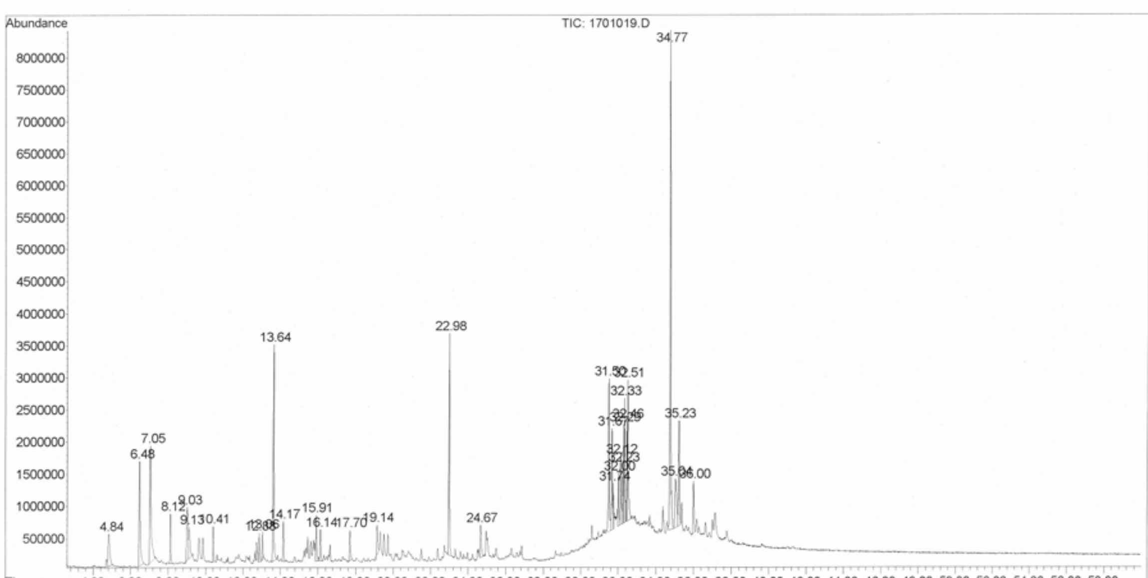

Figure-4. Chromatogram of methanol extract of citronella grass (*Cymbopogon nardus*) by using GC-MS.

Table-2. Chemical composition of methanol extracts of *Cymbopogon nardus* base on GC-MS analysis.

No.	Retention time	Compounds	Relative concentration (%)	Similarity (%)
1	4.84	Citronellal	2.42	98
2	6.48	Citronellol	5.27	98
3	7.05	trans.Geraniol	6.58	95
4	8.13	Pyronene (.alpha. and .beta.)/Phenol, 2-(1-methylethyl)	1.63	22
5	9.03	Cis-2, 7-Dimethyl-4-octene-2, 7-diol	2.47	59
6	9.13	Cyclohexane, 1-methyl-4- (1-methylethenyl)-, trans-	1.84	83
7	10.41	trans-Carryophyllene	1.11	99
8	12.88	.delta.-Cadinene	0.89	99
9	13.06	.delta.-Cadinene	0.73	64
10	13.64	Elemol	7.61	98
11	14.17	Caryophyllene oxide	1.23	99
12	15.92	T-Muurolol	1.77	95
13	16.14	Tricyclo [3.3.1.1 (3.7)] decane-2-carboxamide, 4,8-dioxo-	1.17	30
14	17.70	7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo [4.3.0] nonane	1.16	87
15	19.14	4, 8, 8-Trimethyl-4-acetylcyclooctan	0.95	45
16	22.98	.alpha.-trans-Sesquicyclogeraniol	7.59	50
17	24.67	Cyclohexane, 1,1-dimethyl-2, 4- bis (1 -methylethenyl)-, cis	1.53	78
18	31.50	Citronellyl propionate/Butanoic acid, 3, 7-dimethyl-6-octene	4.38	52
19	31.67	Butanoic acid, 3, 7-dimethyl-2,6-octadienyl ester, (E)-	3.13	78
20	31.75	Bicyclo (10.3.0) pentadec-1 (12)-en-13-one tosylhydrazone	1.04	47
21	32.00	1, 5, 9-Decatriene, 2, 3, 5, 8-Tetramethyl-	1.36	70
22	32.13	Dimethyl 4,6-dioxo-5, 6-dihydro-4H-pyrido [3, 2, 1-jk-carbazole-5-spirocyclohexane-1, 3-dicarboxylate	2.08	46
23	32.23	Dimethyl 4, 6-dioxo-5, 6-dihydro-4H-pyrido [3, 2, 1-jk-carbazole-5-spirocyclohexane-1, 3-dicarboxylate	1.46	59
24	32.29	Citronellyl acetate	2.19	83
25	32.33	d-Nerolidol	3.29	46
26	32.46	1, 5, 9-Decatriene, 2, 3, 5, 8-Tetramethyl-	2.28	55
27	32.51	Ethanol, 2- (3,3-dimethylcyclohexylidene)-	4.54	68
28	34.78	Silane, [[(3.beta)-lanosta-8, 24-dien-3-yl]oxy]trimethyl	18.58	53
29	35.04	2-[.alpha.-(p-bromophenyl)-.beta.-mercaptoethenyl] isiquinolin-1-(2H)-one	3.69	64
30	35.23	(24S)-3beta-methoxy-24-methylanosta-9(11), 25-diene	3.98	91
31	36.00	Benzo [a] heptalen-10 (5H) -one, 11-am	2.06	46
		Total	100	

DISCUSSIONS

Methanol extract of citronella grass had a deterrent activity against *B. carambolae*, meanwhile water and n-hexane extracts were considered to have a neutral activity. Methanol extract was the most effective extract in preventing mango from *B. carambolae* to deposit her eggs than either water or n-hexane extracts. Since the extract strongly deterred the fruit fly to deposit her eggs, it is suggested that the dominant compound might act as a deterrent. Selective solvent markedly influence the activity of the extract because the solvent influence the quantity and quality of the extracted compound (Jaroszynska, 2003). In this case, methanol was more selective qualitatively and quantitatively in dissolving addressed compounds from citronella grass than water and n-hexane.

The deterrent activity of methanol extract to gravid females increased when the concentration of extract was increased from 1,000 to 2,500 ppm. However, its activity decreased at the concentration of 5,000 ppm. This changing may be due to fact that the relative concentration of main compound of methanol extract increased when concentration of extract was increased from 1,000 to 2,500 ppm, but then its relative concentration decreased when the concentration of extract was further increased to 5,000 ppm. Schroth *et al*. (2001) and Burguiere *et al*. (2001) stated that insect only detect a compound when concentration of compound upper threshold limit. Based on those views, sensory organ of *B. carambolae* probably recognizes the unfavorable compounds up to the certain concentration, but when the concentration of extract was continuously increased, the effect of the unfavorable compound was masked by other compounds. A plant has many compounds which have different activity and some may have the contradicting function (Ericsson *et al*., 2007). The use of solvent for extraction would determine what compound would be diluted that eventually determines its activity to the insects. Therefore, choosing the right solvent for the purpose that is being researched is essential.

The olfactory test revealed that the preference of gravid female of *B. carambolae* to methanol extract was not significantly different than that to the control. These results indicate that mechanism of the methanol extract in inhibiting oviposition of the fruit fly mostly by means of deterrent activity rather than repellency. In this case, female of *B. carambolae* probably use their gustatory organ to recognize and discriminate whether the host is suitable or not for their offspring. Female's *B. carambolae* may come to the fruits because of its odor. However, after probing they left the fruit, without deposit their eggs because of unfavorable taste as a result of methanol extract of citronella grass. Previous researchers reported that fruit flies usually use the odors as important cue to deposit eggs (Jang and light, 1991; Prokopy and Vargas, 1996).

The most abundant compound in methanol extract of citronella grass is silane, [[(3.beta)-lanosta-8, 24-dien-3-yl] oxy] trimethyl followed by elemol. Those compounds were likely responsible in deterring oviposition of *B. carambolae*. There is no previous report about biological activity of silane, [[(3.beta)-lanosta-8, 24-dien-3-yl] oxy] trimethyl, meanwhile some previous researchs reported various biological activities of some plant extracts consisted of elemol. The essential oil of *Schinus molle*, which elemol was one of the main compound, had biological activity as repellent and had to be applied as fumigant for controlling *Sitophilus orizae* (Benzi, *et al*., 2009; Upadhyay and Ahmad, 2011). Moreover, the essential oil of *Cymbopogan martini*, which elemol was also apart of this oil, acted as repellent for *Anopheles sundaicus* (Das and Ansari, 2003). Elemol was also known as larvicidal against *Culex quinquefasciatus* (Ranaweera and Dayananda, 1996). Leaves oil of *Cryptomeria japonica*, which was contained of 19% elemol, acted as larvicidal for *Aedes aegypti* and *Aedes albopictus* (Cheng *et al*., 2009). Furthermore, El-Moaty (2010) was also found that essential oil of *Nepeta septemcrenata* consisted of 13.8% elemol acted as antimicrobial against *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*.

CONCLUSIONS

Methanol extract of citronella grass at the concentration of 2,500 ppm was effective in preventing mango from the oviposition of carambolae fruit fly through deterrent mechanism.

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