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Toxicity and sub-lethal effect of endemic plants from family Anacardiaceae on oviposition behavior of *Aedes albopictus*

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ABSTRACT

Objective: To evaluate the lethal concentration, oviposition deterrence and ovicidal activity of acetone extracts of *Melanochyla fasciculiflora* (*M. fasciculiflora*) leaf and *Gluta renghas* (*G. renghas*) leaf against *Aedes albopictus* (*Ae. albopictus*).

Methods: To determine the lethal concentration of Anacardiaceae, ten test concentrations of the extracts ranging from 200 to 650 mg/L were selected for larvicidal bioassays and 25 early fourth instar larvae were exposed to the extracts for 24 h. The sub-lethal concentrations used for oviposition deterrence was the value of LC_{25}, LC_{50} and LC_{75} from above study which is 235 mg/L, 470 mg/L and 705 mg/L for *M. fasciculiflora* extract and 187.5 mg/L, 375 mg/L and 562.5 mg/L for *G. renghas* extract, respectively. Twenty gravid *Ae. albopictus* were allowed to oviposit in different treated concentrations. For oviciding procedure, a total of 300 eggs of *Ae. albopictus* were soaked in solution with each treated concentration as mentioned above for 24 h. After 24 h, eggs were sieved and soaked in seasoned water, and hatching rates were calculated. For comparison, only seasoned water was used in control experiment.

Results: *G. renghas* demonstrated lower LC_{50} value of 372.80 mg/L compared to *M. fasciculiflora* (467.90 mg/L). The activity index of negative oviposition revealed the deterrent effect and thus, caused a remarkable negative response resulting in oviposition of fewer eggs compared with control (without plant extract). The acetone extract of *M. fasciculiflora* was more effective than *G. renghas* extract in displaying oviposition deterrence potential since the latter did not possess the deterring effect within the concentration range tested. However, both plant extracts exhibited excellent oviciding effect as 92.33% of eggs failed to be hatched when treated with 705.0 mg/L of *M. fasciculiflora* and 86.67% with 562.5 mg/L of *G. renghas*. The oviposition deterrence and percentage of egg mortality were directly proportional to the concentrations of extracts in both plants tested.

Conclusions: These results clearly indicate that the acetone extract of *G. renghas* could be served as potential larvicide, whereas *M. fasciculiflora* has better sub-lethal effect for oviposition deterrence and against *Ae. albopictus* as an oviciding agent.

1. Introduction

To date, *Aedes albopictus* (*Ae. albopictus*) is capable of transmitting 26 arboviruses, which comprise of genus *Flavivirus*, genus *Alphavirus*, genus *Orbivirus*, genus *Picornavirus*, genus *Bunyavirus* and genus *Phlebovirus* [1]. Most of the vector controls in the world are assisted by synthetic chemical insecticides since insecticides are the cheapest, easiest and most rapidly effective in control approach. However, when the effectiveness of residual house-spraying dichlorodiphenyltrichloroethane was greatly reduced in controlling mosquito populations, scientists began to realize the problem of resistance. As of now, the resistance problem (more than 100 mosquito species were reported as resistant to one or more insecticides) still persists, and even the vector control programs have been

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switched to use of other insecticides such as pyrethroids, organophosphates, and carbamates [2]. During the past decades, the main factor driving the widespread resistance is the heavy reliance on a single class of insecticides, the pyrethroids [3].

Due to insistence of insecticide resistance problem, the new control agent obtained from natural products has played a vital role in controlling insect vector. Botanical insecticides or so-called naturally occurring insecticides such as neem, rotenone and so forth have been proven to be effective in vector control. Botanical insecticides are relatively safe, rapidly effective, decomposable, and readily available sources which can be obtained from the nature [4]. Due to these factors, many researches are investigated by utilizing the plant extraction as the approach to control mosquitoes for advance use. Plants have been studied due to biochemical properties as an alternative agent for pest control because plant can produce diverse organic chemical compounds regardless of involvement in growth and development of plants, and these compounds are simplistically called secondary metabolites [5,6]. The most effective way to deal with the mosquitoes is to use their biological behaviors as a weapon to control the mosquito population. It is obviously clear that distribution of oviposition sites is relevant to disease propagation [7,8]. When the process of oviposition is inhibited, the population of mosquitoes will be reduced and thereby the probability of getting infected with mosquito-borne disease will be reduced. Hence, nowadays, research on oviposition has become the focus in the concept of integrated vector control management [9,10].

The selected plants chosen to be used in this study are commonly known as Renghas in Malaysia for both species of *Gluta renghas* (*G. renghas*) and *Melanochyla fasciculiflora* (*M. fasciculiflora*) [11]. Both species belong to family Anacardiaceae; with endemic taxa, this family are naturally occurring and widely distributed in Peninsular Malaysia and Borneo [12]. Rengas trees can be very hazardous because they can secrete noxious substances which will cause dermatitis [13]. The detrimental substances in *Gluta* spp. can be found in the fruits, leaves, roots, sap and even timbers [14]. These substances are usually made up of mono- or di-hydric phenols or monohydric phenolic [15]. Most of the variety of phenols is originated from phenylalanine, tyrosine or tryptophan which is one of the secondary metabolites [16,17]. Hence, the noxious substance may prove that the plants in family Anacardiaceae can possibly become one of the biopesticides due to the organic chemicals or secondary metabolites. Apart from these, both species are endemic in Malaysia, hence the source is available and ready to prepare.

Therefore, our study aimed to find the lethal concentration from two endemic species of plants found in Malaysia, namely, *M. fasciculiflora* and *G. renghas* as one of the sources of biopesticides. We also investigated the sub-lethal effects for oviposition deterrence and ovicidal activity against *Aedes* (*Stegomyia*) *albopictus* (Skuse). To the best of our knowledge, no information is available on the efficacy of these two plants.

2. Materials and methods

2.1. Wild strain of mosquito colonies

Ae. albopictus wild strain was collected from secondary forest located at main campus area of Universiti Sains Malaysia (5°21′ N, 100°18′ E) by using ovitrap method. Ovitraps with wooden paddle were placed in the area which will not be disturbed by children or pets, away from excess rainwater, close to the accumulated trash or any expected breeding sites and where direct sunlight is avoided. The wild strain was collected after four days. Collected larvae and paddles were brought back to laboratory and the larvae were reared until adults. The eggs on paddles were immersed in an enamel tray containing chlorine-free water. Hatching occurred within 24 h. The larval food made of dog biscuit, bovine liver, yeast and milk powder at the ratio of 2:1:1:1 was given at 1 mg daily. After pupation, the pupae were transferred and placed in the standard mosquito rearing cage (30 cm × 30 cm × 30 cm). Once the adults emerged, cotton soaked with 10% sucrose solution with B-complex was provided continuously before blood feeding and they were allowed to mate. After two to five days, female adults were then offered with blood feeding of white mouse confined in a wire. After fed with blood, the mosquitoes were allowed to rest for 2 days for the development of eggs before the experiment was carried out. The culture and all experiment were maintained and ran at (28 ± 2) °C, 70%–85% relative humidity with a photo period of 14 h light: 10 h dark.

2.2. Extraction of plant leaves

Two species of plants from family Anacardiaceae were chosen for this study. *G. renghas* and *M. fasciculiflora*, both of which are endemic plants in Malaysia, were chosen. Both plant leaves were collected from Penang National Park, Penang (5°27′ N, 100°12′ E). The leaves were rinsed with tap water and shade dried at the normal environment temperature. The dried leaves were powdered mechanically by using commercial electrical stainless steel blender. The powdered plant leaves were extracted with acetone solvent by using the Soxhlet apparatus. A total of 40 g of powdered plant leaves were inserted into paper thimble (43 mm × 123 mm) and mixed with pebbles in order to ensure that optimum solvent flows through the plant powder. The thimble was closed with cotton wool and extraction was started by using Soxhlet apparatus. The boiling point of acetone was set at 50.5 °C. The apparatus ran for 3 to 4 cycles and the procedure was repeated twice by replacing the plant powder for each round in the paper thimble. The extract yield then went through evaporation process by using rotary evaporator under 80 to 100 r/min at 60 °C to evaporate the excess acetone solvent. The crude extract was stored in oven at 37 °C for further drying process.

2.3. Mosquito larvicidal bioassay

To prepare stock solution, 1 g of crude extract was weighed and dissolved in 100 mL of acetone to produce 10 000 mg/L of stock solution [18]. Larvicidal bioassays were performed as per standard of World Health Organization larval susceptibility test method [19]. Bioassay was performed in 350 mL paper cups containing 250 mL of test medium (distilled water and plant extract solution) and 25 *Ae. albopictus* of early fourth instar larvae were exposed to it for 24 h. A homogenous population of late third instar larvae (5 days old and 4–5 mm in length) were chosen for this study [19]. Initially, the mosquito larvae were exposed to a wide range of test concentrations to find out the activity range of the extract solutions. Ten concentrations ranging from 200 to 650 mg/L yielding between 0% and 100% mortality in 24 h of exposure were selected for larvicidal bioassays. A total of three

replicates were set for each concentration. One control of distilled water with 1 mL of 10% of acetone solvent was kept with each set of experiment. Solvent was added into the control containers to ensure it was identical to the test solutions which may have also contained the solvent and not affected the mortality rate of *Ae. albopictus* larvae. Larval mortality was recorded after 24 h. Larvae with total absence of movement, even after touched, were considered dead.

2.4. Oviposition deterrence assay

The sub-lethal concentrations used for oviposition deterrence were the values of LC_{25}, LC_{50} and LC_{75} determined in the above study. A series of dilution was carried out in order to obtain desired concentration of 235 mg/L, 470 mg/L and 705 mg/L for *M. fasciculiflora* extract and 187.5 mg/L, 375 mg/L and 562.5 mg/L for *G. renghas* extract, respectively. The lowest concentration was always prepared first when a series of dilution was carried out [19].

To investigate the oviposition deterrent effect and the number of eggs deposited in the plant extracts with different concentrations, method by Elango *et al.* was performed with a slight modification on the concentration used [18]. Twenty gravid *Ae. albopictus* were placed in a cage with oviposition substrate. Each cage was equipped with four black bowls together with paddle in it. The paddles were made of hardwood with both smooth and rough surfaces which provided a site for egg attachment. Bowls were filled with 100 mL of distilled water mixed with plant extracts at a concentration of 235 mg/L, 470 mg/L and 705 mg/L for *M. fasciculiflora* extract and 187.5 mg/L, 375 mg/L and 562.5 mg/L for *G. renghas* extract. The fourth bowl only contained 100 mL of distilled water without any plant extracts and served as a control. Both experiments for *G. renghas* and *M. fasciculiflora* were run separately. A total of three replicates for each concentration were carried out at the same day. The positions of bowls in different replicates were alternated randomly in order to eliminate any effect of position on oviposition behavior. After 24 h, the number of eggs which were laid on the paddles of each concentration and control bowls were counted under dissecting microscope.

2.5. Ovicidal activity assay

The methodology for ovicidal activity assay was modified from the study by Chenniappan and Kadarkarai by using the eggs of older age [9]. Filter paper in the Petri dish was offered in the cages for two days after the female *Ae. albopictus* were fed with blood. The eggs were laid on the lining of filter paper provided. Freshly laid eggs, which were 0–6 h old, were the most effective to trigger higher percentage of mortality [9,20,21]. However, we used three-day-old eggs (embryonated eggs or eggs at resistance stage) instead of freshly laid eggs in order to yield enough number of eggs for further experiment. Besides that, the dormant eggs were one of the factors causing wide spread of *Aedes* mosquitoes in the world.

The concentrations used in this study were 235 mg/L, 470 mg/L and 705 mg/L for *M. fasciculiflora* extract and 187.5 mg/L, 375 mg/L and 562.5 mg/L for *G. renghas* extract. Whereas, distilled water without any plant extracts served as control. A total of 100 eggs were used for each tested concentration, and the experiment was replicated for three times. Experiments were run separately for both plant extracts. After scoring, the filter papers containing the eggs were exposed to the treated and control solutions for 24 h. After 24 h, the eggs were sieved through muslin cloth and thoroughly rinsed with seasoned water. Then, the eggs were left in the enamel pan filled with 500 mL of seasoned water for hatching purpose.

2.6. Statistical analysis

The data from mosquito larvicidal bioassay were subjected to log probit analysis for calculating the LC_{50} and LC_{75} with 95% confidence limit by using Statistical Package for the Social Sciences 20.0 software.

The oviposition experiment results were expressed as mean number of eggs and oviposition activity index (OAI). The value of OAI was calculated by the formula below:

$$\mathrm{OAI} = \frac{\mathrm{NT} - \mathrm{NC}}{\mathrm{NT} + \mathrm{NC}}$$

where NT is total number of eggs in the test solution and NC is total number of eggs in the control solution. The range of OAI index was from +1 to −1. The positive sign means attractancy, and more eggs were deposited in the treated solution than control solution. On the other hand, the negative sign means repellency, hence, more eggs were deposited in the control solution rather than treated solution [9].

Whereas, the percentage of effective repellency (ER) was expressed by the formula below:

$$\mathrm{ER} = \frac{\mathrm{NC} - \mathrm{NT}}{\mathrm{NC}} \times 100$$

where NC is total number of eggs in control and NT is total number of eggs in treatment.

The percentage of eggs mortality in ovicidal activity assay was calculated by the formula:

$$\%\ \text{of egg mortality} = \frac{\mathrm{TE} - \mathrm{TH}}{\mathrm{TE}} \times 100$$

where TE is total number of eggs and TH is total number of hatching.

One-way analysis of variance was used to analyze multiple concentrations of plant extracts of each plant species for both oviposition deterrence and ovicidal activity assays. Results with $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Mosquito larvicidal bioassay

The results of larvicidal bioassay indicated that the LC_{50} and LC_{95} of *G. renghas* on *Ae. albopictus* larvae were 372.80 mg/L and 579.68 mg/L respectively. Whereas the LC_{50} for *M. fasciculiflora* was 467.90 mg/L and LC_{95} was 645.00 mg/L (Table 1). Our results indicated that less concentration of *G. renghas* crude extract (550 mg/L) was needed to kill 100% larvae of *Ae. albopictus* compared to *M. fasciculiflora* (650 mg/L) (Figure 1). Based on this finding, the values of LC_{25}, LC_{50} and LC_{95} can be calculated and these concentrations can be used for the next two studies.

Table 1
Mean LC_{50} and LC_{95} of larval efficacy on *Ae. albopictus* for two endemic crude plant extracts of *G. renghas* and *M. fasciculiflora* (mg/L).

Crude extracts	LC_{50} (95% confidence limit)	LC_{95} (95% confidence limit)	Regression equation
G. renghas	372.80 (336.07–417.60)	579.68 (493.42–833.37)	$y = -22.06 + 8.58x$
M. fasciculiflora	467.90 (403.83–545.83)	645.00 (550.37–1610.03)	$y = -31.51 + 11.80x$

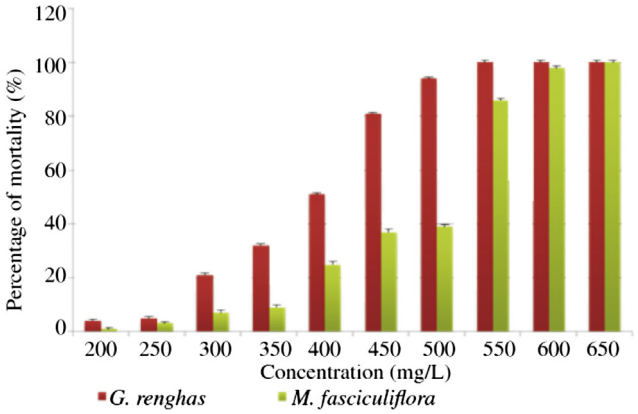

Figure 1. Percentage of *Ae. albopictus* larval mortality after 24 h exposure to *G. renghas* and *M. fasciculiflora* crude extracts (mean ± SE).

3.2. Oviposition deterrence assay

In the oviposition deterrence assay of *M. fasciculiflora* against *Ae. albopictus*, gravid mosquito preferred to lay eggs in the control solution rather than treated solutions (Table 2). There was a clear-cut difference in the number of eggs laid when the control solution was in comparison with the treated solutions. The number of eggs laid by *Ae. albopictus* was decreased with the rise of concentrations of *M. fasciculiflora* and *G. renghas*. However, only *M. fasciculiflora* extract treatment showed significantly less eggs than those laid in the control treatment ($df = 3$, $F = 28.602$, $P < 0.05$), but not for *G. renghas* ($df = 3$, $F = 3.251$, $P > 0.05$). At the lowest dose of LC_{25} of *M. fasciculiflora*, the ER reached 57.01%. However, *G. renghas* extract needed higher concentration than LC_{50} to reach 50% ER. Both results from *M. fasciculiflora* and *G. renghas* clearly indicated that all the treated solutions showed a negative sign in each OAI, which meant that the gravid mosquito were deterred to carry out oviposition process in offered containers. There was an obvious difference with higher number of eggs laid in control solution compared to treated solutions.

3.3. Ovicidal activity assay

It was clearly indicated that the ovicidal activity of both plant extracts was concentration-dependent since the percentage of egg mortality increased with increase in concentrations (Table 3).

There was statistically significant difference in the egg hatchability in each concentration of *M. fasciculiflora* ($df = 3$, $F = 100.129$, $P < 0.05$) and *G. renghas* ($df = 3$, $F = 16.039$, $P < 0.05$). The acetone extract of *M. fasciculiflora* exerted a high number of egg mortality at LC_{75} that 92.33% of eggs failed to hatch into the first instar larvae. While, 86.67% of eggs failed to hatch at LC_{75} of acetone extract of *G. renghas*. At LC_{50}, higher percentage of mortality was noted in *G. renghas* compared to that in *M. fasciculiflora* even the LC_{50} of extract was lower in *G. renghas*. Less than 5% mortality of *Ae. albopictus* larvae was noted in control treatment.

Table 2
Oviposition deterrence assay of *M. fasciculiflora* and *G. renghas* on *Ae. albopictus*.

Plants	Concentration (mg/L)	Number of eggs (mean ± SE)		ER (%)	OAI
		Control	Treated		
M. fasciculiflora	235.0	656.00 ± 19.98[a]	282.00 ± 76.07[b]	57.01	−0.41
	470.0		142.33 ± 52.90[b]	78.30	−0.64
	705.0		88.67 ± 12.99[b]	86.48	−0.76
G. renghas	187.5	236.67 ± 14.85[a]	152.67 ± 72.45[a]	35.49	−0.22
	375.0		120.00 ± 32.70[a]	49.30	−0.33
	562.5		61.00 ± 8.50[a]	74.23	−0.59

Different superscript letters represent the significant difference (One-way ANOVA followed by Turkey's test, $P < 0.05$).

Table 3
Ovicidal activity of *M. fasciculiflora* and *G. renghas* against eggs of *Ae. albopictus*.

Plants	Concentration (mg/L)	No. of egg hatched (mean ± SE)	Percentage of egg mortality (%)	*df*	*F*	*P*
M. fasciculiflora	Control	97.67 ± 1.45[a]	2.33	3	100.129	0.000
	235.0	89.67 ± 4.91[a]	10.33			
	470.0	66.67 ± 5.24[b]	33.33			
	705.0	7.67 ± 3.53[c]	92.33			
G. renghas	Control	95.00 ± 2.65[a]	5.00	3	16.039	0.001
	187.5	56.67 ± 12.44[ab]	43.33			
	375.0	32.67 ± 11.98[bc]	67.33			
	562.5	13.33 ± 1.86[c]	86.67			

Different superscript letters represent the significant difference (One-way ANOVA followed by Turkey's test, $P < 0.05$).

4. Discussion

In this study, the best plant crude extract to possess 100% 1ethal effect was *G. renghas* with lower lethal concentration at 500 mg/L. However, the best plant that has performed dual properties of oviposition deterrence and ovicidal activity against *Ae. albopictus* was *M. fasciculiflora*, whereas *G. renghas* only possessed significant ovicidal activity. The bioactive compounds in plants that induced larvicidal or adulticidal response might be from various compounds including phenolics, terpenoids, flavonoids, and alkaloids as single compound or as joint compounds [22]. Flavonoids, phenolic lipids and triterpenes are the main substances in several species of the Anacardiaceae family [23]. Therefore, we suspected that the same active compound that caused the mortality of *Ae. albopictus* larvae might be found in *M. fasciculiflora* and *G. renghas*. Previous study found that the higher concentration was needed to totally kill another species of *Aedes* named *Aedes aegypti* and *M. fasciculiflora* worked better than *G. renghas* [24].

The plant-based insecticides have been well-known in mosquito control for a long time. As naturally occurring insecticides, these plants could effectively restrict the mosquito population by reduction in egg number through oviposition deterrence and ovicidal activity. Besides that, there are similarities between mosquito host searching and oviposition behaviors as both involve complex integration of physical and chemical factors, for example, the physical factors, like, visual cues, allowing mosquito to target the specific oviposition habitat or host, whereas the chemical factors, like, olfactory cues, allowing mosquito to evaluate suitableness to oviposit or detect the CO_2 odor released from host [25]. Hence, if the oviposition of mosquitoes is inhibited successfully, there might be possible to cause poor performance of mosquito host seeking.

Since the distribution of oviposition sites is relevant to disease propagation [7,8], prevention of oviposition should be the best method to prevent disease propagation. Even though the eggs were laid in *G. renghas* and *M. fasciculiflora* containers, the hatching process was hindered by strong ovicidal effect which still effectively inhibited disease propagation by *Ae. albopictus*. A lot of experiments proved the oviposition deterrence and ovicidal potential of a wide range of plants [9,20,18,26,27]. Most of the investigated plants may possess dual properties of oviposition deterrence and ovicidal activity, for example, the hexane extract of *Andrographis lineata*, an indigenous Indian plant, which is probable in *Anopheles subpictus* control even at low concentration and short exposure time [26].

Oviposition site selection by mosquito often involves in the synergism of chemical factors in the water and physical factors which is associated with the microorganism [28]. The chemical factors could be induced by secondary metabolites, for example numerous mixed phenolics which perform defensive roles or possess characteristic tastes and odors on plant materials [5]. On the other hand, physical factors are the majority depending on olfactory, visual and thermal cues such as color of container, color of oviposition water, size of container, reflection coefficient from water surface, light–dark contrasts, presence of intraspecific larvae or pupae and environment [29–31]. Females always evaluate the oviposition sites and the best site is usually described as larger water surface, larger container volume and presence of chemical compounds that will attract and stimulate oviposition [29,32]. In our study, both crude plant extracts presented chemical compounds that repelled the mosquitoes to oviposit in the containers. The selection of oviposition site is very important because it has a direct relationship with larval survival, development rate, population dynamics and vector-related disease propagation [33,34]. From our observation on both plants, the inserted gravid mosquito flew and rested on the margin of the cages instead of straightly carrying out the process of laying eggs in the first few hours. Since the physical factors were maintained under laboratory conditions especially the size and color of containers, we can assume that the observation responses may be caused by olfactory or gustatory stimuli or both.

In our oviposition deterrence assay, it was noted that significant OAI was increased with the increment of tested plant extract concentrations. Increase in concentrations may induce higher deterring effect and the OAI of *Artemisia annua* at 500 mg/L against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* [27]. Our current findings suggested higher concentration up to 705 mg/L for *M. fasciculiflora* but less for *G. renghas* in order to achieve 100% of oviposition deterrence for *Ae. albopictus*. Continuous effects had been shown by these two plant extracts on eggs laid by the female *Ae. albopictus*. Hence, even though the tested plants cannot provide 100% deterring effect, they are still promising in mosquito control since the laid eggs failed to hatch.

Organic infusions from plants can demonstrate either positive or negative oviposition as a result of attracting, stimulating, repelling or deterring effect [33]. From our study, *M. fasciculiflora* exhibits deterring effect which means that each female only oviposits less than 60 eggs in average. Phenol which is found in the Anacardiaceae, can be used to prevent the attack from insects and herbivores and act as a defense of plants from pests such as growth of fungi [6]. Since *M. fasciculiflora* and *G. renghas* belong to Anacardiaceae family, it might be possible that phenol is the main oviposition deterring and ovicidal agent. Besides that, plants can produce other secondary metabolites. Therefore, these secondary metabolites may act independently or synergistically to prohibit the oviposition process and elicit ovicidal activity. However, the action modes of these organic compounds are poorly understood and this is a very difficult task to distinguish the secondary metabolites and therefore difficult to confirm that which organic compound carries out the most roles in oviposition deterrence and ovicidal activity. Moreover, the possible factor that affects oviposition deterrence could be volatiles of plant extract. In oviposition deterrence assay, we can assume that *M. fasciculiflora* has more easily detectable volatiles than *G. renghas*, hence, females can immediately recognize volatile compounds via olfactory mechanism and then assess the possibility of oviposition in that site.

Freshly laid eggs, which are 0–6 h old, are the most effective to trigger higher percentage of mortality [9,20,21]. However, in this practice, 3-day-old eggs which are embryonated eggs or at resistance stage were used instead of freshly laid eggs. This is because dormant eggs are one of the most important factors causing wide spread of *Aedes* mosquitoes in the world [35,36]. From our observation on the immersed eggs' morphology, there was no any abnormality in structure, hence it is suggested that both plant extracts' chemicals were strong enough to be absorbed and the effects of extracts could be

elicited through the eggshell layer. After submerging the eggs for 1 day in treated solution, only a few larvae hatched after 24 h.

The exposure of eggs to various concentrations of *M. fasciculiflora* and *G. renghas* induced different degrees of egg mortality. As the concentration increased, the failure of egg hatching increased. In addition, the efficient penetration and exposure period are the two important factors that influence the efficiency of the ovicide agent to act on the embryo within the egg shell through highly hydrophobic cuticle [21,37]. Since we cannot evaluate the efficient penetration, we only determined the exposure period of ovicide based on the time of exposure. Our study has indicated that during the 24-h exposure, the extract of *M. fasciculiflora* at 705 mg/L caused more that 90% mortality of *Ae. albopictus*' eggs. Hence, from our observation, hatching of eggs did not occur at all if the eggs were submerged in treated water for more than 120 h. Longer exposure period may aid increment in penetration of plant chemicals into cells.

The screening results suggested that the acetone extract of *M. fasciculiflora* should be further assessed in field trials, for isolation of bioactive constituents and its related mode of action for better understanding and ideal outcomes. Further investigations about the evaporation rate of plant extract, the lasting of odor to repel mosquitoes from oviposition and so on, are encouraged. In the future, the experiments should be oriented toward eggs. Once the oviposition is banned or the hatching process fails, the mosquito population will be greatly dropped down and the probability of infection of vector-related diseases would be decreased.

Conflict of interest statement

We declare that we have no conflict of interest.

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