Evaluation of Attractants and Egg-Laying Substrate Preference for Oviposition by *Aedes albopictus* (Diptera: Culicidae)

Usavadee Thavara, Apiwat Tawatsin, Jakkrawarn Chompoosri

National Institute of Health, Department of Medical Sciences, Ministry of Public Health


**Abstract**

Evaluation of oviposition attractants and substrate preferences of *Aedes albopictus* was carried out under laboratory and field conditions. To obtain candidate oviposition substances we used a water rinse of 3 mollusk species: blood cockle (*Anadara granosa*), carpet shell (*Paphia undulata*), sea mussel (*Mytilus smaragdinus*), and the giant tiger prawn (*Penaeus monodon*). The rinse water of carpet shell and giant tiger prawn showed higher attractiveness for oviposition than the other candidate attractants. The filter paper substrate received fewer eggs than the other two substrates. There was no significant difference between the mean number of eggs laid on hardboard paddles and sponge sheets. The hatching rate of *Ae. albopictus* eggs laid on hardboard paddles was higher than those from the filter papers and sponge sheets. The sponge had lethal effects on *Ae. albopictus* eggs, and very few eggs laid on sponge hatched. In field experiments, evaluation of attractiveness of carpet shell rinse in ovitraps lined with sponge sheet as egg-laying substrate was carried out in various habitats and different areas of Thailand. The mean number of eggs in traps containing carpet shell rinse was significantly higher than those laid in rainwater traps. These studies reveal that the carpet shell and giant tiger prawn rinses are sources of oviposition attractant for *Ae. albopictus* under both laboratory and field conditions and could possibly be used as attractant in surveillance and control.

**Keywords**

Oviposition attractants, mosquitoes, *Aedes albopictus*, oviposition substrates, ovitraps
Introduction

*Aedes albopictus* (Skuse), the Asian Tiger Mosquito, is a vector of dengue haemorrhagic fever (DHF), and is capable of breeding in a wide range of container types and water holding habitats. In Thailand, *Ae. albopictus* has been found in forested habitats ranging in elevation from 450 to 1,800 m as well as in a variety of other habitats in rural and suburban areas (Scanlon and Esah 1965, Gould et al. 1970, Thavara et al. 1996). Ubiquitous breeding sites, such as tree holes, coconut shells, fruit peels, water jars, unused and discarded tires and boats holding water have been found to contain *Ae. albopictus* larvae. Because of the diverse breeding sites of *Ae. albopictus*, especially in the forested areas, they may be hard to reach to monitor larval populations. Detection and measuring mosquito abundance through their egg-laying activities using ovitraps is the most common surveillance or sampling method for this and some other *Aedes* mosquitoes, especially *Ae. aegypti* (Service 1992). Yap et al. (1995) pointed out the importance of oviposition site preferences in planning vector control programs against *Aedes* mosquitoes. However, information on oviposition attractants for *Ae. albopictus* is rather limited at the present time. Sucharit et al. (1980) studied the oviposition behavior of *Ae. aegypti* and *Ae. albopictus* to be influenced by their own larval holding water or that of other species. They found that larval holding water of *Ae. albopictus* significantly increased oviposition by *Ae. aegypti*, but there was no oviposition attractancy for *Ae. albopictus*. Thavara et al. (1989) demonstrated that *Ae. albopictus* (*Ae. aegypti* absent) prefer to lay eggs in the field in containers with conditioned water that was left outside for a long period and with a stable flora together with the immature stages of this species. The present study was carried out to investigate a range of attractant materials and egg-laying substrates for oviposition of *Ae. albopictus* under both laboratory and field conditions.

Materials and methods

Laboratory evaluations

Mosquito colony

*Ae. albopictus* mosquitoes used in this study were taken from the colony maintained in the insectary of the Biology and Ecology Section, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. The colony was established from eggs collected from
Samui Island, Surat Thani Province, in 1989 by the authors. To obtain larvae, a filter paper substrate with conditioned attached eggs was submerged in water for four hours in a plastic tray (23x32x5 cm) containing 500 ml of deionized water. After hatching, larvae were fed with mouse-food powder (~ 0.5 g at a time) twice a day until completion of larval development. All pupae were removed daily and placed in a beaker with 300 ml of deionized water and then kept in a screen cage (30x30x30 cm) for adult emergence. The mosquito colony was kept in an environmentally controlled insectary, temperature of 26±2 °C, relative humidity of 70±10% and photoperiod of 14L:10D. A piece of cotton wool soaked in 10% sugar syrup was placed in each cage to provide food for the adults. Three days post-emergence, restrained 1-month-old mice were provided for adult females for blood feeding for four hours during daytime. Thereafter, the females were fed with mouse blood at 3-day intervals after the first feeding. A few days after blood meal, the gravid mosquitoes were allowed to lay their eggs in a 400 ml beaker, containing 200 ml of deionized water, the inside of which was lined with a strip of filter paper (Whatman No.1) 27-cm long and 6-cm wide for oviposition. The water level in the beakers reached the middle of the filter paper strip. After oviposition, the filter paper strips were removed and dried at room temperature (25-29 °C) for three days in order to facilitate embryonic development and conditioning of the eggs under dry conditions.

**Attractants for ovipositions**

The hypothesis that mollusks may be producing odors that attract mosquitoes originated from preliminary observations on the attractancy of mollusks to adult mosquitoes. After purchasing some carpet shells, we put them in a bucket of water to wash and clean them for cooking but observed numerous adult mosquitoes hovering over the bucket. This stimulated our interest in testing several mollusks for *Ae. albopictus* ovipositional activity. Three mollusk species: *Anadara granosa* Linnaeus (blood cockle), *Paphia undulata* Bom (carpet shell), *Mytilus smaragdinus* Chemnitz (sea mussel) and one prawn species: *Penaeus monodon* Fabricius (giant tiger prawn) were used for obtaining oviposition attractant agents for *Ae. albopictus* in tests conducted under laboratory conditions. The three mollusk species were caught in the sea whereas the giant tiger prawn was cultured in an aquaculture farm. The fresh
marine animals were purchased from a local market in Nonthaburi for obtaining attractants before start of each test. One kg of each species was submerged in one L of distilled water in a plastic tray and left for 30 minutes. The animals were then removed by netting from the trays and the rinse waters were used as candidate sources of attractants. During the netting most, if not all, of the sediments were removed. The rinse water was used immediately in oviposition bioassays.

For oviposition testing, 250 *Ae. albopictus* gravid females (aged 5-7 days, 3-4 days after blood meal) were released in a mosquito screen cage (40x40x40 cm), where five white plastic cups (15-cm high and 12 cm rim-diameter) were placed for oviposition. Each cup was filled with 300 ml of one of the five test waters: blood cockle rinse, carpet shell rinse, sea mussel rinse, giant tiger prawn rinse, and distilled water control. These cups were randomly located in a circle of 15-cm diameter. The inside of each cup was lined with a strip of white filter paper (Whatman No.1, 7-cm long and 6-cm wide) where the water level reached the middle of the strip for mosquito oviposition. A piece of cotton wool soaked in 10% sugar syrup was placed in the cage to provide food for the adult mosquitoes. The cage was kept for 48 h in an environmentally controlled room with photoperiod (14L:10D), relative humidity (70±10%) and temperature (26±2 °C). After oviposition for two days, all oviposition cups were taken from the cage and the strips were removed and dried at room temperature (25-29 °C) for one day. The numbers of eggs deposited in each strip were then counted using a stereomicroscope. Each experiment was carried out in one cage for a total of nine replications.

**Egg-laying substrate preferences**

Filter papers, sponge sheets and hardboard paddles were studied for oviposition by *Ae. albopictus*. The white filter paper (Whatman No.1) and pale-yellow sponge sheets (approximately 2 mm thick, mostly used as shoulder pad in lady garments) were 27 cm long and 6 cm wide, whereas the dark-brown hardboard paddles (made from compressed sawdust) were 12 cm long and 2 cm wide. All were placed in cups containing distilled water. The filter paper strip and sponge sheet lined the inside of the cup where the water level reached the half way mark. The paddle was clipped to the inside of the trap with one half submerged and the other half out of water.
For oviposition, 50 *Ae. albopictus* gravid females (aged 5-7 days, 3-4 days after blood meal) were released into each of three small mosquito screen cages (30x30x30 cm) where three white plastic cups (15 cm high and 12 cm rim-diameter) were used as before for oviposition. Each cup was filled with 300 ml of distilled water and supplied with different substrates (i.e., filter paper, sponge sheet, and hardboard paddle). The water level reached the middle of the filter paper and sponge sheet strip, whereas the hardboard paddle was clipped to the inside of the cup with the rough side exposed for mosquito oviposition. The oviposition cups were randomly located in a 15-cm diameter circle. A piece of cotton wool soaked in 10% sugar syrup was placed in the cage to provide food for adult mosquitoes. The cage was kept for two days in an environmentally controlled room with photoperiod (14L:10D), relative humidity of 70±10% and temperature of 26±2°C. The numbers of eggs deposited on various substrates in the cups were counted under a stereomicroscope. The substrates containing eggs were dried at room temperature for three days for embryonic development and conditioning of the eggs, and then each substrate was submerged in water for four h in a plastic tray (23x32x5 cm) containing 500 ml of deionized water for hatching observations. Two days after hatching, the numbers of larvae hatched from each substrate were counted and recorded. Each experiment was carried out in three cages on seven occasions for a total of 21 replications.

**Laboratory evaluations of ovitraps with carpet shell rinse water as attractant and sponge sheet as substrate**

Because the carpet shell rinse and sponge sheet substrate received greater ovipositional activity, we wanted to test these two factors jointly. An ovitrap using carpet shell rinse water as an attractant and dechlorinated water as control with the sponge sheet as egg-laying substrate was evaluated for ovipositional activity under laboratory conditions. The test procedures used were similar to the study of egg-laying substrate preferences for ovipositions and hatching observations as described above. Each experiment was carried out in four cages on nine different occasions for a total of 36 replications.
Field evaluations

Evaluations of the attractiveness of carpet shell rinse were carried out in the field by using modified ovitraps of Pratt and Jacob (1967). The ovitraps used were 450-ml capacity black flower-pots (9 cm high and 10.5 cm in diameter at the top) that had no drain holes. A total of 120 ovitrap pairs (i.e., one trap filled with 300 ml of carpet shell rinse and the other trap with 300 ml of rainwater) were set at a time in various habitats, including an orchard, palm plantation, rubber plantation, waterfall area, and a public park on the hill, in four southern provinces: Surat Thani, Nakhon Si Thammarat, Trang and Songkhla. These traps were set on the ground in shady areas protected from intense rain and wind. The carpet shell rinse was prepared as described in the laboratory studies and the fresh rinse was used in the ovitraps. The inside of each ovitrap was lined with a strip of the sponge sheet (27x6 cm) for mosquito oviposition. Three days after their placement in the different field sites, the sponge sheet strips were collected, dried, and examined for *Aedes* eggs. If eggs were present, the eggs were counted under a stereomicroscope about two or three days after drying. For species compositions, the egg strips were brought to the laboratory and after conditioning for a week were flooded and the eggs hatched. The larvae were reared to the adult stage and identified to species. These field trials were carried out on three occasions once a month in July 1998, April and July 1999.

Data analysis

The numbers of *Ae. albopictus* eggs obtained from various attractants, substrates and hatching rates were transformed to $\sqrt{x} + 0.5$ to normalize the data prior to statistical analysis. They were compared for mean numbers using the one-way analysis of variance (ANOVA) and Duncan’s Multiple Range Test. For the laboratory and field trials of ovitrap using carpet shell rinse water as an attractant and sponge sheet as egg-laying substrate, the *t*-test analysis was used to compare treatments with controls. The accepted level of significance for all comparisons was $P \leq 0.05$. Analysis was carried out using the SPSS program for windows version 10.0 (SPSS 1999).
Results

Laboratory evaluations of oviposition attractants

The average numbers (± S.E.) of mosquito eggs in cups holding rinse water from blood cockle, carpet shell, giant tiger prawn, sea mussel and distilled water (control) were: 1,081 ± 60, 2,604 ± 128, 2,455 ± 94, 1,655 ± 104 and 861 ± 102, respectively. There was no significant difference between mean numbers of eggs collected from the carpet shell and the giant tiger prawn rinses (P > 0.05), but both received significantly higher number of eggs than the sea mussel, the blood cockle rinse and distilled water (P < 0.01). There was also no significant difference between the blood cockle rinse and distilled water (P > 0.1), but both were significantly lower than the sea mussel (P < 0.05).

Egg-laying substrate preferences

The substrate preference study revealed that *Ae. albopictus* preferred to lay eggs in the cups provided with sponge sheets and hardboard paddles over the filter paper strips used in laboratory colonies. The mean (± S.E.) number of eggs collected was: 169.7 ± 21.6, 277.9 ± 27.5 and 364.1 ± 40.9 for filter paper, sponge sheet and hardboard paddle, respectively. The mean number of eggs collected from filter papers was significantly lower than those collected from the hardboard paddles and sponge sheets. However, there was no significant difference between the mean numbers of eggs collected from hardboard paddles and sponge sheets. The average hatching rate of *Ae. albopictus* eggs obtained on the hardboard paddles was higher than the hatches on the filter papers and sponge sheets, with mean (± S.E.) hatching of 54 ± 3.7%, 31.6 ± 2.8%, and 3.7 ± 0.8%, respectively. Significant differences in hatching rates from each other were observed among the three substrates (P < 0.01).

Evaluation of carpet shell attractant and sponge sheet ovitraps

An experiment was carried out to compare oviposition of *Ae. albopictus* in carpet shell rinse water and dechlorinated tap water using sponge sheets as egg-laying substrates. The results showed that *Ae. albopictus* laid significantly more eggs in carpet shell water about twice as many eggs as in dechlorinated tap water (P < 0.001), the mean numbers (± S.E.) of eggs collected were 854 ± 86 and 425 ± 55, respectively. As in the previous hatching rate study, the hatching rate of eggs laid on sponge sheets was very low and there was no
significant difference between the hatching rates of the eggs obtained from both carpet shell rinse water and dechlorinated tap water \((P > 0.05)\). The mean hatching rates (± S.E.) of \(Ae.\ albopictus\) eggs obtained from the ovitraps containing carpet shell rinse water and dechlorinated tap water were 5.3 ± 1.2% and 4.2 ± 1.0%, respectively.

Field evaluations

We used a total of 360 ovitrap pairs, with each pair was filled with 300 ml of either carpet shell rinse or rainwater was used. The substrate for oviposition was sponge sheet. In the two treatments run on three occasions (120 ovitrap pairs in each occasion), the positive rate of oviposition in the ovitrap having carpet shell rinse was higher than that of the rainwater in all instances. A total of 10,588 eggs were collected from 243 positive traps filled with the carpet shell rinse, whereas a total of 6,606 eggs was obtained from 222 positive traps filled with rainwater (control). In the group of traps with carpet shell rinse, the eggs found in each trap ranged from a single egg to 225, whereas those in the control group ranged from a single egg to 175. The mean number of eggs \((43.6 ± 2.8)\) collected in the traps containing carpet shell rinse was significantly higher than that of the control group \((29.8 ± 2.0)\) containing rainwater only \((P < 0.01)\). In hatching experiments, very few \(Ae.\ albopictus\) larvae hatched from eggs collected by sponge sheets in the traps. Hatching rates of the eggs obtained from the traps containing carpet shell rinse and rainwater were equal and less than 1%. This again showed the lethal effects of the sponge sheets as before. All the hatched larvae reared to adults were identified to be \(Ae.\ albopictus\).

Discussion

The carpet shell and giant tiger prawn rinses increased the numbers of \(Ae.\ albopictus\) eggs collected in the ovitraps under both laboratory and field conditions. The oviposition attractants in these rinses may be chemical stimuli released from the carpet shell and giant tiger prawn. This is the first report on the ovipositional activity of water rinse obtained by submergence of carpet shell and giant tiger prawn in water. It would be interesting to chemically identify the specific compound(s) responsible for attractant activity in these rinses. Nonetheless, the carpet shell or giant tiger prawn rinses could be used as an
effective attractant sources in ovitraps used in monitoring and management of *Ae. albopictus* populations.

The sponge sheet had two interesting features that should encourage its use as an effective egg-laying substrate. First, although the sponge sheet collected lower numbers of eggs than did the hardboard paddle, there was no significant difference between the two means. This may imply that the sponge sheet is equally effective in receiving eggs and it could be used as the egg-laying substrate instead of the hardboard paddle. Second, the sponge sheet demonstrated highly significant inhibitory effect on the hatching of *Ae. albopictus* eggs as compared to the other substrates. This lethal effect on eggs will be an important strategy for use in the control of *Ae. albopictus* mosquitoes because it requires no insecticidal treatments. This novel strategy offers a viable option for sampling *Ae. albopictus* that also kills them, with a mortality ranging from 95 to 99%.

An ovitrap with a combination of the carpet shell rinse as the attractant and the sponge sheet as the egg-laying substrate could constitute a lethal ovitrap system for *Ae. albopictus* mosquitoes in the field. This type of trap could reduce mosquito populations substantially as it could induce more oviposition of the mosquitoes in baited ovitraps in natural sites and subsequently, due to the lethal effects of the substrate, very few eggs laid in the traps would hatch. Further research is needed to clarify these aspects of this novel lethal trap system.

In conclusion, these studies reveal that the carpet shell and giant tiger prawn rinses exhibit a good level of attractiveness for oviposition of *Ae. albopictus* under both laboratory and field conditions. If the attractant principles can be improved by blending and concentration, the strategy could provide a viable and practical tool for use in surveillance and management of *Ae. albopictus* populations. The rinses may contain one or more chemical stimuli inducing oviposition in *Ae. albopictus*. Further research for identifying the specific compound(s) responsible for attractancy in the carpet shell and giant tiger prawn rinses is warranted. As for the egg-laying substrate study, the sponge sheet showed excellent hatching-inhibition effects against *Ae. albopictus* eggs under both laboratory and field conditions. With further refinement of this technique, it could become an effectively lethal ovitrap for *Ae. albopictus* without the use of insecticides. Further studies on the factors causing hatching-inhibition of eggs on the sponge sheet could lead to the development of new strategies for reducing the hatch of eggs of *Ae. albopictus*. 
Acknowledgements

The authors are grateful to the National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health, Thailand for financial support in this study. We thank Dr. Pathom Sawanpanyalert, Director of NIH, and Dr. Motoyoshi Mogi, JICA expert for their kind review of the manuscript. Special thanks are given to Prof. Mir S. Mulla, University of California, Riverside, for his comments and revision on this manuscript. We also appreciate assistance in laboratory and fieldwork of Mr. Yutthana Phusap, Mr. Sumas Janthamas, Mr. Nares Junthornnuan, Mrs. Laddawan Wansopa, Mr. Sathit Wannasri and Mr. Dusit Noree.

References


