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ASSESSMENT OF POTENTIAL KAIROMONES AND COMBINATION WITH THE PRESENCE OF INSECTICIDES IN ATTRACTING *Aedes aegypti* (L.) (DIPTERA: CULICIDAE)

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ABSTRACT

Kairomone play crucial role for *Aedes aegypti* (L) in the host seeking behaviour. Despite numerous researches had attempted kairomore in the formulation of "lethal lure"; however, lacking study of the interaction between the kairomore and lethal agent making the development of the strategy retard. In this study, attractiveness of selected kairomones and the effect of malathion and deltamethrin were tested in a modified single tunnel olfactometer. Ammonia-acetone and ammonia-acetic acid attracted significantly greater number of *Ae. agypti* and their attractiveness performed no significance different with the present of 5% of malathion impregnated paper; but demonstrated significantly lower attraction to mosquitoes when combined with 0.05% deltamethrin. This indicated malathion might be the suitable candidates for the formulation of lethal lure and future study should be focused on other possible lethal agent in combining with kairomones and field trial also required to test the efficiency of the formulation.

Keywords: *Aedes aegypti*, kairomone, lethal lure, malathion, deltamethrin

ABSTRAK

Kairomon mempunyai peranan penting untuk *Aedes aegypti* (L) dalam kelakuan mencari hos. Walaupun banyak penyelidikan telah mencuba kairomon dalam perumusan umpan pemati; Walau bagaimanapun, tidak ada hubungkait antara interaksi kairomon dan agen pemati yang menjadikan perkembangan terhambat. Di dalam kajian ini, daya tarikan kairomon terpilih dan kesan malathion dan deltamethrin diuji di olfaktometer satu lubang yang diubahsuai. Ammonia-aseton dan asid ammonia-asetik menarik lebih banyak *Ae aegypti* dan daya tarikan mereka tidak menunjukkan perbezaan yang nyata dengan kehadiran 5% kertas malathion yang telah diresapi; tetapi secara signifikan menunjukkan tarikan yang lebih rendah kepada nyamuk apabila digabungkan dengan deltametrin 0,05%. Ini menunjukkan bahawa malathion mungkin calon yang sesuai untuk perumusan umpan pemati dan kajian di masa hadapan harus difokuskan kepada agen lain yang mungkin mematikan dengan menggabungkan kairomon dan percubaan lapangan juga diperlukan untuk menguji keberkesanan formula tersebut.

Kata kunci: *Aedes aegypti*, kairomone, umpan pemati, malathion, deltamethrin

INTRODUCTION

Applying synthetic insecticides actively to the environment has created undesired problems such as insecticide resistance and killing of non-target organisms (WHO 1967; 1988). Currently, cost effective chemical control methods aim to reduce the doses of the insecticides yet maintain the efficacy in order to sustain the control program (Michaelakis *et al*., 2007). Improvement of the insecticides delivery method was certainly achieved the goal of that purpose, for example lethal lure that attract the mosquito using lure agent and deliver the lethal insecticides to the mosquito (Bernier *et al.*, 2003; Michaelakis *et al.*, 2007). However, studies of the lure agent and the interaction with insecticides still insufficient.

Kairomones were proposed as the lure agent for the lethal lure formulation (William *et al*., 2006). They could be the excrement of human or mammals such as carbon dioxide and acetone in exhaled breath and some volatile organic acid such as acetic acid that produced by the microorganisms on the human skin (Bosch, 2000; Smallegange *et al.*, 2011) and used by *Ae. aegypti* in host seeking. Bernier et al. (2003) in attempt to design the lethal lure formulation by studied the attractiveness of kairomones and the effect of the synergists dichloromethane, acetone and dimethyl disulphide on L-lactic acid, and the binary blend showed significantly higher percentage of attractiveness compared to when emitted the component singly.

Although the carbon dioxide and lactic acid were the major components in human excrement; however, kairomones for mosquitoes might be complicated and not only consist of these compounds. This was also hypothesised by William *et al*. (2006) that studied the attractiveness of kairomones using blend of lactic acid, ammonia and caproic acid and USDA-blend (acetone, lactic acid and dimethyl disulphide) on 4 strains of *Ae.*

aegypti and concluded that both the blends and modification (when combined with the synergist) were significantly attractive even without the carbon dioxide. Most of the researches were focused on the application of kairomones in the mosquito trap; however, for the combination of kairomones and insecticides, the effect of insecticides on the attractiveness was insufficient study. Therefore, in this study the attractiveness of kairomones-lactic acid, ammonia, acetone and acetic acid was to compare and the effect of insecticide on the kairomones have also been investigated.

MATERIALS AND METHODS

Insect

Susceptible strain of *Ae. aegypti* (World Health Organization/Vector Control Research Unit (WHO/VCRU) strain) was used for laboratory testing. The mosquitoes were reared at 25±3°C, 67±5% relative humidity (RH), and a photoperiod of 8:16 (Light:Dark) h at the VCRU at University Science Malaysia. Four to five-days old (emerged from pupae) female adult mosquitoes were used and the mosquitoes were blood meal deprived before proceeding to the attractiveness test.

Chemicals

The interaction study of kairomones and insecticides was studied by releasing the kairomones by vaporisation with the present of insecticide impregnated paper. Technical-grade of ammonia (40%, Merck, Germany) and L-lactic acid (87.6% R & M Chemicals, U.K.) were used to prepare 10% (v/v) ammonia and 20% (v/v) L-lactic acid, respectively by dilution. Analytical-grade (99.9%) of acetone (BDH, England) and acetic acid (BDH, England) were used in this study. The preparation and concentration of malathion and deltamethrin was adapted from WHO (2009) diagnostic dose, in which insecticides impregnated papers were prepared by pipetting 5% of malathion and 0.05% of deltamethrin on a white paper (25 x 17.6 cm), respectively.

Olfactometer

The attractiveness of the kairomones and the effect of the insecticides on kairomones was test using olfactometer that modified from Klowden & Lea (1978) and Foster & Lutes (1985). The olfactometer (Figure 1) consists of four sections: release chamber (A); flight tunnel (B); trap and dispersion chamber (C) and lure chamber (D). The release chamber consisted of a polyethene (PET) cylindrical-shaped container (Figure 1A, diameter 14cm; long 30cm) that attached with a PVC adaptor. The PVC adaptor contains a rotatable door that served as a temporary barrier between tested mosquitoes and the vaporized kairomones, it was connecting to the flight tunnel (Figure 1B, diameter 11cm and long 100cm) that linked to the trap chamber (Figure 1C, 18 x 18 x 20cm). The trap chamber consisted of a cuboids-shape removable cage and left and right of interior wall of the trap chamber equipped with a holder for insecticides impregnated paper. The dispersion chamber (Figure 1C) contained a 5V fan (AVF, Selangor Malaysia, 1500 rpm and 2.5m/s speed) that functioned as an air-flow generator to disperse the kairomones. The lure chamber (Figure 1D) was a combination of water bath (Shel Lab SWB715 7L, United States) and a 100ml conical flask that filled with kairomones. The temperature of the water bath that used for vaporizing the kairomones was set at 40±1ºC for the purpose of imitating the mammal body temperature.

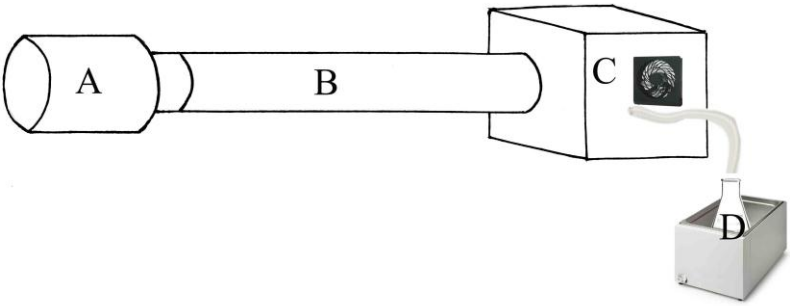

Figure 1. (A) release chamber; (B) flight tunnel; (C) trap and dispersion chamber; (D) lure chamber.

Interaction between kairomones and insecticides

Due to the temperature to vaporise the kairomones was 40±1ºC, the negative control of the experiment consisted of 40±1ºC heated water vapour from distilled water and positive control was a human left palm placed behind the fan of dispersion chamber. For attractiveness tests on kairomones, 5ml of kairomone was transferred into the conical flask of lure chamber and warmed at 40 ºC in the water bath for 2 minutes, the release rate was determined by measuring the mass of kairomone before and after the test (Bernier, 2003). Twenty 4-to 5-days old mated female *Ae. aegypti* were prior transferred to the release chamber using an electrical aspirator and allowed the mosquito to adapt the chamber for 20 minutes. The fan of dispersion chamber was turned on and the rotating screen in the trap chamber was then opened. The mosquitoes were given 2 minutes to response and allowed to fly against the wind current through the flight tunnel; the mosquitoes that caught in the trap chamber were counted.

The effect of insecticides on the attractiveness of kairomones was studied by placing insecticide impregnated paper in the holder of the trap chamber and carried out the similar attractiveness testing. The control for the effect of insecticides were the impregnated paper with the solvent (for malathion, silicon oil was used; for deltramethrin, olive oil was used). Each of the kairomone and binary blend were repeated 6 times. All the tests were carried out at an indoor environment temperature of 26±2ºC and 70±5% humidity and all the air conditioning and fan were turned off while running the tests. From the previous studies, mosquitoes were sensitive to the hand-touch contamination on the inner walls of the test chamber. This was carefully avoided by wearing hand gloves while carrying out the test and each part of the test chamber was washed with detergent after each assay.

Statistical analysis

The attractiveness to the mosquitoes of the kairomones and binary-blends, and the kairomones with the presence of insecticides were prior transformed by arcsine-square root, and the attractiveness was compared using one-way analysis of variance (ANOVA) at the significance level of $\alpha = 0.05$ and least significant differences (LSD) as the post-hoc test using SPSS 17.0.

RESULT

In this study, the heated air attracted 4.58 ± 1.15% of the mosquitoes; whereas bare palm that acts as the "natural host" had attracted 75.42 ± 3.17% of female *Ae. aegypti*. Table 1 showed the attractiveness of kairomones and release rate. Lactic acid was the most attractive kairomone, in which lactic acid attracted 46.25 ± 1.52% of female *Ae. aegypti*. Acetic acid, ammonia and acetone have attracted 43.75 ± 2.72%, 27.50±0.75% and 29.17±1.61% of mosquitoes, respectively. In general, binary blending of kairomones showed better lure effect compared to the single kairomone. Ammonia-acetone and ammonia-acetic acid demonstrated the highest attraction which had attracted 59.58±4.10% and 57.50±2.80% of mosquitoes, respectively.

For the effect of insecticides on kairomones attractiveness toward the mosquitoes, both binary blends-ammonia-acetone and ammonia-acetic showed no significant differences in attracting *Ae. aegypti* with the solvent of the insecticides (silicone oil and olive oil). The attractiveness of the kairomones and with the presence of insecticides were detailed in Table 2. The attractiveness of ammonia-acetone and ammonia-acetic on mosquitoes had no significant differences with 5% of malathion but showed significant low percentage when the kairomones combined with 0.05% deltamethrin impregnated paper.

Table 1 Response of *Aedes aegypti* towards on single and binary kairomones.

Kairomone	Attractiveness (%)*	Release rate at 40±1ºC (mg/s)
Control	4.58±1.15 a	
Host (bare hand)	75.42±3.17 f	
Lactic acid	46.25±1.52 d	0.02±0.02a
Ammonia	27.50±0.75 a	0.40±0.11b
Acetone	29.17±1.61 ab	0.47±0.09c
Acetic acid	43.75±3.49 cd	0.08±0.01d
Lactic acid-Ammonia	40.42±2.72 bcd	
Lactic acid- acetone	35.00±2.54 abcd	
Lactic acid- acetic acid	32.50±3.56 abc	
Ammonia- acetone	59.58±4.10 e	
Ammonia- acetic acid	57.50±2.81 e	
Acetone- acetic acid	44.17±2.81 d	

n=6, $p < 0.05$, different symbol indicating they are significantly different.
*Mean percentage of attractiveness of 20 female *Aedes aegypti* for six replicates.

Table 2 Response of *Aedes aegypti* towards on kairomones with the presence of insecticides

Lethal Lure		Attractiveness ± S.E. (%)
Kairomones	Lethal agent	
Ammonia- acetone	None	59.58 ± 4.10a
	Silicon oil	54.17 ± 2.01a
	5% malathion	53.33 ± 4.22a
	Olive oil	54.17 ± 2.39a
	0.05% deltametrin	46.67 ± 2.58b
Ammonia-acetic acid	None	57.50 ± 2.81a
	Silicon oil	51.67 ± 2.79a
	5% malathion	53.33 ± 4.01a
	Olive oil	52.50 ± 2.81a
	0.05% deltametrin	47.20 ± 2.58b

n=6, $p < 0.05$, different symbol indicating they are significantly different
*Means percentage of attractiveness of 20 female *Aedes aegypti* for six replicates

DISCUSSION

Lactic acid was the main compound in the human sweat (Geier *et al*, 1999) and the compound has been tested by many researches to prove its attractiveness to females *Ae. aegypti* (Acree, 1968; Geier *et al*, 1999); however, in this study acetic acid attracted no significant differences compared with lactic acid. This suggested the carboxylic acid played similar role to lactic acid and this was supported by the study of Bosch *et al*. (2000), in which two fatty acid groups, 1-3 and 5-8 carbons-chains were studied, and they were significantly attractive to *Ae. aegypti*. The mosquito showed positive attractiveness towards the acetic acid could be explained by the microbial activities that on human skin, in which carboxylic acids were often generated by the microbes (Smallegange et al. 2010). The importance of acetic acid was also emphasized by the studies of Folk et al (1991) and Wilson (2008) that L-Lactic acid and acetic acid were the compounds existed on human skin and played vital role for mosquitoes in host-seeking behavior.

Despite lactic acid was proposed as the main component in designing a kairomones lure (Bernier 2003); however, the binary kairomones blends attracted more *Ae. aegypti* than single kariomones generally. This was paralleled with the studies of Bernier *et al.* (2003) and William *et al.* (2006), in which the studies have demonstrated the effect of a kairomone can be enhanced by combining with other synergists. Similar result had also been demonstrated by the studies of Willians *et al*. (2006) that lactic acid absent USDA-blend significant attractive to *Ae. aegypti* and was enhanced when combined with ammonia. Smallegance (2010) had showed the important of ammonia instead of lactic acid when ammonia was attracted significantly higher number of female *An. gambiae*. Ammonia was the key kairomone could be due to the volatile ammonia that relatively easier to vaporize and detected by the mosquitoes (Carson *et al*, 2008; Shakhashiri, 2008) and this was supported by the significantly higher released rate of ammonia from the result of this study. Synergetic effect of acetone and acetic acid on ammonia in this study were paralleled with the studies of Takken *et al*. (1997) and Bernier *et al* (2003) that suggested the trace amount of acetone in human breath play a similar role to carbon dioxide for mosquitoes and the carboxylic acid that evaporated from human skin allowed the mosquitoes to allocate the host.

For the effect of insecticides on the attractiveness of the kairomones, attractiveness of ammonia-acetone and ammonia-acetic acid combination showed no significant differences when the 5% of malathion impregnated paper were present. The tolerances of malathion with attractant on insect had also been demonstrated by California Department of Food and Agriculture (CDFA) in a program that controlling the fruit flies using malathion-bait (CDFA, 1987). Yee and Phillips (2004) had also evaluate malathion-bait towards Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Ventura County, California, U.S.A., it is found that the malathion-bait is not repellent to the fly and gave no harm to the natural enemies of the fly. In contrast, combination of the kairomones blends with deltamethrin demonstrated significantly lower attracted mosquitoes than the control and malathion combination. This indicated deltamethrin exhibited relatively stronger repellence and this was also observed from the studies of Achee *et al*. (2009), which applied three different concentrations of deltamethrin to *Ae. aegypti* and the mosquitoes showed repelling behaviour for all the concentrations.

CONCLUSION

In general, kairomones in binary-blend had more effective lure effect than single kairomone. Besides lactic acid, ammonia could be the key kairomone for *Ae. aegypti* in host-seeking. Malathion was more tolerant than deltamethrin as the lethal agent in formulation of lethal lure. Upon the field test, the formulation was potentially become a part of the integrated program for controlling of *Ae. aegypti*.

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