BEHAVIORAL RESPONSE OF *AEDES AEGYPTI* (L.) TO ITS SEMIOCHEMICALS

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Abstract. Semiochemicals are chemical substances or mixtures of chemicals substances, which act as messengers between or within the insect species, regulating the behavior of other individuals. Identification of specific semiochemicals and their behavior regulating property can provide leads for designing target specific, nontoxic, environmentally less persistent insecticide which is difficult for insects to develop resistance against. This study was conducted to identify semiochemicals of the Selangor strain of *Aedes aegypti* (L.), a vector of dengue in Malaysia, and to observe its behavioral response toward its n-hexane extract from various age groups. Behavioral bioassay was conducted in a customized Y-tube olfactometer. Male mosquitoes 3 days to 6 days post-emergence were found to be attracted to 6 days to 15 days post-emergence male n-hexane extracts and 1 day to 9 days post-emergence n-hexane extracts of females. On the other hand, female mosquitoes 3 days to 6 days post-emergence were attracted to 1 day to 3 days post-emergence male n-hexane extracts and 6 days to 15 days n-hexane extracts of females. n-Hexane extract of *Ae. aegypti* of various age groups was analyzed via Gas Chromatography Mass Spectrometry (GCMS). Chromatographic analysis revealed that n-alkanes contributed toward 40% of the total chemical composition in the n-hexane extracts of mosquitoes of various ages. n-Heptacosane was detected in greatest abundance in both male and female n-hexane extracts, followed by n-tetratriacontane. Chemical analysis of extracts and secretions of the *Ae. aegypti* throughout its life revealed that n-alkanes were the major compounds. Longer carbon chains (>C20) extracted from the insect cuticle were found to dominate *Ae. aegypti* n-hexane extracts from various age groups.

Keywords: *Aedes aegypti*, behavioral response, GCMS, n-hexane extract, semiochemicals

INTRODUCTION

Aedes aegypti (L.) is a domestic mosquito species found in or around residential areas and prefers breeding indoors. It is a vector transmitting dengue fever, dengue hemorrhagic fever (Christophers, 1960; Gubler and Clark, 1996; Rigau-Perez *et al*, 1998; Guzman and Kouri, 2002) and chikungunya virus (Nero, 2008; Moro *et al*, 2010). It is an insect species which is closely associated with humans (Rigau-Perez *et al*, 1998) and breeds in man made artificial containers in urban areas such as

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tire depots, vase, discarded cans and cups, flower pots, tree holes, water pots or water tanks that are able to support a successful reproduction cycle (Christophers, 1960; Cheong, 1986). It is originally found in tropical and subtropical zones with the moist warm climate, high humidity and rainfall, which appear to suit the propagation of the mosquitoes (Christophers, 1960; Cheong, 1986).

Semiochemicals are naturally produced substances and comprises a single or a series of chemical signals released and received by insects or animals and trigger a certain behavior response from insects in inter- and intra-species communication (Law and Reignier, 1971; Metcalf, 1998; Gordh and Headrick, 2002; Wyatt, 2003). These chemical messages can be transmitted through air, or transferred to receivers via substrates such as food, nest material or the bodies of colony members (Gilley *et al*, 2006). The semiochemicals can be secreted by the glands and also by the cuticle of the insect. Desena *et al* (1999) reported that the semiochemical extracted from the adult *Ae. aegypti* was used to determine the age of the mosquito. Various age-related semiochemicals are usually extracted from the insect cuticle. Such extracts contain the chemical compounds, which can evoke certain behavioral responses to the adult mosquitoes. This study was conducted to extract and identify the semiochemicals from *Ae. aegypti* aged for various period of time. The extracts were evaluated in the Y-tube olfactometer against *Ae. aegypti* to observe their behavioral response towards the extracts.

MATERIALS AND METHODS

Insect rearing

The mosquito, *Aedes aegypti* (Selangor strain) used originated from a colony maintained in the insectary of Institute for Medical Research, Kuala Lumpur for more than 20 years. *Ae. aegypti* eggs were hatched in the plastic basin containing seasoned water at 26 ± 1°C and RH 60 ± 10% with photoperiod 12:12 (L:D). A pinch of ox liver powder was added to the basin until the larvae emerged to pupae. To obtain virgin mosquitoes, pupae were separately isolated into 5 ml glass vials containing 1 ml distilled water and plugged with a cotton wool. Emerged adults were sorted by sex and released into separate cages. They were allowed to feed on 10% sucrose-B-complex solution and distilled water ad libitum.

Preparation of the mosquito extract

One hundred male or female mosquitoes from six age groups, namely, 1 day, 3 days, 6 days, 9 days, 12 days, and 15 days post-emergence were used. The samples were extracted using the non-polar n-hexane whereby only non-polar compounds were extracted. Mosquitoes were collected using an aspirator and knocked down by exposing to - 4°C for 2 minutes. The mosquitoes were transferred into empty amber vials (4 ml) and n-hexane (1 ml) was added and the vials were slowly agitated for 30 seconds at room temperature. Following that, the n-hexane extract was filtered and collected in a fresh vial. The extract was dried under a gentle steam of nitrogen. The weight of the extract was recorded and kept in -4°C for further analysis.

Gas chromatography – Mass spectrometry analysis (GCMS)

CGMS analysis was conducted using an Agilent 7890GC equipped with 5975 MSD, CTC auto injector and a DB5-MS UI (30 m x 0.25 mm x 0.25 mm; 5% polydimethylsiloxane) capillary column. The GCMS conditions were as follows: injector temperature was set at 220°C in

a pulsed splitless mode; injection volume was 3 µl. The GC oven was programmed to 50°C; then ramped at a flow rate of 7°C/ minute to 290°C and held for 10 minutes. The column flow was set at 1 ml/minute. The electron impact mode was at 70 eV and scanned from m/z 25 to 400 at one scan per second with source temperature held at 250°C and quadrupole at 150°C. n-Decane (10 ppm) was used as a standard in setting up the Retention Time Locking (RTL) method. While in the GCMS analyses 21 hydrocarbon standards (C10-C22, C24-C31, C14H28O2, C18H26O2) were used to obtain the hydrocarbon library. The data was analyzed by software (Chemstation®; Agilent Technologies: Santa Clara, CA), and the compounds were identified by comparing their spectra to those of the libraries of hydrocarbons and libraries of the NIST installed in the GCMS. The mass spectral with the quality >90% matched compared with the library was accepted for further analysis.

Experimental set-up

Mosquito behavior towards the compounds was tested using a custom-made Perspex Y-tube olfactometer. A Y-tube olfactometer was fabricated using plexiglass based on a modified design (Geier and Boeckh, 1999; Blackmer *et al*, 2004; Bray and Hamilton, 2007). The dimension of the inner diameter of the test, control and release chambers was 4 cm x 4 cm x 4 cm each while the inner thickness was 0.5 cm. The length of the release arm was 20.0 cm while the control and test arm were each 19.5 cm. The fan was located at the end of the release arm. Before the test was conducted, the Y-tube was wiped clean with 70% ethanol using lint free tissue paper. The set up was placed in a closed room at relative humidity of 60±10% and 26 ± 1 °C. At higher or lower ranges of temperatures and relative humidity, experiments were discarded. Experiments were not conducted on rainy days, as the behavior of the mosquitoes would be affected because mosquitoes were inactive during rainy and cold conditions. An aliquot of mosquito extract (10 ppm, 20 ml) was pipetted onto a filter paper disc (ø 13 mm). Then the sample was left for 2 minutes to dry. The sample-loaded disc was then placed inside the test chamber arm while the negative control of 20 ml n-hexane was placed inside the control arm.

Behavioral test

Both male and female mosquitoes aged 3-to-6 days post-emergence were tested against the extract. The mosquitoes at this age have a well-developed and functional organ system (Christophers, 1960), and hence they are ready to mate, copulate and have their first blood meal (the female mosquitoes). Five mosquitoes of same gender were placed in the release chamber. Before the door of the release chamber was opened, the fan was turned on for 30 seconds at a minimum speed of 1.2 m/s at the release chamber and 0.5 m/s at the test and control chambers. The airflow was set in the direction from sample and control chambers, exiting through the release chamber.

After this, the fan was turned off and all three doors of the release chamber, control chamber and test chamber were opened. Subsequently, the mosquitoes were allowed to fly to their choice of direction. Once all the mosquitoes had made their choices, the experiment was stopped. If after two minutes the mosquito did not make any choice or decision, the experiment was stopped and the result recorded as 'no response' (NR). Thirty replicates were repeated for each experiment. After two replicates the Y-tube was cleaned and

the discs replaced with new ones. The test chamber treatment was swapped every two experiments.

RESULTS

Chemical study

Table 1 summarizes the name and amount of chemical compounds identified from male *Aedes aegypti* n-hexane extract at selected age. There were nine n-alkanes ranging from C17 until C34 identified in the male extracts. The major compound identified in male *Ae. aegypti* from 1 day post-emergence to 15 days post-emergence was n-heptacosane which constituted 35-47% of the male extract composition. The percentage of n-heptacosane was higher in the 6 days post-emergence while the 3 days post-emergence extract had the least percentage of this compound.

The n-tetratriacontane was the second highest compound, which appeared in all male *Ae. aegypti* n-hexane extracts. The 6 days post-emergence extract contained the highest percentage of this compound (21.2±0.79%) compared to other extracts from different mosquito age, namely, 3 days post-emergence (19.6±1.47%) and 1 day post-emergence (18.7±0.69%). n-Heptadecane (0.86 ± 0.04%) and n-heneicosane (0.79 ± 0.04%) were unique to the 1 day post-emergence male. n-Tricosane was detected in two samples; 1 day (1.74 ± 0.10%) and 3 days (1.57 ± 0.14%) post-emergence extract. The percentage of n-pentacosane was higher in the 3 days post-emergence and 6 days post-emergence extract.

However, in the 12 days and 15 days post-emergence extract, the amount had declined to 5.60±0.27% and 4.84 ± 0.66%, respectively. n-Hexacosane was present in all male extracts and the amount was detected at ≈4.0% in all the extracts tested. The percentage of n-octacosane was

Table 1

Percentage (±SD) of chemical compounds identified in n-hexane extracts of male *Aedes aegypti* of various age groups.

Compounds	% Area (±SD)					
	M1	M3	M6	M9	M12	M15
n-Heptadecane (C17H36)	0.86 (0.04)	nd	nd	nd	nd	nd
n-Heneicosane (C21H44)	0.79 (0.04)	nd	nd	nd	nd	nd
n-Tricosane (C23H48)	1.74 (0.10)	1.57 (0.14)	nd	nd	nd	nd
n-Pentacosane (C25H52)	7.04 (0.23)	10.31 (0.67)	10.53 (0.18)	8.00 (0.15)	5.60 (0.27)	4.84 (0.66)
n-Hexacosane (C26H54)	4.09 (0.12)	3.89 (0.28)	4.21 (0.27)	4.02 (0.05)	3.40 (0.07)	3.02 (0.21)
n-Heptacosane (C27H56)	36.18 (1.50)	35.05 (3.03)	47.22 (2.89)	44.96 (0.27)	46.80 (0.21)	43.22 (4.18)
n-Octacosane (C28H58)	1.76 (0.04)	1.76 (0.12)	2.19 (0.06)	2.48 (0.02)	2.60 (0.00)	2.41 (0.28)
n-Nonacosane (C29H60)	4.74 (0.11)	6.27 (0.27)	11.03 (0.28)	8.80 (4.50)	9.70 (3.52)	8.38 (3.32)
n-Tetratriacontane (C34H70)	18.74 (0.69)	19.60 (1.47)	21.19 (0.79)	12.93 (1.69)	15.30 (0.67)	14.17 (1.73)

nd, not detected. M1, 1 day; M3, 3 day; M6, 6 day; M9, 9 day; M12, 12 day; and M15, 15 day post-emergence of males.

found to correlate positively with increased mosquito age, that is, from 1.76±0.04-to-2.60±0.00%. The percentage of n-nonacosane was higher in the 6 days post-emergence extract (11.03±0.28%) and was detectable in all male extracts tested.

Table 2 shows compounds identified in the n-hexane extracts of the female *Ae. aegypti*. Eleven compounds were identified in female extracts of *Aedes aegypti* of various age groups. In the female, n-heptacosane was found to be the most abundant compound in all extracts. The percentage of n-heptacosane was higher in the 15 days post-emergence sample at 40.14±3.06%. This was followed by n-tetratriacontane identified as the compound in second highest percentage in the extracts. The percentage of the n-heptacosane showed that this compound was increasing with the age of the mosquito, while for n-pentacosane and n-tetratriacontane the amount of the compounds was observed to increase in the younger mosquitoes and then decreased in the 9 days post-emergence. They reached the highest percentage in the 6-day post-emergence but decreased after that. n-Hexacosane occurred in the range of 3.18-4.83% and was not affected by the increasing age of the mosquito.

Meanwhile, the percentages of n-octacosane and n-nonacosane had a linear relationship with the age of the mosquito tested. The amount of these two compounds increased with the age of the female mosquito. The 3-day post-emergence contained the lowest percentages of n-hexacosane, n-heptacosane and

Table 2
Percentage (± SD) of chemical compounds identified in n-hexane extracts of female *Aedes aegypti* of various age groups.

Compounds	% Area (±SD)					
	F1	F3	F6	F9	F12	F15
n-Hexadecane (C17H34)	1.39 (0.73)	nd	nd	nd	nd	nd
n-Heptadecane (C17H36)	1.14 (0.43)	nd	nd	nd	nd	nd
n-Heneicosane (C21H46)	nd	nd	0.97 (0.20)	nd	nd	nd
n-Tricosane (C23H48)	1.76 (0.03)	nd	1.78 (0.63)	1.10 (0.23)	1.59 (0.64)	nd
n-Pentacosane (C25H52)	10.15 (0.19)	9.86 (0.30)	8.51 (0.61)	6.94 (0.22)	5.42 (0.44)	5.82 (0.36)
n-Hexacosane (C26H54)	4.83 (0.92)	4.01 (0.10)	3.63 (0.08)	3.18 (0.10)	3.23 (0.04)	3.53 (0.26)
n-Heptacosane (C27H56)	34.42 (1.29)	29.21 (0.81)	31.28 (0.74)	29.96 (0.35)	33.42 (0.47)	40.14 (3.06)
n-Octacosane (C28H58)	1.71 (0.37)	1.80 (0.03)	1.98 (0.01)	1.76 (0.06)	2.20 (0.04)	2.58 (0.18)
n-Nonacosane (C29H60)	5.34 (0.74)	3.25 (1.40)	4.21 (1.75)	7.35 (1.34)	6.88 (2.90)	10.65 (1.06)
n-Triacontane (C30H62)	nd	2.22 (0.48)	2.07 (0.46)	1.79 (0.15)	1.67 (0.35)	1.26 (0.28)
n-Tetratriacontane (C34H70)	17.50 (1.15)	22.86 (0.55)	21.17 (1.03)	17.88 (0.16)	18.37 (0.91)	15.48 (1.61)

nd, not detected. F1, 1 day; F3, 3 day; F6, 6 day; F9, 9 day; F12, 12 day; F15, 15 day post-emergence of females.

n-nonacosane. The analysis identified the compounds present in the extracts as a class of hydrocarbons with most of them appearing to be n-alkanes. In the female extract, n-heneicosane was observed in the 6 days post-emergence (0.97±0.20%) while in the male, n-heneicosane was found in the 1 day post-emergence. n-Tricosane was present in all samples except 3-day and 15-day post-emergence female mosquitoes. In the 3-day post-emergence extract, the n-triacontane was found ranging from 1.3-to-2.2%. However, the presence of this compound was not consistent throughout the study.

Behavioral study

The behavioral study was conducted on 6 days post-emergence *Aedes aegypti*. On the 6th day both genders would have fully developed organs for mating, copulation, blood feeding and ovipositioning (Christophers, 1960). Six male and female extracts were prepared for behavioral observation: M1, M3, M6, M9, M12, and M15 for males; and F1, F3, F6, F9, F12, and F15 for females. The responses of male and female mosquitoes to the male n-hexane extract are shown in Table 3.

Male extracts on male mosquito

The mean value of the male mosquito attracted to the M1 chamber was lower compared to the control chamber. The control and 'NR' showed the same mean value (1.70). The male mosquito, however, showed a different response towards M3. The mean value for the control (2.00±0.12) was higher compared to the M3 sample (1.80±0.25). The sample M6 has the highest mean of attraction at 2.50±0.23. The lowest mean value for the 'NR' choices was for the M6 extract (0.85±0.21). In comparison, M9 showed different values for the control and sample. The male was attracted to the sample with a mean value of 1.80±0.17.

Male mosquitoes were attracted to the extract of the older male starting from sample M6. The sample M12 registered as the second highest mean value for attraction to the sample with a mean value of 2.25±0.19. M15 was also attractive to the male mosquito. These results showed that the male mosquito was likely to choose the sample chamber containing male extracts ranging in age from 6 days to 15 days post-emergence. All results were found to be not significant ($p>0.05$). There was a negative correlation value between all data ($p<0.05$), that is, it was inversely proportional. As the control mean increased the sample mean was found to decrease.

Male extracts on female mosquito

Female showed different behavior towards the male extracts. The highest mean of female attraction towards male extracts was observed in M1, M3, and M6. The sample, M3 (3.00±0.23), prevailed the highest mean value in captivating the attention of female mosquitoes compared to the rest of the samples. However females beyond 6 days post-emergence did not show interest in choosing the sample chamber containing extracts of older males (>9 days post-emergence). Sample M9 had the highest mean (2.25±0.22) to drive away females from the sample chamber. The female response to male extract showed no significant relationship between the sample and the control ($p>0.05$) except M3 showed significant value when comparing the means for both parameters. The correlation tests showed that all the samples have negative and significant value except M12. In comparison, the mean value for 'NR' in M15 showed the female has a dilemma in choosing between the sample chamber and control chamber, as the value for 'NR' and sample chamber have the same mean

Table 3
Behavioral response based on mean (± SE) number of male and female mosquitoes toward n-hexane extracts of males of various age groups[a].

Extract code	Male			Female		
	Control chamber	Sample chamber	NR	Control chamber	Sample chamber	NR
M1	1.70 (0.25)	1.60 (0.27)	1.70 (0.26)	1.80 (0.30)	2.10 (0.22)	1.10 (0.22)
M3	2.00 (0.12)	1.80 (0.25)	1.20 (0.23)	1.75 (0.26)	3.00 (0.23)	0.25 (0.10)
M6	1.65 (0.27)	2.50 (0.23)	0.85 (0.21)	2.05 (0.22)	2.15 (0.27)	0.80 (0.19)
M9	1.75 (0.22)	1.80 (0.17)	1.45 (0.22)	2.25 (0.22)	1.95 (0.21)	0.80 (0.20)
M12	1.70 (0.21)	2.25 (0.19)	1.05 (0.17)	1.85 (0.18)	1.80 (0.21)	1.45 (0.22)
M15	1.55 (0.22)	2.10 (0.20)	1.35 (0.17)	1.70 (0.21)	1.65 (0.26)	1.65 (0.21)

[a]150 mosquitoes were used in this test. NR, no response.

Table 4
Behavioral response based on mean (±SE) number of male and female mosquitoes toward n-hexane extracts of females of various age groups[a].

Extract code	Male			Female		
	Control chamber	Sample chamber	NR	Control chamber	Sample chamber	NR
F1	2.10 (0.19)	2.40 (0.29)	0.55 (0.17)	1.55 (0.20)	1.25 (0.20)	2.20 (0.32)
F3	1.70 (0.24)	2.90 (0.25)	0.40 (0.13)	2.35 (0.25)	2.35 (0.24)	0.30 (0.11)
F6	1.80 (0.16)	2.45 (0.15)	0.75 (0.12)	1.65 (0.18)	2.20 (0.19)	1.15 (0.17)
F9	2.40 (0.21)	2.05 (0.22)	0.55 (0.11)	1.90 (0.23)	2.30 (0.24)	0.80 (0.20)
F12	2.45 (0.19)	1.60 (0.17)	0.95 (0.15)	1.70 (0.27)	1.85 (0.20)	1.45 (0.30)
F15	2.10 (0.22)	1.85 (0.22)	1.05 (0.19)	1.85 (0.20)	2.10 (0.19)	1.05 (0.21)

[a]N, 150 mosquitoes were used in this study. NR, no response.

value. M3, M6, and M9 showed the lowest mean value for 'NR'. These compounds have the properties that enable the female to choose the control chamber and sample chamber without any difficulty.

Female extracts on male mosquito

The male mosquito's responses to female extracts of various age groups are shown in Table 4. The highest mean value in the sample chamber was observed for F3 (2.90±0.25), followed by F6 (2.45±0.15) and F1 (2.40±0.29). The sample F3 showed a significant value between the mean of control and sample ($p<0.05$). This sample has the capability to attract the mosquito into the sample chamber. It also showed a strong mean value compared to "NR" results. Meanwhile, for F9, F12, and F15, the male mosquitoes were attracted to the control chamber and the highest mean value was observed for F12 (2.45±0.19). Male mosquitoes also showed the lowest

mean value of ≤1.00 for the 'NR' choice. These results showed that the males were attracted to the sample chamber containing female extracts of 1 day, 3 days, and 6 days post-emergence. F1, F3, and F6 contained compound(s) with the capability of luring males into choosing the sample chamber. There were negative correlations for all results between the control and the sample, and the values were significant ($p<0.05$).

Female extracts on female mosquito

The mean±SE of female mosquito behavior towards various age of female extract was present in Table 4. The highest mean value was when the female mosquito was stimulated to choose the chamber with sample F9 (2.30±0.24). The F1 had the highest mean of the 'NR' (2.25±0.32) compared to the control chamber and the sample chamber. n-Hexane and F1 could not lure the female mosquito into the chambers. The F1 chamber had the lowest mean value compared to all the samples tested in this study. F3 showed the same mean value between control chamber and the sample chamber (2.35) and has the lowest mean for 'NR'. The pattern of attraction changed when F6 was tested. The mean in the sample chamber was higher than the control and 'NR' chamber. F9 (2.30±0.24) had the highest mean value when compared to mean values obtained from the test. The statistical test showed F3, F6, and F12 mean values were significant ($p>0.05$). There is not much difference in the F12 and F15 data obtained from the behavior test. The female was still attracted to the sample chamber; however, F12 had a low mean value. All data had negative correlation values except for F1, which had showed positive correlation. However, correlation showed that there was significant relationship between sample chamber and control chamber in all samples.

DISCUSSION

The chromatographic analyses showed that n-alkanes group is the most abundant compounds identified in both male and female of the *Aedes aegypti* extracts. These linear n-alkanes appeared as odd and even numbered carbon compounds. The percentage of odd numbered carbon n-alkanes present in male and female extracts was higher compared to the even numbered carbon n-alkanes. They contributed to nearly 50.0% of all compounds identified. However there was very little or no difference in the percentages of odd numbered carbon n-alkanes in the 1 day and 15 days post-emergence male and female n-hexane extracts. However, 3 days, 6 days, 9 days and 12 days post-emergence males had more odd numbered carbon n-alkanes compared to females. The highest percentage in male was observed in the 6 days post-emergence. Conversely, in the females, this was detected in the 15 days post-emergence.

Lockey (1980) suggested that the odd numbered carbon n-alkanes dominated over cuticular hydrocarbons such as n-tricosane, n-pentacosane, n-heptacosane, and n-nonacosane. Six compounds from n-alkanes group appeared in all male and female *Aedes aegypti* extract. These compounds were n-pentacosane, n-hexacosane, n-heptacosane, n-octacosane n-nonacosane, and n-tetratriacontane. Three compounds, namely, n-pentacosane, n-heptacosane, and n-tetratriacontane were found at the highest percentages in all male and female *Ae. aegypti* extracts analyzed.

The study by Horne and Priestman (2002) reported that n-heptacosane was

found as the most abundant compound in Rock strain *Aedes aegypti*, and n-alkanes found in the Rock strain were also detected in the Selangor strain of *Ae. aegypti*. These compounds can be found in n-hexane extracts of 1 day to 15 days post-emergence male *Ae. aegypti*, which elicited behavioral responses. The compounds were present throughout the first fifteen days post-emerging from the pupal stage. The major compound, n-heptacosane, was shown to increase with the mosquito age. Similarly n-octacosane and n-nonacosane both also increased with the age of the mosquito. In contrast, n-pentacosane level was lower in the younger male. The composition started to increase in 3 days post-emergence reaching the highest percentage at 6 days post-emergence. Subsequently, n-pentacosane composition in older male extract showed decreasing pattern from 9 to 15 days post-emergence.

This linearly decreased pattern was more obvious in the female groups. These indicated that pentacosane is more needed or secreted in early days of the mosquito development and can be indicated as an aging compound that was also reported by Desena *et al* (1999). n-Pentacosane with 9-methylpentacosane and 3-methylpentacosane was produced by Coleopteran beetle Xylotrechus colonus as a contact pheromone to attract the male beetle (Ginzell *et al*, 2003).

Christophers (1960) suggested that the mating behavior of male *Aedes aegypti* begun after emergence from the pupa stage to the adult stage. Males will seek out females and try to mate with them. In this study the males used were between 3-6 days post-emergence, which is the mating age for the male mosquito. Results for behavioral analysis suggested that male mosquitoes were attracted to older male extract and younger female extract. The extracts obtained from female at 1 day post-emergence to 6 days post-emergence possessed the characteristics to attract the males into the sample chamber. However, the male response towards the older male is still uncertain.

Laboratory observation showed when many males were caged together; rivalry occurred resulting in several deaths of the males. Females 3 days post-emergence attracted toward extract of male 1 day post-emergence compared to extract of same age females. The females are less attracted to older than 6 days post-emergence extracts. The pattern of attraction was found to change in the female for the 9 days post-emergence. At this point female mosquitoes become more attracted to female extracts compared to male extracts. Both male and female *Aedes aegypti* attracted towards extracts of 6 days post-emergence.

The behavioral analysis also suggested that male *Aedes aegypti*, 3-6 days post-emergence were attracted to extracts of males at 6-15 days post-emergence. The extracts of other age groups did not lure males into the sample chamber compared to females. The lowest percentage of attraction in male extracts was observed in the 1 day post-emergence. The female extract was more attractive to male *Ae. aegypti* compared to the male extract. Male mosquitoes showed the highest percentage of attraction to the extract of females 3 days post-emergence. The active ingredient(s) in the female extract managed to attract the male mosquito into the sample chamber. However, males 12 days and 15 days post-emergence were attracted more towards male extracts compared to female extracts.

In this study, the chromatographic re-

sults that n-alkanes were identified as the most abundant compounds with n-heptacosane and n-tetratriacontane occurred as the highest percentages in both males and female extracts. The behavioral study showed male *Aedes aegypti* (<6-day-old) were attracted to extracts from younger females while female *Ae. aegypti* were attracted to extract of older males.

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