

**IDENTIFICATION OF SEMIOCHEMICALS  
EVOKING BEHAVIORAL RESPONSE IN THE  
SELANGOR STRAIN *Aedes Aegypti* (L.)**

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BEHAVIORAL RESPONSE IN THE SELANGOR  
STRAIN *Aedes Aegypti* (L.)

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**Dedicated to my father, mother, brother and sisters**

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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 its 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 *Aedes aegypti* (L.), an important vector for dengue in Malaysia, and to observe their behavioral response toward *n*-hexane extract of mosquitoes of 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. Meanwhile, 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 extracts of *Aedes aegypti* of various age groups and separate body parts of unmated and mated insects were analyzed via GC-FID and 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 and the C<sub>30</sub> mixture. 3 $\beta$ -Cholest-5-en-3-ol (cholesterol), followed by *n*-heptacosane occurred in abundance in *n*-hexane extracts of the various male body parts. 3 $\beta$ -Cholest-5-en-3-ol (cholesterol) was the major compound detected in the *n*-hexane extract of female body parts. The percentage of a number of identified compounds, viz. *n*-pentacosane, *n*-heptacosane and 3 $\beta$ -cholest-5-en-3-ol, in the *n*-hexane body parts extract was found to have increased in mated groups of the mosquitoes. HS-SPME-GCMS on live *Aedes aegypti* detected *n*-heptadecane as the most abundant compound secreted by both males and females during the 16 hours incubation period. Chemical analysis of extracts and secretions of the *Aedes aegypti* throughout its life revealed mostly *n*-alkanes occupied as major compounds. Longer carbon chains (> C<sub>20</sub>) extracted from the insect cuticular were found to dominate the *n*-hexane extracts. On the contrary HS-SPME-GCMS analyses were dominated by shorter chain carbons (< C<sub>20</sub>) secreted from the *Aedes aegypti* glands.

## ABSTRAK

Semiokimia adalah suatu bahan atau campuran sesuatu bahan yang berfungsi sebagai pengantara dan bertindak menghantar suatu mesej di antara serangga daripada spesies yang sama atau spesies yang berlainan. Ia juga berfungsi mengawalatur pelakuan serangga tersebut. Pengenalpastian semiokimia spesifik yang mampu merangsang tidak balas pelakuan sesuatu serangga boleh digunakan bagi menghasilkan suatu racun serangga baru yang bersifat lebih spesifik, tidak toksik, mesra alam dan mampu mengelakkan daripada berlakunya kerintangan serangga terhadap racun serangga. Kajian ini dijalankan bagi mengenalpasti bahan semiokimia yang terdapat di dalam nyamuk *Aedes aegypti* (L.) strain Selangor yang merupakan vektor utama pembawa virus denggi. Selain daripada itu tindak balas tingkah laku nyamuk tersebut terhadap ekstrak *n*-heksana nyamuk pada pelbagai peringkat umur akan diperhatikan. Bioassai tingkah laku telah dijalankan dengan menggunakan tiub Y-olfaktometer yang telah direka khas untuk tujuan ini. *Aedes aegypti* jantan yang berumur 3 hari - 6 hari lebih tertarik kepada sampel ekstrak *n*-heksana nyamuk jantan pada umur 6 hari - 15 hari manakala bagi pemerhatian ke atas ekstrak *n*-heksana nyamuk betina, ia lebih tertarik kepada sampel berumur 1 hari - 9 hari. Nyamuk betina pada umur yang sama lebih tertarik kepada ekstrak *n*-heksana nyamuk jantan yang berumur 1 hari - 3 hari dan ekstrak *n*-heksana nyamuk betina pada 6 hari - 15 hari. Ekstrak *n*-heksana kumpulan pelbagai umur dan ekstrak *n*-heksana bahagian badan nyamuk telah dianalisis dengan menggunakan GC-FID dan GCMS. Analisis kromatografi menunjukkan bahawa 40 % kumpulan *n*-alkana telah dikenalpasti dan merupakan bahan yang paling banyak ditemui dalam komposisi ekstrak *n*-heksana nyamuk jantan dan betina pada kumpulan pelbagai umur. *n*-Heptakosana adalah bahan yang paling banyak ditemui dalam ekstrak *n*-heksana nyamuk jantan dan betina dan kemudian ini diikuti oleh *n*-tetratriakontana dan Campuran C<sub>30</sub>. 3 $\beta$ -Cholest-5-en-3-ol (kolesterol) dan *n*-heptakosana pula wujud dalam ekstrak *n*-heksana bahagian badan nyamuk jantan. 3 $\beta$ -cholest-5-en-3-ol adalah bahan utama yang terdapat dalam ekstrak *n*-heksana bahagian badan nyamuk betina. Peratus bagi beberapa bahan yang dikenalpasti seperti *n*-pentakosana, *n*-heptakosana dan 3 $\beta$ -cholest-5-en-3-ol (kolesterol) dalam ekstrak *n*-heksana bahagian badan nyamuk meningkat pada kumpulan nyamuk yang telah mengawan. Analisis menggunakan HS-SPME-GCMS menunjukkan *Aedes aegypti* merembeskan lebih banyak *n*-heptadekana semasa proses inkubasi selama 16 jam. Analisis kimia pada ekstrak dan rembesan *Aedes aegypti* menunjukkan kumpulan *n*-alkana wujud sebagai bahan utama sepanjang hidupnya. Ekstrak *n*-heksana pada bahagian kutikel serangga ini didominasi oleh rangkaian karbon (> C<sub>20</sub>) yang panjang sebaliknya keputusan yang diperolehi daripada analisis HS-SPME-GCMS pada rembesan kelenjar *Aedes aegypti* menunjukkan lebih banyak rangkaian karbon pendek (< C<sub>20</sub>) dan mendominasi sebahagian besar sebatian kimia yang telah dikenalpasti.



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## LIST OF SYMBOLS

-	Minus
%	Percent
∅	Diameter
''	Inch
<	Less than
>	Greater than
±	Plus-minus
≈	Almost equal to
≤	Lesser than or equal to
°C	Degrees Celcius
μl	Microliter
μm	Micrometer
cm	Centimeter
cps	Cycles per second
eV	Electron volt
g	Gram
g/mol	Gram per mol
m	Meter
m/s	Meter per second
m/z	Mass to charge ratio
mg	Miligram
mg/ml	Milligram per mililiter
min	Minutes
ml	Mililiter
mm	Millimeter
ppm	Parts per million
psi	Pound-force per square inch
β	Beta

## LIST OF ABBREVIATION

AA	Antibiotic assay
CAR	Carboxen
CW	Carbowax
DDT	Dichloro Diphenyl Trichloroethane
DVB	Divinyl benzene
GC	Gas chromatography
GC-FID	Gas chromatography – Flame ionization detector
GCMS	Gas chromatography Mass Spectrometry
HS-GC	Headspace – Gas Chromatography
HS-SPME	Headspace - Solid Phase Microextraction
HS-SPME-GCMS	Headspace - Solid Phase Microextraction- Gas Chromatography
MW	Molecular Weight
PDMS	Polydimethyl siloxane
NR	No Respond
SD	Standard deviation
SE	Standard error
SPME	Solid phase microextraction
TAS	Terminal abdominal segment
VOC	Volatile organic compounds
WHO	World Health Organization

## GLOSSARY

<i>ad libitum</i>	Without restraint or imposed limit; as much as often as is wanted
Cephalothorax	The fused head and thorax
Chordotonal	A sensory organ for insect and crustacean
Copulatory	The bond or linkage between male and female during copulation in which semen and other elements are transmitted from male and female
Ejaculatory duct	A duct form by joining of vas deferens from each testis and involved in the propulsion of semen
Epideictic	A name of a pheromone group which is used to mark the territories especially for the female to lay their eggs and mark it to avoid the predator.
Holometabolic	Insect with a complete transformation during metamorphosis with eggs, larval, pupal and adult stages distinctly separated by profoundly different morph at each state.
Hypipharynx	A component of the insect mouth-part arising behind the mouth and just behind the labium and lower lip. Usually short and tongue-like species with biting jaws, but often drawn out to form a tube for the salivary glands duct in those species with sucking mouth.
Hypopygium	In Diptera, the male sexual organ and terminal segment of the abdomen
<i>in-vivo</i>	Taking place in living organism
Mandible	The anterior most pair of oral appendages on the insect head
Mechanosensory	Sensory receptor that respond to the mechanical deflection of a sensillum's surfaces
Maxillary palps	Appendage arising from maxilla
Metamorphosis	Changing in form



Oviducts	The distal tubular portion of the female reproductive system which transmit the eggs outside the body.
Oviposit	Process of laying egg by a female insect
Palps	A segmented leg-like structure arising on the maxilla or labium, have a sensory function and play a major role in tasting food.
Phytophagus	Plant-eating
Proboscis	Any extended or extensible mouth structure
Rostrus	A snout-like prolongation of the head
Saculli	A small sac or pouch and a part of the genitalia
Salivary glands	Exocrine glands typically associated with the labial segment; highly variable in size, structure and function
Sclerites	The hardened plates of the insect skeleton
Sensillum	Specialized structure that collect sensilla from the external and internal environment and transmit their information to the central nervous system
Spermathecae	Out pocketing of the vagina in which spermatozoa are store prior to fertilization
Tarsi	Distal, segmented part of the insect legs attached to the tibia.

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