Identification of Chemical Signals that Influence the Oviposition Behaviour of *Anopheles Gambiae* and Exploration of their Potential in Mosquito Control

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A Thesis Submitted in Fulfilment for the Degree of Doctor of Philosophy in Chemistry in the Jomo Kenyatta University of Agriculture and Technology

2009

ABSTRACT

The African mosquito species *Anopheles gambiae* and *An. funestus* are ranked high among the world's most efficient vectors of human malaria. Their juvenile stages develop in aquatic environments while adults are terrestrial. Chemical signals guide gravid females of these vectors to their egg-laying sites. Several attributes of a pond including presence of other organisms influence egg hatching success and larval survival. Gravid *An. gambiae* females strongly discriminate among potential egg-laying sites to ensure viability of their offsprings. This study is based on the hypothesis that gravid *An. gambiae* females use chemical cues from microbial activity and/or those associated with competitors as interspecific cues as well as intraspecific signals associated with their own eggs or larvae to select suitable habitats for oviposition.

The main aim of this study was to identify the chemicals that guide gravid *An. gambiae* to their oviposition site and to find out their effect on oviposition behaviour. To achieve this, behavioural responses of caged gravid *An. gambiae* on two choice assay of test water consisting of *Culex quinquifasciatus* egg rafts and/or larvae and test water as control were compared. We found out that *An. gambiae* is deterred or avoids laying eggs in the sites where there is *C. quinquifasciatus* egg rafts, larvae or both. *C. quinquifasciatus* larvae deterred the oviposition by gravid *An. gambiae* even at low density. Moreover, when both *C. quinquifasciatus* larvae and egg rafts were used with varying density of egg rafts and constant number of larvae the deterrence was more than when the two were used separately.

Dynamic and static trapping systems were used to collect volatiles emanating from larvae,

extract from test water with *C. quinquefasciatus* larvae, test water extract (supernatant of muddy soil mixed with double-distilled water and allowed to settle for 3-7days), *An. gambiae* egg extract, *C. quinquefasciatus* egg rafts extract, soil and cultured soil bacteria. Gas chromatograph-mass spectrometry (GC-MS) was used to characterize the chemical constituents of the volatiles.

Eleven compounds were identified from *C. quinquefasciatus* larval volatiles; dimethyl disulfide, dimethyl trisulfide, 3,5-dimethylbenzaldehyde, 2,4-bis(1,1-dimethylethyl)phenol, 1-chlorotetradecane, isopropyl myristate, isopropyl palmitate, 4-phenylmorpholine, 3-phenyl-1-azabicyclo[2.2.2]octane, eicosane and 2,4-bis(1-methyl-1-phenylethyl)phenol.

Six compounds were identified from the extract of test water with *C. quinquefasciatus* larvae; 4-methylphenol, 4-(1,1,3,3-tetramethyl butyl)phenol, 4-(1,1-dimethylpropyl)phenol, 2[(4-hydroxyphenyl)methyl]phenol, N,N-dimethylthiocarbamoylphenyltrithiocarbonate, 2,4-bis(1-methyl-1-phenylethyl)phenol and (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane.

The test water was extracted with dichloromethane and nine compounds were identified; 2,4-bis(1,1-dimethylethyl)phenol, 4-(1,1-dimethylpropyl)phenol, 6,10,14-trimethyl-2-pentadecanone, 2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)phenol, phytol, 2,4-bis(1-methyl-1-phenylethyl)phenol, (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene and 4-octyl-N-(4-octylphenyl)benzenamine.

Tetradecanoic acid, Z-11-hexadecenoic acid, n-hexadecanoic acid, (Z)-9-octadecenoic acid, octadecanoic acid, docosane, (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene were obtained from *An. gambiae* eggs extract. Z-11- hexadecenoic acid, n-hexadecanoic acid, (Z)-9-octadecenoic acid, octadecanoic acid, N-butyl-4,9-decadien-2-amine, arachidonic acid and 1,2,3-propanetriyl ester hexanoic acid were found in *C. quinquefasciatus* egg rafts extract.

Volatiles trapped from the muddy soil used for preparation of test water yielded eleven compounds; d-limonene, [3aR-(3a.alpha.,4.beta.,7.alpha.)]-2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-3H-3a,7-methanoazulene, 2,6-bis(1-methylethyl)benzeneamine, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)naphthalene, (1S-cis)-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene, [R-[R*,R*-(E)]]-3,7,11,15-tetramethyl-2-hexadecene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (1-methyldodecyl)benzene, 2[(4-hydroxy phenyl)methyl]phenol, 2-phenyl-2-(phenylmethyl)-1,3-dioxolane and 2,4-bis(1-methyl-1-phenylethyl)phenol. The same soil was cultured for bacteria and the trapped volatiles thereof yielded twelve compounds identified as: dimethyl disulfide, dimethyl trisulfide, 2-ethyl-1-hexanol, 2-phenoxyethanol, tetradecane, 2,6-bis(1,1- dimethylethyl)- 2,5-cyclohexadiene-1,4-dione, hexadecane, octadecane, isopropyl myristate, 4-hydroxy-4-methyl-2-pentanone, 1-undecene and 4-phenylmorpholine.

Some of the compounds identified were evaluated for their effect on oviposition behaviour against gravid females of *An. gambiae* mosquitoes at different concentrations. Dimethyl dilsulfide and 1:1 mixture of N-hexadecanoic acid and octadecanoic acid had behavioural effect on gravid *An. gambiae*. At low concentrations the compounds showed positive oviposition response and as the concentration increased there was a negative oviposition effect. Erythro-6-acetoxy-5-hexadecanolide, previously isolated from *C. quinquifasciatus* egg rafts, showed a negative oviposition effect.

This study showed that interspecific chemical signals mediate the oviposition of gravid *An. gambiae* to a specific site. The presence of *C. quinqufasciatus* larvae and/or egg rafts in a pond deters gravid *An. gambiae* from ovipositing in that specific pond. The microorganisms in the soil influence to a great extent the decision of gravid *An. gambiae* to oviposit on a given site. The chemical cues believed to mediate oviposition behaviour by *An. gambiae* have been identified and characterized. This provides the basis of understanding the behavioural effect of individual and blended compounds and this may be used to develop alternative methods of controlling malaria vectors