PHYSIOLOGICAL AND MOLECULAR RESPONSES IN ADULT AND LARVAE *ANOPHELES GAMBIAE* S.S. TO *MURRAYA KOENIGII* PHYTOCHEMICALS

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Abstract

Continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non-target organisms including human health. Naturally occurring plant compounds and their derivatives are, therefore, of increasing interest for the development of new compounds against the malaria vector. In this study, adulticidal and larvicidal activity of essential oil and crude extracts isolated from the leaves of *Murraya koenigii* against mosquito vectors will be investigated. Essential oil will be isolated from the leaves of *M. koenigii* using hydro-distillation method. Different compounds will be identified by gas chromatography-mass spectrometry analysis. Larvicidal activity of crude chloroform, dichloromethane and methanol extracts of the leaves will be tested against the early fourth instar larvae of *Anopheles gambiae* s.s. and larval mortality observed after 24 h of exposure relative to the positive control Temephos, an effective emulsifiable concentrate larvicide. Susceptibility tests on adult mosquitos will be carried out using WHO insecticide susceptibility standard procedures. The fumigant toxicity assays will be repeated with commercially available constituents of the essential oils and blends of these at their natural proportions in the oils and in amounts present in the minimum 100% lethal dose of the oils. Each component will then be subtracted from the blend in turn to determine its relative contribution to the overall toxicity of the natural oil. RNA will be extracted from mosquito stages exhibiting unique response to the plant compounds, allowing detection of genes that might show differential expression in a constitutive and/or inducible way. The extracted RNA will be treated with DNase and the RNA quantified. Three replicate experiments will be performed for each observation. The cDNA libraries will be developed from the extracted RNA and sequenced on illumina platform using established protocols. The libraries will be made from three independent collections from each observation, and will be barcorded for sequencing. Resultant transcriptome sequences will be subjected to quality control checks using FastQC software following which the reads will be mapped to their respective reference genomes and splice junctions between exons identified using combinations of TopHat and Bowtie software. Differential expression and regulation among and between samples from different species will be determined using cufflinks software. Specific methods adopted will be determined by the inherent nature of the data, following standard biometric procedures (e.g normality test where appropriate), and software. Our approach employs the repellent, toxic or otherwise bioactive effects of major components of essential oils through subtraction assays that will provide an additional insight into the relative contribution of these components and potentially explore new targets in the malaria vector that will spur research into novel synthetic formulations.

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List of Abbreviations

AchE	Acetylcholinerase
BTI	*Bacillus thuringiensis israelensis*
DDT	Dichlorodiphenyltrichloroethane
GPS	Global Positioning System
HPLC	High Performance Liquid Chromatography
IGRs	Insect growth regulators
IR	Infrared
IRS	Indoor residual spraying
ITN	Insecticide treated Nets
LLINs	long-lasting insecticide Treated Nets
LSM	Larval Source Management
NMR	Nuclear magnetic Resonance
UV	Ultraviolet