Tropical Biomedicine 30(1): 125–130 (2013)

Species composition and pyrethroid susceptibility status of *Anopheles* mosquitoes from two different locations in Malaysia

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Received 23 October 2012; received in revised form 11 January 2013; accepted 21 January 2013

Abstract. A 14-months survey was carried out to identify the species composition of *Anopheles* mosquitoes from Kampung Bongor, Grik, Perak. Adding to that, a preliminary one month mosquito population screening was done at Kampung Tepin, Serian, Sarawak. Consequently, the insecticide susceptibility status of a pyrethroid was tested against two selected species of *Anopheles* collected from these two locations in Malaysia. A total of 4,497 *Anopheles* from 11 species were identified from collections in Kampung Bongor, whereas 2,654 *An. letifer* were collected from Kampung Tepin. The *An. maculatus* of Kampung Bongor and *An. letifer* of Kampung Tepin were then selected and tested using WHO standard diagnostic test kits and impregnated papers with 0.75% permethrin. The response values of KT_{50} and KT_{95} for *An. maculatus* were recorded at 28.09 minutes and 62.98 minutes respectively. *Anopheles letifer* recorded much slower response values of KT_{50} and KT_{95}, which was at 35.09 minutes and 73.03 minutes respectively. Both *An. maculatus* and *An. letifer* showed 100% mortality after 24 hours holding period. The results indicate that both species were still susceptible to the tested pyrethroid. For effective vector control and resistance management, accurate and periodic insecticide resistance monitoring should be undertaken especially in rural areas with agricultural usage of insecticides.

INTRODUCTION

Kampung Bongor of Grik (101° 11'E, 5° 30'N) in the district of Hulu Perak is known to have a significantly abundant population of *Anopheles* mosquitoes with *Anopheles barbirostris* and *Anopheles aconitus* being in the top ranks, as noted in previous researches conducted by Rahman *et al.* (1995; 2002) and Abu Hassan *et al.* (2001). Due to its locality which is in close proximity to the Thailand border, this small village had been implicated as being a malarious prone area. Marina (2007) noted a malaria outbreak in Southern Thailand in early 2007 had subsequently led to an increase of malaria cases in Hulu Perak as well.

Random mosquito population surveys conducted by public health officials in Kampung Tepin of Balai Ringin in the district of Serian, Sarawak (110º 43'E, 1º 4'N) recorded high populations of *Anopheles* mosquitoes. Although malaria cases in this village of less than 100 occupancies for the past decade have been extremely low, however, as preventative measures, fogging activities were conducted periodically and the villagers were also provided with insecticide treated bed nets (ITNs). Essentially, the insecticide susceptibility status of this large number of *Anopheles* mosquitoes should be inspected. As they are continuously being subjected to insecticidal controls, there should be frequent checks on development of resistance to the used chemicals.

In Malaysia, control of *Anopheles* mosquitoes relies heavily on the use of insecticides. Chemical method remains as one of the indispensable strategies used in vector control due to the speedier response time and wider area of coverage. For personal protection, household insecticide products such as mosquito coils, liquid vaporizer and aerosol are used. Usage of ITNs is also one type of personal protection. As for larger scale vector control of *Anopheles* mosquitoes, residual spray on wall surfaces are used (Lee & Yap, 2003). Chemical control can be considered the easiest and most efficient way of controlling malaria vector populations. However, there are limited numbers of safe and cost effective chemicals (WHO, 1998a). Moreover, there is also the tendency for development of insecticides resistance in the *Anopheles* populations (WHO, 2005; Yadouleton, 2010). As such, malaria still remains as an unresolved health issue in many countries.

Insecticide resistance has been developing among malaria vectors which are being controlled using existing insecticides. This is due to the high usage frequency and subjecting of the insect population to insecticide selective pressure for a long duration of time (Lee *et al.*, 2003). It is of great concern especially towards the compounds in pyrethroid group because they are applied in almost all chemical control methods. Permethrin, a Class 1 compound of synthetic pyrethroid, is used in agriculture to protect stored grain and in aerial application for forest protection. Together with many other pyrethroid compounds, permethrin was also used in ITNs as vector control to replace residual spraying using DDT and malathion in many Asian countries (WHO, 1998a). As it exhibits low mammalian toxicity, permethrin is widely used as a surface residual spray and space spray in vector control (Yap *et al.*, 2003), thus making it an insecticide which *Anopheles* mosquitoes are frequently exposed to. Early detection of insecticide resistance forewarns existence of resistance (WHO, 1998a). Thus, constant monitoring of the susceptibility status of mosquito vectors is essential to keep check on any signs of selection occurring, especially toward frequently used insecticides.

MATERIALS AND METHODS

Mosquito samples

The collections at Kampung Bongor, Grik, Perak were conducted twice per month for 14 months. Most *Anopheles* mosquitoes are not exclusively anthropophilic or zoophilic (Abu Hassan & Yap, 2003), thus the cow baited trap (CBT) method was adopted in sampling the *Anopheles* mosquito population in Kampung Bongor. An animal, in this case, a young cow was tied and placed inside a mosquito net to attract the entry of blood-seeking female mosquitoes but prevent their escape (Silver, 2008). The mosquito net was set up a few meters away from inhabited dwelling, on ground level, at an open area with slightly dense vegetations and fruit trees. Hourly collections of adult *Anopheles* mosquitoes resting on the net after their blood meal were done by a two-man team using aspirators.

The more convenient human baited trap (HBT) method was used in the short sampling period for the population of *Anopheles* mosquitoes in Kampung Tepin, Serian, Sarawak. The human bait will be seated inside a mosquito net which serves to attract host-seeking female mosquitoes but prevents their escape, and the collections were done by conducting human landing-biting catches. This research was carried out weekly for a period of one month. A mosquito net was set up at a cleared, open location on ground level, located a few metres away from inhabited dwelling and within the vicinity of a small durian farm. Mosquitoes that landed and tried to bite the bait were collected by two collectors using glass vials and aspirators (Silver, 2008).

All collected *Anopheles* mosquitoes were transferred and kept in portable mosquito cages provided with 10% sucrose solution and brought back to the laboratory to be counted and identified using a dissecting microscope. Field strains of *Anopheles maculatus* from Kampung Bongor, and

Anopheles letifer from Kampung Tepin, both species being incriminated as vectors of malaria (Abu Hassan & Yap, 2003; MOH, 2008), were separated to be used for the bioassay testing.

WHO standard diagnostic tests

Standard diagnostic test kits used in the bioassay tests of adult mosquitoes were as determined by WHO (1998b). The impregnated papers with 0.75% concentration of permethrin were used for exposure, as it is a commonly used discriminating dosage for testing insecticide susceptibility. This insecticide was chosen based on its wide usage in the agricultural industry for crop protection, in vector control as ITNs, and for space spraying (Yap *et al.*, 2003).

The test procedures for determining insecticide susceptibility of adult mosquitoes were based on the methodology described by WHO (1981). Each test was conducted using 20 sugar-fed female adult *Anopheles* mosquitoes. The age and size of these field collected test samples were not measured prior to testing. At the end of the exposure period, the tested mosquitoes were transferred into paper cups and covered with mesh cloths. They were provided with cotton wool soaked with 10% sucrose solution and kept for 24 hours under laboratory condition at a temperature of 23–25ºC and relative humidity of 75–80%. Total mortality was determined after a 24 hour holding period.

Each test was replicated five times. Negative control test using silicon oil treated papers (pyrethroid control) was conducted twice. When control mortality was between 5% and 20%, the average observed percentage mortalities were corrected by Abbott's Formula:

$$\frac{\text{\% Test Mortality} - \text{\% Control Mortality}}{100 - \text{\% Control Mortality}} \times 100$$

Probit analysis using a Statistical Package for the Social Sciences (SPSS) software was done to calculate and determine the 50% (KT_{50}) and 95% (KT_{95}) knockdown time of the tested insecticide towards the field collected *An. maculatus* and *An. letifer* mosquitoes respectively.

RESULTS

A total number of 4,497 *Anopheles* mosquitoes consisting of 11 types of species were caught and identified in Kampung Bongor, Grik, Perak. Out of these 11 species, seven species were from the *Anopheles* (*Cellia*) subgenus, whereas the remaining four species were identified to be under the *Anopheles* (*Anopheles*) subgenus (Table 1). As for Kampung Tepin, of Serian district in Sarawak, a total number of 2,654 *An. letifer* from the *Anopheles* (*Anopheles*) subgenus were caught. No other *Anopheles* species were found in this research site for the whole duration of collection.

Table 1. Species composition of *Anopheles* mosquitoes of Kampung Bongor, Grik, Perak

Subgenus	Species	Percentage (%)
Anopheles (*Anopheles*)	*An. barbirostris*	5.14
	An. separatus	3.58
	An. umbrosus	2.13
	An. nigerrimus	2.29
Anopheles (*Cellia*)	*An. aconitus*	31.49
	An. maculatus	17.99
	An. philippinensis	16.86
	An. vagus	7.47
	An. kochi	4.96
	An. subpictus	3.96
	An. tessellatus	4.14

For the results of insecticide susceptibility testing using probit analysis, the comparative time–response values for *An. maculatus* from Kampung Bongor were notably faster as compared to *An. letifer* from Kampung Tepin. The KT_{50} and KT_{95} values for *An. maculatus* were at 28.09 minutes and 62.98 minutes respectively. As for *An. letifer*, the KT_{50} and KT_{95} values were at 35.09 minutes and 73.03 minutes respectively ($P<0.001$) (Table 2; Figure 1).

Table 2. Comparative time–response values of permethrin 0.75% against *An. maculatus* from Kampung Bongor, Grik and *An. letifer* from Kampung Tepin, Balai Ringin

Mosquitoes	Knockdown Time (Confidence Limit)* (min) KT_{50}	Knockdown Time (Confidence Limit)* (min) KT_{95}	Regression Slope ± Standard Error (min)
An. maculatus	28.09 (26.90 – 29.26)	62.98 (58.66 – 68.45)	4.69 ± 0.11
An. letifer	35.09 (34.23 – 35.96)	73.03 (69.44 – 77.32)	5.17 ± 0.08

* A heterogeneity factor is used in the calculation of confidence limits

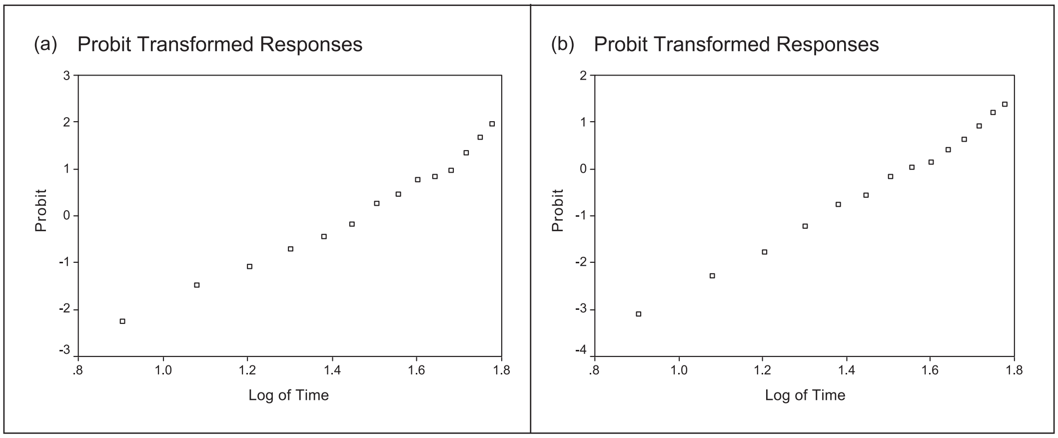

Figure 1. Regression slopes for probit transformed responses for (a) *An. maculatus* and (b) *An. letifer*

The tested samples of *An. maculatus* and *An. letifer* showed heterogeneity factor, indicating a non-homogenous sample. This may be due to the slight variation in age and size of the female adults collected during the sampling period. Both *An. maculatus* and *An. letifer* showed 100% mortality after the 24 hours holding period.

DISCUSSION

The *Anopheles* mosquitoes from both sample sites have shown more than 50% knockdown at the first half an hour exposure and 100% mortality after 24 hours holding period. Based on use of discriminating dosage in the assay, results indicated that the field strains of both *An. maculatus* of Kampung Bongor and *An. letifer* of Kampung Tepin were considered still susceptible towards permethrin. Yet, their lack of resistance should not be taken lightly as selective pressure and behavioural changes can occur through their constant exposure to agriculture pesticides.

Majority of the villagers in Kampung Bongor and Kampung Tepin were involved in agricultural activities. As the villages were surrounded by rubber plantations and vegetable and fruit farms in the midst of dense tropical rain forests, the population of *Anopheles* mosquitoes which breeds abundantly would have higher probability of exposure to indefinite amount of insecticides. In rural areas where agriculture is the main source of income and there is large-scale usage of insecticides, selective pressure may be exerted among the vectors as they are not only subjected to agricultural chemicals but also chemicals from vector control. Insecticide resistances have a higher probability of developing from long term exposure in areas where agriculture is a major activity and frequent usage of insecticides is unavoidable (WHO, 1998a). Inevitably, it is important to determine the possible influence of agricultural pesticides on *Anopheles* vector resistance before conducting any form of vector insecticide resistance programmes (WHO, 1998a).

Besides the concern of developing insecticide cross-resistance mechanism, there is also the possibility of changes in the bionomics of *Anopheles* mosquitoes, such as their resting places and biting preferences when they start developing insecticide avoidance behaviours (Chareonviriyaphap *et al*., 2002). As insecticide resistance is not confined to the control area of particular *Anopheles* mosquitoes, susceptibility studies should cover other species of *Anopheles* mosquitoes which are known vectors but there have been no records of malaria transmissions.

The number of insecticides for public health is limited and likely to remain as so due to the rise in cost of development and registration of new compounds (WHO, 1998a). It is imperative for the current insecticides to be used optimally to ensure we will still be able to chemically control vectors of diseases in the near future. Periodic inspections and detections of insecticide resistance will help public health personnel to formulate suitable vector management programmes to improve the effectiveness of their current control methods and counter any emerging selective pressure through resistance management. Alternatives such as reducing use of chemicals, changing and alternating effective chemicals, or substituting chemicals with more environmentally friendly options, for instance adopting biological control methods, should be considered as forms of resistance management tools. Hence, there is great importance to have timely, systematic and accurate collection of data and information on insecticides resistance.

Acknowledgements. The authors wish to thank the staff of Vector Control Research Unit, USM Penang for providing technical support, to Pak Yah and family of Kampung Bongor, Grik for their hospitality and support during the whole collection period, and to Tn. Hj. Asmad Matusop, Mr. David Lubim, staff and lab officers of the Vector Borne Diseases Control Unit of the Sarawak General Hospital for permitting the use of their facilities and for their kind assistance throughout the research duration in Sarawak.

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