Tropical Biomedicine 31(3): 441–448 (2014)

Surveillance and resistance status of *Aedes* population in two suburban residential areas in Kampar town, Perak, Malaysia

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Received 20 November 2013; received in revised form 22 February 2014; accepted 23 February 2014

Abstract. In this study, 13 weeks (October to December 2012) of ovitrap surveillance was conducted in two suburban residential areas in Kampar town, Perak. A total of 17,310 *Aedes* mosquitoes were found in Taman Kampar Jaya, whereas Taman Juloong recorded a higher number at 19,042. Less than 1% of these were identified as *Aedes aegypti*, with the remaining confirmed as *Aedes albopictus*. The female *Ae. albopictus* were subsequently subjected to WHO standard diagnostic test kits against two pyrethroids (0.05% deltamethrin and 0.75% permethrin) and two organophosphates (1% fenitrothion and 5% malathion). The *Ae. albopictus* from both research sites were the most susceptible to deltamethrin, recording KT_{50} and KT_{95} response values of 15.84 minutes and 16.18 minutes; and 48.18 minutes and 49.44 minutes respectively. This was followed by permethrin (20.57 minutes and 17.52 minutes; 29.54 minutes and 54.54 minutes) and malathion (48.46 minutes and 62.69 minutes; 87.72 minutes and 141.04 minutes). Fenitrothion was found to be least effective towards *Ae. albopictus*; recording KT_{50} and KT_{95} response values of 150.29 minutes and 293.41 minutes for Taman Kampar Jaya, and 203.32 minutes and 408.07 minutes respectively for Taman Juloong. All tested *Ae. albopictus* showed 100% mortality after 24 hours post exposure. As both residential areas were fogged periodically by the municipal council; alternating between organophosphates and pyrethroids, thus, constant monitoring is crucial in light of the emergence of resistance noted in *Ae. albopictus* towards fenitrothion.

INTRODUCTION

Malaysia's tropical climate and dense rainforest allows it to house more than 434 species of mosquitoes from at least 20 genera (Rahman *et al.*, 1997). Approximately 10% of them are involved in the transmission of diseases; with *Anopheles* sp. being the most important vectors of malaria, *Culex* sp. for filariasis and Japanese Encephalitis, and *Aedes* sp. for dengue (Harun, 2007). Besides dengue, *Aedes* sp. has also been implicated in the transmission of chikungunya, yellow fever and Eastern Equine Encephalitis (EEE), especially within the Southeast Asian regions (Estrada-Franco & Craig, 1995).

The process of rapid urbanization and growth of cities promotes continuous breeding of an abundance of disease vectors (Chen *et al.*, 2005; Hidayati *et al.*, 2011), in particular the *Aedes* mosquitoes; which thrive well in urban, suburban, rural and forested areas (Harun, 2007), and in any form of water-containing receptacles (Estrada-Franco & Craig, 1995). Being successful vectors, the *Aedes* mosquitoes are widely found in human settlements; greatly escalating the chances of vector and human contact, thus leading to the potential increase in rate of disease transmission (Moore & Mitchell, 1997). Many surveillance activities on mosquitoes have been conducted and are still ongoing due to the high mosquito population, particularly in urban cities (Abu Hassan *et al.*, 2010).

Control of vector-borne diseases is crucial especially in populated areas which are susceptible to high risk of exposure and infection (McCall & Kittayapong, 2007). There are several vector control methods; ranging from chemical and biological control, to environmental management. Out of these, chemical control; being the fastest, cheapest and most effective method, is widely used.

The major classes of chemical insecticides used in vector control included pyrethroids, carbamates, organophosphates, and organochlorines (Nauen, 2007). Usage of the infamous dichlorodiphenyltrichloroethane (DDT) in agriculture and vector-borne disease control in the 1950s was eventually terminated 20 years later due to its high bioaccumulation effect (CDC, 2009; Becker *et al.*, 2010). The World Health Organization (WHO) recommends pyrethroids for use on insecticide treated (bed)nets (ITNs), and malathion and carbamates for indoor residual spraying (IRS), as these chemicals do not cause as much pollution to the environment as compared to DDT (Becker *et al.*, 2010).

Since the 1950s, development of potential resistance in vectors has been apparent but was poorly documented (Hemingway & Ranson, 2000). Insecticide resistance is usually induced in mosquitoes due to extensive applications of similar chemicals over extended periods of time (Surendran *et al.*, 2007). In tropical countries, resistance issue was often found to be highest as there were higher population and shorter generation time of mosquitoes, with less effective vector control programs (Head & Savinelli, 2008). Emergence of resistance among the *Aedes* sp. mosquito populations in Malaysia began due to fogging operations with malathion since early 1970s and permethrin since early 1996 (Hamdan *et al.*, 2005). These were popular chemicals, with malathion being the cheapest, and permethrin being the most commonly used insecticide in mosquito control (Chan *et al.*, 2011).

Efficacy of vector control is threatened with the emergence of insecticide resistance, which makes it even harder to control vector-borne diseases (Vythilingam *et al.*, 1992). The level and progression of resistance development in mosquito population is dependant on the frequency and volume of insecticides used against them (Hemingway & Ranson, 2000). As such, the main defense against resistance is close surveillance of the insecticides' efficacy towards controlling the mosquito populations. Constant monitoring is vital to detect resistance at an early stage, as insufficient data could interfere with the effectiveness of disease control programs. Furthermore, this provides baseline data for program planning and pesticide selection before the commencement of control operations (Brogdon & McAllister, 1998).

MATERIALS AND METHODS

Study sites

Kampar (101^o 09' 0" E 4^o 18' 0" N) is a former tin mining town located within the Kinta Valley of Perak state. The district of Kampar is comprised of Kampar town and its surrounding areas within 20–50 km^2 radiuses, with a population of more than 90,000 (Department of Statistics Malaysia, 2010). There were a total of 66 dengue cases with no fatality reported by the Kampar district council in the year 2012 alone. Kampar town; broadly divided into the 'old town and 'new town' areas, is the largest town in Kampar district with a total area of about 15 km^2. Two suburban residential areas; Taman Kampar Jaya and Taman Juloong, which are located within the 'old town' area of Kampar town, were chosen for this study.

Taman Kampar Jaya, which is located on the north end of Kampar's 'old town' is comprised of 18 rows of single- and double-storey terrace houses, a field, and two rows of shop houses. Towards the south end of 'old town' is Taman Juloong, a moderately populated suburban residential area located on a hill. There are only three bungalows and six rows of double-storey semi-detached houses on the hilltop, and three blocks of fourstorey flats on the foothill. Both residential areas are surrounded by many lush tall trees and plants.

Most of the residents of both residential areas maintained a well-kept garden within the compounds of their own houses; however, their surrounding vicinities lacked much care. There were numerous sites which seemed to have become common dumping grounds for the residents to dispose off their rubbish and unwanted items and furniture.

Ovitrap surveillance

The ovitrap is a well-known economic tool in the detection and surveillance of vector populations as it is sensitive and reliable (Yap *et al.*, 1995). It consists of a 300 ml black plastic container (H: 9.0 cm x D: 7.8 cm), and a piece of hardboard paddle (H: 10.0 cm x L: 2.5 cm x W: 0.3 cm) with a rough surface on one side and smooth surface on the other. The paddle is placed diagonally inside with the rough surface facing up, and the container is half-filled with dechlorinated tap water. Sixty ovitraps were hung outdoors on tree trunks randomly selected in each of these residential areas. All paddles were collected and replaced thrice weekly for a total of 13 weeks from the months of October to December 2012.

All collected paddles were brought back to the laboratory and air dried overnight before being immersed into dechlorinated tap water the following day. Emerged larvae will be fed with ground cat food and allowed to grow until pupae stage, then transferred into mosquito cages provided with 10% sucrose solution for adult emergence. All emerged adults were collected using aspirator and identified. Subsequently, the identified females were separated for bioassay testing.

WHO standard diagnostic tests

The adult bioassay procedures and methods used were as described by WHO (1981). Four types of insecticide impregnated papers which consisted of two pyrethroids (0.05% deltamethrin and 0.75% permethrin), and two organophosphates (1% fenitrothion and 5% malathion) were used. The dosages chosen are commonly used discriminating dosages for testing insecticide susceptibility.

Each test was conducted using 20 sugar-fed female adult *Ae. albopictus* mosquitoes. All tested samples were of one to two days of age after emergence. 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 controls were done using silicon oil- (pyrethroid control) and olive oil- (organophosphate control) treated papers. When control mortality was between 5% and 20%, the average observed percentage mortalities were corrected by Abbott's Formula:

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

Data analyses

The following parameters were included in the analyses. All levels of statistical significance were determined at $p < 0.05$ using t-test:

I. Total number of mosquitoes

II. Ovitrap Index (OI) =

$$\frac{\text{Number of Positive Ovitraps}}{\text{Total Number of Ovitraps Examined}} \text{ X } 100$$

III. Mean number of mosquito species =

$$\frac{\text{Total Number of Mosquitoes}}{\text{Total Number of Ovitraps Examined}}$$

Probit analysis was done using SPSS software to calculate and determine the 50% (KT_{50}) and 95% (KT_{95}) knockdown time of the four types of insecticides towards the *Ae. albopictus* from both research sites.

RESULTS

Taman Juloong recorded the highest number of *Aedes* mosquitoes at 19,042; followed by Taman Kampar Jaya with a total of 17,310. Out of these, 0.07% and 0.28% respectively were identified to be *Ae. aegypti*, with the remaining collections confirmed as *Ae. albopictus*. However, the total number of collected *Ae. albopictus* from Taman Kampar Jaya (M = 1327.85, SD = 367.10) and Taman Juloong (M = 1463.77, SD = 327.15) were not significantly different (t(24) = -1.00, p = 0.329). Similarly, the collected *Ae. aegypti* from both residential areas were also not significantly different (t(24) = 1.05, p = 0.306).

Both Taman Kampar Jaya and Taman Juloong attained an Ovitrap Index (OI) within the ranges of 90 – 100% for *Ae. albopictus* (t(24) = 0.12, p = 0.909) and 0–2% for *Ae. aegypti* (t(24) = 0.19, p = 0.852) with no significant difference between both areas (Table 1). Although the mean number of mosquitoes per ovitrap for the *Ae. albopictus* of Taman Juloong was recorded higher than Taman Kampar Jaya, it was not significantly different (t(24) = -1.00, p = 0.329). Conversely, the mean number of mosquitoes per ovitrap for *Ae. aegypti* of Taman Kampar Jaya was higher, but also not significantly different from Taman Juloong (t(24) = 1.05, p = 0.307).

In the insecticides susceptibility tests, *Ae. albopictus* from both Taman Kampar Jaya and Taman Juloong were the most susceptible to deltamethrin, recording the lowest response values of 50% and 95% knockdown time (Table 2). The KT_{50} and KT_{95} of *Ae. albopictus* from Taman Kampar Jaya were at 15.84 minutes and 48.18 minutes respectively, whereas the KT_{50} and KT_{95} of *Ae. albopictus* from Taman Juloong were slightly higher at 16.18 minutes and 49.44 minutes respectively. The tested *Ae. albopictus* of both research sites were also shown to be susceptible to permethrin and malathion, both having KT_{50} response values of 1 hour or less.

Table 1. Ovitrap Index (OI) (%) and mean number of mosquitoes (± SE) per ovitrap observed

Location	Ovitrap Index (OI) (%)		Mean number of mosquitoes per ovitrap (Mean number ± SE)	
	Ae. albopictus	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*
Taman Kampar Jaya	94.69 ± 1.05	1.15 ± 0.39	22.13 ± 1.70	0.06 ± 0.04
Taman Juloong	94.49 ± 1.43	1.03 ± 0.55	24.40 ± 1.51	0.02 ± 0.01

Table 2. Comparative time–response values of four types of insecticides against the *Ae. albopictus* from Taman Kampar Jaya and Taman Juloong

Insecticides	Taman Kampar Jaya			Taman Juloong		
	Knockdown Time (KT) (min) KT_{50}	Knockdown Time (KT) (min) KT_{95}	Regression slope ± SE	Knockdown Time (KT) (min) KT_{50}	Knockdown Time (KT) (min) KT_{95}	Regression slope ± SE
0.05% Deltamethrin	15.84 (14.99 – 16.68)	48.18 (45.70 – 51.00)	3.41 ± 0.76	16.18 (15.26 – 17.07)	49.44 (46.80 – 52.46)	3.39 ± 0.08
0.75% Permethrin	20.57 (20.17 – 20.96)	29.54 (28.89 – 30.27)	0.18 ± 0.01	17.52 (16.66 – 18.36)	54.54 (52.02 – 57.36)	3.34 ± 0.08
1% Fenitrothion	150.29 (148.53 – 152.03)	293.41 (288.71 – 298.37)	5.66 ± 0.06	203.32 (194.60 – 212.00)	408.07 (381.62 – 441.64)	5.44 ± 0.06
5% Malathion	48.46 (47.71 – 49.20)	87.72 (85.91 – 89.67)	6.38 ± 0.12	62.69 (61.39 – 63.98)	141.04 (137.71 – 144.61)	4.67 ± 0.07

However, fenitrothion was found to be the least effective towards *Ae. albopictus* from both residential areas. The KT_{50} and KT_{95} response values for *Ae. albopictus* of Taman Kampar Jaya were 150.29 minutes and 293.41 minutes respectively, whereas the *Ae. albopictus* of Taman Juloong were shown to be even more resistant, with KT_{50} and KT_{95} response values of 203.32 minutes and 408.07 minutes respectively. All tested *Ae. albopictus* from both research sites showed 100% mortality after 24 hours post exposure.

DISCUSSION

The Ovitrap Index (OI) acquired for both Taman Kampar Jaya and Taman Juloong exceeded 95%, with mean number of mosquitoes recording more than 20 per ovitrap; an indication of an exceptionally high activity of mosquito breeding in both residential areas. There were two species of *Aedes* identified from both research sites; namely *Ae. albopictus* and *Ae. aegypti*. The majority species found were *Ae. albopictus* as they are generally exophilic; preferring a wide variety of outdoor habitats ranging from natural receptacles to artificial manmade containers (Lee, 2000).

Taman Kampar Jaya and Taman Juloong are both suburban residential areas, which provide for more favorable habitat conditions for *Ae. albopictus* (O'Meara *et al.*, 1995). Conversely, the *Ae. aegypti* which seemed to be more persistent in a wide range of highly urbanized habitats (Cox *et al.*, 2007) were only found in low numbers. Previous researches have demonstrated *Ae. aegypti* sharing similar breeding places with *Ae. albopictus* (Lee, 2000; Chua *et al.*, 2005; Dieng *et al.*, 2012). However, as the *Ae. aegypti* are better known to be indoor breeders and anthropophilic (Miyagi & Toma, 2000), they are more like to be found breeding in assorted water receptacles in and around homes. This may account for the lower numbers of *Ae. aegypti* detected in both research sites as all the ovitraps were placed outdoors. The positive ovitraps with *Ae. aegypti* were mostly from those placed nearer to the residential houses. Moreover, the higher number of this vector of dengue detected in Taman Kampar Jaya can be an important indication of a higher risk of a dengue outbreak to occur among residents of the area.

Results of the WHO adulticidal bioassay have shown both deltamethrin and permethrin of the class of pyrethroids to be more effective as compared to organophosphates malathion and fenitrothion against the *Ae. albopictus* of Taman Kampar Jaya and Taman Juloong. This finding is similar to the works done in many other countries such as Thailand, India, Greece, Italy and Cameroon, where the exophilic *Ae. albopictus* were found to remain susceptible to both of these pyrethroids (Vontas *et al.*, 2012). Conversely, their counterpart *Ae. aegypti* which is highly anthropophilic has been exhibiting resistance to pyrethroids which are the most common insecticides used in vector control programs in many areas (Lima *et al.*, 2003; Sathantriphop *et al.*, 2006; Loke *et al.*, 2012).

In this study, the *Ae. albopictus* from both Taman Kampar Jaya and Taman Juloong displayed evidence of resistance towards fenitrothion, with the KT_{50} response values of more than 2 hours. With reference to information given by the Kampar district health division and municipal council, the mosquito populations of Taman Kampar Jaya were commonly exposed to alternating insecticides of permethrin and fenitrothion, whereas those in Taman Juloong were usually being subjected to malathion and fenitrothion. The extensive usage of these organophosphates may have been one of the contributing factors to the presence of resistance, as noted in the *Ae. albopictus* population of Taman Juloong.

Unlike most pyrethroids which were introduced two decades ago, organophosphates such as malathion and fenitrothion, and carbamates which included bendiocarb and propoxur, have been heavily used in vector control programs for many decades (Chareonviriyaphap *et al.*, 2013). The earlier exposure to the latter might have accounted for the higher rate of resistance observed. Nazni and colleagues (2005) in their study have reported that the progress of selection pressure of insecticides against mosquito population can be reduced and resistance can even be reversed, if the mosquitoes were kept insecticide-free for a long period. As such, the importance of adequate planning and the alternative use of active ingredients (Nazni *et al.*, 2005) in vector control programs should be stressed. Although permethrin resistance is found to develop at a faster rate as compared with other insecticides (Loke *et al.*, 2012), since the mosquito populations of both Taman Kampar Jaya and Taman Juloong were not constantly exposed to permethrin, we therefore found no indications of resistance.

The results obtained in this study can provide useful information in making judicious management decisions on the choice of pesticides in a vector control program. Routine surveillance on the resistance status of mosquito populations is crucial to implement fitting strategies to prevent untoward disease outbreaks. As indicators of insecticides efficiency, susceptibility status of mosquitoes should be evaluated from time to time for better resistance management and control. Moreover, rotational use of insecticides has to consider the mode of action of the insecticides of choice to avoid possible occurrence of cross resistance.

Acknowledgements. The authors wish to thank En. Azrul Nizam who is the officer in charge of the Vector Borne Diseases Control Unit of Kampar Health Division and Mr. Goh Seng Chee from the Kampar District Municipal Council for their kind assistance, staff of the Vector Control Research Unit, USM Penang for providing technical supports, and residents of Taman Kampar Jaya and Taman Juloong for their hospitality, cooperation, and understanding throughout the research duration.

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