Insecticide susceptibility and selection for resistance in a population of *Aedes aegypti* from Ratchaburi Province, Thailand

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Abstract. The insecticide susceptibility of *Aedes aegypti* larvae and adults from four areas of Ratchaburi Province, Thailand was investigated using World Health Organization standard procedures. The larvae of *Ae. aegypti* in all areas were found to be susceptible to temephos. The strain with the highest level of temephos resistance was subjected to temephos selection. The resistance ratios increased to 2.74-fold from their parental non selected strain after 5 generations of selection. For the adult of *Ae. aegypti*, the study was conducted to determine the effects of 0.75 % permethrin and 0.05 % deltamethrin impregnated paper selection on the strain having the highest with the highest LT_{50} level. The results showed the increase in the LT_{50} to 7.46 and 1.18-fold after the third selected generation with 0.75 % permethrin and 0.05 % deltamethrin respectively. Mortality rates after exposing adults to discriminating concentrations showed that field populations in Ratchaburi Province were resistant to permethrin and deltamethrin. Alternative non-chemical measures need to be used in new of the emergence of resistance in the mosquito population.

INTRODUCTION

Dengue is the most important arthropod-borne viral disease in Southeast Asia (WHO, 2000). The principal vector of dengue virus in Thailand is the mosquito *Aedes (Stegomia) aegypti (L.)*. Until an effective, safe and affordable vaccine become available, no adequate prevention or control measures other than control of vector, *Ae. aegypti*, are available to deal with epidemics of disease(Gratz,1993). A vaccine against dengue is currently not available and the control of is likely to remain vector control(Pan-American Health Organization, 1998). Although reduction of sources in which the mosquitoes develop can keep vector populations low, the use of insecticides is often necessary. The primary insecticides used against dengue vector in

Thailand is temephos, applied as 1 % sand granules into household water containers and other breeding foci for larval control. Fenitrothion, malathion, deltamethrin, tetramethrin and permethrin are mainly used in thermal fogging and ultra-low volume sprays for routine control of adult or when outbreak of this diseases has appeared. In Thailand, the yearly use of insecticide is determined by the average cases of dengue fever, dengue haemorrhagic fever and dengue shock syndrome in 0-14 years old children per 100,000 population in the past 5 years. If average cases are more than 350, that area is defined as a high risk area and so the distribution of insecticide is increased. Ratchaburi is a province in the central part of Thailand that was determined in the year 2002 to be a high risk area by its yearly average cases. Hence Ratchaburi was designated as our study area. The development of resistance in mosquitoes to the insecticide used against them as larvicides and adulticides is an important issue to be considered as difficulty with control would be encountered due to the emergence of insecticide-resistant mosquitoes.

This study has two main objectives : Characterization of the resistance to temephos, deltamethrin and permethrin in *Ae. aegypti* in Ratchaburi Province and to determine whether selection with temephos, deltamethrin and permethrin will result in tolerance or resistance in those population. Such knowledge is essential in defining future control strategies against this medically important mosquito.

MATERIALS AND METHODS

Mosquitoes Sampling

Sampling of mosquito populations from four areas in Ratchaburi Province were chosen according to the number of dengue cases and the intensity of insecticide use. The locations are illustrated in Fig. 1.

Larval bioassay;

Larvae of *Ae. aegypti* were collected from 4 areas in Rachaburi Province ; and colonized in the laboratory. The F1 or F2 larvae were used in bioassay to determine their susceptibility to temephos (WHO 1963, 1981) compared with a laboratory susceptible strain colonized at the Department of Medical Sciences, Ministry of Public Health, Thailand to determine the strain with the highest resistance ratio based on the median lethal concentration(LC_{50}).

Insecticide

Technical grade (90 % purity) temephos (Abate[R]) was obtained from Cyanamid Co., U.S.A.

Bioassay procedures

The late third or early fourth instar larvae were used for bioassay test. The procedures was followed briefly, 25 larvae in 249 ml of declorinated-tap water with 1 ml of each concentration of temephos were tested. Mortality counts were made after 24 hours. The results were analyzed to obtain the LC_{50} by probit analysis using a computer program (Raymond, 1985).

Selection procedures

The strain which showed the highest resistance ratio(RR) to temephos would proceed for selection pressure. This strain would be selected for 5 generations by exposing late third or early fourth instars to the concentrations which produced 60-70% mortality.

Adult bioassay;

The adults of F1 or F2 from 3 areas of Ratchaburi Province were used for bioassay by determining their susceptibility to 0.75% permethrin and 0.05% deltamethrin impregnated paper (WHO, 1981). This was compared with a laboratory susceptible strain colonized at the Department of Medical Sciences, Ministry of Public Health, Thailand to determine the strain which showed the highest resistance ratio of the median lethal time(LT_{50}).

Insecticides

0.75% Permethrin and 0.05% deltamethrin impregnated paper were purchased from Vector Control Research Unit, Penang, Malaysia.

Bioassay procedures

Non-blood fed females aged 2-3 days old were used for bioassay test. A minimum number of 100 mosquitoes should be used for each insecticide, with 4-5 replicates of 20-25 mosquitoes per test. Treatments contained paper impregnated with insecticide whereas control tubes contained oil-treated papers without insecticide. Mortaity was recorded at the end of 24 hours holding period. The results were analyzed to obtain the LT_{50} by using computerised probit analysis (Raymond, 1985).

Selection procedures

The strain which showed the highest RR to 0.75% permethrin and 0.05% deltamethrin were used for selection. These strains were continuously selected for 3 generations by exposing non-blood fed females aged 2-3 days old for a certain period of time that would produce 60-70% mortality against these insecticides.

Diagnostic dosage testing: In addition to complete bioassays, a single dose diagnostic tests were conducted (WHO, 1981)in order to separate the susceptible and the resistant individuals.

Larvae: The third and early fourth instar larvae of the strain with had the highest RR to temephos were exposed to the diagnostic concentration of temephos (0.02 mg/L).

Adult: Non-blood fed females aged 2-3 days old of the strains with the highest RR to 0.75% permethrin and to 0.05% deltamethrin were exposed to the diagnostic concentrations of permethrin (0.75%) and deltamethrin (0.05%) impregnated paper for 1 hour and the mortality was recorded at the end of 24 hours holding period.

Those strains that had been selected by permethrin and deltamethrin were also tested in the same manner. Cross resistance between the two insecticides was studied based on the results obtained.

RESULTS

Larval bioassay;

The resistance ratios at 50% lethal concentration (LC_{50}) from each localites in Ratchaburi showed various susceptibility to temephos when compared with the laboratory susceptible strain. This varied from 2.19 to 5.61 as shown in Table 1. *Ae. aegypti* from Tambon Thanat, Damnoen Saduak district has the highest level of temephos resistance (LC_{50} = 0.00202mg/L). This strain was subjected to temephos selection. The resistance ratios after 5 generations of selection increased to 15.39 and 2.74-fold compared with laboratory susceptible strain and with the Tambon Thanat non selected strain, respectively. The LC_{50} of Tambon Thanat strain after five generations of temephos selection was 0.00554 mg/L.

Adult bioassay for permethrin;

The susceptibility test of adult mosquitoes to 0.75% permethrin impregnated paper showed the variety of the susceptibity to permethrin when compared with the laboratory susceptible strain. The resistance ratio of the 50% lethal time (LT_{50}) compared with laboratory susceptible strain varied from 23.19 to 68.92 as shown in Table 2. The adult of *Ae. aegypti* from Tambon Pongsawai, Muang Ratchaburi District has the highest level of permethrin resistance. So this strain was subjected to permethrin selection pressure. The resistance ratios after 3 generations of selection increased to 513.93 and 7.46-fold compared with laboratory susceptible strain and with Tambon Pongsawai non selected strain, respectively.

Adult bioassay for Deltamethrin;

The resistance ratios at 50% lethal time (LT_{50}) from each locality in Ratchaburi showed various susceptibily to deltamethrin impregnated paper compared with laboratory susceptible strain. This varied from 21.47 to 52.74 as shown in Table 3. Adult of *Ae. aegypti* from Wat KhuBua, Tambon KhuBua, Muang Ratchaburi District showed the highest level of deltamethrin resistance. This strain was subjected to deltamethrin selection pressure. The resistance ratios after 3 generations of selection increased to 62.29 and 1.18-fold when compared with laboratory susceptible strain and with Wat KhuBua non selected strain respectively.

Diagnostic dose test:

Larvae: The larvae of Tambon Thanat non-selected strain and F_5 generation after selection were exposed to the diagnostic dose of temephos at 0.02 mg/L . The result indicated that all the laboratory susceptibility strain, Tambon Thanat non-selected and selected strains showed complete larval mortality (Table 4).

Adult: The laboratory susceptible strain showed complete adult mortality to the diagnostic concentration of permethrin (0.75%) and deltamethrin (0.05%) impregnated paper. Twenty two percent mortality was observed from Tumbon Pongsawai non-selected strain and 7.27 % mortality was observed from Tumbon Pongsawai F_3 permethrin-selected strain for expose against 0.75% permethrin. When these two strains were exposed to diagnostic concentration of deltamethrin (0.05%), both showed the same mortality percentage (31%). On the other hand, the adults from Wat Khu Bua non-selected and F_3 deltamethrin selected strains showed 17% and 16% mortality when exposed to diagnostic concentration of deltamethrin. The test also showed that there was 46% and 30% mortality in Wat Khu Bua non-selected and F_3 deltamethrin selected strains respectively when exposed to permethrin at diagnostic concentration. These suggested the resistance to permethrin and deltamethrin was present in these population.

DISCUSSION

All field strains of *Ae. aegypti* were susceptible to temephos with RR varying from 2.11 to 5.61 when compared with the laboratory susceptible strain. Their susceptibilities were confirmed by complete mortality after exposing to WHO diagnostic concentration of 0.02 mg/L (table 4). Even the larvae from Tambon Thanat, five generations after temephos selection pressure, with resistance ratio of 15.39, showed complete mortality by this diagnostic dose. It was possible that the F_5 selected generation was not resistant enough to survive the diagnostic concentration of temephos.

In Thailand, the recommended dosage of Abate^R sand granules(Abate^R 1-S.G.) applied to domestic stored water is 1 g/10 liters water which is equivalent to 1 mg/L of active ingredient. This is very much in excess of the calculated LC_{99} values of the Tambon Thanat (0.01149 mg/L), and if applied accordingly, is undoubtedly still highly effective(Lee, 1989). However, if the LC_{99} of any strain, especially field strains were beyond 1 mg/L, an alternative control method for larvae is needed.

Resistance is defined as the acquired ability of an insect population to tolerate doses of insecticide which can kill the majority of individuals in a normal population of the same species (WHO, 1975). Our results indicated a rather high resistance to permethrin with resistance ratio varying from 23.19 to 68.92, and to deltamethrin with the resistance ratio of 21.47 to 52.74 in the adults of *Ae. aegypti* from 3 study areas (Table 2 and 3). The presence of resistance in the natural population was probably due to the impacts from pyrethroids used in household insecticides, fogging for mosquito control and agricultural practices. Moreover, a high level of resistance to permethrin in *Ae. aegypti* from Tambon Pongsawai can be developed under selection pressure in the laboratory. According to the WHO protocol; a population is considered resistant if more than 20% of the population survives the diagnostic dose compared to the susceptible strains (WHO, 1981). In our experiment, the mortality percentage decreased from 22% to 7.27% after 3 generations selection with permethrin. No change in mortality to diagnostic dose of deltamethrin was observed for the same strain. For the adult from Tambon Khubua after 3 generations of deltamethrin selection, the mortality to diagnostic dose decreased only by 1% (Table 5). However, the F_3 deltamethrin-selected showed decreased mortality to permethrin diagnostic dosage from 46% in non-selected strain to 30%. It was likely that the cross resistance from deltamethrin to permethrin had occurred because of the same mode of action of both pyrethroids on the voltage-dependent sodium channel of nerve axons (Brook et al., 1996). However, the cross resistance from permethrin to deltamethrin was not evident in Tambon Pongsawai F_3 permethrin-selected. It was possible that the resistance level of permethrin was too low to produce cross resistance to deltamethrin. Therefor permethrin selections were needed in the further generations in order to elucidate the actual mechanism of resistance.

Detection of resistance will help public health personnel to formulate appropriate steps to counter reductions in effectiveness of control effort that may accompany with the emerging problems of insecticide resistance. Furthermore, cross resistance or resistance as a result of agricultural uses of insecticides may evolve and adversely impact the options to switch to an alternative method or insecticides for disease control. Biological control agents such as *Bacillus thuringiensis* variety *israelensis*, larvivorous fishs, other natural enemies of mosquito larvae, e.g. water boatman, *Toxorhynchites* larvae, nymphs of dragonflies and certain copepods that are commonly found in man-made water containers, should be incorporated into *Ae. aegypti* larval control programs.

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