Determination of insecticides diagnostic doses in susceptible *Culex quinquefasciatus* (Diptera: Culicidae)

A Bukar1,2, and S N Hamzah*

1School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
2Department of Biology Education Federal College of Education (Technical) Potiskum Yobe State, Nigeria

*E-mail: sitinasuh@usm.my

**Abstract.** Despite Mass Drug Administration cases of Lymphatic filariasis still exist in several developing countries such as, China, India, Indonesia, Malaysia and several Southeast Asian countries. *Culex quinquefasciatus* is the major vector that is dominant and widespread mosquito distributed in rural and urban areas. This study aimed at determining the diagnostic doses of LC50 and LC90 (lethal concentrations that causes 50% and 90% mortality respectively) of commonly used insecticides in Malaysia for dengue vector control by Malaysian Ministry of Health. The diagnostic LC50 of the Malathion, Pirimiphos methyl, Temephos and Deltamethrin insecticides were determined at 0.019mg/L, 0.0060 mg/L, 0.0011 mg/L and 0.0032 mg/L as well as LC90 0.033 mg/L, 0.012 mg/L, 0.0033 mg/L and 0.0010 mg/ml respectively. It is concluded that various LC50 and LC90 diagnostic doses of these insecticides were effective against the susceptible population of *Cx. quinquefasciatus* that also carries infective third instar larvae (L3) microfilariae. We recommend the use of these doses for effective control of *Cx. quinquefasciatus* population and constant monitoring of its susceptibility status will provide more information on the possible resistance that may affect the effort in the elimination of Lymphatic filariasis in affected countries.

1. **Introduction**

*Culex quinquefasciatus* is one of the dominant mosquito species in Malaysia, creating biting nuisance at night for long hours and transmit filarial disease (*Wucheraria bancrofti*), exposing the human population to the risk of public health emergency that demands urgent attention for control [1-3]. It is also the most dominant and widespread mosquitoes distributed in the rural, urban, suburban and remote settlements in Malaysia, breeding in stagnant polluted water near residential settlement at a breeding index at high value favored by the environmental condition adapted for the nutrition of the larvae and the adult [4]. *Culex quinquefasciatus* larvae was also found co-breeding with *Aedes albopictus* larvae in Johor, Selangor, Pahang, Kedah, Negeri Sembilan and Kuala Lumpur at palm oil plantation areas, agricultural areas and residential areas with proper water supplies, drainage system and waste management [5]. This mosquito species has a longer biting hour of almost seven hours in Malaysia with different locations having varying peaks of biting hours at night, making it a nocturnal feeder from 1800 to 0700 hours [1]. In a study conducted in nine different locations in Peninsular Malaysia on insects
bitching cycles involving Culex, Aedes, Mansonia and Armigeres, the findings showed that they all have the same biting peak hours from 1900 to 2000 hours [2].

Studies also showed that cases of Lymphatic filariasis still exist in Malaysia despite the Mass Drug Administration conducted for a long period of time to eliminate the disease, but it is still an endemic in Sabah, Sarawak, Pahang, Terengganu, Johor, Kelantan and Selangor [6,7]. These cities were previously at high risk of confirmed urban transmission of W. bancrofti following the discovery of third instar larvae (L3) microfilariae in a Burmese in Negeri Sembilan. The finding of the study shows that the susceptible strain of Cx. quinquefasciatus carries the infective stage of microfilaria on their head at a higher infectivity rate of 33.3%. The study further indicates that the susceptible strain of Cx. quinquefasciatus that harbor microfilariae can develop to L3 infective and transmission stages [8].

Globally, over 120 million individuals are suffering from lymphatic filariasis with the greatest burden of the diseases in tropical and subtropical countries of Africa constituting over 40% of the burden, then India, Southeast Asia, the Caribbean which account for almost 50% of all lymphatic filariasis infections in humans and Brugia malayi and B. timori constituting the remaining 10% of the global infection burden mainly in Malaysia, China, Indonesia and several Southeast Asian countries [9].

+The World Health Assembly enacted a resolution WHA50.29 emphasizing the need for its member states to eradicate lymphatic filariasis as a public health menace. In 2000, WHO launched its Global Program to Eliminate Lymphatic Filariasis in 2020 by stopping the large-scale transmission of the infection by annual treatments of patients and reducing the suffering of the infection through provisions of basic palliatives for the patients [10]. Pathological manifestation of signs and symptoms of the disease begins when the parasite migrates to the lymphatic system and blocks the lymph vessels leading to development of a chronic case referred to as elephantiasis, i.e. the swelling of skin due to microfilaria infection. Other symptoms such as lymphedema, lymphadenitis, cellulitis, hydrocele, lymphangitis, funiculitis, chyluria and acute-dermato-lymphangio-adenitis may also occur [11].

Since there is no particular lymphatic filariasis vector control, the Ministry of Health Malaysia Dengue Vector Control which targets at larvae on grasses and contaminated stagnant waters bodies in the breeding sites of Aedes in the dengue hotspot areas primarily use organophosphates and pyrethroids insecticides since both Culex and Aedes breed in the same site [5,12]. Therefore, understanding the diagnostic dose of these insecticides towards Cx. quinquefasciatus control will help in the significant reduction of the field strains of Cx. quinquefasciatus population in Malaysian cities and enhance the efforts of the Global Program for Elimination of Lymphatic filariasis by 2020 in Malaysia.

2. Materials and Methods
2.1. Susceptible Mosquito Strain
Susceptible Cx. quinquefasciatus first instar larvae used were supplied by the Vector Control Research Unit (VCRU) of School of Biological Sciences, Universiti Sains Malaysia. The strain was maintained for over 300 generations without coming into contact with insecticides. The larvae were reared in a metal tray in a seasoned water at a density of 200 larvae per tray, larvae were provided with larval food. The late third instar and early fourth larvae were collected and used for the bioassay.

2.2. Insecticides
Serial dilutions of the technical grade insecticides (96.1% Malathion, 90.52% Pirimiphos methyl, 98% Deltamethrin and 92.6% Temephos) were used in this study to determine the diagnostic dosage to kill 50% and 90% of mosquito larvae population (LC50 and LC90, respectively).

2.3. Larval Bioassay Susceptibility Test
Larval bioassay was conducted according to [13] procedure. Twenty-five late third instar and early fourth instar larvae were collected using 1ml hand pick pipette and placed into a 500 ml white plastic cup. Mosquitoes were collected and exposed to a wide range of insecticides test concentrations doses and control to determine the mortality and a small dose of 4 to 5 concentrations were also conducted to determine the LC50 and LC90 values. Each white plastic cup contains 200 ml of seasoned water at a
particular dose of insecticide prior to the exposure to achieve the homogeneous mixture of the insecticide and the seasoned water. Larvae and 50 ml of seasoned water were later added to make a homogenous mixture of 250 ml. The tests were conducted starting with the least concentrations at four replicates. Ten percent ethanol was used at the controls and larval food was added to both the tests replicates and the control. The tests were conducted at a controlled room temperature of 25°C to 28°C and photoperiod of 12 Light and 12 dark. Larval mortality was recorded after 24 hours exposure and moribund larvae were also counted as death.

2.4. Statistical Analysis
The results of the larval bioassay were subjected to statistical analysis using SPSS statistical software version 24 to determine the LC50 and LC90 of the test concentrations of the insecticides.

3. Results and Discussions
The results of the bioassay subjected to statistical analysis using SPSS Probit to determine LC50 and LC90 diagnostic doses of the insecticides tested are presented in Table 1. Temephos was calculated with LC50 diagnostic dose of 0.0011 mg/L and LC90 of 0.0033 mg/L among the three organophosphates tested in the study, while Malathion was calculated at LC50 0.019 mg/L and LC90 0.033 mg/L and Pirimiphos methyl at LC50 0.060 mg/L and LC90 of 0.012 mg/L. Deltamethrin, the only pyrethroid tested in the study, was calculated to have the diagnostic LC50 dose of 0.0032 mg/L and LC90 of 0.0010 mg/L at the upper bound of 0.0035 mg/L and lower bound of 0.0029 mg/L respectively.

Among the insecticides tested in the study, Temephos was the most effective at both LC50 and LC90 at the least concentrations of 0.0011 mg/L and 0.0033 mg/L causing mortality of the 50% and 90% of the Cx. quinquefasciatus larvae tested. It is followed by Deltamethrin causing 50% mortality at 0.0032 mg/L and 90% mortality at the least concentration of 0.00103 mg/L. Pirimiphos methyl also showed a lethal toxicity causing 50% mortality at the concentration of 0.0060 mg/L and 90% mortality at the 0.012 mg/L. The insecticide that was least effective causing mortality of Cx. quinquefasciatus larvae tested in this study was Malathion causing 50% mortality at the concentration of 0.019 mg/L and 90% mortality at the highest concentration of 0.033 mg/L respectively.

Table 1. Diagnostic doses of different insecticides against the susceptible Culex quinquefasciatus.

| Insecticides     | LC50 (mg/L) | 95% Confidence Limit (mg/L) | LC90 (mg/L) | 95% Confidence Limit (mg/L) | χ² Value | Intercept |
|------------------|-------------|----------------------------|-------------|----------------------------|----------|-----------|
|                  |             | LCL | UCL | LCL | UCL |             |             |
| Malathion        | 0.019       | 0.020 | 0.017 | 0.033 | 0.037 | 0.031 | 40.795 | 8.692 |
| Pirimiphos-methyl| 0.0060      | 0.0070 | 0.0050 | 0.012 | 0.017 | 0.009 | 10.882 | 10.457 |
| Temephos         | 0.0011      | 0.00191 | 0.0078 | 0.0033 | 0.001776 | 0.00286 | 48.860 | 1.804 |
| Deltamethrin     | 0.0032      | 0.0035 | 0.0029 | 0.00108 | 0.000123 | 0.0087 | 19.312 | 3.805 |

The value represents the mean of eight replications, mortality of the larvae observed after 24 hours of the exposure period, (WHO, 2005).

LC50: Lethal concentration that causes 50% mortality.
LC90: Lethal Concentration that causes 90% mortality.
LCL: Lower Confidence Limit.
UCL: Upper Confidence Limit.
χ² Value: Chi square value.

Efforts on Lymphatic filariasis control cantered on the elimination of the infective stage, third instar (L3) microfilariae in the body of the infected persons through Mass Drug Administration while no much attention toward the main vector, Cx. quinquefasciatus control in most of the endemic countries including Malaysia. The determination of diagnostic doses of insecticides is important in the control
efforts using various levels of insecticides concentrations to manage resistance in mosquitoes of various species [14]. The result of LC50 and LC90 of Temephos in this study was highly effective against the susceptible larvae of *Culex quinquefasciatus*. The findings of this study were in support of the susceptibility study of Temephos conducted in four locations in Brazil and found that all the three locations were susceptible to Temephos. The study further showed that *Culex* mosquitoes did not have prior exposure to Temephos prior to the study [15]. This was also in agreement with the result of the study conducted in Tamil Nadu, India, where Temephos was found to be susceptible against *Aedes aegypti* tested at the same level of insecticide concentration used in this study. On the contrary, the study in Morocco showed that *Cx. pipiens* were resistant against Temephos suggests the influence of selective pressure due to excessive use of Temephos towards vector control of the Bancroftian filariasis in the state [16]. Similarly, in Brazil, *Ae. aegypti* larvae were reported to be resistant to Temephos [17].

Deltamethrin, a pyrethroid was found to be resistant against the field population of *Cx. pipiens* due to its wider use [18]. However, a recent study in Columbia showed a degree of susceptibility to pyrethroids in *Cx. quinquefasciatus* [19]. In Iran, *Cx. quinquefasciatus* was previously susceptible to Deltamethrin before the recent study that first recorded its resistance to Deltamethrin and suspected a *krd* gene responsible for cross resistance of organochlorine and pyrethroid [20]. This finding was also the same with *Anopheles stephensi* which showed resistance to Deltamethrin in Iran due its excessive use in malaria control [21]. In Tunisia, the population of *Cx. pipiens* was reported to be susceptible against Pirimiphos methyl in support of the finding of this study [22].

Moreover, Pirimiphos methyl was used in adult susceptibility test at 1% showing significant effects in malaria control against *An. gambiae* mosquitoes [23]. Recent study also found that *An. gambiae* was resistant to Pirimiphos methyl in Côte d'Ivoire [24]. Furthermore, *An. arabiensis* also showed resistance to Pirimiphos methyl in Tanzania with high elevation in Glutathion S-Transferases (GSTs) and Esterases detoxification enzymes [25]. Malathion was the least effective among the insecticides tested in this study and the findings support the previous study on trans-generation and the comparison among ten various generations of the exposed larvae in Kuala Lumpur [26]. Malathion susceptibility was also observed in adult *Cx. quinquefasciatus* in India at various diagnostic doses [27]. However, a recent study showed that Malathion resistance in Malaysia was detected due to its excessive use in dengue vector control [28].

4. Conclusion
The diagnostic doses (LC50 and LC95) of Malathion, Pirimiphos-methyl, Temephos and Deltamethrin determined in this study was effective towards susceptible population of *Cx. quinquefasciatus* at least doses in this study and in previous studies in various countries endemic with Lymphatic filariasis. Therefore, much attention is needed in the control of lymphatic filariasis vector, *Cx. quinquefasciatus* for successful achievement of its elimination alone with insecticide resistance monitoring for sustainability and less effects against non-target organisms in their ecosystem.

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