The Effectiveness of Organotin (IV) Benzylisopropyldithiocarbamate Compounds as Insecticide Against Aedes aegypti Linn. (Diptera: Culicidae) in Laboratory

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Abstract: The widespread use of insecticides has resulted in insecticide resistance of vectors of dengue as well as polluting the environment. Organotin (IV) compounds have the potential to be developed as the insecticides to overcome the existing problem. The aim of this study was to examine the insecticidal effects which is larvicidal and adulticidal effects of organotin (IV) benzylisopropyldithiocarbamate compounds against Aedes aegypti Linn. in laboratory. Larvicidal bioassay test of a series of three organotin (IV) benzylisopropyldithiocarbamate compounds on third instar larvae of Aedes aegypti had been carried out. The study found that compound B showed the best larvicidal effect with the LC50 and LC90 values of 0.004 ppm and 0.007 ppm, respectively. The compound C also displayed good larvicidal effect with the LC50 and LC90 values of 0.029 ppm and 0.108 ppm, respectively. While, compound I showed the least larvicidal effect with the LC50 and LC90 values of 0.404 ppm and 0.749 ppm, respectively. Further testing was conducted on compound B on adults of Aedes aegypti female to investigate its adulticidal property. The result showed that compound B displayed good adulticidal activity with LC50 dan LC90 of 4.277 ppm and 27.653 ppm, respectively. In conclusion, compound B is the most effective compound among three organotin(IV) benzylisopropyldithiocarbamate compounds tested against the dengue vector Aedes aegypti and has potential to be explored as an insecticide to control the spread of dengue fever.

Key words: Adulticidal activity · Aedes aegypti · Dithiocarbamates · Larvae · Larvicidal activity · Mosquito · Organotin (IV)

INTRODUCTION

Dengue fever is a mosquito-borne disease that threatens international public health. The disease is found in many tropical and subtropical countries throughout the world, especially in urban areas and semi urban [1]. Dengue fever is caused by dengue virus. There are four dengue viruses serotype known as DEN-1, DEN-2, DEN-3 and DEN-4 [2]. Aedes aegypti and Aedes albopictus is the main vector of this disease, but the Aedes aegypti is the most efficient vector of dengue fever since it live in the house and surrounding houses [3].

Until now no vaccine is completely appropriate to be given to society for the prevention of further spread of dengue fever [4]. Thus, vector control is the most effective method of controlling the spread of the disease [5] and chemical insecticide is a method that is believed to reduce the Aedes population and as the result incidence of dengue fever can be reduced [6].

Unfortunately, the uncontrolled use of insecticide has resulted in problems of insecticide resistance [7]. In addition, the effect of degradation of a toxic insecticide metabolites in the environment leading to environmental pollution such as pollution of surface water and ground water [8] as well as negative effects on non-target organisms, including human [9]. Recognizing this problem, scientists seek new alternatives in the production of environmentally friendly insecticide and at the same time preventing resistance in the vector.
Wide application of organotin(IV) compounds in the industry [10] and its potential in biological activity has attracted researchers to conduct studies on this compound [11]. Among the biological activities of organotin(IV) compound are this compounds have been identified as a potential antiviral agent [12], antineoplastic agents, antituberculosis [13-16], antibacterial agent [17], anticancer agent [18] and so on.

Use of organotin(IV) compounds in the field of entomology are also now getting attention [19]. Several studied showed organotin(IV) compounds were effective against some pests like Periplanata Americana, Musca domestica, Spodoptera litura and Tetranychus urticae [19] and a few species of larvae and adult mosquitoes like Aedes aegypti and Aenopheles stephensi [20-23]. Group and the nature of organic group that bound to tin atom, such as the monoalkyl, dialkyl and triphenyl is basic factors determining the biological activity [10, 18, 24].

Until now there has been report of Ae. aegypti resistance to organotin(IV) compound and reported that this compound will be degrade to non-toxic inorganic compound in environment [25, 26].

In this paper, we report the insecticidal activity of three compound of organotin (IV) benzylisopropylthiocarbamate that were dimethyltin (IV) (compound A), dibutyltin(IV) (compound B) and triphenyltin(IV) benzylisopropylthio-carbamate (compound C) against Ae. aegypti mosquito in laboratory.

**MATERIALS AND METHODS**

**Materials:** Organotin(IV) benzylisopropylthiocarbamate compounds (A-C).

**Larvae and Adults Mosquito:** The Aedes aegypti mosquito larvae and adult mosquitoes were obtained from the colonies that had been reared continuously for generations in a laboratory free of exposure to pathogens and insecticides. They were maintained at 25-30°C and 80-90% relative humidity under a photoperiod of 12:12 h (light/dark) in t the Insectarium of the Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia. The larvae were fed with ground beef liver that have been dried and grind after reaching the 1st instar. The dechlorinated water that contains the larvae and the beef liver must be changed regularly to ensure the water condition is always clean and clear. The adults were reared in cages and was provided with 10% sucrose added with multivitamins.

Female mosquitoes were periodically blood-fed on restrained guinea pigs to obtain protein used principally for egg production. Under these conditions, the full development from egg to adult lasted about 3-4 weeks. Batches of 2-5 day-old healthy female mosquitoes were used in the adulticidal bioassay.

**Preparation of the Organotin(iv) Benzylisopropylthiocarbamate Stock Solutions:** Stock solutions of the organotin(IV) benzylisopropylthiocarbamate were prepared in either 95% ethanol, dimethyl sulfoxide (DMSO) or acetone depending on the solubility of the compound at concentration 100 parts per million (ppm). The dissolution of the organotin(IV) benzylisopropylthiocarbamate in water was to facilitate the dispersion of the compounds in water. The acetone and DMSO was spectrograde quality while the 95% ethanol was reagent grade.

**Larvicidal Bioassay Testing:** The larvicidal bioassay testing was followed the method from Basu Baul et al. (2010) [23] with slight modification. This testing was performed in 30 ml disposable cup using ten larvae of Aedes aegypti in the 3rd instar stage. Solution of compounds 1-3 were added to 15 ml of distilled water that had been prepared in disposable cup. The Aedes aegypti larvae were then introduced into the solution and distilled water to give the desired concentration of solution. The total assay volume in each case was 20 ml. Solution containing distilled water and DMSO, but without the organotin(IV) solution, served as a negative control and temephos was used as a positive control. Mortalities were recorded at 24 h of exposure. The moribund and dead larvae in three replicates were combined and expresses as a percentage mortality of each concentration. The larvae were considered dead if they showed no sign of swimming movements even after probing with a needle.

**Adulticidal Bioassay Testing:** Further testing on the adulticidal activity of the most effective compound in the larvicidal bioassay testing was conducted on adults of Aedes aegypti female. The adulticidal bioassay testing was performed by topical application of the compound, following slightly modified versions of the WHO standard protocols - [27]. Adulticidal activity of the compound was evaluated at four concentrations and 25 females were used for each concentration of the most effective compound. Non-blood-fed females’ mosquitoes (2-5 days old) were briefly anesthetized with extreme...
temperature for 30 seconds and placed on a cold plate. The selected compound solution (1µl) dissolved in DMSO was applied onto the upper part of the immobilized mosquito's pronotum using multipette plus (Eppendorf Research, Jerman model no. 4981950). Solution containing distilled water and DMSO, but without the compound solution, served as a negative control and malathion was used as a positive control for comparison. Both groups were treated in a similar manner to that treated groups. After each test, females were transferred into disposable cups and 10% sucrose solutions with added multivitamin on cotton wools were provided. After application, the females in all groups were maintained at 27±3°C and 80% ± 10% RH in plastic cups. At the end of a 24-hour recovery period, the mosquitoes were considered dead if they showed no sign of movement such as lying on the bottom of the plastic cup and not responding to mechanical stimulation. Three replicates were carried out with mosquitoes from different rearing batches and the results were pooled.

**Statistical Analysis of Data:** Tests with more than 20% control mortality were discarded and the repeated. However, if the mortality of control were between 5-20%, the observed percentage mortality was corrected by Abbot’s formula [28].

\[
\% \text{ mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

LC50 and LC90, with their 95% confidence limits of the compound was determined using computerized Log probit analysis test [29].

**RESULTS AND DISCUSSION**

**Larvicidal Bioassay Testing:** The LC50 and LC90 values expressed in parts per million and their 95% confidence limit for compounds A-C screened against the third instar larvae of *Ae. aegypti* mosquitoes are given in Table 1. As can be seen from Table 1, compounds A, B and C had the good larvicidal activity on third instar larvae of *Ae. aegypti* mosquitoes because all LC50 and LC90 values for these three compounds were less than 1 ppm. The data showed that there were differences in larvicidal effect of compounds which compound B displayed the best larvicidal effect with the LC50 and LC90 values of 0.004 ppm and 0.007 ppm, respectively. Compound C showed good larvicidal effect with the LC50 and LC90 values of 0.029 ppm and 0.108 ppm, respectively while the least larvicidal effect showed by compound A with the LC50 and LC90 values of 0.404 ppm and 0.749 ppm, respectively. From the results, the order of larvicidal activity based on the organic group attached to the tin atom can be observed which compound that attached with dibutyl group (compound B) was the most effective larvicide, followed by compound that attached with triphenyl group (compound C) and compound that attached with dimethyl group (compound A) to the tin atom. However, when compared to the LC50 and LC90 values of temephos which was gold standard larvicidal testing, only compound B could equal the effectiveness of temephos as a larvicide against *Ae. aegypti* mosquito larvae while the effectiveness of compound A and C as larvicide against *Ae. aegypti* mosquito larvae is less than temephos.

When compared with other larvicidal activity studies of organotin(IV) compounds, these compounds which used in this study were among organotin(IV) compounds that had good larvicidal effect (reference). The range of LC50 values of triorganotin 2-(p-chlorophenyl)-3-methylbutyrate was 0.32 - 3.13 ppm [21]. This value indicated that compound B and C were more effective as larvicide against *Ae. aegypti* mosquito larvae than these compounds. For triphenyltin *para*-substituted benzoate and tricyclohexyltin *para*-substituted benzoate compound, the average of the LC50 values were 0.62 ppm and 1.16 ppm, respectively [30]. Based on the LC50 values, compound A-C had more potential to be explored as larvicide on *Ae. aegypti* mosquito larvae compared to triphenyltin and tricyclohexyltin *para*-substituted benzoate compounds. Larvicidal effects of compounds A-C on *Ae. aegypti* mosquito larvae also was better than triorganotin (IV) complex compound because compound A-C have higher LC50 values [22]. Organic group and ligand that attached to tin atom were the main factor that influence biological activity of organotin(IV) [10, 18, 24].

| Compound | LC50 (ppm) (Confidence interval 95%) | LC90 (ppm) (Confidence interval 95%) | Gradient |
|----------|--------------------------------------|--------------------------------------|----------|
| A        | 0.404 (0.377-0.438)                  | 0.749 (0.671-0.867)                  | 4.779±8.473 |
| B        | 0.004 (0.004-0.005)                  | 0.007 (0.007-0.009)                  | 5.822±0.580 |
| C        | 0.029 (0.024-0.034)                  | 0.108 (0.086-0.147)                  | 2.240±0.207 |
| Temephos | 0.011 (0.010-0.013)                  | 0.026 (0.021-0.036)                  | 3.483±1.180 |
Table 2: Lethal concentration of compound B and malathion against adults of *Ae. aegypti* mosquito after 24 hours exposure

| Compound   | LC₉₀ (ppm) (Confidence interval 95%) | LC₅₀ (ppm) (Confidence interval 95%) | Gradient      |
|------------|-------------------------------------|-------------------------------------|---------------|
| 2          | 4.277 (3.221-6.377)                 | 27.653 (15.318-72.478)             | 1.581±0.204   |
| Malathion  | 4.368 (3.313-6.597)                 | 38.310 (19.408-124.021)            | 1.359±0.191   |

However, further study should be conducted to clarify the mechanism of toxicity of compound A-C to the *Ae. aegypti* mosquito larvae.

**Adulcicidal Bioassay Testing:** From the larvicidal bioassay testing, it was showed that compound B performed the best larvicidal activity against third instar *Ae. aegypti* mosquito larvae compared to compound A and C. Therefore, further study on compound B on adults of *Ae. aegypti* mosquito was conducted to investigate its adulcicidal property by WHO topical application method. The purpose of this method was to determine the intrinsic activity of this compound to a target species to isolate toxicity of from confounding effects resulting from insect behavior [27]. The result of adulcicidal bioassay testing was summarized in Table 2. The result showed compound B also had good adulcicidal effect against adults of *Ae. aegypti* mosquito with LC₉₀ and LC₅₀ values of 4.277 ppm and 27.653 ppm, respectively. Remarkably, the effectiveness of the compound B was comparable to the effectiveness of malathion which was gold standard for mosquito adulticide.

The advantages of organotin(IV) compound are it will biodegrade into a non-toxic inorganic compound in environment and there is no reported resistance of this compound towards *Ae. aegypti*. With these advantages, this organotin(IV) compounds has potentials to be explored as insecticide in the effort to control the widespread of dengue.

**CONCLUSION**

In conclusion, compound B is the most effective compound among three organotin(IV) compound tested against *Ae. aegypti*. The larvicidal effect of the compound towards *Ae. aegypti* mosquito larvae is superior than temephos while its adulcicidal effect towards adults of *Ae. aegypti* mosquito was comparable with malathion. Further study of this compound should be conducted in order to explore its potential as an insecticide to control the dengue vectors in addition to control the spread of dengue.

**ACKNOWLEDGEMENT**

We would like to thank the Malaysia of Higher Education (MOHE) for the financial support of the FRGS grant (Project code: UKM-NN-06-FRGS0003-2007). Technical support from staffs of Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia is gratefully acknowledged.

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