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

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Abstract: Problem statement: The widespread use of insecticides had resulted in insecticide resistance of vectors of dengue as well as polluting the environment. Organotin (IV) compounds had the potential to be developed as the insecticides to overcome the existing problem. **Approach:** The aim of this study was to examine the insecticidal effects which were 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. **Results:** The study was found that compound B showed the best larvicidal effect with the LC_{50} and LC_{90} values of 0.004 ppm and 0.007 ppm, respectively. Compound C was also displayed good larvicidal effect with the LC_{50} and LC_{90} values of 0.029 ppm and 0.108 ppm, respectively. While, compound A was shown the least larvicidal effect with the LC_{50} and LC_{90} values of 0.404 ppm and 0.749 ppm, respectively. Further testing was conducted on compound B on adults of *Aedes ageypti* female to investigate its adulticidal property. The result showed that compound B displayed good adulticidal activity with LC_{50} dan LC_{90} of 4.277 ppm and 27.653 ppm, respectively. **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: Dithiocarbamates, benzylisopropyldithiocarbamate, adulticidal activity, *aedes aegypti*, organotin (IV), mosquito, larvicidal activity, larvae,

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 (WHO, 2007). Dengue fever is caused by dengue virus. There are four dengue viruses stereotype known as DEN-1, DEN-2, DEN-3 and DEN-4 (WHO, 1999). *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 (Rohani *et al*., 1997).

Until now no vaccine is completely appropriate to be given to society for the prevention of further spread of dengue fever (Chapagain *et al*., 2008). Thus, vector control is the most effective method of controlling the spread of the disease (Zaim and Guillet, 2002) 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 (Choocote *et al*., 2006).

Unfortunately, the uncontrolled use of insecticide has resulted in problems of insecticide resistance (Somboon *et al*., 2003). 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 (Dua *et al*., 2010) as well as negative effects on non-target organisms, including

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human (Yang *et al*., 2002). 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 (Boraiko and Batt, 2005) and its potential in biological activity has attracted researchers to conduct studies on this compound (Wiecek *et al*., 2010). Among the biological activities of organotin (IV) compound have these compounds identified as a potential antiviral agent (Sreeja and Kurup, 2005), antineoplastic agents, antituberculosis (Gielen, 2002; Dokorou *et al*., 2004; Kovala-Demertzi *et al*., 2005; Kovala-Demertzi, 2006), antibacterial agent (Jamil *et al*., 2009), anticancer agent (Awang *et al*., 2010) and so on.

Use of organotin (IV) compounds in the field of entomology is also now getting attention (Shukla *et al*., 2010). Several studies showed organotin (IV) compounds were effective against some pests like periplanata Americana, musca domestica, Spodoptera litura and Tetranychus urticae (Shukla *et al*., 2010) and a few species of larvae and adult mosquitoes like *Aedes Aegypti* and *Aenopheles stephensi* (Eng *et al*., 2003; 2007; Baul *et al*., 2005; 2010). Group and the nature of organic group that bound to tin atom, such as the monoalkyl, dialkyl and trphenyl are the basic factors determining the biological activity (Boraiko and Batt, 2005; Awang *et al*.,2010; Pellerito *et al*., 2006).

Until now there has been reports of *Ae. aegypti* resistance to organotin (IV) compound and reported that this compound will be degraded to non-toxic inorganic compound in the environment (Blunden and Chapman,1986; Buck-Koehntop *et al*., 2006).

In this study, we report the insecticidal activity of three compounds of organotin (IV) bezylisopropyldithiocarbamate that were dimethyltin (IV) (compound A), dibutyltin (IV) (compound B) and triphenyltin (IV) benzylisopropyldithiocarbamate (compound C) against *Ae. aegypti* mosquito in laboratory.

MATERIALS AND METHODS

Materials: Organotin (IV) benzylisopropyl-dithiocarbamate compounds (A-C).

Larvae and adult 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 the Insectarium of the Program in Biomedical Science, Faculty of Health Sciences, Universiti Kebangsaan Malaysia. The larvae were fed with a 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 were provided with 10% sucrose added with multivitamins. Female mosquitoes were periodically blood-fed to 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) benzyl-isopropyldithiocarbamate stock solutions: Stock solutions of the organotin(IV) benzylisopropyldithiocarbamate 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) benzylisopropyldithiocarbamate in the organic media was to facilitate the dispersion of the compounds in water. The acetone and DMSO were spectrograde quality while the 95% ethanol was reagent grade.

Larvicidal bioassay testing: The larvicidal bioassay testing was followed the method from Baul *et al*. (2010) with slight modification. This testing was performed in 30 ml disposable cups using ten larvae of *Ae. aegypti* in the 3rd instar stage. Solution of compounds 1-3 were added to 15 ml of distilled water that had been prepared in a disposable cup. The *Ae. aegypti* larvae were then transferred into the solution and distilled water to give the desired concentration of solution. The total assay volume in each case was 20 mL. A 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 *Ae. aegypti* female. The adulticidal bioassay testing was performed by topical application of the compound, following slightly modified versions of the WHO standard protocols (WHO, 2006). The 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 female mosquitoes (2-5 days old) were briefly anesthetized with extreme temperature for 30 seconds and placed on a cold plate. The selected compound solution (1 µL) dissolved in DMSO was applied onto the upper part of the immobilized mosquito's pronotum using multipette plus (Eppendorf Research, Jerman model no. 4981950). A 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 group. 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-h 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 was between 5-20%, the observed percentage mortality was corrected by Abbot's formula (Abbott, 1925).

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

LC_{50} and LC_{90} with their 95% confidence limits of the compound was determined using computerized Log probit analysis test (Raymond, 1985).

RESULTS

Larvicidal bioassay testing: The LC_{50} and LC_{90} 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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values of 0.004 and 0.007 ppm, respectively.

Table 1: Lethal concentration of compound A-C and temephos against third instar *Ae. aegypti* mosquito larvae after 24 h exposure

Compound	LC_{50} (ppm) (Confidence interval 95%)	LC_{90} (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 h exposure

Compound	LC_{50} (ppm) interval 95%) (Confidence	LC_{90} (ppm) (Confidence interval 95%)	Gradient
B	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

Compound C showed a good larvicidal effect with the LC_{50} and LC_{90} values of 0.029 ppm and 0.108 ppm, respectively while the least larvicidal effect showed by compound A with the LC_{50} and LC_{90} values of 0.404 and 0.749 ppm, respectively.

Adulticidal bioassay testing: The result of adulticidal bioassay testing was summarized in Table 2. The result showed compound B also had good adulticidal effective against adults of *Ae. aegypti* mosquito with LC_{50} and LC_{90} values of 4.277 and 27.653 ppm, respectively.

DISCUSSION

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 to a dibutyl group (compound B) was the most effective larvicide, followed by a compound that attached with triphenyl group (compound C) and compound that attached to a dimethyl group (compound A) to the tin atom. However, when compared to the LC_{50} and LC_{90} values of temephos which was the 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 a good larvicidal effect. The range

of LC_{50} values of triorganotin 2-(*p*-chlorophenyl)-3-metylbutyrate was 0.32-3.13 ppm (Eng *et al*., 2007). 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 compound and tricyclohexyltin para-substituted benzoate compound, the average of the LC_{50} values were 0.62 and 1.16 ppm, respectively (Duong *et al*., 2006). Based on the LC_{50} 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 had also been better than triorganotin (IV) complex compound because compound A-C have higher LC_{50} values (Baul *et al*., 2005). Organic group and ligand that attached to the tin atom were the main factor that influence the biological activity of organotin (IV) (Boraiko and Batt, 2005; Awang *et al*., 2010; Pellerito *et al*., 2006). However, further study should be conducted to clarify the mechanism of toxicity of compound A-C to the *Ae. aegypti* mosquito larvae.

From the larvicidal bioassay testing, it was shown that compound B performed the best larvicidal activity against third instar *A.e 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 adulticidal property by a WHO topical application method. The purpose of this method was to determine the intrinsic activity of this compound of a target species to isolate toxicity of from the confounding effects resulting from insect behavior (WHO, 2006). Remarkably, the effectiveness of the compound B was comparable to the effectiveness of malathion which was the gold standard for mosquito adulticide.

The advantages of organotin (IV) compound are it will biodegrade into a non-toxic inorganic compound in the environment and there is no reported resistance of this compound towards *Ae. aegypti.* With these advantages, these organotin (IV) compounds have potential to be explored as an 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 to temephos while its adulticidal 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.

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