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Research Article

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The effectiveness of bismuth(III) dithiocarbamate compounds as a larvicide against *Aedes aegypti* (Linn.) (Diptera: Culicidae) in laboratory

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ABSTRACT

The uncontrolled use of insecticides has caused major problems including resistance of mosquito towards the insecticides, environmental pollution, and effects on non-target organisms. Therefore, the potential of bismuth(III) dithiocarbamate compounds to be developed as insecticides to overcome these problem was explored in this study. The aim of this study was to examine the larvicidal effects of bismuth(III) dithiocarbamate compounds against Aedes aegypti (Linn.). The larvicidal bioassay test of a series of three bismuth(III) dithiocarbamate compounds was carried out on third instar larvae of A. aegypti in the laboratory. Bismuth(III) chloro-ethylaminoethanoldithiocarbamate showed the highest larvicidal effect with the LC_{50} and LC_{90} values of 7.53 ppm and 14.43 ppm, respectively. Bismuth(III) chloro-metylcyclohexyldithiocarbamate also displayed a good larvicidal effect with the LC_{50} and LC_{90} values of 14.29 ppm and 23.11 ppm, respectively. On the other hand, bismuth(III) chloro-butylmethyldithiocarbamate showed the lowest larvicidal effect with the LC_{50} and LC_{90} values of 28.30 ppm and 49.75 ppm, respectively. Morphological changes on the larvae were observed and recorded during the larvicidal bioassay test, for instances destructed digestive tract, shortened length, and elongated neck region. The order of larvicidal activity was based on the group attached to the dithiocarbamate compounds. Bismuth(III) chloro-ethylaminoethanoldithiocarbamate is the most effective compound among the seven bismuth(III) dithiocarbamate compounds tested against the dengue vector A. aegypti and has potential to be explored as a larvicide to control the spread of dengue fever.

Keywords: Dithiocarbamates, *Aedes aegypti*, bismuth(III), larvicidal activity, larvae

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INTRODUCTION

Mosquito is a vector in many diseases including dengue [14]. Dengue cases have been recorded in Malaysia since 1902 [10]. Dengue is transmitted through a mosquito that has been infected with any one of the serotypes of dengue virus, which are DEN-1, DEN-2, DEN-3, and DEN-4 [17]. Until now, there is still no vaccine or a specific drug to treat dengue [5]. In the absence of a vaccine, therapeutic treatment or cure, the best protection against dengue fever is thus to control the mosquito vector, *Aedes aegypti* [21].

Vector control is an important step to reduce morbidity caused by dengue [19]. There are several strategies used in vector control, which include chemical control, environmental control, biological control, and education [8]. Until now, chemical control remains an important control method in controlling the vector population [18] as insecticides can provide rapid and maximum impact. Effective chemical insecticides are effective in reducing the population of *A. aegypti* and dengue incidence [6].

Due to these problems, scientists are trying to find new alternatives to produce insecticides that are more environmentally friendly and at the same time prevent problems including resistance in vector and interference on non-target organisms. There are various alternative sources for synthesising new insecticides, and among them are bismuth(III) dithiocarbamate compounds. Bismuth compounds are less toxic than other heavy metals such as antimony and lead. Bismuth compounds also have low solubility in blood, are eliminated through urine, and have no carcinogenic, mutagenic, and teratogenic effects in long-term tests on animals [9]. Besides that, bismuth salicylate has been found to inhibit the growth of a range of bacteria and yeast [15].

In this study, the larvicidal activity of the newly synthesised bismuth(III) dithiocarbamate compounds namely bismuth(III) chloro-methylcyclohexyldithiocarbamate, bismuth(III) chloro-etylaminoethanoldithiocarbamate, and bismuth(III) chloro-butylmethyldithiocarbamate was evaluated.

EXPERIMENTAL SECTION

Test compounds: Bismuth(III) dithiocarbamate compounds namely bismuth(III) chloro-methylcyclohexyldithiocarbamate, bismuth(III) chloro-etylaminoethanoldithiocarbamate, and bismuth(III) chloro-butylmethyldithiocarbamate.

Larvae: The *A. aegypti* mosquito larvae were obtained from the colonies that were reared continuously for generations in a laboratory free of exposure to pathogens and insecticides. The larvae were maintained at 25–30°C and 80–90% relative humidity under a photoperiod of 12:12h(light/dark) in the Insectarium of the Biomedical Science Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur. The larvae were fed with a beef liver that was dried and ground after reaching the first instar. The dechlorinated water containing the larvae and the beef liver was changed regularly to ensure clean water. The adults were reared in cages and were provided with 10% of sucrose solution added with vitamin B. Female mosquitoes were periodically blood-fed to guinea pig used principally for egg production. Under these conditions, the full development from egg to adult lasted about 3–4 weeks. Third instar larvae were used in this study.

Preparation stock solution of bismuth(III) dithiocarbamate: Stock solutions of bismuth(III) dithiocarbamate compounds were prepared in 1 mL of dimethyl sulphoxide (DMSO) at a concentration of 10,000 parts per million (ppm). The dissolution of the bismuth(III) dithiocarbamate compounds in the organic media was to facilitate the dispersion of the compounds in water.

Larvicidal bioassay testing: The larvicidal bioassay testing was done following the method from WHO (1981) with a slight modification [16]. This testing was performed in 12 oz. of disposable cups using ten larvae of *A. aegypti* in the third instar stage. Solution of tested compounds was added to 90mL of distilled water that was prepared in a disposable cup. The *A. 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 100mL. A solution containing distilled water and DMSO, but without the bismuth(III) dithiocarbamate compounds, 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 six replicates were combined and expressed as a percentage mortality of each concentration. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface.

Statistical analysis of data: If there were larvae that turned into pupae during the larvicidal bioassay testing, the test was discarded and repeated. However, if the mortality of the larvae was 5–20%, the observed percentage mortality was corrected by Abbot's formula [1]. The following is the formula used in the Abbott's correction.

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

LC_{50} and LC_{90} with 95% confidence limits of the compound were determined using computerised log-probit analysis test [13].

Observation of morphological changes of *A. aegypti* larvae: During the course of lethal experiments, the morphological features of larvae from treated and control media were compared after 48 h of exposure. Any notable difference in appearance between treated and control media was recorded as anomaly. Morphology of larvae was observed under 32× magnification using dissecting microscope.

RESULTS AND DISCUSSION

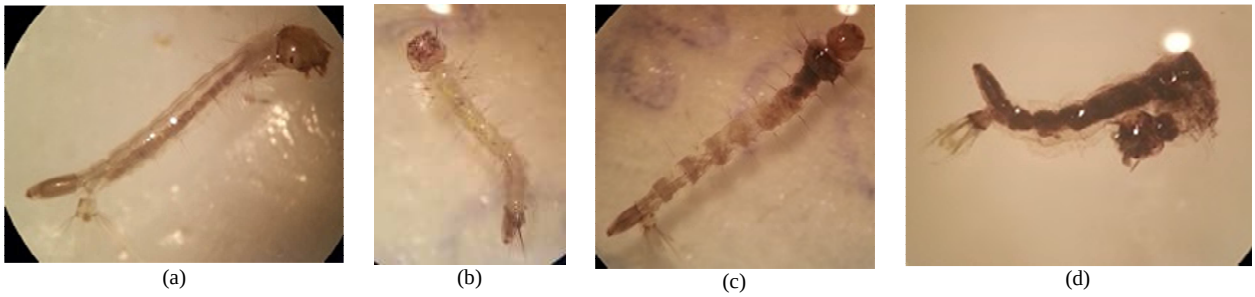
Larvicidal bioassay testing: Table 1 shows the LC_{50} and LC_{90} values expressed in ppm and the 95% confidence limit for bismuth(III) dithiocarbamate compounds screened against the third instar larvae of *A. aegypti.*

Table 1: Lethal concentration of the first three compounds in the series of bismuth(III) dithiocarbamate and temephos against third instar *A. aegypti* mosquito larvae after 24 h of exposure

Compound	LC_{50} (ppm) (95% confidence interval)	LC_{90} (ppm) (95% confidence interval)	Gradient
Bismuth(III) chloro-metylcyclohexyldithiocarbamate	14.29 (13.44–15.17)	23.11 (20.93–26.73)	6.14±0.68
Bismuth(III) chloro-etylaminoethanoldithiocarbamate	7.53 (6.99–8.14)	14.43 (12.67–17.28)	4.54±0.42
Bismuth(III) chloro-butylmethyldithiocarbamate	28.30 (26.40–30.27)	49.75 (44.85–57.30)	5.23±0.49
Temephos	0.01 (0.01–0.01)	0.03 (0.02–0.05)	2.82±0.41

Table 2: Morphological changes of larvae of *Aedes aegypti* caused by bismuth(III) dithiocarbamate compounds after 48 h of exposure

Compound	Morphology Changes
Bismuth(III) chloro-methylcyclohexyldithiocarbamate	Destruction of digestive tract
Bismuth(III) chloro-etylaminoethanoldithiocarbamate Bismuth(III) chloro-butylmethyldithiocarbamate	Deposition of compound Blackened larvae



(a) (b) (c) (d)

Fig. 1: Morphological changes of larvae of *A. aegypti* caused by bismuth(III) dithiocarbamate compounds after 48 h of exposure under 32× magnification with (a) negative control, (b) destruction of digestive tract, (c) deposition of compound, and (d) blackened larvae

As shown in Table 1, the three compounds in the series of bismuth(III) dithiocarbamate exceeded the LC_{50} and LC_{90} of temephos. Among the three compounds, bismuth(III) chloro-etylaminoethanol was the most effective with LC_{50} and LC_{90} of 7.53 ppm and 14.43 ppm, respectively. Second effective was bismuth(III) chloro-metylcyclohexyldithiocarbamate with LC_{50} of 14.29 ppm and LC_{90} of 23.11 ppm. The least effective compound was bismuth(III) chloro-butylmethyldithiocarbamate, which recorded LC_{50} and LC_{90} of 28.30 ppm and 49.75 ppm, respectively.

Observation of morphological changes of *A. aegypti* larvae: Treatment of third instar larvae with bismuth(III) dithiocarbamate compounds produced various morphogenetic anomalies. As all of the morphogenetic aberrations cannot be described here, only the more common and notable aberrations are shown and described. Table 2 shows the morphological changes recorded by bismuth(III) dithiocarbamate compounds after 48 h of exposure.

As shown in Table 2, all the compounds were deposited in the larvae. Bismuth(III) chloro-metylcyclohexyldithiocarbamate caused destruction of digestive tract. Bismuth(III) chloro-etylaminoethanoldithiocarbamate was deposited in the larvae. Lastly, bismuth(III) chloro-butylmethyldithiocarbamate caused the larvae to become blackened and lost suppleness of the body.
From the results, the larvicidal activity was ordered based on the group attached to the dithiocarbamate compounds. The hydroxyl group attached to the dithiocarbamate compound, i.e., bismuth(III) chloro-etylaminoethanoldithiocarbamate was the most effective larvicide, followed by the compound attached to a cycloalkyl group, i.e., bismuth(III) chloro-metylcyclohexyldithiocarbamate and the compound attached to an alkyl group, i.e., bismuth(III) chloro-butylmethyldithiocarbamate. However, when compared to the LC_{50} and LC_{90} values of temephos, which was the gold standard for larvicidal testing, none of the three compounds could be as effective as temephos as a larvicide against *A. aegypti* mosquito larvae as they exceeded the concentration of temephos within a range of hundreds to thousands fold.

Bismuth(III) chloro-etylaminoethanoldithiocarbamate was found to have the highest larvicidal acitivity due to the presence of hydroxyl group in its structure. Hydroxyl group is associated with an antimicrobial activity [11]. The evaluation of hydroxyl as an antioxidant and antimicrobial has been studied, and the assessment showed that the group can be used for these purposes [3].

The cycloalkyl group in bismuth(III) chloro-metylcyclohexyldithiocarbamate increases its potential as a larvicide. Cyclohexyl complexes have been found to possess potent antioxidant [20] and anticandidal activities [4]. As for bismuth(III) chloro-butylmethyldithiocarbamate, the methyl butyl it contains may act as an antiinflammatory and analgesics, and the group has antipyretic activities [12].

Based on the morphological observation, bismuth(III) chloro-etylaminoethanoldithiocarbamate was deposited in the *A. aegypti* larvae tested. In a study, same morphological anomalies of the mosquito larvae were observed when tested with dibutyltin(IV) ethyl-phenyl dithiocarbamate [2]. Destruction of digestive tract occurred when tested with di-tert-butyltin(IV) ethylphenyl dithiocarbamate and bismuth(III) chloro-methylcyclohexyldithiocarbamate.
In other study, same morphological anomalies of mosquito larvae were observed. For example, larvae tested with an essential oil, *Piper aduncum* showed the same morphological anomalies like the larvae tested with bismuth(III) chloro-butylmethyldithiocarbamate [7]. The treatment resulted in blackened larvae, and the larvae lost suppleness of their body.

Morphological changes in larvae subjected to treatment with bismuth(III) dithiocarbamate compounds are very important to be observed as these observations will probably lead to an explanation of the toxic action of the compounds on mosquito larvae. These changes provide indications of the mechanism of action of bismuth(III) dithiocarbamate compounds in the body of the larvae.

CONCLUSION

In conclusion, bismuth(III) chloro-etylaminoethanoldithiocarbamate is the most effective compound against the dengue vector, *A. aegypti* in the series of bismuth(III) dithiocarbamate compounds and has the potential to be explored as a larvicide. Further study is needed to elucidate and ensure that this compound can be safely used as a larvicide to control dengue vector in addition to control the spread of dengue.

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