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Research Article

The Effectiveness of Dibutyltin (IV) and Di-tert-butyltin (IV) Alkyl-phenyl Dithiocarbamate Compounds as Larvicide against *Aedes aegypti* Linn. (Diptera: Culicidae) in Laboratory

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Abstract: Extensive use of insecticides to control dengue vectors has led to the development of mosquito resistance, environmental pollution and undesirable effects on non target organisms. Thus, the potential of organotin (IV) compounds has been explored to be developed as insecticides to obviate the existing problems. The aim of this study was to examine the larvicidal effect of dibutylt in (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate compounds against *Aedes aegypti* Linn. in laboratory. Larvicidal bioassay test of four compounds dibutyltin (IV) ethyl phenyl dithiocarbamate, di-tert-butyltin (IV) ethyl phenyl dithiocarbamate, dibutyltin (IV) butyl phenyl dithiocarbamate and di-tert-butyltin (IV) butyl phenyl dithiocarbamate against third instar larvae of *A. aegypti* was carried out. The study indicated that dibutyltin (IV) butyl phenyl dithiocarbamate showed the highest larvicidal effect with LC_{50} and LC_{90} values of 0.59 and 3.91 ppm, respectively. However, the other three compounds showed lower larvicidal effects. Dibutyltin (IV) ethyl phenyl dithiocarbamate showed moderate larvicidal effect with LC_{50} and LC_{90} values of 4.45 and 11.08 ppm, respectively, followed by di-tert-butyltin (IV) butyl phenyl dithiocarbamate compound with LC_{50} and LC_{90} values of 5.08 and 11.50 ppm, respectively. Di-tert-butyltin (IV) ethyl phenyl dithiocarbamate compound showed the least larvicidal effect with LC_{50} and LC_{90} values of 8.59 and 36.74 ppm, respectively. Morphological anomalies such as elongated neck region, shortened length and destructed digestive tract in the larvae were observed and recorded during the larvicidal bioassay testing. As a conclusion, dibutyltin (IV) butyl phenyl dithiocarbamate compound is the most effective compound against the dengue vector, *A. aegypti* and has the potential to be explored as a larvicide. Further study is needed to ensure that this compound can be used safely as a larvicide.

Keywords: *Aedes aegypti*, dithiocarbamate, insecticides, larvicidal, organotin (IV)

INTRODUCTION

Dengue fever is ranked by the World Health Organisation (WHO) as the most important mosquito-borne and the most rapidly spreading viral disease in the world with a 30-fold increase in global incidence over the past 50 years (WHO, 2014). Until now, no vaccine is completely appropriate to be given to society for the prevention of further spread of dengue fever (CDC, 2012). Effective vector control measures are important to achieve and sustain reduction of morbidity attributable to dengue (WHO, 2012) and chemical insecticide is a method that is believed to reduce the *Aedes* population (Choocote *et al*., 2006; Awang *et al*., 2012).

According to Saranya *et al*. (2013), the control methods should aim at the weakest link of the life cycle of the mosquito, which is the larval stage. In principle, vector control of mosquitoes can be divided into two stages namely when they are immature and adult (Yap *et al*., 1994). During the immature stage, mosquitoes are relatively immobile and remain more concentrated than they are in the adult stage. Therefore, effective larviciding can reduce the number of adult mosquitoes available to disperse, potentially spread disease, create a nuisance and lays eggs, all of which leads to more mosquitoes.

However, the uncontrolled use of insecticide can reduce the effectiveness of chemical control and result in several problems including resistance of *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes albopictus* mosquitoes against malathion, permethrin and temephos (Hamdan *et al*., 2005), distruption of biological control of non target organisms (Magar and Shaikh, 2013) and environmental pollution such as insecticide residues in the rice paddies (Arjmandi *et al*., 2010).

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Hence, some approaches have been introduced to overcome these problems, including production of novel types of insecticide that prevent insects' resistance and that are environmentally friendly. Organotin (IV) compounds have shown potential in biological activities. These compounds have been identified as potential antiviral agents (Singh *et al*., 2000), anticancer agents (Awang *et al*., 2011; Balas *et al*., 2012), anti-microbe and antifungal (Jamil *et al*., 2009; Rehman *et al*., 2004), anti-tuberculosis (Dokorou *et al*., 2004) and insecticide against several species of larvae and mosquitoes such as *A. aegypti* and *Anopheles stephensi* (Awang *et al*., 2012; Baul *et al*., 2005; Eng *et al*., 2003). Biological activities of organotin (IV) compounds are greatly influenced by their various molecular structures and most of thesecompounds are generally very toxic even at very low concentrations (Pellerito *et al*., 2006).

The major advantage of evaluating organotin (IV) compounds as potential insecticides against mosquitoes is that there have been no reports of resistance of *A. aegypti* to organotin (IV) compounds. Instead, it is reported that these compounds will be degraded to nontoxicinorganic compounds in the environment (Buck-Koehntop *et al*., 2006; Awang *et al*., 2012).

In this study, the larvicidal activity of the newly synthesised organotin (IV) compounds namely dibutyltin (IV) ethyl phenyl dithiocarbamate, di-tert-butyltin (IV) ethyl phenyl dithiocarbamate, dibutyltin (IV) butyl phenyl dithiocarbamate and di-tert-butyltin (IV) butyl phenyl dithiocarbamate against *A. aegypti* mosquito in laboratory was assessed.

MATERIALS AND METHODS

Test compounds: Organotin (IV) compounds namely dibutyltin (IV) ethyl phenyl dithiocarbamate, di-tert-butyltin (IV) ethyl phenyl dithiocarbamate, dibutyltin (IV) butyl phenyl dithiocarbamate and di-tert-butyltin (IV) butyl phenyl dithiocarbamate were synthesised at the School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi.

Larvae: The *A. aegypti* mosquito larvae were obtained from the colonies that had been 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 12:12 h (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 that contained 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 argus used principally for egg production. Under these conditions, the full development from egg to adult lasted about 3-4 weeks. Third instar larvae was used in this study.

Preparation stock solution of dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate: Stock solutions of the dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate compounds were prepared in 1 mL of Dimethyl Sulphoxide (DMSO) at concentration 5000 parts per million (ppm). The dissolution of the dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate in the organic media was to facilitate the dispersion of the compounds in water.

Larvicidal bioassay testing: The larvicidal bioassay testing was based on the method from WHO (1981) with slight modification. This testing was performed in 12 oz disposable cups using ten larvae of *A. aegypti* in the third instar stage. Solution of tested compounds was added to 90 mL of distilled water that was prepared in a disposable cup. The *A. aegypti* larvae were then transferred into the solution and distilled water was added to give the desired concentration of solution. The total assay volume in each case was 100 mL. A solution containing distilled water and DMSO but without the organotin (IV) solution that 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 percentage mortality for 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: Tests with more than 20% control mortality were discarded and repeated. However, if the mortality of the control was between 5-20%, the observed percentage of mortality was corrected by Abbot's formula (Abbott, 1925) as follows:

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

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

Observation of morphological anomalies 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 exposure. Any notable difference in appearance between treated and control was recorded as anomaly. Morphology of larvae was observed under 32× magnification using dissecting microscope.

RESULTS

Larvicidal bioassay testing: Table 1 shows the LC_{50} and LC_{90} values expressed in parts per million and their

Table 1: Lethal concentration of dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate and temephos against third instar *A. aegypti* mosquito larvae after 24 h exposure

Compound	LC_{50} (ppm) (95% confidence interval)	LC_{90} (ppm) (95% confidence interval)	Gradient
DB EF	4.45 (3.82-5.05)	11.08 (9.19-14.77)	3.23±0.42
DB BF	0.59 (0.44-0.77)	3.91 (2.63-7.04)	1.56±0.18
DtB EF	8.59 (6.58-10.47)	36.74 (26.71-64.42)	2.03±0.32
DtB EF	5.08 (4.48-5.66)	11.50 (9.54-15.70)	3.61±0.51
Temephos	0.01 (0.01-0.01)	0.03 (0.02-0.05)	2.82±0.41

DB EF: Dibutyltin (IV) ethyl-phenyl dithiocarbamate; DB BF: Dibutyltin (IV) butyl phenyl dithiocarbamate; DtB EF: Di-tert-butyltin (IV) ethyl-phenyl dithiocarbamate; DtB BF: Di-tert-butyltin (IV) butyl phenyl dithiocarbamat

Table 2: Classification of morphological anomalies of larvae of *A. aegypti* caused by dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate compounds after 48 h exposure

	Compound			
Morphological anomalies category	DB EF	DB BF	DtB EF	DtB BF
Shortened length	Yes	Yes	Yes	Yes
Deposition of compound in digestive tract	Yes	Yes	Yes	Yes
Loose suppleness of body	No	No	Yes	Yes
Destructed digestive tract	Yes	No	Yes	No
Larvae became blackened	Yes	No	Yes	No
Shortened length and twisted abdomen	No	No	No	Yes
Elongated neck region	No	Yes	No	No

DB EF: Dibutyltin (IV) ethyl-phenyl dithiocarbamate; DB BF: Dibutyltin (IV) butyl phenyl dithiocarbamate; DtB EF: Di-tert-butyltin (IV) ethyl-phenyl dithiocarbamate; DtB BF: Di-tert-butyltin (IV) butyl phenyl dithiocarbamate

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

Fig. 1: Morphological anomalies of larvae of *A. aegypti* caused by dibutyltin (IV) butyl phenyl dithiocarbamate compound after 48 h exposure under 32× magnification (a) negative control (b) shortened length of larvae (c) deposition of compound in the digestive tract of larvae (d) elongated neck region with deposition of compound in digestive tract of larvae (e) elongated neck region with heavy deposition of compound in digestive tract of larvae

95% confidence limit for dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate compounds screened against the third instar larvae of *A. aegypti*.

Based on Table 1 only dibutyltin (IV) butyl phenyl dithiocarbamate showed good larvicidal activity on third in star larvae of *A. aegypti* mosquito because its LC_{50} values for this compound were less than 1 ppm. The data show that there were differences in larvicidal effect among the tested compounds. Dibutyltin (IV) butyl phenyl dithiocarbamate displayed the best larvicidal effect with LC_{50} and LC_{90} values of 0.59 and 3.91 ppm, respectively.

The other three compounds showed less effective larvicidal activity. Dibutyltin (IV) ethyl-phenyl dithiocarbamate showed LC_{50} and LC_{90} values of 4.45 and 11.08 ppm, respectively. Next, di-tert-butyltin (IV) butyl phenyl dithiocarbamate showed LC_{50} and LC_{90} values of 5.08 and 11.50 ppm, respectively. Lastly, di-tert-butyltin (IV) ethyl-phenyl dithiocarbamate showed the least effective larvicidal activity with LC_{50} and LC_{90} values of 8.59 and 36.74 ppm, respectively.

Observation of morphological anomalies of *A. aegypti* larvae: Treatment of third instar larvae with dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl 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 classification of morphological anomalies of *A. aegypti* larvae caused by dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate after 48 h exposure.

As shown in Table 2, all the tested compounds shortened the length of larvae and deposition of compound at digestive tract of *A. aegypti* larvae. Di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate caused

loose suppleness of larvae body, while di-tert-butyltin (IV) butyl phenyl dithiocarbamate compound shortened the length and twisted the larvae's abdomen. Other than that, dibutyltin (IV) and di-tert-butyltin (IV) ethyl phenyl dithiocarbamate caused the larvae to became blackened and destructed the indigestive tract. Apart from that, only dibutyltin (IV) butyl phenyl dithiocarbamate compound caused elongated neck region of the *A. aegypti* larvae (Fig. 1).

DISCUSSION

It is well documented that the organotin (IV) compounds display strong biocidal activities (Duong *et al*., 2006). Organic group and ligand attached to the tin atom are the main factors that influence the biological activity of organotin (IV) (Awang *et al*., 2012). According to Jenkins *et al*. (2004), organotin (IV) toxicity is directly related to the number and nature of organic moiety, in which highly substituted organotin compounds are more toxic than other neurotoxicants. However, alkyl chain reduces the toxicity of these compounds.

This study found that dibutyltin (IV) butyl phenyl dithiocarbamate showed the highest larvicidal effect among the four tested compounds. Dibutyl group showed the most effective larvicidal activity, while di-tert-butyl group showed lesser activity, thus less effective. However, the effectiveness of all compounds as larvicide against *A. aegypti* mosquito larvae was lower than temephos, which is the gold standard of larvicidal testing.

Other than that, organotin (IV) with ligand butyl phenyl dithiocarbamate is more toxic compared to ethyl phenyl dithiocarbamate. The increased alkyl chain length of organic group may play a role in the toxicity of the dithiocarbamates as larger ligand may dissociate more easily to give action towards the *A. Aegypti* larvae (Eng *et al*., 2003).

When compared with larvicidal activities of other organotin (IV) compounds, the compounds used in this study were among those that had better larvicidal effect. For example, as found by Rosalina (2012), the LC_{50} values of diphenyltin (IV) ethyl phenyl dithiocarbamate and diphenyltin (IV) butyl phenyl dithiocarbamate are 8.89 and 18.78 ppm, respectively. These values are lower than the LC_{50} values of dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate tested in this present study.

For triphenyltin and tricyclohexyltin para-substituted benzoate, their average LC_{50} values are 0.62 and 1.16 ppm, respectively (Duong *et al*., 2006). In comparison with the compounds in this present study, dibutyltin (IV) butyl phenyl dithiocarbamate had more potential to be explored as a larvicide on *A. aegypti* mosquito larvae compared to triphenyltin and tricyclohexyltin para-substituted benzoate.

Based on the morphological observation, larvae of *A. aegypti* tested with dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate became shortened and blackened and showed elongation at the neck region, destruction of digestive tract and deposition of compound in digestive tract.

There were several other biocide compounds that caused the same morphological anomalies of mosquito larvae, such as dibutyltin (IV) and di-tert-butyltin (IV) alkyl-phenyl dithiocarbamate. For example, *A. aegypti* larvae tested with aqueous leaf extract of *Spathodea campanulata* showed the same morphological anomalies as the larvae tested with dibutyltin (IV) and di-tert-butyltin (IV) ethyl phenyl dithiocarbamate. The treatment results in the destruction of digestive tract of larvae (Saranya *et al*., 2013). Besides, *A. aegypti* larvae tested with an extract of *Annona coriacea* fruits also showed the same morphological change (Costa *et al*., 2012).

Other than that, *Culex quinquefasciatus* larvae tested with *Bacillus thuringiensis* ser. H-14 also became shortened, partially black and elongated at the neck region (Mulla and Singh, 1991).

Morphological changes in larvae subjected to treatments with organotin (IV) compounds are very important because these observations will probably lead to a full explanation of the toxic action of compounds on larvae of mosquito. These changes provide indications of the way of action of organotin (IV) compounds in the body of the larvae.

CONCLUSION

In conclusion, dibutyltin (IV) butyl phenyl dithiocarbamate compound is the most effective compound against the dengue vector *A. aegypti* and has the potential to be explored as a larvicide. However, 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 controlling the spread of dengue.

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REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-266.

Arjmandi, R., M. Tavakol and M. Shayeghi, 2010. Determination of diazinon insecticide residues in rice paddies. Int. J. Environ. Sci. Technol., 12(2): 19-28.

Awang, N., N.F. Kamaludin and A.R. Ghazali, 2011. Cytotoxic effect of organotin (IV) benzylisopropyldithiocarbamate compounds on Chang liver cell and hepatocarcinoma HepG2 cell. Pak. J. Biol. Sci., 14(15): 768-774.

Awang, N., N.A. Kosnon, H. Othman and N.F. Kamaludin, 2012. The effectiveness of organotin (IV) benzylisopropyldithiocarbamate compounds as insecticide against *Aedes aegypti* Linn (Diptera: Culicidae) in laboratory. Am. J. Appl. Sci., 9(8): 1214-1218.

Balas, V.I., N.C. Banti, N. Kourkoumelis, S.K. Hadjikakou, G.D. Geromichalos, D. Sahpazidou, L. Male, M.B. Hursthouse, B. Bednarz, M. Kubicki, K. Charalabopoulos and N. Hadjiliadis, 2012. Structural and *in vitro* biological studies of organotin(IV) precursors; selective inhibitory activity against human breast cancer cells, positive to estrogen receptors. Aust. J. Chem., 65: 1625-1637.

Baul, B.T.S., K.S. Singh, M. Holcapek, R. Jirasko, A. Linden, X. Song, A. Zapata and G. Eng, 2005. Electrospray ionization mass spectrometry of tributyltin(IV) complexes and their larvicidal activity on mosquito larvae: Crystal and molecular structure of polymeric (Bu3Sn[O2CC6H4{NN (C6H3-4-OH(C(H) N C6H4OCH3-4))}-o])$_n$. Appl. Organomet. Chem., 19: 935-944.

Buck-Koehntop, B.A., F. Porcelli, J. Lewin, C.J. Cramer and G. Veglia, 2006. Biological chemistry of organotin compounds: Interactions and dealkylation by dithiols. J. Organomet. Chem., 691: 1748-1755.

CDC, 2012. Dengue. Centre for Dieses Control and Prevention, Atlanta.

Choocote, W., U. Chaitong, K. Kamsuk, E. Rayyanachanpichal, A. Jitpakdi *et al*., 2006. Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three *Piper spp*. Rev. Inst. Med. Trop. Sao Paulo., 48: 33-37.

Costa, M.S., D.O. Pinheiro, J.E. Serrão and M.J.B. Pereira, 2012. Morphological changes in the midgut of *Aedes aegypti* L. (Diptera: Culicidae) larvae following exposure to an *Annona coriacea* (Magnoliales: Annonaceae) extract. Neotrop. Entomol., 41: 311-314.

Dokorou, V., D. Koyala-Demertzi, J.P. Jasinki, A. Galani *et al*., 2004. Synthesis, spectroscopic studies, and crystal structures of phenylorganotin derivatives with [Bis(2,6-dimethylphenyl) amino]benzoic acid: Novel antituberculosis agents. Helv. Chim. Acta, 87: 1940-1950.

Duong, Q., X. Song, E. Mitrojorgji, S. Gordon and G. Eng, 2006. Larvicidal and structural studies of some triphenyl-and tricyclohexyltin para-substituted benzoates. J. Organomet. Chem., 691: 1775-1779.

Eng, G., X. Song, Q. Duong, D. Strickman, J. Glass and L. May, 2003. Synthesis, structure characterization and insecticidal activity of some triorganotin dithiocarbamates. Appl. Organomet. Chem., 17(4): 218-225.

Hamdan, H., M.A. Sofian, W.A. Nazni and H.L. Lee, 2005. Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. Trop. Biomed., 22(1): 45-52.

Jamil, K., W. Rehman, B. Muhammad, M. Danish and N. Bukhari, 2009. Biologically active organotin (IV) schiff bases derived from indoline-2,3-dione and 2-aminobenzoic acid. World Appl. Sci. J., 6: 563-1568.

Jenkins, S.M., K. Ehman and S.J. Barone, 2004. Structure-activity comparison of organotin species: Dibutyltin is a developmental neurotoxicant in vitro and in vivo. Dev. Brain Res., 151(1-2): 1-12.

Magar, R.S. and A. Shaikh, 2013. Effect of malathion toxicity on detoxifying organ of fresh water fish *Channa punctatus*. Int. J. Pharm. Chem. Biol. Sci., 3(3): 723-728.

Mulla, M.S. and N. Singh, 1991. Delayed mortality and morphogenetic anomalies induced by the microbial control agent *Bacillus thuringiensis* Ser. (H-14) in *Culex quinquefasciatus*. J. Am. Mosquito. Contr., 7(3): 420-3.

Pellerito, C., L. Nagy, L. Pellerito and A. Szorcsik, 2006. Biological activity studies on organotin $(IV)^{n+}$ complexes and parent compounds. J. Organomet. Chem., 691: 1733-1747.

Raymond, M., 1985. Log-probit analysis basic programme of microcomputer. Cahiers ORSTOM series. Entomol. Med. Parasitol., 22: 117-121.

Rehman, W., K.B. Musa, M. Bakhtiar, B. Amin and M.K. Khalid, 2004. Characteristic spectral studies and in vitro antifungal activity of some Schiff bases and their organotin complexes. Chinese Sci. Bull., 49: 119-122.

Rosalina, 2012. Keberkesanansebati and ifenilstanum (IV) dantrifenilstanum (IV) alkilfenilditiokarbamat sebagai insektisid ke atas *Aedes aegypti* Linn. (Diptera: Culicidae) di makmal. Tesis Program Kesihatan Persekitaran, Fakulti Sains Kesihatan, University Kebangsaan Malaysia.

Saranya, M., R.S. Mohanraj and B. Dhanakkodi, 2013. Larvicidal, pupicidal activities and morphological deformities of *Spathodea campanulata* aqueous leaf extract against the dengue vector *Aedes aegypti*. Eur. J. Exp. Biol., 3(2): 205-213.

Singh, N.K., A. Srivastava, A. Sodhi and P. Ranjan, 2000. *In vitro* and *in vivo* antitumour studies of a new thiosemicarbazide derivative and its complexes with 3d-metal ions. Transit. Metal Chem., 25: 133-140.

WHO, 1981. Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticide. World Health Organization, Geneva.

WHO, 2012. Global Strategy for Dengue Prevention and Vector Control. World Health Organization, Geneva.

WHO, 2014. Impact of Dengue. World Health Organization, Geneva.

Yap, H.N., N.L. Chong, A.E. Foo and C.Y. Lee, 1994. Dengue vector control: Present status and future prospect. Kaohsiung J. Med. Sci., 10: 102-108.