Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:54:(e20200146): 2021
https://doi.org/10.1590/0037-8682-0146-2020

Short Communication

Selective activity of diselenides against *Aedes aegypti* (Diptera: Culicidae) larvae

***Sabrina Ketrin Targanski*[1], *Janaina Rosa Sousa*[1], *André Luiz Agnes Stein*[2] *and Marcos Antônio Soares*[1]**

[1]. Universidade Federal de Mato Grosso, Departamento de Botânica e Ecologia, Cuiabá, MT, Brasil.
[2]. Universidade Federal de Mato Grosso, Faculdade de Engenharia, Cuiabá, MT, Brasil.

Abstract

Introduction: *Aedes aegypti* (L.) is the major vector of arboviruses that causes serious public health concerns in tropical and subtropical countries. **Methods:** We examined the larvicidal activity of 1,2-diphenyldiselenide [$(PhSe)_2$] and 1,2-bis(4-chlorophenyl) diselenide [$(p\text{-}ClPhSe)_2$] and determine its toxicity to different non-target organisms. **Results:** $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ killed *Ae. aegypti* L3 larvae with $LC_{50/24h}$ values of 65.63 µM (20.48 mg/L) and 355.19 µM (135.33 mg/L), respectively. $(PhSe)_2$ was not toxic to the four model organisms. **Conclusions:** $(PhSe)_2$ is a larvicidal compound with selective action against *Ae. aegypti* larvae. The mechanisms of action of $(PhSe)_2$ under field conditions remain to be investigated.

Keywords: Selenium. 1,2-diphenyldiselenide. 1,2-bis(4-chlorophenyl) diselenide. *Caenorhabditis elegans. Chlorella vulgaris. Galleria mellonela.*

Aedes aegypti (L.) is a mosquito widely distributed in tropical, subtropical, and temperate regions during warm seasons. The mosquito transmits human arboviruses, including dengue, chikungunya, and Zika virus[1]. It is estimated that the dengue virus infects over 390 million people in at least 100 countries on different continents and causes significant number of deaths and public health concerns. Zika virus infection has been associated with malformations in human fetal development[1]. The circulation of and co-infection by the dengue, chikungunya, and Zika viruses create a worrying scenario that demands effective measures to control the mosquito vector population[2].

The *Ae. aegypti* populations can be controlled by different methods, such as the physical elimination of containers with larvae and pulp, chemical larvicides, and insecticides; the use of biological control agents such as *Bacillus thuringiensis* var. *israelensis* (Bti), *Wolbachia* bacteria, fish, and genetically modified male mosquitoes[2]. Larvicides are among the most important control agents because they eliminate immature forms and prevent the development of the adult form that transmits arboviruses. Bti is a larvicide of limited use because of its low persistence and the need for reapplication for effectiveness[3]. The chemical agent Pyriproxyfen acts more like a growth regulator than larvicide, and the agricultural pests *Bemisia tabaci* and *Musca domestica* (L.) are resistant to its action[4]. The development of new and efficient larvicides is important to broaden the arsenal of methods to control *Ae. aegypti* population. Larvicides must cause rapid larval death at low concentrations and no toxicity to non-target organisms. Sodium selenite suppresses the growth of *Culex quinquefasciatus* larvae and kills 50% of the larvae population at a concentration of 11 mg/L[5]. Selenium is a trace element essential for the antioxidant activity of selenoenzymes but can be toxic at high doses. Selenium compounds such as 1,2-diphenyldiselenide [$(PhSe)_2$] exert various biological activities, including neuroprotective, anti-inflammatory, antiulcer, antidepressant, and anxiolytic activities. Its structural analog 1,2-bis(4-chlorophenyl) diselenide [$(p\text{-}ClPhSe)_2$] prevents memory loss and treats metabolic disorders[6]. We hypothesize that low concentrations of selenium compounds $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ are toxic to *Ae. aegypti* larvae, but not other non-target organisms.

Ae. aegypti eggs were provided by Professor Margareth de Lara Capurro Guimarães, Ph.D., from the Institute of Biomedical Sciences of the University of São Paulo (USP), São Paulo, SP, Brazil. The eggs were hatched in glass jars containing autoclaved distilled water at 28 °C exposed to a 12 h photoperiod and fed with

Corresponding author: Marcos Antônio Soares.
e-mail: drmasoares@gmail.com
https://orcid.org/0000-0002-8938-3188
Received 25 March 2020
Accepted 3 July 2020

crushed fish feed (Tetramin®). Newly hatched larvae (L1) and third instar larvae (L3) were obtained approximately 12 h and 72 h after egg contact with water, respectively[7].

The selenium compounds, $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$, were synthesized according to a modified literature procedure[8]. The identity and purity of the compounds were confirmed by nuclear magnetic resonance (NMR) spectroscopy. ^1H and ^{13}C NMR spectra were recorded on a Bruker Ascend™ 500 MHz instrument with tetramethylsilane as an internal standard. Column chromatography was performed using Merck silica gel (230-400 mesh). Thin-layer chromatography was performed using Merck silica gel GF254 of 0.25 mm thickness, and the plates were revealed with either iodine vapor or acidic vanillin. $(PhSe)_2$: yellow solid; yield: 74%. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.61 – 7.57 (m, 2H), 7.25 – 7.21 (m, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 127.2, 129.3, 131.1, 132.9. $(p\text{-}ClPhSe)_2$: yellow solid; yield: 72%; ^1H NMR ($CDCl_3$, 500 MHz): δ = 7.53 (d, *J*= 8.6 Hz, 2H), 7.25 (d, *J*= 8.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 134.22, 133.18, 129.55, 129.29. Both compounds were dissolved in 10% dimethyl sulfoxide (DMSO) for biological assays.

The tests were performed in plastic cups (180 mL) containing 20 mL of tap water and 20 L3 of *Ae. Aegypti*[7], in the presence of different concentrations of $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ or 10% DMSO (control). The cups were incubated at 28°C with a 12 h photoperiod. The number of dead larvae were counted after 24 h and 48 h, and the larval mortality percentage was calculated. The $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ concentrations capable of killing 50% (LC_{50}) and 90% (LC_{90}) of the L3 larval population were estimated from the concentration-dependent mortality curves.

The eggs were kept in vials containing selenium compounds at a concentration corresponding to the $LC_{90/24h}$ or 10% DMSO (control) to determine the death time of L1. The time from egg hatching to 100% L1 larvae death was recorded.

The toxicity of $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ to different model organisms was assessed at concentrations corresponding to the $LC_{90/24h}$. The compounds' environmental toxicity was estimated from the growth rate of the algae *Chlorella vulgaris* BR017 and the viability of the protozoan *Tetrahymena pyriformis*. The toxicity to non-target organisms was determined in *Caenorhabditis elegans* N2 and *Galleria mellonella*[9].

Despite the availability of chemical control methods, the incidence of arboviruses transmitted by *Ae. aegypti*, such as dengue, chikungunya, and Zika, has increased annually, demanding the development of new larvicidal agents with low environmental toxicity and high selectivity and efficiency. Here, we report the selective larvicidal action of diphenyldiselenide $(PhSe)_2$ against *Ae. aegypti* larvae and its low environmental impact.

The selenium compounds $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ killed *Ae. aegypti* L3 larvae and provided the $LC_{50/24h}$ values of 65.63 µM (20.48 mg/L) and 355.19 µM (135.33 mg/L), respectively (**Table 1**). The World Health Organization proposes that promising larvicidal compounds are active at concentrations lower than 100 mg/L[7]. Compound $(PhSe)_2$ fitted this criterion, as the concentrations required to kill 50% ($LC_{50/24h}$), 90% ($LC_{90/24h}$ = 108.14 µM or 33.74 mg/L), and 100% (160.25 µM or 50 mg/L) of L3 larvae within 24 h were lower than 100 mg/L (**Table 1**). Compounds $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ significantly differed (T-test, $p < 0.01$) concerning the time required to kill *Ae. aegypti* L1 larvae: their $LC_{50/24h}$ values were 32 ± 0 min and 380 ± 17.32 min, respectively.

N-substituted methyl maleamates, especially N-hexyl methyl maleamate at $LC_{50/24h}$ = 0.36 mg/L (1.69 µM), probably affected *Ae. aegypti* larvae development through inhibition of sulfhydryl groups present in various key receptors essential for larval survival[10]. These compounds are efficient, but their toxicity has not been determined yet. Indole derivatives effectively control *Ae. aegypti* larvae population with low environmental toxicity, such as 6-bromo-2,3,4,9-tetrahydro-1H-carbazole with $LC_{50/24h}$ = 1.5 mg/L (5.88 µM)[9]. Although the presence of chlorine or bromine groups in the aromatic ring usually improves the larvicidal activity of indole derivatives[9], the addition of chlorine groups to $(PhSe)_2$ to produce $(p\text{-}ClPhSe)_2$ considerably reduced larvicidal activity

TABLE 1: Concentration of selenium compounds capable of killing 50% (LC_{50}) and 90% (LC_{90}) of *Aedes aegypti* L3 larvae after 24 h and 48 h of treatment.

Parameter	$(PhSe)_2$		$(p\text{-}ClPhSe)_2$	
	µM	mg/L	µM	mg/L
$LC_{50/24h}$	65.63	20.48	355.19	135.33
	(58.03-73.24)*	(18.10-22.85)	(313.99-396.4)	(119.63-151.03)
$LC_{90/24h}$	108.14	33.74	602.28	229.47
	(96.96-129.26)	(30.25-40.33)	(540.71-712.24)	(206.01-271.36)
$LC_{50/48h}$	52.44	16.36	191.72	73.04
	(46.9-57.98)	(14.63-18.09)	(164.52-218.92)	(62.68-83.41)
$LC_{90/48h}$	84.54	26.38	393.42	14.89
	(76.65-98.46)	(23.91-30.72)	(345.76-474.6)	(131.735-180.82)

$(PhSe)_2$: 1,2-diphenyldiselenide; **$(p\text{-}ClPhSe)_2$:** 1,2-bis(4-chlorophenyl) diselenide. *The values in parentheses represent the upper and lower limits at the 95% confidence interval.

(**Table 1**). The LC_{50} value of $(PhSe)_2$ and its analogs in mice range from 278 to 381 mg/kg[11]. Compared with the control, treatment with $(PhSe)_2$ at $LC_{90/24h}$ (108.14 µM or 33.74 mg/L) did not alter *C. elegans* and *G. mellonella* survival rate. At the same concentration, $(PhSe)_2$ did not affect: (i) *Chlorella vulgaris* BR017 cell growth rate ($1.15\times10^6 \pm 4.02\times10^5$ cells/mL) when compared with the control ($7.83\times10^5 \pm 1.54\times10^5$ cells/mL) on the seventh analysis (T-test, $p < 0.01$); and (ii) *Tetrahymena* sp. viability ($1.53\times10^6 \pm 5.05\times10^4$ cells/mL) when compared with the control ($1.0\times10^6 \pm 2.0\times10^4$ cells/mL) (T-test, $p < 0.01$). Therefore, $(PhSe)_2$ was not toxic to the four organisms studied, at least under the conditions assessed. Diorganoyldiselenides - $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ - reduce food intake in animals, resulting in a dose-dependent decrease in body weight[12]. The larvicidal activity of $(PhSe)_2$ in *Ae. aegypti* larvae may be associated with the free radical generation and oxidation of thiol groups that usually mediate these compounds' toxicity to mammals[13,14] and *Drosophila melanogaster*[15]. Our findings help to develop larvicidal products from $(PhSe)_2$ with selective action against *Ae. aegypti* larvae and low environmental toxicity, but their mechanisms of action remain to be determined.

ACKNOWLEDGMENTS

We thank the researchers Dr. Margareth de Lara Capurro Guimarães from Institute of Biomedical Sciences of the University of São Paulo (ICB-USP, São Paulo, SP, Brazil) for supplying *Ae. aegypti* eggs; Dr. Adriano Nunes Nesi from Department of Plant Biology of the Federal University of Viçosa (UFV, Viçosa, MG, Brazil) for providing the microalgae species; Dr. Marcia Cristina de Azevedo Prata from Embrapa Milk Cattle (Brasília, DF, Brazil) for furnishing *G. mellonella* eggs; and Dr. Tiago Campos Pereira from Department of Biology of the University of São Paulo (FFCLRP-USP, Ribeirão Preto, SP, Brazil) for providing *C.elegans* samples.

FINANCIAL SUPPORT

This work was supported by the Fundação de Amparo à Pesquisa do Estado de Mato Grosso – FAPEMAT, MT, Brazil [grant number 0415241/2016] and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ, Brazil [grant number 307288/2018-8].

AUTHORS' CONTRIBUTION

SKT: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing, Final approval of the version to be submitted; JRS: Methodology, Final approval of the version to be submitted; ALAS: Methodology, Final approval of the version to be submitted; MAS: Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Roles/Writing - original draft; Writing - review & editing, Final approval of the version to be submitted.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest during the development of the study.

REFERENCES

1. Brady OJ, Hay SI. The Global Expansion of Dengue: How *Aedes aegypti* Mosquitoes Enabled the First Pandemic Arbovirus. Annu Rev Entomol. 2019;65(1):1–18.
2. World Health Organization (WHO). Dengue: guidelines for diagnosis, treatment, prevention and control. New Ed. 2009;528(1):160.
3. Mulla MS. Activity, Field Efficacy, and Use of *Bacillus thuringiensis israelensis* against Mosquitoes. In 1990. p. 134–60.
4. Shah RM, Shad SA, Abbas N. Mechanism, stability and fitness cost of resistance to pyriproxyfen in the house fly, *Musca domestica* L. (Diptera: Muscidae). Pestic Biochem Physiol. 2015;119(1):67–73.
5. Jensen PD, Sorensen MA, Walton WE, Trumble JT. Lethal and sublethal responses of an aquatic insect *Culex quinquefasciatus* (Diptera: Culicidae) challenged with individual and joint exposure to dissolved sodium selenate and methylmercury chloride. Environ Toxicol. 2007;22(3):287–94.
6. Lenardão EJ, Santi C, Sancineto L. New Frontiers in Organoselenium Compounds. 1 ed. Springer; 2019. 200p.
7. World Health Organization (WHO). Guidelines for laboratory and field testing of mosquito larvicides. World Heal Organ. 2005;1–41.
8. Singh D, Deobald AM, Camargo LRS, Tabarelli G, Rodrigues OED, Braga AL. An efficient one-pot synthesis of symmetrical diselenides or ditellurides from halides with CuO nanopowder/Se0 or Te0/base. Org Lett. 2010;12(15):3288–91.
9. Sousa JR, Silva FA, Targanski SK, Fazolo BR, Souza JM, Campos MG, et al. Synthesis and larvicidal activity of indole derivatives against *Aedes aegypti* (Diptera: Culicidae). J Appl Entomol. 2019;1–10.
10. Harburguer L, Gonzalez P V., Gonzalez Audino P, Zerba E, Masuh H. N-substituted methyl maleamates as larvicidal compounds against *Aedes aegypti* (Diptera: Culicidae). Parasitol Res. 2018;117(2):611–5.
11. Savegnago L, Jesse CR, Nogueira CW. Structural modifications into diphenyl diselenide molecule do not cause toxicity in mice. Environ Toxicol Pharmacol. 2009;27(2):271–6.
12. Bortolatto CF, Heck SO, Gai BM, Zborowski VA, Neto JSS, Nogueira CW. Effects of diphenyl and p-chloro-diphenyl diselenides on feeding behavior of rats. Psychopharmacology (Berl). 2015;232(13):2239–49.
13. Rocha JBT, Saraiva RA, Garcia SC, Gravina FS, Nogueira CW. Aminolevulinate dehydratase (δ-ALA-D) as marker protein of intoxication with metals and other pro-oxidant situations. Toxicol Res (Camb). 2012;1(2):85–102.
14. Barbosa NV, Nogueira CW, Nogara PA, De Bem AF, Aschner M, Rocha JBT. Organoselenium compounds as mimics of selenoproteins and thiol modifier agents. Metallomics. 2017;9(12):1703–34.
15. Occai BK, Hassan W, da Rocha JBT. Gender-based behavioral and biochemical effects of diphenyl diselenide in *Drosophila melanogaster*. Chem Biol Interact. 2018;279:196–202.