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

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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-ClPhSe)$_2$] and determine its toxicity to different non-target organisms. **Results:** (PhSe)$_2$ and (p-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 mellonella*.

*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. 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. 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.

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. 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. 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. 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-ClPhSe)$_2$] prevents memory loss and treats metabolic disorders. We hypothesize that low concentrations of selenium compounds (PhSe)$_2$, and (p-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...
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.

The selenium compounds, (PhSe)$_2$, and (p-ClPhSe)$_2$, were synthesized according to a modified literature procedure. The identity and purity of the compounds were confirmed by nuclear magnetic resonance (NMR) spectroscopy. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AscendTM 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%. $^{1}$H 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 = 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$, in the presence of different concentrations of (PhSe)$_2$, and (p-ClPhSe)$_2$, or 10% DMSO (control). The cups were incubated at 28℃ with a 12 h photoperiod. The number of dead larvae was counted after 24 h and 48 h, and the larval mortality percentage was calculated. The (PhSe)$_2$ and (p-ClPhSe)$_2$, concentrations capable of killing 50% (LC$_{50/24h}$) and 90% (LC$_{90/24h}$) 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$_{50/24h}$ 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-ClPhSe)$_2$, to different model organisms was assessed at concentrations corresponding to the LC$_{50/24h}$. 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.

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-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-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$^9$. 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, the addition of chlorine groups to (PhSe)$_2$ to produce (p-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-ClPhSe)$_2$ |
|-----------|-----------|----------------|
| 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$_{50/24h}$ | 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$_{50/48h}$ | 84.54 | 26.38 | 393.42 | 14.89 |
| | (76.65-98.46) | (23.91-30.72) | (345.76-474.6) | (131.73-180.82) |

(PhSe)$_2$: 1,2-diphenyldiselenide; (p-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), and its analogs in mice range from 278 to 381 mg/kg\(^{11}\). Compared with the control, treatment with (PhSe), at LC_{99/25}(108.14 \mu M or 33.74 mg/L) did not alter *C. elegans* and *G. mellonella* survival rate. At the same concentration, (PhSe), 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\times10^6 \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), was not toxic to the four organisms studied, at least under the conditions assessed.

**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.

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