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Cytotoxicity of Piperamides Towards *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT The effectiveness of the amides piplartine and piperlonguminine isolated from *Piper* species for controlling L3 and L4 of *Aedes aegypti* (L.) was assessed through bioassays at concentrations ranging from 1 to 300 μg/ml. Piplartine reduced the mosquito development period and caused larval mortality only at concentrations >100 μg/ml, whereas piperlonguminine resulted in an extended period of mosquito development (10 μg/ml) and caused 100% larval mortality (30 μg/ml) within 24 h. The toxicity and cytotoxic effects of piperlonguminine on epithelial cells of the digestive system of *Ae. aegypti* were viewed using transmission electron microscopy, which indicated vacuolization of cytoplasm, mitochondrial swelling and leaking of nuclear material. Piperlonguminine was the more effective amide, showing toxic activity with LD_{50} of ≈12 μg/ml against the larvae of *Ae. aegypti*.

KEY WORDS cytotoxicity, Culicidae, larvicide, piperamide

Several studies have drawn attention to larvicidal natural products that could be useful for controlling many vectors (Consoli et al. 1988, Park et al. 2002, Silva et al. 2003), including *Aedes aegypti* (L.) (Angerilli 1980). Natural insecticides have a variety of modes of action. Although some inhibit normal growth and development (Cabral et al. 2007a,b; 2008; 2010; Park et al. 2002), others inhibit the synthesis of tyrosinase, which is a key enzyme in cuticle sclerotization for larval mosquitoes such as *Culex quinquefasciatus* Say (Evans and Kaleysa 1992). Additional mechanisms of action involve an antidiuretic effect caused by hormones (Cabral et al. 2000), reduction of reproductive capacity (Nogueira et al. 2009), appetite suppression (Edwards and Wratten 1981), and larvicidal activity (Cabral et al. 2009). The appeal of using natural products from plants results from their relatively shorter persistence in the environment and greater specificity in comparison with classical insecticides. In addition, many of these natural insecticides present low toxicity to mammals (Dantas 1993, Vendramin 2000).

Amides are a class of natural products with considerable potential for biological activity, especially as insecticides (Barbieri et al. 2007, Scott et al. 2008), fungicides (Navickiene et al. 2000, Marques et al. 2010, Lago et al. 2012), parasiticides (Cotinguiba et al. 2009, Moraes et al. 2013), and molluscicides (de Moraes et al. 2011, Rapado et al. 2013). The toxic activity of the amide tetrahydropiperine has been observed among flies, with LC_{50} = 16.25 μg per fly against *Lucilia cuprina* Wiedemann within 24 h, and with LC_{50} = 7.65 μg per fly against *Musca domestica* L. (Barbieri et al. 2007).

Ae. aegypti is the primary vector of the dengue virus and is widely distributed in tropical urban areas (Teixeira et al. 2002). It has been responsible for frequent epidemics of the four dengue serotypes throughout the Americas (World Health Organization [WHO] 1997), infecting 50–100 million people a year and exposing 2.5 billion people to the risk of infection (Flauzino et al. 2009). The adaptive fitness of this mosquito for coping with adverse conditions includes long viability of its eggs in harsh environments (Silva and Silva 1999) and the ability to develop in polluted waters (Silva et al. 1999).

Previous studies on the lignan grandisin have shown that it has larvicidal activity against *Ae. aegypti* (Cabral et al. 2009) and has stimulated a search for new bioactive compounds from *Piper* species. Because amides are the class of compounds that most typically have presented insecticide activity in *Piper* species, our study evaluated the effects of piplartine and piperlonguminine amides on the development of *Ae. aegypti*.

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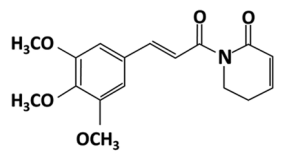
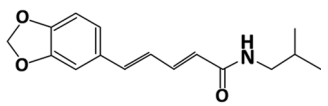


1 (piplartine)



2 (piperlonguminine)

Fig. 1. Chemical structures of piperamides 1 and 2.

Materials and Methods

Natural Products. Two piperamides, piplartine and piperlonguminine (Fig. 1), were isolated from *Piper tuberculatum* Jacq. and *Piper scutifolium* Yunck., respectively (Navickiene et al. 2000, Silva et al. 2002, Marques et al. 2007). Roots of *P. tuberculatum* and *P. scutifolium* were collected from specimens cultivated at the Institute of Chemistry, University of São Paulo (USP), São Paulo, Brazil. Samples of piplartine and piperlonguminine were isolated as previously described (Silva et al. 2002, Marques et al. 2007).

Ae. aegypti. *Ae. aegypti* eggs were obtained from the Vector Research and Support Center (NApVE; partnership DIRAC-IOC-VPAAPS), Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, and the colony was maintained in the Diptera Laboratory, Oswaldo Cruz Institute, FIOCRUZ. All experiments were carried out on third- (L3) and fourth-instar (L4) larvae from the F_1 to F_5 generations. For bioassays, eggs were placed in a receptacle containing mineral water with fish food added daily (0.3 mg per larva; Beserra et al. 2006) until pupation (Cabral et al. 2009, Narciso 2009, Leite 2012).

Bioassays. Solutions of piplartine and piperlonguminine were dissolved in acetone at 1–300 μg/ml and applied at final concentrations of 1, 10, 30, 50, 70, 100, and 300 μg/ml in glass containers (4.0 cm in diameter by 4.5 cm in height) containing mineral water (25 ml). After one hour of treatment, a diet of fish meal (Alcon Guppy, Alcon, Camboriú, Santa Catarina, Brazil) at 0.3 mg per larva, was added daily to the medium (Cabral et al. 2009, Narciso 2009, Leite et al. 2012). *Ae. aegypti* larvae (L3) groups (F1–F5; 25 larvae per group) were evaluated in triplicate with three repetitions. Two control groups were used: one sample with acetone solution (without piperamides) and another untreated sample in mineral water. The development of *Ae. aegypti* larvae was observed until the end of the experiments. The larvae were maintained in a climate-controlled chamber at 27 ± 1°C, 70 ± 10% relative humidity, and a photoperiod of 12:12 (L:D) h. The bioassays followed the methodology of Cabral et al. (2009) and Narciso (2009), which was adapted from WHO (1970). The data were analyzed using one-way analysis of variance with means separated using the Tukey's test with a significance level of 5% (Sokal and Rohlf 1979). Standard deviations were calculated using the averages from the experiments using the GraphPad InStat 3.05 software (Motulsky 2002) and BioEstat 5.0 software (Ayres et al. 2007). LD_{50} was calculated by means of trimmed Spearman–Karber analysis (Hamilton et al. 1978).

Table 1. Number of days taken for *Ae. aegypti* to develop (L3 to adult; 1A) and larval mortality (1B) when treated with piplartine at concentrations of 1–300 μg/ml

	1A		1B		%
	X ± SD	VI	X ± SD	VI	
Control	13.2 ± 4a	5–19	0	0	0
Control 2	14.4 ± 4a	6–20	0	0	0
1	11.8 ± 3.4b***	6–19	0	0	0
10	11.4 ± 3.3c***	6–18	0	0	0
30	NO	NO	NO	NO	NO
50	14.3 ± 3.4a	7–19	0	0	0
70	NO	NO	NO	NO	NO
100	12.5 ± 3a	6–20	3 ± 3.6a	1–1	12
300	0	0	25 ± 0b***	1–1	100

Piplartine treatments on *Ae. aegypti* at concentrations of 1–300 μg/ml. Values are mean ± SD (X ± SD), as an average of three replicates of 25 (L3) larvae per group. Values followed by the same letter in the same column did not significantly differ from each other by Tukey test with $P > 0.05$. Significance levels comparing compound versus control 2 (acetone control) are represented as ***, $P < 0.001$. VI, range of variation; NO, not observed.

Transmission Electron Microscopy on the Digestive System of L4 Larvae of *Ae. aegypti*. Midgut samples ($n = 3$) obtained from L4 larvae of *Ae. aegypti* were fixed in solution containing 2.5% glutaraldehyde, 4% paraformaldehyde (Karnovsky 1965), and 0.5 mM calcium chloride in 0.1 M sodium cacodylate buffer solution (pH 7.2). The samples were then washed twice for 5 min in 0.1 M sodium cacodylate buffer solution (pH 7.2) and postfixed in 1% osmium tetroxide, 0.8% potassium ferricyanide, and 0.5 mM calcium chloride in 0.1 M sodium cacodylate buffer solution (pH 7.2) for 1 h at room temperature in the dark. The samples were dehydrated in increasing gradients of acetone: 50, 70, and 90% for 5 min each, and 100% twice for 10 min. Subsequently, the material was infiltrated in Epon followed by definitive inclusion in plastic shapes for polymerization in an oven at 60°C for 48 h. Ultrathin sections (50–80 nm thick) were collected on grids and contrasted with 5% uranyl acetate in water for 15 min and then 2% lead citrate for two min, for observations in a Jeol transmission electron microscope (JEM-1011, JEOL, Akishima, Tokyo, Japan).

Results

Bioassays. *Ae. aegypti* larvae (L3) treated with piplartine showed decreased L3-adult development time at the concentrations of 1 μg/ml (3.15 μM) and 10 μg/ml (31.51 μM; Table 1A). The larvicidal activity level using piplartine was 100% ($P < 0.001$) at 300 μg/ml (945 μM; Table 1B) and the LD_{50} was 155.5 μg/ml (490 μM).

Ae. aegypti larvae (L3) treated with piperlonguminine showed significant extension of the L3-adult development period at the concentration of 10 μg/ml

Table 2. Number of days taken for *Ae. aegypti* to develop (L3 to adult; 2A) and larval mortalilty (2B) when treated with piperlonguminine at concentrations of 1–100 μg/ml

	2A		2B		
	X ± SD	VI	X ± SD	VI	%
Control	13.2 ± 4a	5–19	0	0	0
Control 2	14.4 ± 4a	6–20	0	0	0
1	15.4 ± 4a	3–19	0	0	0
10	17.6 ± 1.4b***	15–22	5.6 ± 6.6b*	1–2	22.6
30	0	0	25 ± 0c***	1–4	100
50	0	0	25 ± 0c***	1–3	100
70	0	0	25 ± 0c***	1–1	100
100	0	0	25 ± 0c***	1–1	100
300	NO	NO	NO	NO	NO

Piperlonguminine treatments on *Ae. aegypti* at concentrations of 1–100 μg/ml. Values are mean ± SD (X ± SD), as an average of three replicates of 25 (L3) larvae per group. Values followed by the same letter in the same column did not significantly differ from each other by Tukey test with $P > 0.05$. Significance levels comparing compound versus control 2 (acetone control) are represented as ***, $P < 0.001$ and *, $P < 0.05$. VI, range of variation; NO, not observed.

(36.5 μM; $P < 0.001$), with a longer length of time for development (15–22 d), in comparison with the controls (5–19 and 6–20 d; Table 2A).

The treatment with 10 μg/ml (36.5 μM) of piperlonguminine resulted in 23% larval mortality. A 100% larval mortality was observed within 4 d at 30 μg/ml (109.7 μM; $P < 0.001$; Table 2B), with $LD_{50} = 12$ μg/ml (43 μM). The assays at concentrations higher than 30 μg/ml (109.7 μM) gave 100% larval mortality ($P < 0.001$), and concentrations higher than 50 μg/ml (182.9 μM) displayed nearly the same mortality within 24 h (Table 2B).

Electron Microscopy. Morphological analysis of L4 larvae by means of transmission electron microscopy, on the *Ae. aegypti* groups, controls, and acetone controls, showed normal features without apical, medial, or basal morphological alterations to the midgut cells (Fig. 2A). The epithelial cells were arranged in a single layer, with an apical surface covered with numerous well-preserved elongated microvilli, nuclei with normal chromatin condensation, preserved intercellular junctions, and homogeneous cytoplasm appearance (Fig. 2B).

Nevertheless, in cases of L4 *Ae. aegypti* larvae treated with 50 μg/ml (182.9 μM) of piperlonguminine, several changes in the digestive tube cells were observed, with low and wide microvilli (Fig. 2C) and intense vacuolization of the cytoplasm (Fig. 2 F). The presence of myelin figures (Fig. 2E) and disorganized structures indicated cell stress, which is one of the signs of cell degeneration (Fig. 2C). In some nuclei, extraction of DNA material from the midgut cells was observed (Fig. 2D).

Discussion

The effects of amides from *Piper* species on *Ae. aegypti* were evaluated taking into account various biological parameters, such as the inhibition of developmental processes and toxicity. The most significant data regarding toxicity were shown by piperlonguminine, which caused 100% larval mortality within 24–96 h, at 30 μg/ml (109.7 μM). The treatment with piplartine at the concentration of 10 μg/ml (36.5 μM) delayed mosquito development and presented larval mortality only at concentrations higher than that observed for piperlonguminine.

The results from this study corroborated the findings of piperamide toxicity toward insects that had previously been observed in relation to piplartine (LC_{50} = 40 ppm) against *Anopheles darlingi* Root (Trindade et al. 2012). In addition, larvicidal activity was displayed by the mixture *N*-phenylethyl-2Z-nonen-6,8-diinoic amide and *N*-phenylethyl-2Z-decen-6,8-diinoic amide against *Ae. aegypti*, with LC_{50} = 1.46 ppm (Simas 2003). Also, piperine amide has shown larvicidal activity against *Ae. aegypti*, with LC_{50} = 1.53 ppm (Simas et al. 2007). The toxicity of piptigrine, an amide isolated from *Piper nigrum* L., was LC_{50} = 15 ppm (Siddiqui et al. 2004), while the toxicity of pipernonaline, isolated from *Piper longum* L., was LC_{50} = 25 ppm (Yang et al. 2002), against *Ae. aegypti.* In the current study, piperlonguminine was confirmed to have larvicidal activity against *Ae. aegypti* at the lower concentrations that were observed with pipernonaline (Yang et al. 2002).

The toxic activity of 3,4-methylenedioxy cinnamoyl piperidine amide, piperine, and tetrahydropiperine have been investigated against flies, but only tetrahydropiperine showed significant activity against *Lucilia cuprina* Wiedemann and *Musca domestica* L., with LC_{50} = 16.25 μg per fly and 7.65 μg per fly in 24 h, respectively (Barbieri et al. 2007).

In our evaluations of amides against *Ae. aegypti*, the isobutyl amide piperlonguminine was found to have the highest larvicidal effect. Piperlonguminine has methylenedioxyphenyl moiety similar to piperine, pipernonaline, and piptigrine. It has been recognized that this pharmacophore group is involved in several synergistic agents, such as in piperonyl butoxide and analogs, promoting larvicidal, antifeedant, and insecticidal activity (Casida 1970, Scott 1999, Waliwitiya et al. 2012). In general, methylenedioxyphenyl compounds act by competitively inhibiting P450 mixed-function oxidases. In the case of piperine, the inhibitory effect on monoamine oxidase has been demonstrated in relation to many targets (Rahman and Rahmatullah 2010), with possible use in Parkinson's disease (Al-Baghdadi et al. 2012).

The treatment of L4 larvae of *Ae. aegypti* with piperlonguminine caused effects on the ultrastructure of epithelial cells, in which intense cytoplasmic vacuolization and changes to the structure of the microvilli were observed similarly to previous descriptions (Sharma and Rai 1969, Cocke et al. 1979, Davidson 1979, Charles 1987, Flores and Lamorena 1991).

In a study on plant extracts, Arruda et al. (2008) reported that in the midgut cells of larvae of *Ae. aegypti*, the microvilli were poorly developed with various mitochondrion sizes. In addition, the cell nuclei had a very distinct appearance and large numbers of vacuoles indicating intense lysis. Those findings were similar to the results from the current study. Accord-

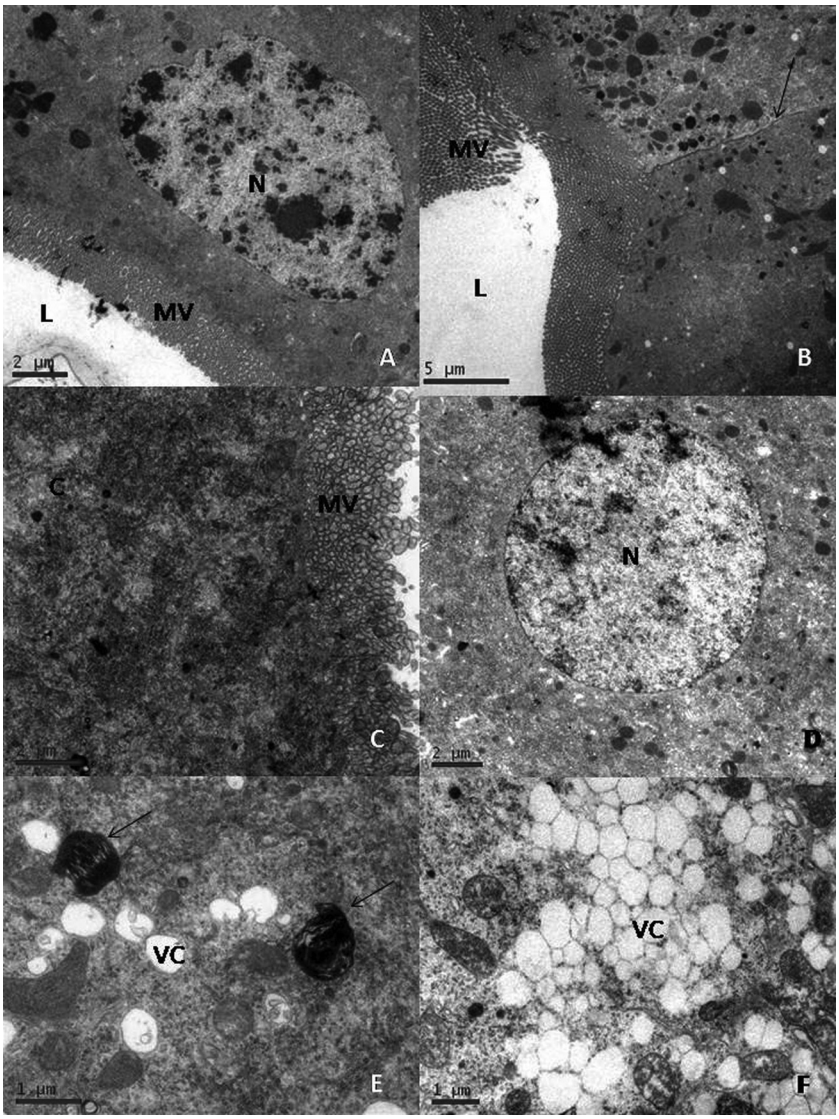


Fig. 2. (A) Epithelial cell of digestive tube of L4 larva of *Ae. aegypti* in control group, showing nucleus (N), basal membrane (MB), and microvilli (MV). (B) Apical region of digestive tube cell of L4 larva of *Ae. aegypti* in acetone control group: lumen (L), microvilli (MV), and preserved intercellular junction (arrow) are visible. (C) Cytoplasmic (C) and microvilli (MV) disorganization of digestive tube cells of L4 larvae of *Ae. aegypti* treated with piperlonguminine. (D) View of cytoplasm (C) and nucleus (N) showing extraction of DNA material from digestive tube cells of L4 larvae of *Ae. aegypti* treated with piperlonguminine. (E) Myelin figure formations (arrow) and cytoplasmic vacuolization (VC) of digestive tube cells of L4 larvae of *Ae. aegypti* treated with piperlonguminine. (F) Intense cytoplasmic vacuolization (VC) of digestive tube cells of L4 larvae of *Ae. aegypti* treated with piperlonguminine.

ing to the authors cited above, the toxic effects of various compounds caused changes to the midgut cells of larvae of *Ae. aegypti* and other mosquitoes. Therefore, regardless of the source of the toxic substances tested as larvicides, they will act on the midgut epithelial cells, thereby causing irreparable damage and indicating the location of absorption of these compounds.

In conclusion, the amides exhibit toxicity against L3 larvae of *Ae. aegypti.* Piperlonguminine was the most effective compound: it increased the length of development and finally caused high mortality among the larvae of *Ae. aegypti.* Moreover, piperlonguminine caused changes to the digestive tube cells, as revealed by transmission electron microscopy. This indicated that it was absorbed by the midgut epithelial cells, thereby causing cell destruction and leading to larval death.

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References Cited

Al-Baghdadi, O. B., N. I. Prater, C. J. Van der Schyf, and W. J. Geldenhuys. 2012. Inhibition of monoamine oxidase by derivatives of piperine, an alkaloid from the pepper plant *Piper nigrum,* for possible use in Parkinson's disease. Bioorg. Med. Chem. Lett. 22: 7183–7188.

Angerilli, N.P.D. 1980. Influences of extracts of fresh water vegetation on the survival and oviposition by *Aedes aegypti* (Diptera: Culicidae). Can. Entomol. 112: 1249–1252.

Arruda, W., G. M. Cavasin, and I. G. Silva. 2008. Estudo ultra-estrutural do efeito da toxicidade do extrato da *Magonia pubescens* (St. Hil.) no mesêntero de larvas de *Aedes aegypti* (L) (Diptera:Culicidae). Rev. Patol. Trop. 37: 255–267.

Ayres, M., M. Ayres Júnior, D. I. Ayres, and A. S. Santos. 2007. BioEstat 5.0: aplicações estatísticas nas áreas das ciências biológicas e médicas, 5 ed. Sociedade Civil Mamirauá, Belém, PA.

Barbieri, E. J., C. B. Barreto, R. C. Ribeiro, V. H. Oliveira, L.M.E. Freire, and G. E. Moya-Borja. 2007. Efeito inseticida de amidas naturais de *Piper* e do derivado sintético tetraidropiperina sobre *Lucilia cuprina* (Diptera: calliphoridae) e *Musca domestica* (Diptera: muscidae). Rev. Bras. Parasitol. Vet. 16: 87–91.

Beserra, E., F. P. Castro Jr., J. W. Santos, T. S. Santos, and C.R.M. Fernandes. 2006. Biologia e Exigências Térmicas de *Aedes aegypti* (L.) (Diptera: Culicidae) Provenientes de Quatro Regiões Bioclimáticas da Paraíba. Neotrop. Entomol. 35: 853–860.

Cabral, M.M.O., A. H. Kollien, T. Kleffmann, P. Azambuja, O. R. Gottlieb, and E. S. Garcia. 2000. *Rhodnius prolixus:* effects of the neolignan burchellin on *in vivo* and *in vitro* diuresis. Parasitol. Res. 86:710–716.

Cabral, M.M.O., P. M. Mendonça, C.M.S. Gomes, J. M. Barbosa-Filho, M.M.C. Queiroz, and R. P. Mello. 2007a. Biological activity of neolignans on the post-embrionic development of *Chrysomya megacephala.* Fitoterapia 78: 20–24.

Cabral, M.M.O., P. M. Mendonça, C.M.S. Gomes, J. M. Barbosa-Filho, C. S. Dias, and M. J. Soares. 2007b. Biological activity of yangambin on the postembryonic development of *Chrysomya megacephala* (Diptera: Calliphoridae). J. Med. Entomol. 44: 249–255.

Cabral, M.M.O., E.R.F. Crescente, P. M. Mendonça, C.M.S. Gomes, V. C. Oliveira, and A. Kelecom. 2008. *Melia azedarach* L. extract and their activity on *Musca domestica* L. (Diptera: Muscidae). Rev. Bras. Farmacog. 18: 699–702.

Cabral, M.M.O., J. A. Alencar, E. A. Guimarães, and M. J. Kato. 2009. Larvicidal activity of grandisin against *Aedes aegypti.* J. Am. Mosq. Control Assoc. 25: 103–105.

Cabral, M.M.O., G.L.A. Maia, M.C.O. Chaves, M. V. Braga, W. De Souza, and R.O.A. Soares. 2010. Neolignans from plants in northeastern Brazil (Lauraceae) with activity against *Trypanosoma cruzi.* Exp. Parasitol. 124: 319–324.

Casida, J. E. 1970. Mixed-function oxidase involvement in the biochemistry of insecticide synergists. J. Agric. Food. Chem. 18: 753–759.

Charles, J. F. 1987. Ultrastructural midgut events in culicidae larvae fed with *Bacillus sphaericus* 2297 spoke/crystal complex. Ann. Inst. Pasteur Microbiol. 138: 471–484.

Cocke, J., A. C. Bridges, R. T. Mayer, and J. K. Olson. 1979. Morphological effects of insect growth regulating compounds on *Aedes aegypti* (Diptera: Culicidae) larvae. Life Sci. 24: 817–832.

Consoli, R.A.G.B., N. M. Mendes, J. P. Pereira, B. S. Santos, and M. A. Lamounier. 1988. Influência de diversos derivados de vegetais na sobrevida das larvas de *Aedes fluviatilis* (Lutz) (Diptera: Culicidae) em laboratório. Mem. Inst. Oswaldo Cruz 83: 87–93.

Cotinguiba, F., L. O. Regasini, V. D. Bolzani, H. M. Debonsi, G. D. Passerini, R.M.B. Cicarelli, M. J. Kato, and M. Furlan. 2009. Piperamides and their derivatives as potential anti-trypanosomal agents. Med. Chem. Res. 18: 703–711.

Dantas, I. M. 1993. Toxicidade de isoflavonóides de sementes de *Pachyrrhizu stuberosus* (Lam.) Spreng (Leguminosae) var. Preta, sobre adultos de Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae). [Tese Mestrado - Agronomia]. Lavras: Escola Superior de Agricultura de Lavras, Lavras. 50p.

Davidson, E. W. 1979. Ultrastructure of midgut events in the pathogenesis of *Bacillus sphaericus* strain SSII-1 infection of *Culex pipiens quinquefasciatus* larvae. Can. J. Microbiol. 25: 178–184.

De Moraes, J., C. Nascimento, P. Lopes, E. Nakano, L. F. Yamaguchi, M. J. Kato, and T. Kawano. 2011. *Schistosoma mansoni:* in vitro schistosomicidal activity of piplartine. Exp. Parasitol. 127: 357–364.

Edwards, P. J., and S. D. Wratten. 1981. Ecologia das interações entre insetos e plantas. São Paulo. E.P.U. 71pp.

Evans, D. A., and R. Kaleysa. 1992. Effect of quassin on the metabolism of catecholamines in different life cycle stages of *Culex quinquefasciatus.* Indian J. Biochem. Biophys. 29: 360–363.

Flauzino, R. F., R. Souza-Santos, and R. M. Oliveira. 2009. Dengue, geoprocessamento e indicadores socioeconômicos e ambientais: um estudo de revisão. Rev. Panam. Salud Publica 25: 456–461.

Flores, J. G., and M. B. Lamorena. 1991. Ultrastructure study of *Bacillus thuringiensis* treated *Aedes aegypti* larvae. Acta Manilana 39: 25–29.

Hamilton, M. A., R. C. Russo, and R. V. Trurston. 1978. Trimmed spearman-karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 12: 714–719.

Karnovsky, M. J. 1965. The formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell. Biol. 27: 137–138.

Lago, J.H.G., A. T. Ito, C. M. Fernandes, M.C.M. Young, and M. J. Kato. 2012. Secondary metabolites isolated from *Piper chimonantifolium* and their antifungal activity. Nat. Prod. Res. 26: 770–773.

Leite, A.C.F.C., M. J. Kato, R.O.A. Soares, A. E. Guimarães, J. R. Santos-Mallet, and M.M.O. Cabral. 2012. Grandisin caused morphological changes larval and toxicity on *Aedes aegypti.* Rev. Bras. Farmacog. 22: 517–521.

Marques, J. V., R.O.S. Kitamura, J.H.G. Lago, M.C.M. Young, E. F. Guimarães, and M. J. Kato. 2007. Antifungal amides from *Piper scutifolium* and *Piper hoffmanseggianum.* J. Nat. Prod. 70: 2036–2039.

Marques, J. V., A. de Oliveira, L. Raggi, M.C.M. Young, and M. J. Kato. 2010. Antifungal activity of natural and synthetic amides from *Piper species.* J. Braz. Chem. Soc. 21: 1807–1813.

Moraes, J., J. Keiser, K. Ingram, C. Nascimento, L. F. Yamaguchi, C. R. Bittencourt, M. P. Bemquerer, J. R. Leite, M. J. Kato, and E. Nakano. 2013. In vitro synergistic interaction between amide piplartine and antimicrobial peptide dermaseptin against *Schistosoma mansoni* schistosomula and adult worms. Curr. Med. Chem. 20:301–309.

Motulsky, H. J. 2002. Analyzing data with Graphpad Prism. GraphPad Software Inc, San Diego, CA.

Narciso, J.O.A. 2009. Atividade larvicida de neolignana isolada de *Ocotea cymbarum* sobre *Aedes aegypti* L. (Diptera: Culicidae). [Monografia de Especialização em Entomologia Médica], Instituto Oswaldo Cruz. Rio de Janeiro, Brasil. 63p.

Navickiene, H.M.D., A. C. Arrival, M. J. Kato, V. S. Haddad, M.C.M. Young, and A. J. Gentleman. 2000. Antifungal amides from *Piper hispidum* and *Piper tuberculatum.* Phytochemistry 55: 621–626.

Nogueira, C.D.R., R. P. Mello, M. J. Kato, and M.M.O. Cabral. 2009. Disruption of growth caused by lignin grandisin. J. Med. Entomol. 46: 281–283.

Park, I. K., S. G. Lee, S. C. Shin, J. D. Park, and Y. D. Ahn. 2002. Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. J. Agric. Food Chem. 50: 1866–1870.

Rahman, T., and M. Rahmatullah. 2010. Proposed structural basis of interaction of piperine and related compounds with monoamine oxidases. Bioorg. Med. Chem. Lett. 20: 537–540.

Rapado, L. N., A. S. Pinheiro, P.O.M.V. Lopes, H. H. Fokoue, M. T. Scotti, J. V. Marques, F. P. Ohlweiler, S. I. Borrely, C.A.B. Pereira, M. J. Kato, et al. 2013. Schistosomiasis control using piplartine against *Biomphalaria glabrata* at different developmental stages. PloS One Negl. Trop. Dis. 7: e2251.

Scott, J. G. 1999. Cytochrome P450 and insecticide resistance. Insect Biochem. Mol. Biol. 29: 757–777.

Scott, I. M., H. R. Jensen, B.I.R. Philogène, and J. T. Arnason. 2008. A review of *Piper s*pp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. Phytochem. Rev. 7: 65–75.

Siddiqui, B. S., T. Gulzar, A. Mahmood, S. Begum, B. Khan, and F. Afshan. 2004. New insecticidal amides from petroleum ether extract of dried *Piper nigrum* l. Whole Fruits. Chem. Pharm. Bull. 52: 1349–1352.

Sharma, V. P., and K. S. Rai. 1969. Histopathological and developmental effects of the chemosteril an tapholate, on the mosquito *Aedes aegypti.* Mosq. News 29: 9–14.

Silva, H.H.G., and I. G. Silva. 1999. Influência do período de quiescência dos ovos sobre o cicloevolutivo de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) em condições de laboratório. Rev. Soc. Bras. Med. Trop. 32: 349–355.

Silva, H.H.G., I. G. Silva, C.L.N.S. Oliveira, and C. N. Elias. 1999. Adaptação do *Aedes aegypti* (Linnaeus, 1762) em criadouros artificiais com água poluída. Entomol. Vectores 6: 383–391.

Silva, R. V., H.M.D. Navickiene, M. J. Kato, V. S. Haddad, C. I. Méda, and M.C.M. Young. 2002. Antifungal amides from *Piper arboreum* and *Piper tuberculatum.* Phytochemistry 59: 521–527.

Silva, I. G., V. P. Guimarães, C. G. Lima, H.H.G. Silva, C. N. Elias, and C. M. Mady. 2003. Efeito larvicida e toxicológico do extrato bruto etanólico da casca do caule de *Magonia pubescens* sobre *Aedes aegypti* (Diptera: Culicidae) em criadouros artificiais. Rev. Patol. Trop. 32: 73–86.

Simas, N. K. 2003. Análise química, biológica e biotecnológica de espéciesvegetais com atividade larvicida em *Aedes aegypti.* [Tese de Doutorado]. Universidade Federal do Rio de Janeiro. Rio de Janeiro, RJ.

Simas, N. K., E. C. Lima, R. M. Kuster, C.L.S. Lage, and A.M.O. Filho. 2007. Potential use of *Piper nigrum* ethanol extract against pyrethroid-resistant *Aedes aegypti* larvae. Rev. Soc. Bras. Med. Trop. 40: 405–407.

Sokal, R. R., and F. J. Rohlf. 1979. Principios y Metodos Estadísticos em La Investigación Biológica. H. Blume Ed., Madri, España. 223.

Teixeira, M. G., M. L. Barreto, M.C.N. Costa, L.D.A. Ferreira, P.F.C. Vasconcelos, and S. Caincross. 2002. Dynamics of dengue vírus circulation: a silent epidemic in a complex urban area. Trop. Med. Int. Health 7: 757–762.

Trindade, F.T.T., R. G. Stabeli, V. A. Facundo, C. T. Cardoso, M. A. Silva, L.H.S. Gil, I. S. Silva-Jardim, and A. A. Silva. 2012. Evaluation of larvicidal activity of the methanolic extracts of *Piper alatabaccum* branches and *P. tuberculatum* leaves and compounds isolated against *Anopheles darling.* Rev. Bras. Farmacog. 22: 979–984.

Vendramim, D. J. 2000. Plantas inseticidas e controle de pragas. Informativo da Sociedade Entomológica do Brasil 25: 1–5.

Waliwitiya, R., R. A. Nicholson, C. J. Kennedy, and C. A. Lowenberger. 2012. The synergistic effects of insecticidal essential oils and piperonyl butoxide on biotransformational enzyme activities in *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 49: 614–623.

[WHO] World Health Organization. 1970. Insecticide resistance and vector control. Technical Reports Series. 443.

[WHO] World Health Organization. 1997. Dengue bulletin: epidemic dengue/dengue haemorrhagic fever: global public health problem in the 21st century.

Yang, Y. C., S. G. Lee, H. K. Lee, M. K. Kim, S. H. Lee, and H. S. Lee. 2002. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J. Agric. Food Chem. 50: 3765–3767.

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