Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
ISSN 1984-6428

| Vol 7 | | No. 4 | | October-December 2015 |

Full Paper

Insecticidal Activity of $[Cu(H_2NTA)_2].2H_2O$ in *Aedes aegypti* Larvae (Diptera: Culicidae)

Alessandra Ramos Lima[a], Eduardo José de Arruda[a*], Cláudio Teodoro de Carvalho[a], Cícera Maria da Silva[a], Magda Freitas Fernandes[b], Mika Fumie Haiashi Kato[a], Isaías Cabrini[a*], Carlos Fernando S. Andrade[c]

[a]*Faculty of Exact Sciences and Technology, Federal University of Grande Dourados (UFGD), 79804-970, Dourados, Brazil.*
[b]*Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados (UFGD), 79804-970, Dourados, Brazil.*
[c]*Department of Animal Biology, Institute of Biology, State University of Campinas (UNICAMP), 13083-862, Campinas, SP, Brazil.*

Article history: Received: 04 May 2015; revised: 21 October 2015; accepted: 08 November 2015. Available online: 30 December 2015. DOI: http://dx.doi.org/10.17807/orbital.v7i4.735

Abstract: The demand for new insecticides and alternative strategies for the population control of *Aedes aegypti* has stimulated research to obtain new compounds with broad biological activity. Accordingly, the dihydrogen nitrilotriacetate complex of copper (II) dihydrate, $[Cu(H_2NTA)_2].2H_2O$, was synthesized by the stoichiometric reaction of nitrilotriacetic acid (H_3NTA) hydroxide with basic copper carbonate (II) ($Cu_2(OH)_2CO_3$) and characterized by the spectroscopic techniques UV-Vis and FT-IR. The biological toxicity in *A. aegypti* was determined by bioassay using concentrations ranging from 90.0 mg L^{-1} to 897.4 mg L^{-1}. The LC_{50} obtained was 146.11 mg L^{-1} [132.18–160.10] and the LT_{50} obtained at a concentration of 897.4 mg L^{-1} was 70.61 min [38.21–94.90]. The results showed that the complex obtained in this work is a potential metal-insecticide.

Keywords: copper; mosquitoes; metal-insecticide; biological activity; chemical control

1. INTRODUCTION

Aedes aegypti is the vector of dengue. Reported cases of this disease have reached epidemic proportions worldwide. Insecticides are a major strategy for population control of this vector and containment of disease transmission. However, the indiscriminate use of insecticides, application errors, short periods between applications, lack of assessment of the susceptibility of the target, and/or regular exchange of insecticide populations are the main factors leading to resistance and environmental problems [1]. The conjunction of the fact that the development of new insecticides that can be applied in public health is costly and that it demands long periods of research, as well as the resistance of insects to the old, previously efficient, insecticides, has led to a health crisis scenario [2]. This has stimulated the development of innovative research for the synthesis of substances with broad biological activity for vector control.

Cu(II) ions are micronutrients required for the metabolism of plants and animals and are considered a biological catalyst. The range of toxicity of these ions is narrow, and an excess or lack may cause pathological features. Metal complexation has been used as a way to decrease reactivity, enhance solubility and/or mobility, and facilitate metal removal in living tissues. In the class of chelating agents, aminopolycarboxylates, such as ethylenediaminetetra-acetic acid (EDTA), have been used as molecules to regulate the absorption of Cu(II) as its chelate complex [3]. Studies on the larvae of *A. aegypti* have shown the potential application of transition metals as active insecticides owing to their toxicity. The results showed that the coordination of such compounds, particularly with Cu(II), leads to the induction of physical and metabolic damage to mosquito larvae and eggs when administered at the median lethal concentration (LC_{50})

*Corresponding author. E-mail: ejarruda@gmail.com, isaiascabrini@gmail.com

of 33 mg L^{-1} [4]. Other studies have demonstrated that Cu(II) in the form of metal complex Na_2[Cu(EDTA)] produces satisfactory results with the same LC_{50} values as Cu(II) and 32.65 mg L^{-1} [5]. Thus, metal complexes can be proposed as a comprehensive activity of insecticides for immature insects. It is assumed that metals interfere with the food chain and in the reproductive process of insects, causing toxic effects and damaging multiplication of microorganisms in the breeding of insects.

In addition, metal complexation may potentiate the insecticidal activity from a binder with or without biological activity and facilitate the conduction of the metal ion in the vector body, promoting toxicity by oxidative stress. This situation causes the production of free radicals with the formation of oxidizing species in the target organism [3,6]. Cu(II)-induced hyperpolymerization of the cellular microtubules of *A. albopictus*, followed by cell aggregation and massive apoptosis. Cell death occurs by necrosis [7]. Extended 1st instar quiescence results in a significant decrease in lipid reserves and negatively affected larval fitness and development. AaMtn transcription and metal tolerance were compromised in the first instars emerging from eggs that had undergone an extended quiescence. These findings suggest that newly emerged mosquito larvae that had survived a relatively long pharate 1st instar quiescence (as might occur during a dry season) are more vulnerable to environmental stress [8].

Other studies using copper complexes also support the action of this material, for example, acetate-Cu (II), which has shown toxicity at LC_{50} 22.81 mg L^{-1} [9]. A recent study showed the insecticidal activity of four copper complexes: ([*Cu(phen)(L-Thr)(U)](ClO4)*], [*Cu(phen)(L-Thr)(Sc)](ClO4)*], [*Cu(phen)(L-Thr)(TU)](ClO4)*], and [*Cu(phen)(L-Thr)(H2O)](ClO4)*]. These compounds 1–4 were active, with LC_{50} varying in the range of 0.89 to 1.88 mg L^{-1} for larvae *Anopheles subpictus* and 0.61 to 2.09 mg L^{-1} for larvae *Culex quinquefasciatus*. All four complexes showed larvicidal activity, while complex 3–4 showed larvicidal activity for C*ulex quinquefasciatus* larvae at lower doses when compared with complexes 1–2. Complex 3 had better larvicidal activity for larvae *A. subpictus* and *C. quinquefasciatus* [10]. Such work is relevant in that it demonstrates that, in addition to the metal ion, the ligand (amino acids, alkaloids, colloidal complex, or other simple molecules) with biological activity can determine the range of toxicity and the intensity of damage to various metabolic sites of the target insect [11,12]. Bordeaux mixture demonstrated toxicity in *A. aegypti* larvae, with LC_{50} 3.06 mg L^{-1} (2.73–3.35) [13].

The studies demonstrate the potential of metal complexes of Cu(II) as the active compound feasible for insecticidal activity and low toxicity in humans and in the environmental [14]. Other transition metals, such as Cd(II) and Hg(II), also have insecticidal activity, but are toxic to human health and the environment. Furthermore, the molecular organization and non-toxic labile metal complexes for the environment can be better exploited as metal-complexing ligands that will provide various understanding of the mechanism of toxicity to the target organism, the permeation and/or transfer/induction via oxidative stress, which occurs mainly in the insect's digestive system, increasing insecticidal activity.

In this paper, the proposed synthesis and characterization of complex copper (II) and insecticide evaluation using *A. aegypti* was mainly concerned with implementing the production of a new substance that has broad biological activity for vector control.

2. MATERIALS AND METHODS

Synthesis of metal complex

The reagents used were nitrile triacetic acid (Sigma–Aldrich), basic copper carbonate hydroxide (II) (Sigma–Aldrich) analytical grade. The metal complex was synthesized using the methodology described by Dung and coworkers [15]. The acid–base reaction was performed in an aqueous medium with nitrilotriacetic acid (H_3NTA) hydroxide and basic copper carbonate ($Cu_2(OH)_2CO_3$) molar at a ratio of 4:1. After mixing the solution, it was heated to 60°C for 30 min and maintained at room temperature (25±2°C). It was then filtered and concentrated in a water bath at 40°C for crystallization. The product was stored in amber glass inside a desiccator to perform the characterization and toxicity bioassays.

Characterization of the metal complex

The absorption spectroscopy in the UV-Vis measurements were performed using Varian® spectrometer Cary model 50, to scan 200–800 nm. All measurements were performed in an aqueous medium at concentrations of 6.40 10^{-5} mol L^{-1} for the complex $[Cu(H_2NTA)_2].2H_2O$ and 1.70 10^{-5} mol L^{-1} for H_3NTA binder.

The absorption spectroscopy measurement in

the mid-infrared region (FT-IR) spectrometer were performed using Jasco®, FT-IR model 4100, with potassium bromide pellets (KBr) Scan range 400–4000 cm^{-1}.

Toxicity bioassays in *A. aegypti* larvae

Solutions in distilled water and different concentrations were prepared: 90.0 mg L^{-1}, 142.0 mg L^{-1}, 225.0 mg L^{-1}, 357.0 mg L^{-1}, 566.5 mg L^{-1} and 897.4 mg L^{-1}. For each concentration, four replicates of 20 larvae of the third stage of *A. aegypti* (Rockefeller strain) in 45 mL of solution. The negative control was distilled water; the positive control, Temephos® 0.012 mg L^{-1}. Mortality readings were recorded at two, four, eight and 24 h after application. The mortality criterion was no reaction of *A. aegypti* at the touch of a brush [16].

Statistical analysis

Data were analyzed using Bioestat 5.3 software for performing ANOVA. Comparisons between means were made by conducting Duncan's multiple comparison test. PC Polo software was used to conduct a Probit analysis to calculate the concentrations lethal and lethal time of 50% and 95% mortality of the larvae [17]. Results were considered significant at the level of $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

Synthesis and characterization of the metal complex

The metal complex, $[Cu(H_2NTA)_2].2H_2O$, was synthesized and purified by recrystallization. The material was obtained as crystalline blue needles with an 80% yield. This yield is satisfactory when compared with the values reported in the literature [18].

Figure 1 shows the UV-Vis spectrum for the complex and the ligand aminopolycarboxylic H_3NTA. The characteristic bands are observed in the 235 nm region for the complex, while the two bands are observed in the region of 207 nm and 250 nm for the binder (H_3NTA).

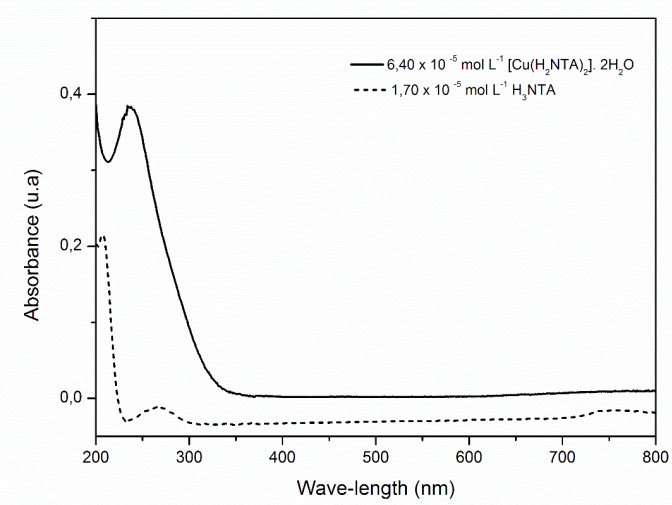

Figure 1. Absorption in the UV-Vis spectrum for the metal complex (—) $[Cu(H_2NTA)_2].2H_2O$ and ligand H_3NTA (----).

In the spectra of Figure 1, it can be seen that the band related to the complex showed a hipsocromic shift. This band, corresponding to the $n \rightarrow \pi^*$ transition, which is shifted to a smaller wavelength, was initially centered on 250 nm for the ligand. Additionally, a discrete signal, at 207 nm, characteristic of absorption of organic compounds groups, was present -COOR, -COOH and the complex was observed at 235 nm. The offset of the observed bands in the ultraviolet region to the complex relative to the ligand provides evidence for the formation of the metal complex with the carboxylate groups [18].

The interaction of metal carboxylate groups with the metal ion was evaluated through the symmetric and asymmetric stretching of these groups.

The comparative FT-IR spectra of the ligand (----), and the metal complex (___) is shown in Figure 2.

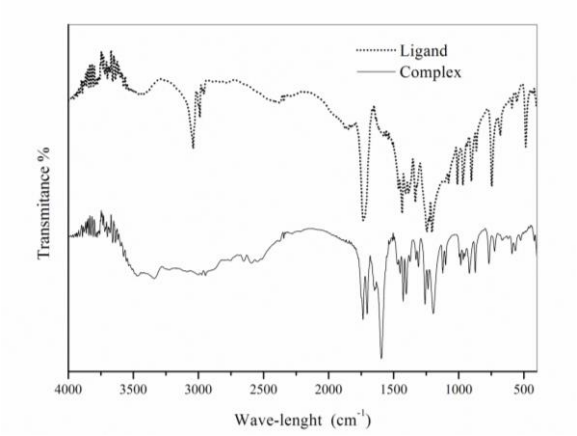

Figure 2. FT-IR comparative spectra of (----) the ligand (H_3NTA) and (—) for the metal complex $[Cu(H_2NTA)_2].2H_2O$.

In these spectra, it is possible to observe the stretches of the carbonyl groups (C=O), as well as other groups participating in the chemical bonding. Thus, the principal absorption bands of the ligand complex are summarized in Table 1 and compared to the values described in the literature [18].

The bands, as shown in Table 1, in the region of 1735 cm^{-1} and around 1434 cm^{-1}, can be assigned to asymmetric and symmetric stretching of the carboxylic group in the ligand, respectively, according to expected behavior in these groups [11,12]. According to Rajaballe [18], the carboxylate group coordinated with the Cu(II) ion has a characteristic stretching between 1650 cm^{-1} and 1620 cm^{-1}. These bands are absent in the infrared spectrum H_3NTA, confirming the previous assignment. For the complex observed the presence of two bands: one of medium intensity at 1647 cm^{-1} in the shoulder region and another shape in the region of 1638 cm^{-1} on the metal-ligand coordination. The bands in the regions of 1735 cm^{-1} and 1703 cm^{-1} suggest the presence of two carboxylic acid groups (R-COOH) of the binder that will probably not be coordinated, consistent with values found in the literature [12,18,19].

Such analyses suggest that the complex synthesized is consistent with the one obtained by Dung et al. [12]. They reported that the Cu(II) is coordinated by two $[H_2NTA]^-$ ligands, with one carboxylate and the amine group, and two water molecules, completing the coordination sphere [12,20].

Table 1. Assignment of the FT-IR spectra of the ligand (H_3NTA) and the metal complex $[Cu(H_2NTA)_2].2H_2O$.

Group	Ligand		Metal complex	
	n° reference wave	Experimental	n° reference wave	Experimental
-OH, (H_2O, -COOH)	2600–2100w	2703–2159w	3480sm	3469 m
	-	-	3320wm	334im
	-	-	2700–2500w	2693–2496w
-COO-H	1728s	1735s	1735s	1735s
	-	-	1700s	1702s
-COO-M	-	-	1640vs	1647vs
	-	-	1600sh	1638sh
$-COO^-$	-	-	1588vs	1694vs
$-COO^-$	1434s	1434s	1450s	1494s
	-	-	1428s	1425s
	-	-	1404s	1400s
$-COO^-$	1332vs	1333vs	1329wm	1328 m
	1320vs	1320vs	1310sm	1308 m
-C-N	-	1078vs	1122sm	1122sm
	-	-	1011 m	1011 m
	-	-	-	-
$-COO^-$	1010vs	1010 m	994w	992w
$-COO^-$	967vs	968vs	982 m	965w
	900vs	903vs	963w	963w
	-	-	950w	947w
	-	-	917sh	917sh

w = weak; m = moderate; s = strong; sh = shoulder; wm = weak moderate; sm = strong moderate; vs = very strong.

Toxicity Bioassays in *A. aegypti* larvae

The mean percentage mortality of the larvae of *A. aegypti* in different concentrations and exposure times are shown in Table 2. There was no mortality in the negative control but 100% mortality in the positive

control. The range of concentrations allowed average mortality rates between 1.25% (±2.16) for 2 h and 100% 24 h after exposure. An initial toxic effect of the complex is most noticeable only at concentrations above 200 mg L^{-1}, in which there was more than 50% mortality. However, after 4 h of exposure to the concentration of 566.5 mg L^{-1}, there was been an average mortality rate of over 90%, and 97.61% (±4.12) 24 h after exposure. At higher concentrations (897.4 mg L^{-1}), the insecticidal activity reached over 90% at 4 h exposure, reaching 97.67% (±2.33) at 8 h and 100% at 24 h. These results indicate that the insecticidal activity of the metal complex may be evaluated 8 h after exposure to the product, as we observed little increase in mortality at all concentrations, after this evaluation with 8 h of exposure.

The concentrations covered a characteristic mortality assessed interval for determining baseline sensitivity, in reference to which it is possible to calculate LC_{50} (Table 3). Statistical analysis showed a significant difference ($p < 0.05$) between the lethal concentrations for 2, 4, and 8 h after exposure; 4 h LC_{50} value was not within the confidence interval of 8 h LC_{50}. Already, the LC_{50} 8 h is within the confidence interval for the LC_{50} 24 h, with no significant difference. These results show that the toxic effect of the complex increases gradually up to 8 h after exposure, and does not change significantly after this period of exposure.

It is noted that the complexation of Cu(II) provides 50% mortality after 2 h of exposure from 142.5 mg L^{-1} and total mortality, only the highest concentration (897.4 mg L^{-1}) after 24 h, whilst in Temephós® (the positive control), larval mortality allows full for a much lower concentration (0.012 mg L^{-1}).

Table 2. Mean Percentage mortality (±SD) of *A. aegypti* larvae exposed to different concentrations of metal complex $[Cu(H_2NTA)_2].2H_2O$. Mortality readings between 2 h and 24 h.

	Concentration (mg L^{-1})					
Hours	90	142.5	225.8	357	566.5	897.4
2	1.25 (±2.16)	16.25 (±6.49)	53.45 (±16.47)	61.59 (±21.79)	63.4 (±1.95)	67.73 (±8.08)
4	6.19 (±2.20)	30.0 (±7.90)	76.6 (±15.36)	77.38 (±14.96)	92.6 (±5,45)	95.34 (±4.65)
8	11.01 (±6.17)	41.25 (±4.14)	88.92 (±11.43)	91.5 (±9.01)	94.98 (±5.02)	97.67 (±2.33)
24	12.26 (±5.27)	46.25 (±2.16)	93.92 (±4.05)	94.25 (±4.14)	97.61 (±4.12)	100

This difference may be related to the mechanism of toxicity of each active ingredient. The organophosphates act by inhibiting acetylcholinesterase (AChE), an important enzyme in the central nervous system. This enzyme is phosphorylated by the insecticide, being irreversibly inactivated. AChE inhibition results in the accumulation of acetylcholine in nerve junctions (or synapses), which prevents disruption of electrical impulse propagation. Consequently, the central nervous system will continue to be stimulated, triggering the process of paralysis, which can lead to insect death [1]. Cu(II) initially acts in the insect's digestive system, by destroying the peritrophic matrix (PM), causing continuous cell damage by oxidative stress. In chemical terms, oxidative stress represents a significant increase in cell reduction potential, or a significant decrease in the reducing capacity of cellular redox couples, such as glutathione [21]. The effects of oxidative stress depend on the intensity of these variations. Generally, a cell is able to overcome the adverse effects of oxidative stress through the oxidation mechanism, if the disturbance is not sharp, resulting in the restoration of intracellular equilibrium. However, higher disorders may lead to death, apoptosis, and even cell necrosis [22]. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species such as free radicals and oxidizing species (peroxides). Some of these species with low reactivity, such as the superoxide anion, can be converted into more reactive species through redox reactions involving transition metals or other species

capable of varying its oxidation state (such as quinones), species which can cause severe cell damage [23]. Most of these species are produced through the reduction of molecular oxygen, which is produced in small amounts by aerobic metabolism and any damage is repaired steadily. However, in extreme conditions of oxidative stress, damage causes depletion of ATP levels, which prevents a controlled cell apoptosis and causes total failure of cell operation, causing necrosis [24,25].

Metals such as iron, copper, chromium, vanadium, and cobalt can undergo redox cycling in which a single electron is accepted or donated by a metal ion. This action is found in reactions that produce radical species and consequently produce reactive oxygen species. The most important reactions are the reactions of Fenton and Haber-Weiss, wherein the radical production from the iron in the reduced state (Fe(II)) is hydrogen peroxide. The hydroxyl radicals can cause changes in amino acids (for example, ortho- and meta-forming tyrosine from phenylalanine); carbohydrates and lipid peroxidation start to oxidize nitrogen bases. The presence of such metals in biological systems, complexed to proteins or other protective molecules, can significantly increase levels of oxidative stress [24,25,26].

Table 4 presents lethal concentrations when exposed to the median length of time (LT_{50}). The analysis shows that the toxic effect of the three highest concentrations provides significant mortality within 2 h. This is an important factor in the analysis of insecticide substances, for use in the event of the metal-insecticide for control of immature forms of A. aegypti. A short time to toxic effect make it possible to obtain faster mortality results with consequent effect on adult vector population. This is one of the goals in dengue epidemic programs control. The speed of insecticidal effect is probably related to permeation of the complex and plasma membrane lipid solubility and/or to permeation of cell channels. The complex $[Cu(H_2NTA)_2].2H_2O$ is adduced more efficiently first to the surface of the larvae then permeation/complexed metal transport into cells to induce oxidative stress and the production of radicals and oxidant species to cell damage in the digestive system of the larvae [19]. Statistical analysis showed a significant difference ($p < 0.05$), except for the two highest values of the comparisons, confirming that each concentration exerts a toxic effect to cause 50% mortality at various time intervals after exposure of the larvae of A. aegypti.Na Figura 2 estão representados os valores dos efeitos no rendimento de adsorção de Cr (VI). Uma mudança entre os níveis mínimo e máximo das variáveis *pH*, *S*, *P*, *m*, *C*, *t* e *v*, resulta em variações de, respectivamente, –3,95; +18,31; –10,74; +13,88; –21,46; +7,08 e +26,67% na eficiência de remoção.

Table 3. Lethal concentrations: median (LC_{50}) and confidence intervals ($CI_{0.05}$) for different periods of exposure of *A. aegypti* larvae to metal complex $[Cu(H_2NTA)_2].2H_20$.

Time (h)	LC_{50} ($CI_{0.05}$)	B ± SD	χ_2	df
2	358.25 (298.45–437.38)	2.02 ± 0.088	167.76	22
4	198.83 (178.14–220.18)	3.24 ± 0.013	103.91	22
8	158.45 (137.74–179.16)	3.67 ± 0.134	163.93	22
24	146.11 (132.18–160.10)	4.66 ± 0.180	121.38	22

b = slope SD = Standard Deviation df = degrees of freedom.

Table 4. Median lethal time (LT_{50}) (minutes) and confidence intervals ($CI_{0.05}$) of metal complex $[Cu(H_2NTA)_2].2H_2O$ in *A. aegypti* larvae.

Concentration (mg L^{-1})	LT_{50} ($CI_{0.05}$)	B ± SD	χ^2	df
142.5	1,058.37 (718.37–2,087.40)	1.00 ± 0.190	7.27	14
225.8	353.47 (251.14–479.04)	1.16 ± 0.199	5.52	12
357.0	109.88 (26.11–194.81)	0.83 ± 0.220	3.02	9
566.5	86.0 (32.18–139.21)	1.08 ± 0.21	7.02	13
897.4	70.61 (38.21– 94.90)	2.72 ± 0.54	10.78	14

b = slope SD = Standard Deviation df = degrees of freedom.

4. CONCLUSION

$[Cu(H_2NTA)_2].2H_2O$ was synthesized with and characterized by FT-IR and UV-Vis spectroscopies. The FT-IR indicates coordination via the carboxylate and amine group, consistent with the literature [18]. Analysis of the UV-Vis shows two bands in the region of 207 nm and 250 nm for the binder (H3NTA) characteristic absorption of compounds -COOR, COOH groups, but in the complex, it is shifted to 235 nm.

The bioassays showed that the toxicity was concentration-dependent, and that there was a reduction in the time to mortality of *A. aegypti* larvae (Rockefeller strain). The results also showed that the metal complex is most likely to be better developed and proposed as a metal-insecticide for the management of the larvae in the breeding of *A. aegypti*. This study indicates the need to carry out new experiments on toxicity to obtain LCs involving field populations and with testing in semi-natural conditions.

5. ACKNOWLEDGMENT

FUNDECT, CAPES/PROCAD, CNPq, UFGD, PIVIC/CNPq, GOA-Group of Applied Optics/Molecular Characterization Laboratory.

6. REFERENCES AND NOTES

[1] Braga, I. P.; Valle, D. *Epidem. Serv. Saúde*, **2007**, *16*, 113.

[2] IVCC - Liverpool School of Tropical Medicine. Accelerating development of new insecticides can save thousands of lives. Available from: http://www.ivcc.com/news-and-media/blog/accelerating-development-of-new-insecticides-can-save-thousands-of-lives. Access March, 2015.

[3] Blum, C. S. E.; Behrens, B. N. *Coord. Chem. Rev*, **2000**, *196*, 3. [CrossRef]

[4] Rayms-Keller, A.; Olson, K. E.; McGaw, M.; Oray, C.; Carlson, J. O.; Beaty, B. J. *Ecotoxicol. Environ. Saf*, **1998**, *39*, 41. [CrossRef]

[5] Arruda, E. J.; Rossi, A. P. L.; Porto, K. R. A.; Oliveira, L. C. S.; Arakaki, A. H.; Silva, L. C.; Scheidt, G. N.; Roel, A. R. *Braz. Arch. Biol. Technol.* **2011**, *53*, 335. [CrossRef]

[6] Lelli, J.; Becks, L.; Dabrowska, M.; Hinshaw, D. *Free Radical Biol. Med.* **1998**, *25*, 694. [CrossRef]

[7] Raes, H.; Braeckman, B. P.; Criel, G. R.; Rzeznik, U.; Vanfleteren, J. R. *J. Exp. Zool*. **2000**, *286*, 1.

[8] Perez, M. H.; Noriega, F. G. *J. Insect Physiol*. **2012**, *58*, 824.

[9] Nardeli, J. V.; Arruda, E. J.; Carvalho, C. T.; Vila Nova, P. C. C.; Cabrini, I.; Arakaki, A. H. *Orbital: Electron. J. Chem.* **2014**, *6*, 122. [Link]

[10] Gopinathan, H.; Arumugham, M. N. *Journal of Taibah University for Science*, **2015,** 9, 27. [CrossRef]

[11] Arruda, W.; Oliveira, G. M. C.; Silva, I. G. *Rev. Patol. Trop.* **2008**, 37, 191. [CrossRef]

[12] Valoto, C. F. B.; Cavasin, C.; Silva, H. H. G.; Geris, R.; Silva, I. G. *Rev. Pat. Trop.* **2010**, *39*, 309.

[13] Silva, A. K.; Arruda, E. J.; Fonseca, G. G.; Carvalho, C. T.; Silva, C. M.; Vila Nova, P. C. C.; Gaban, C. R. G.; Cabrini, I. J. *Mosq. Res.* **2015**, 5. [CrossRef]

[14] Campos, M. L.; Silva, F. N.; Furtini Neto, A. E.; Guilherme, L. R. G.; Marques, J. J.; Antunes, A. S. *Pesq. Agropec. Bras.* **2005**, *40*, 361.

[15] Dung, N. H.; Viossat, B.; Busnot, A.; Perez, J. M. G.; Garcia, S. G.; Gutiérrez, J. N. *Inorg. Chem.* **1998**, *27*, 1227. [CrossRef]

[16] Campos, J.; Andrade, C. F. S. *Caderno Saúde Pública*, **2002**, *18*, 661. [CrossRef]

[17] Leora Software. (1987). POLO-PC: a user's guide to Probit or Logit analysis. LeOra Software, Berkeley, CA.

[18] Rajaballe, F. J. M. *J. Inorg. Nucl. Chem.* **1974**, 36, 557. [CrossRef]

[19] Benite, A. M. C.; Machado, A. P.; Barreiro, E. J. *Quim. Nova* **2007**, *30*, 2062. [CrossRef]

[20] Whitlow, S. H. *Inorg. Chem*. **1973**, *12*, 2286. [CrossRef]

[21] Lennon, S. V.; Martin, S. J.; Cotter, T. G. *Cell Proliferation* **1991**, *24*, 203. [CrossRef]

[22] Valko, M.; Morris, H.; Cronin, M. *Med. Chem.* **2005**, *12*, 1161. [CrossRef]

[23] Lee, Y.; Shacter, E. *J. BiolChem*. **1999**, *274*, 19792.

[24] Stohs, S, J.; Bagchi, D. *Free Radicals Biol. Med.* **1995**, *18*, 321. [CrossRef]

[25] Halliwell, B.; Gutteridge, J. M. C. *Arch. Biochem. Biophys.* **1990**, *280*, 1. [CrossRef]

[26] Halliwell, B. *The American Journal of Medicine* **1991**, *14*. [CrossRef]