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Determination of the larvicidal activity of benzoyl thiosemicarbazone and its Ni(II) complex against *Aedes aegypti* and *Anopheles darlingi* larvae in Amazonas, Brazil

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Due to the resistance of some mosquitoes to pyrethroids insecticides, new synthetic compounds are of great interest for the development of new insecticides against vectors of tropical diseases, especially in the Amazon region. Our aim was to synthesize and evaluate the larvicidal potential of benzoyl thiosemicarbazone and its Ni(II) complex against larvae of *Aedes aegypti*..... and *Anopheles darlingi*..... The compounds were synthesized from thiosemicarbazide according to the literature, and the larvicidal potential was evaluated in triplicate at concentrations of 7 to 500 µg/mL. Benzoyl thiosemicarbazone and its Ni(II) complex showed an LC_{50} of 42.09 and 42.28 µg/mL, respectively, against . *Ae. aegypti* larvae. For .*An. darlingi* larvae, the LC_{50} values of benzoyl thiosemicarbazone (4.77 µg/mL) were lower than its Ni(II) complex (7.33 µg/mL). Benzoyl thiosemicarbazone presented satisfactory results against the larvae, and due to the insecticidal potential of this substance, the development of new chemical insecticides may be possible.

Key words: Benzoyl thiosemicarbazone, nickel (II), larvicidal activity; *Aedes aegypti*, *Anopheles darlingi.*

INTRODUCTION

Many pathogens can be transmitted to humans from infected mosquitoes such as *Aedes aegypti* (Linnaeus, 1762), a species responsible for the transmission of dengue fever, yellow fever, Chikungunya and Zika. In Brazil, this vector is one of the main public health problems, since it is extremely urban, has high population growth rates and is difficult to control (Simon et al., 2008; Puccioni-Sohler et al., 2017).

Anopheles darlingi (Root, 1926) is of great medical relevance, as it is the vector responsible for the transmission of Malaria in Brazil, where it is basically confined to the Amazon region. Among the anopheline mosquitoes found in the region, *A. darlingi* is the species that most benefits from human modifications to the

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environment. It is highly anthropophilic and endophagic (Deane, 1986; Tadei et al., 1998; Maciel-de-Freitas et al., 2012; Sinka et al., 2012). In this case, the control of mosquito populations is performed with insecticides, which, despite numerous records of resistance and high toxicity to nontarget organisms, still provide one of the most effective methods for combatting mosquitoes in endemic areas (Rivero et al., 2010; WHO, 2017).

Thiosemicarbazones belong to the thiourea group, an important class of N and S donor compounds that have high pharmacological potential, and in many cases, the mechanism of action of thiosemicarbazone is associated with complexed transition metals. From a biological point of view, metal complexes are more bioactive than free ligands, particularly thiosemicarbazones, which are active only when complexed with transition metals (Wasi and Singh, 1987; Rosu et al., 2010; Viñuelas-Zahínos et al., 2011; Netalkar et al., 2015).

The pharmacological applications of the different structural derivatives of thiosemicarbazones and their metal complexes include antifungals (Parrilha et al., 2011), cytotoxics (Rebolledo et al., 2005; Braga et al., 2016), antibacterials (Despaigne et al., 2010), antimalarials (Greenbaum et al., 2004; Chellan et al., 2010; Nandal and Deep, 2017) and insecticides (Rayms-Keller et al., 1998; Wang et al., 2010; Silva et al., 2015). Metal complexes or even free metal ions are toxic to aquatic organisms and may be found available at low levels in the environment (Arnold et al., 2005). Thus, due to the high incidence of vector mosquitoes in the region, the permanent and semi-permanent expressions of *Aedes* and *Anopheles* mosquitoes in the urban areas of cities and control actions on immature forms represent a functional alternative for the control of insects (WHO, 2014).

The synthesis and biological activity of the 1-benzoyl analogue have been reported in the literature (Xue et al., 2007; Pingaew et al., 2010). In the present study, we examined the larvicidal activity of benzoyl thiosemicarbazone and its nickel (II) complex in bioassays against *A. aegypti* and *A. darlingi*, for the control of tropical diseases in the Amazon. Our study addresses the process for obtaining the substance and its nickel complex as well as their larvicidal activity against vectors of dengue and malaria in the Amazon region not yet described in the literature.

MATERIALS AND METHODS

Synthesis of Benzoyl thiosemicarbazone (HBzS) and the nickel metal complex [Ni(BzS)2]

All reagents were purchased from the Sigma-Aldrich and used without further purification. The compounds were synthesized (Beraldo et al., 1997 and Pingaew et al., 2010). The benzoyl thiosemicarbazone was prepared using 11 mmols of thiosemicarbazide for 11 mmol of benzaldeyde. The mixture was heated under reflux for 8 h and ethanol as a solvent. The nickel metal complex was prepared using 2 mmols of Benzoyl thiosemicarbazone for nikel chloride II heated under reflux and drops of amonium hidroxide in ethanol. The FT-IR spectra (KBr) were recorded on Perkin Elmer 283B (4000–400 cm^{-1}) spectrometer. The 1H NMR and 13C NMR were obtained on a Unity Inova 500 Varian spectrometer, em DMSO-d6.

Synthesis of compounds

HBzS: yellow crystals; yield (%): 70; melting point (°C): 205; IR (KBr, cm-1): v (C=N): 1575; v (C=S):857; v (N-H): 3176; v (N-H2): 3052. 1H NMR (500 MHz, DMSO-d6) (δ): 8.71 (s, 1H), 7.89 – 7.87 (dd, 2H, J: 2 Hz), 7.52 – 7.48 (m, 2H J: 5 Hz), 7.45 – 7.40 (m, 1H, J: 7.5Hz), 6.85 (s, 3H), 3.40 (s, 2H). 13C NMR (500 MHz, DMSO-d6) (δ):184.14; 161.44; 133.78; 131.37; 128.92; 128.36; 125.76; 76.95.

[Ni(BzS)2]: green solid; yield (%): 65; melting point (°C): > 300; IR (KBr, cm-1): v (C=N): 1575; v (C=S): 751; v (Ni-N): 497; v (Ni-S): 448; v (N-H): 2950; v (N-H2): 3052. 1H NMR (500 MHz, DMSO-d6) (δ): 8.71 (s, 1H), 7.89 - 7.86 (dd, 3H, J: 2 Hz), 7.52 – 7.48 (m, 2H, J: 5Hz), 3.34 (s, 2H). 13C NMR (500 MHz, DMSO-d6) (δ): 162.12; 134.49; 132.05; 129.61; 129.06; 127.98.

The formation of all compounds was confirmed by IR spectroscopy, 1H NMR and 13C NMR. The IR spectrum of Benzoyl thiosemicarbazone showed absorption bands at 3052, 1575 and 857 cm^{-1}, corresponding to the NH, C=N and C=S groups, respectively. The IR spectrum of the nickel metal complex showed absorption bands at 3052-2950, 1625, 751, 497 and 448 cm^{-1}, corresponding to the NH, C=N, C=S, Ni-N and Ni-S groups, respectively. Compounds showed a sharp singlet observed at δ 8.70 and 8.71, which confirmed the presence of the NH to HBzS and [Ni(HBzS)2], H-aromatic rings at δ 7.42 – 7.87 and δ 7.50-7.89, respectively. The 13C NMR spectra of compounds showed peaks at δ 193.24 and δ 161.33, corresponding to C=S carbon to HBzS and [Ni(HBzS)2], respectively. The above values are evident for formation of compounds (Figure 1).

Study site and period

The study was conducted in 2011 in the city of Manaus, Amazonas State, Brazil (-3.096240 latitude, -59.986194 longitude), located in the northern region of the country, which comprises the Brazilian Amazon region. Manaus has a population of 1,802,014 people according to the last census of 2010.

Mosquito collection and maintenance

The larvae were obtained from the insectarium of the Laboratory of Malaria and Dengue, National Institute of Amazonian Research (Instituto Nacional de Pesquisas da Amazônia - INPA), located in Manaus. The larvae remained in trays and were fed with TetraMin® (fish feed); the adult population was kept in cages with cotton soaked in 10% sucrose solution, whereas the females were also fed blood every other day for egg development. Plastic cups with moistened filter paper strips were provided for oviposition by pregnant females. The population was maintained under laboratory conditions of 26 ± 2°C and 70-80% relative humidity.

Larvicidal activity assays

Benzoyl thiosemicarbazone and its Ni(II) complex were synthesized and characterized according to Beraldo et al. (1997) and Pingaew et al. (2010). In the assays, the compounds were dissolved in

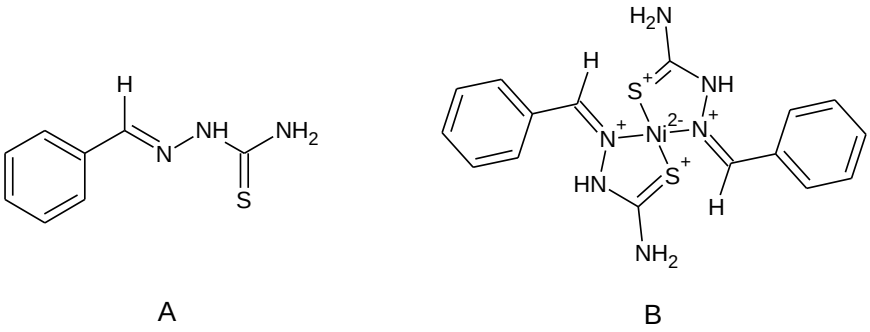

Figure 1. Benzoyl thiosemicarbazones (A) and Nickel complex II (B) structure.

Table 1. Larvicidal bioassays of benzoyl thiosemicarbazone (1) and nickel (II) complex (2) against *A. aegypti* larvae.

A. aegypti				
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 24 h**	**Number of larvae**	χ^2
Benzoyl thiosemicarbazone	y = -1.73 + 4.14 * log x	42.09 (25.94, 66.05)	50	5.97 s
Nickel complex (II)	y = 0.54 + 2.74 * log x	42.28 (23.83, 66.48)	50	4.91 s
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 48 h**	**Number of larvae**	χ^2
Benzoyl thiosemicarbazone	y = -1.73 + 4.24 * log x	38.49 (24.02, 60.08)	50	5.82 s
Nickel complex (II)	y = 0.28 + 3.03 * log x	35.84 (28.52, 44.02)	50	2.16 s

x: concentration; y: probability of mortality; s = significant; χ^2 = chi-square; CI: confidence interval.

dimethyl sulfoxide (DMSO) and evaluated at concentrations of 15.6 to 500 µg/mL against *A. aegypti* larvae and from 7.8 to 250 g/L against *A. darlingi* larvae. The assays were performed in triplicate in plastic cups containing 10 mL of distilled water, 10 larvae, feed and 100 µL of the evaluated concentration. Control was performed with DMSO. After 24 and 48 h, the number of dead larvae was recorded, and the median lethal concentration (LC_{50}) was calculated (Dulmage et al., 1990; WHO, 2005).

Statistical analysis

The results were considered acceptable when the control mortality was less than 10%, and the number of dead larvae in the control was adjusted using the Abbott formula (Abbott, 1925). Mortality data were assessed by probit analysis (Finney, 1971). According to the regression equation, the probability of mortality value corresponds to the y-axis, whereas the tested concentration corresponds to the x-axis. To obtain the LC_{50}, we selected the concentrations that presented larval mortality above 50%. We used the Polo Plus software (Robertson et al., 2003) and a 95% confidence interval (CI); results with $p<0.05$ were considered significant.

RESULTS

The larvae of both species tested were sensitive to benzoyl thiosemicarbazone and to the metal nickel complex after 24 and 48 h of exposure. Table 1 shows that *A. aegypti* larvae were more susceptible to the benzoyl thiosemicarbazone than the nickel complex. The LC_{50} for the benzoyl thiosemicarbazone was 42.09 µg/mL, whereas for the nickel complex, it was 42.28 µg/mL. However, after 48 h, the metal complex showed the highest toxicity, with an LC_{50} of 35.84 µg/mL. In this case, the benzoyl thiosemicarbazone presented an LC_{50} of 38.49 g/L.

A. darlingi larvae were more susceptible than *A. aegypti* larvae for the compounds tested. LC_{50} values for *A. darlingi* were lower at both the 24 and 48 h intervals (Table 2). Benzoyl thiosemicarbazone showed an LC_{50} of 4.77 µg/mL, whereas the nickel complex showed an LC_{50} of 7.33 µg/mL, both after 24 h. After 48 h, the LC_{50} of the metal complex was lower (2.68 µg/mL) than the LC_{50} of benzoyl thiosemicarbazone (2.72 µg/mL). The substance benzoyl thiosemicarbazone presented higher toxicity for both species tested after 24 h. Due to the sensitivity of the larvae, *A. darlingi* was more susceptible than *A. aegypti* larvae.

DISCUSSION

The present study addresses the larvicidal activity of a thiosemicarbazone derivative and its Ni complex against *A. aegypti* and *A. darlingi*, species responsible for the

Table 2. Larvicidal bioassays of benzoyl thiosemicarbazone (1) and nickel (II) complex (2) against *A. darlingi* larvae.

A. darlingi				
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 24 h**	**Number of larvae**	χ^2
Benzoyl thiosemicarbazone	y = 3.44 + 2.30 * log x	4.77 (2.76, 6.79)	30	1.55 s
Nickel complex (II)	y = 3.40 +1.85 * log x	7.33 (4.42, 10.66)	30	5.75 s
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 48 h**	**Number of larvae**	χ^2
Benzoyl thiosemicarbazone	y = 3.73 +2.22 * log x	2.72 (0.61, 4.61)	30	1.78 s
Nickel complex (II)	y = 3.83 +1.78 * log x	2.68 (0.60, 4.48)	30	6.78 s

x: concentration; y: probability of mortality; s = significant; χ^2 = chi-square; CI: confidence interval.

transmission of dengue and malaria, respectively, in the Amazon region. Although the biological properties of the metal complexes of thiosemicarbazones have high toxicity associated with the free ligand (Mendes et al., 2006; Netalkar et al., 2015), we observed that after 24 h, the metal nickel complex was less toxic; that is, this complex displayed a higher lethal concentration against *A. aegypti* (LC_{50} 42.28 µg/L) and *A. darlingi* (7.33 µg/L) than benzoyl thiosemicarbazone, which displayed an LC_{50} of 42.09 and 4.77 µg/mL, respectively.

After 48 h, however, the nickel complex showed better toxicity results, with an LC_{50} of 35.84 µg/mL for *A. Aegypti* and 2.68 µg/mL for *A. darlingi*. After the same period, benzoyl thiosemicarbazone presented an LC_{50} of 38.49 µg/mL for the first species and 2.72 µg/mL for the second.

Gopinathan and Arumugham (2015) evaluated the larvicidal activity of four Cu(II) metal complexes against *Culex quinquefasciatus* (LC_{50} 0.61 to 2.09 mg/L) and *Anopheles subpictus* (LC_{50} 0.89 to 1.88 mg/L). Although all complexes showed high toxicity, urea and thiourea complexed with copper presented the best larvicidal activity results when compared to thiosemicarbazide and semicarbazide. Thiosemicarbazide derivatives showed a broad spectrum of larvicidal activity at different concentrations.

Rayms-Keller et al. (1998) showed that metal ions were highly toxic to *A. aegypti*. For example, copper edetate in nanostructures and chitosan microcapsules showed efficacy against *A. aegypti* larvae, with an LC_{90} of 60 and 20 mg/L, respectively, because nanostructures and microcapsules favour the slow and continuous release of the active ingredient to the environment. Thus, when complexed to the nickel, thiosemicarbazone derivative tested here against *A. aegypti* and *A. darling* showed high toxicity. However, the results of this study indicate that the metal-ligand bond did not significantly favour larvicidal activity at all reading ranges, as observed for the assays against *A. darlingi* (Table 2); that is, the metal complex did not directly affect the simultaneous ion exchange in the biological system due to the reactivity and especially the redox effect caused by transition metals in biological systems (Stohs and Bagchi, 1995; Nguyen et al., 2000).

Beraldo and Gambino (2004) and Al-Amiery et al. (2012) described a series of thiosemicarbazone derivatives and metal complexes with different chemical and biological properties and highlighted the high biological potential of the metal complex relative to the free ligand. The compounds synthesized and evaluated against mosquito larvae in the present study need to be evaluated for toxicity against nontarget insects and especially regarding the accumulation of heavy metals in the environment, which requires special treatment for their removal (Mireji et al., 2010). The search for new active compounds is challenging because of the many cases of insects resistant to the insecticides currently used in vector control campaigns (Rose, 2001). Integrated mosquito management programmes targeting larvae and mosquitoes serve as one of the most effective ways to control insect populations and consequently reduce the number of vector-borne diseases in endemic areas.

Conclusions

Benzoyl thiosemicarbazone and its Ni(II) complex showed larvicidal activity against the larvae of *A. aegypti* and *A. darlingi*, indicating that the thiosemicarbazone metal complex has insecticidal potential. However, elucidating the mode of action of these compounds in larvae and developing new compounds with pharmacological potential are necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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