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BIOLOGICAL SCIENCES

Effects of diflubenzuron on associated insect fauna with *Anopheles* (Diptera: Culicidae) in laboratory, partial-field, and field conditions in the Central Amazon

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Abstract: This study aimed to analyze the possible effects of diflubenzuron on the associated insect fauna under laboratory, semi-field and field conditions. Laboratory bioassays were performed in aquariums with mortality readings (%) every 24h until 96h, and in semi-fields, insects were kept in basins with readings every 24h for up to 12 days, in triplicates and a control. In the field experiment, a collection was performed before the application of diflubenzuron in ten brick factory pits (25m²) and 15 post-application. The values of LC_{50} and LC_{90} for *Chironomus* were 2.77x10-3g/L and 0.86g/L, respectively, and for *Buenoa*, they were 0.019g/L and 0.92g/L, a strong relationship was observed between mortality and exposure time (r^2>0.8) in all concentrations used. In semi-field, similar mean values of emergency inhibition were observed, except for Euthyplociidae, which was more sensitive. There was no significant difference between the data of richness and diversity of aquatic insects in the field experiment, considering an interval of 15 days (p=0.32). Finally, the data suggest that diflubenzuron may have a negative effect on the associated insect fauna, but in the field experiment the environmental conditions of *Anopheles* breeding sites may have affected its efficiency.

Key words: Amazonia, *Chironomus* sp., Chitin, *Anopheles*.

INTRODUCTION

Insects integrate the aquatic macroinvertebrates community, as they play important roles in nutrient cycling within an ecosystem, by converting plant matter into animal tissue, and are energy sources for other trophic levels (Merritt et al. 2008, Albertoni & Palma-Silva 2010). In addition, some insect groups such as Ephemeroptera, Plecoptera, and Trichoptera serve as bioindicators of environmental impacts (Al-Shami et al. 2013, 2014, Wandscheer et al. 2017).

The immature forms of *Anopheles darlingi* Root, 1926 which are the principal vectors of Malaria in Brazil, develop in fresh water and their population density peaks of winged forms is related to the annual rain cycle, as the availability of breeding grounds increases. Such breeding grounds are classified as natural or artificial in the Amazonian environment, and house several other insect groups, which are referred to as the associated insect fauna during mosquito control campaigns (Ferreira et al. 2015, Tadei et al. 2017).

Controlling anopheline larvae is essential to decrease the density of adult anophelines

and, consequently, the number of Malaria cases. Therefore, control strategies, which usually use organophosphates, carbamates, and pyrethroids with neurotoxic action, are necessary (Braga & Valle 2007). However, such compounds are responsible for environmental damage and the development of resistant mosquito populations (Bellinato et al. 2016, Hemingway et al. 2004). Furthermore, these compounds present high residual power, remaining in environments for long periods of time, and wide action spectrum, reaching other organisms, including vertebrate, in aquatic environments (Wilke et al. 2009).

Currently, biological control is usually recommended for natural breeding sites, consisting of biotic agents that act on immature insect forms to reduce the density of disease vectors (Ferreira et al. 2015). In this context, the conservation of natural enemies that act as larvae predators and the entomopathogenic bacteria - *Bacillus thuringiensis* var. *Israelis* (Bti) and *Lysinibacillus sphaericus* - can potentially combat the immature forms of vector mosquitoes (Lobo et al. 2018, Rodrigues et al. 2013, Soares-da-Silva et al. 2015).

However, the use of chemical insecticides is indispensable for certain environmental situations and, in this context, growth regulators gain prominence (Martins & Silva 2004). Such insecticides cause physiological and morphological modifications during insect development, in addition, they have low toxicity for vertebrates and are authorized by the World Health Organization (WHO) for drinking water (Lyra et al. 1998, WHO 2006, 2007).

The morphological modifications to insects are caused by the mode of action of these insecticides, which target the biosynthesis of chitin. In arthropods, chitin is essential for the formation of the exoskeleton, growth, and development of the individual (Merzendorfer & Zimoch 2003). In addition, chitin is a fundamental constituent for the formation of the peritrophic matrix, making the interruption of biosynthesis an attractive target for the development of effective and safe insecticides, since mammals do not biosynthesize chitin (Cohen 2001, Merzendorfer 2006).

Diflubenzuron is an insect growth regulator and acts on Culicidae by interrupting larvae development, causing death during ecdysis, preventing the elimination of the old cuticle, or causing the new cuticle to no longer support the pressure exerted by the muscles, which can allow animals to survive for some time until death (Mulla 1979, Mulla & Darwazeh 1975, 1979). In addition, diflubenzuron may also reduce adult emergence by interrupting the individual's life cycle during the transition between pupa and adult phases (Borges et al. 2012, Costa & Tadei 2012, Mulla 1995).

In Brazil, this insecticide started to be used in 2009 and receives special attention in mosquito control programs where mosquitoes have become resistant to traditional insecticides such as Temephós® (Bellinato et al. 2016, Hemingway & Ranson 2000). Severeal studies have proven the efficiency of diflubenzuron in the field and laboratory conditions on the most globally important mosquito genera: *Aedes* (Borges et al. 2012, Flacio et al. 2015, Machado et al. 2015, Rocha et al. 2015), *Anopheles* (Costa & Tadei 2011, 2012, Zhu et al. 2007) and *Culex* (Msangi et al. 2011, Sadanandane et al. 2012, Zahran et al. 2013).

In the Amazon, the anophelines develop in water collections composed of river basins, permanent and semi-permanent lakes, environments classified as natural breeding sites (Forattini 2002). However, some species of anophelines are adapted to develop in anthropically altered environments, such as fish ponds and clay pits, classified as artificial breeding sites (Arcos et al. 2018). According to

Ferreira et al. (2015), the associated insect fauna present in artificial breeding sites of the fish ponds type is composed of six orders and 23 families, among which stand out Chironomidae and Notonectidae.

Notonectids are small predators that inhabit several types of aquatic ecosystems and, in the Amazon, the genus *Notonecta* and *Buenoa*, can be found in standing water reservoirs with little or moderate amount of aquatic plants, typical characteristics of the artificial breeding sites of *Anopheles darlingi* (López Ruf et al. 2003). In addition to predatory aquatic insects, breeding sites have benthic insects represented by the orders Diptera (Chironomidae), Ephemeroptera and Trichoptera, which act to recycle nutrients and integrate the trophic chain into the aquatic environment, serving as food for fish, amphibians and birds. (Trivinho-Strixino & Strixino 1995, Ayres-Peres et al. 2006).

Aquatic insects are among the most suitable organisms to estimate environmental impacts since they have abundant populations and are distributed in various microhabitats (Callisto & Gonçalves 2005, Rosenberg & Resh 1993). Benthic insects live in the sediment and tend to accumulate pesticide residues, making them potential indicators of environmental change (Martins et al. 2014).

Such changes in the environment often occur due to the implementation of control strategies. The control of mosquito vectors involves strategies for reducing the density of larvae in endemic regions where insecticide chemicals are applied in the environment and threaten other coexisting aquatic organisms.

In view of the importance of the associated insect fauna for the aquatic ecosystem, it is necessary that studies be performed to investigate the effects of insecticides frequently used for the control of Culicidae in the environment. The possible impacts on the associated insect fauna with *Anopheles* in aquatic ecosystems can compromise the survival of higher trophic levels, since insects make up the base of the food chain in this type of environment. In addition, some taxa are known to be predators of Culicidae larvae, promoting a natural control of mosquito vectors, such as the Notonectidae of the genus *Buenoa* and *Notonecta*, as well as immature dragonflies and water bugs.

Diflubenzuron is still used in several control campaigns and no studies have evaluated its effects on the associated insect fauna with *Anopheles* in Brazil. Thus, this study aims to evaluate the effects of diflubenzuron on members of the aquatic associated insect fauna with *Anopheles* in laboratory, partial-field, and artificial breeding site conditions in the Amazon region.

MATERIALS AND METHODS

Sampling of insects

Insects were collected in breeding sites located in the peri-urban region of Manaus, Amazonas State, and were used in bioassays in the laboratory and partial-field conditions. The specimens were collected using aquatic entomological nets and sorted, for laboratory bioassays the following groups were selected: *Buenoa* (Hemiptera: Notonectidae) and *Chironomus* (Diptera: Chironomidae). The partial-field bioassays were carried out using the following groups: Chironomidae (Diptera), Hydropsychidae (Trichoptera), Notonectidae (Hemiptera) and Euthyplociidae (Ephemeroptera).

The field experiment occurred in artificial breeding sites of *Anopheles*, called brick factory pits, located at Km 5 of the AM 070 Highway, which links the municipality of Manaus to Manacapuru (AM). This region has a large number of potteries, which remove clay from the ground and form

pits that become filled with water when the Rio Negro is full and serve as breeding sites for mosquitos and various aquatic insect groups.

To evaluate the possible effects in the field conditions we perform samplings before (pre-application) and after (post-application - 15 days) of diflubenzuron, using aquatic entomological nets for a period of 5 minutes. The sediment collected was stored in plastic bags for further screening and identification. Collected material was sifted and aquatic insects were collected and fixed in alcohol 70% for identification using the appropriate dichotomous keys (Borror & Delong 2011, McCafferty 1981).

Larvicide used

The larvicide diflubenzuron was weighed into 1g/L doses, using a precision balance, and stored in aluminum foil envelopes for transportation to the experiment site. The standard solution was prepared from 5g of Dimilin® containing 1.250mg of diflubenzuron, which was diluted in 1L of water from the commercial product (250g of DFB/Kg of Dimilin®). The insecticide was applied directly to the water, simulating the methods used in mosquito control campaigns, with no water renewal during the period of the experiment. In the field, the dose was calculated according to the size of the breeding site recommended by the manufacturer (1g/L).

Laboratory bioassay

Specimens of *Chironomus* (Insecta: Diptera) and *Buenoa* (Insecta: Notonectidae) were kept in aquariums (15cm height, 14cm width, and 25cm length) containing 1L of water from the breeding site with an aeration system and at room temperature. After the selective bioassays, the following doses were used: 250mg/L, 312.5mg/L, 750mg/L for *Chironomus* and 250mg/L, 750mg/L and 1g/L for *Buenoa*. Readings were performed at intervals of 24, 48, 72, and 96 hours after application, prepared in triplicate and a control. The criteria for bioassay evaluation is described by Dulmage et al. (1990), with modifications: mortality in the control group could not surpass 30% and the confidence interval was 95%.

The assessment of diflubenzuron application in the laboratory on the associated insect fauna was determined from the mortality rate (%), which were subject to regression analysis using the Biostat 5.0 software. This analysis was performed to verify the relationship between exposure time and mortality (%) after the application of the larvicide. Lethal concentrations were obtained using the POLO PC program, generated from the readings of mortality rate (%) obtained from the bioassays of each group tested (LeOra Software 1987).

Partial-field bioassay

The partial-field experiment was conducted by simulating field conditions in a forest fragment area on the campus of the Instituto Nacional de Pesquisas da Amazônia -INPA. Plastic basins (0.50m diameter and 0.20m height) with 20L capacity were installed in a shaded area, near a stream and fish ponds. Just like in the laboratory, we carried out three treatments and a control for each taxa submitted to the bioassays.

Colonization was artificial by transporting insects and introducing them into the basins with water from the breeding sites. Basins were grouped together and protected in by canvas cover to prevent rain from entering and were individually protected using fabric to prevent natural colonization by other organisms.

In this experiment, we applied a 250mg/L dose of diflubenzuron and the methods for evaluating the effects on aquatic insects were performed according to Mulla & Darwazeh (1975), with some modifications: the effects on the tested insect families were analyzed through mortality rates (%) up to the fifteenth day of

bioassay and the emergence inhibition rate (IE = 100 – T/C x 100) was calculated. Where: IE-emergence inhibition; T-emergence or survival in treatment; C-emergence or survival in control.

The relationship between exposure time and mortality (%) of individuals subjected to bioassays in the partial-field was evaluated through regression analysis. To evaluate possible differences in response to the insects analyzed, the emergence inhibition data was submitted to ANOVA using the software Biostat v.5.0. Subsequently, to find groupings with differentiated responses, the data was subjected to a Tukey test ($p<0.05$).

Field application

Ten brick factory pits, with approximately 25 m^2, were selected for the in- field experiment (Figure 1). The larvicide was applied using the dosage recommend by the manufacturer (250 mg/L), with the help of Guarany ® Atomizer at the breeding site borders. The evaluation consisted of samplings (readings) pre-application and 15 days post-application, considering ecological indices of relative abundance, richness and diversity of Shannon (H') and functional trophic feeding groups.

We calculated relative abundance (%), diversity, and richness of the associated insect fauna using the program *Dives* – Diversity of Species v.4.4.8 (Rodrigues 2018) and specimens were classified according to their functional trophic feeding groups as presented by Cummins (2016). We analyzed the effects of diflubenzuron on aquatic insect populations by comparing the average richness and diversity from the pre-application and post-application samples, using a T-test in the Biostat software v.5.0. The breeding sites used in the field bioassay were characterized and the limnological data of temperature, pH, and electrical conductivity were obtained using a field pH meter and a conductivity meter (Orion pH 290A + and VWR "EC METER" 2052).

RESULTS

Bioassays laboratory

The mortality data (%) of *Chironomus* indicated high susceptibility to diflubenzuron at doses 250mg/L and 750mg/L, both resulting in more than 70% mortality in the 96h interval. In addition, a strong relationship between exposure time and mortality (r^2 =0.8, 0.9 and 0.9) was observed considering all doses tested in the laboratory. We verified high susceptibility of *Buenoa* to diflubenzuron at doses of 750mg/L and 1g/L. The effect of diflubenzuron at the dose of 250mg/L started 72h after application and from 96h more than half of the individuals submitted to the bioassay were eliminated. The doses 750mg/L and 1 g/L presented high mortality rates, starting soon after 24h and continuing up to 96h. The peak activity of the larvicide on *Buenoa* occurred in the 96h post-dose interval and, at the highest dose, the mortality was 100% of the individuals submitted to the bioassay. The regression analysis based on the results found for *Buenoa*, showed that there is a strong relation between exposure time and mortality rate (r^2 = 0.8, 0.9 and 0.8) at all doses analyzed in the 96h interval (Table I).

The peak activity of diflubenzuron was greater in 96h post-application for both groups and the mortality rate (%) did not exceed 30% control group. According to the mortality (%) data found for both groups, it was possible to obtain lethal concentrations, where for *Buenoa* the value of LC_{50} obtained was 2.77 x 10^{-3} g/L and LC_{90} was 0.86g/L, whereas for *Chironomus* the LC_{50} was 0.019g/L and the LC_{90} was 0.92g/L. Both values are below the manufacturer's recommended dose for mosquito control, which is 1 g/L (Table I).

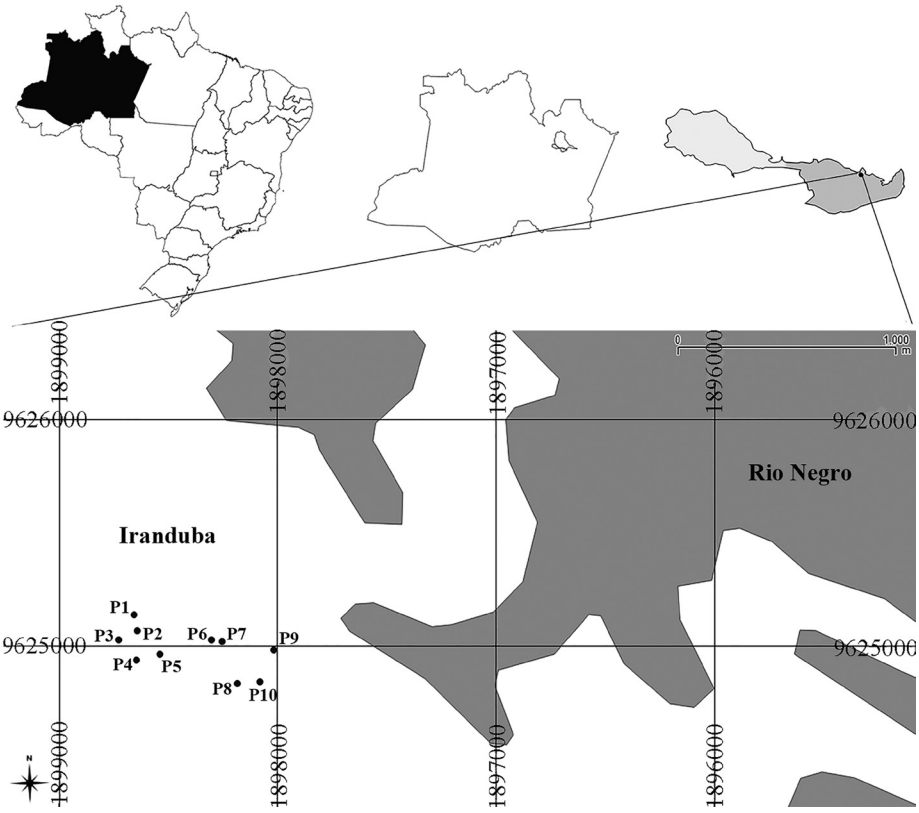

Figure 1. Clay pits (P1 to P10) used in the field experiment with application of diflubenzuron, in the municipality of Iranduba, Metropolitan Region of Manaus Amazonas, Brazil.

Table I. Mortality rate (%) of the groups submitted to laboratory and partial-field bioassays, with readings performed up to 96 hours after application (dose - mg/L; CL - g/L).

Mortality rate (%) - Laboratory bioassay									
Taxon	**n**	**Dose (mg/L)**	**24**	**48**	**72**	**96**	**r^2**	**LC_{50}**	**LC_{90}**
Chironomus	60	250	13	29	53	76	0.8	0.019	0.92
	60	312.5	0	5.5	16.5	32.5	0.9		
	60	750	4	16.5	35.5	73	0.9		
Buenoa	60	750	5	38	76	95	0.9	$2.77x10^{-3}$	0.86
	60	1000	4	12	43	100	0.8		
Mortality rate (%) - Partial-fields bioassay									
Taxon	**n**	**24**	**48**	**72**	**96**	**r^2**	**E.I(%)***	**±SD**	**p**
Chironomidae	60	11	27	48	76	0.9	2.6b	3.7	0.751
Notonectidae	60	0	6.5	25	73.5	0.8	3.7b	6.6	0.378
Euthyplociidae	60	0	21.6	35	62	0.9	2.7a	1.6	0.002
Hydropsychidae	60	8.3	16.5	25	55	0.9	2.5b	2.6	0.072

***E.I (%)** – Emergency Inhibition; letters do not differ from each other considering $p \leq 0.05$; $F = 4.0098$; GL=11; ANOVA: $p = 0.0031$.

Bioassay partial-field

The effect of diflubenzuron on Euthyplociidae (Ephemeroptera) and Notonectidae (Hemiptera) started in the 48-hour post-application interval, and in Chironomidae (Diptera) and Hydropsychidae (Trichoptera), the mortality rate (%) of the individuals submitted to semi-field bioassays. from 24h post-application. All groups analyzed had a mortality rate of >50% in the 96h interval and the regression analysis revealed a strong relationship between the exposure time and the mortality rate (%) of the individuals for all groups analyzed. The most sensitive group during the 4-day interval was Chironomidae (Diptera), with more than 70% mortality, starting 24h after application of diflubenzuron (Table I).

The regression analysis evaluating exposure time and mortality rate (%) of individuals revealed a strong relationship between these variables for Hydropsychidae (r^2=0.9), Notonectidae (r^2=0.8), Euthyplociidae (r^2=0.9) and Chironomidae (r^2=0.9). The highest average emergence inhibition was observed in Notonectidae (3.7%) and the lowest in Hydropsychidae (2.5%). Generally, the inhibition values were estimated with about 1% between the maximum and minimum value found. However, analysis of the emergence inhibition data revealed a significant difference between the groups submitted to bioassays of diflubenzuron in partial-field. Individuals in the Euthyplociidae family showed significant differences when compared to other families, which presented similar responses to each other (Tukey 5%) (Table I).

Field application

We collected a total of 1.102 insects specimens associated with the *Anopheles* sp. in the brick factory pits, distributed in six orders and 20 families. Hemiptera and Diptera were the most abundant orders, representing a little over 50% of all the insects collected, the least abundant was Trichoptera (4%). Among the insect families, Corixidae (21%) was the most abundant and the least abundant were Nepidae (0.05%) and Leptophlebiidae (0.05%) (Table II).

The total Shannon diversity (H') and richness (J 1st) of the brick factory pits was slightly higher in the pre-application reading than the post-application reading. The diversity of total Shannon pre-application was from H'=0.94 and post-application H'=1.0033. The richness of total Jackniffe (J) pre-application was J=15 and post-application J=19. However, the T-test revealed that there was no difference in diversity and richness between pre-and post-application (p=0.32) in the 15-day interval.

The dominant trophic feeding group in these breeding sites were predators, consisting of more than half of the all insects, followed by collectors (Table II). The structure of the insect community according to the functional trophic group pre- and post-application presented differences, with an increase in predators and reduction of other groups (Figure 2).

The physical and chemical characteristics of water in the brick factory pits are typical of isolated environments, i.e. no connection to another water body and exposed to the sun. The temperature of the water varied between 34.7°C and 37.7°C, the PH between 5.2 and 7.1, and the conductivity between 5.5 and 11.1µS/cm (Figure 3). Comparisons of limnological variables showed no differences (p>0.05) between the parameters observed (pH, conductivity, and temperature).

DISCUSSION

The data obtained from the laboratory bioassays indicated an immediate effect of diflubenzuron 24h after application. In addition, the mortality rate (%) was elevated during the first four days, with a peak at 96h post-application, which was

observed for both groups tested, eliminating about 100% of *Buenoa* until this interval (Table I). These findings corroborate with results found by Msangi et al. (2011), who analyzed the effect of diflubenzuron on *Culex* and observed immediate effects on the immature mosquito, suggesting toxicity of diflubenzuron through direct contact.

We also observed that the elevated concentrations of diflubenzuron presented the highest mortality rate values (%) of the groups tested, indicating a relationship between dose and response (Table I). This data corroborates with that found by Chen et al. (2008), who evaluated the effectiveness of two diflubenzuron concentrations on *Aedes aegypti* and noted that the higher the dosage, the more efficient the insecticide was on this mosquito species.

Considering the mortality rate (%) observed for *Chironomus*, the dosage recommended by the manufacturer (250mg/L) eliminated about 76% of individuals in aquariums, indicating sensitivity of these specimens to diflubenzuron. The effect of this larvicide on Chironomidae has already been reported by Ali & Mulla (1977), who verified 96% reduction in populations of *Chironomus utahensis* Malloch, 1915 and 91-100% for *Procladius* (Diptera: Chironomidae), in large artificial lakes in Florida, where these insects are considered pests, and are a nuisance to the human population. Our data agrees with by Steelman et al. (1975), who observed a significant reduction of Chironomidae in the field during a control campaign of *Psorophora columbie* (Dyar & Knab). Later, Ali & Lord (1980) observed the sensitivity of Chironomidae in the field during experiments in small artificial lakes and found a 98% reduction in chironomids six days after application.

In a natural environment the significant reduction in chironomids negatively affects the whole community of aquatic insects and fish since these animals are a base group in the food chain for many other organisms. According to Hansen & Garton (1982), the effectiveness of diflubenzuron is influenced by the generation time of the different groups of aquatic insects, which causes differences in their sensitivity. Therefore, since chironomids have similar generation times as Culicidae, diflubenzuron acts more effectively, eliminating chironomids from the environment in a short period of time.

In partial-field conditions, we found higher mortality rates (%) during the first four days of exposure and after the 5th day no mortality was observed for the organisms tested (Table I). Such fact may be associated with the location where the experiment was assembled, consisting of a shaded area without direct sun rays, which promotes greater permanence of this larvicide in the environment. According to Chen et al. (2008), the residual power of diflubenzuron can be long, up to eight weeks post-application of high doses (100g/ha) in *Aedes aegypti* breeding sites. The breeding sites of these mosquitoes are small containers with water, which are protected from sun and rain. Therefore, the persistence of diflubenzuron in an environment seems to be higher in areas with low incidence of solar rays.

The specimens of Notonectidae, Hydropsychidae, and Chironomidae presented similar responses for emergence inhibition, and Euthyplociidae (Ephemeroptera) was the most sensitive to the larvicide, considering all the groups tested (Table I). These data agree with Farlow et al. (1978), Hurd et al. (1996) and Mulla et al. (1986), who analyzed the effect of diflubenzuron in the field and found that Ephemeroptera was one of the most sensitive groups in the application. This group is admittedly sensitive to environmental changes and it is often used as a clean water bioindicator, which explains its high sensitivity to diflubenzuron.

The structure of the aquatic insect community in *Anopheles* breeding sites is characteristic of

aquatic environments with low rates of dissolved oxygen and high sun exposure. The families Chironomidae and Corixidae were dominant in the sampled brick factory pits. According to Devái (1990), chironomids are dominant in most continental aquatic ecosystems, tolerant to a certain degree of hypoxia, and are treated as an indispensable component of the food chain in the aquatic ecosystem. Studies conducted by Wollmann (2000) indicate that the high density of Corixidae may be related to low pH values, i.e. acidic waters. Of the ten brick factory pits studied, only one presented average pH values > 7, this may have influenced the establishment of a large Corixidae population (Table II).

The richness and diversity data of the associated insect fauna with *Anopheles* in the field presented no significant differences in the pre-and post-application of diflubenzuron during the 15-day interval. Such data corroborates with Wandscheer et al. (2017), who evaluated the richness and diversity of aquatic macroinvertebrates exposed to fungicides and insecticides and found no significant impacts caused by the application of diflubenzuron in a 30-day period.

These results are also similar to those found by Farlow et al. (1978) during a mosquito control campaign in a marshy area in the state of Louisiana (USA). They analyzed the population of non-target organisms exposed to diflubenzuron and observed no significant difference between the populations after six applications over an 18-month period.

The average values of diversity (H') for aquatic insects found in the brick factory pits were close to those found by Ferreira et al. (2015), who studied the effect of *Bacillus sphaericus* on *Anopheles* and associated insect fauna with fish farming ponds in the Central Amazon. However, the richness of aquatic insects in fish farming ponds was higher when compared to the brick factory pits.

In the present study, the dose of diflubenzuron did not significantly impact the associated insect fauna with *Anopheles* in the field during the 15-day period, though, future studies with longer observation times are necessary. However, other studies have found reduced diversity for insects exposed to diflubenzuron. Such reduction was observed in the laboratory by Hansen & Garton (1982), where they simulated a complex interaction network including fungi, bacteria, oligochaetes, and aquatic insects.

The proportion of functional trophic groups in the pre and post-application samples suggests less predators and more organisms from other groups (Figure 2). However, previous studies show that predators do not have high sensitivity to diflubenzuron concentrations used to control Diptera, therefore, we cannot support that this reduction was the result of diflubenzuron application in the environment (Balog et al. 2011).

The temperature of the brick factory pits ranged from 35°C to 40°C, which is typical for aquatic environments completely exposed to the sun (Figure 3). The physical and chemical parameters of the water presented acidic pH and electrical conductivity values typical for aquatic environments with influence of darker waters from the Rio Negro, according to Junk (1983).

The data obtained in this study suggest that diflubenzuron could act by direct contact, causing the death of individuals before acting on the biosynthesis of chitin, within the first four days of exposure, which we observed both in the laboratory and in partial- field conditions. All groups submitted to bioassays were sensitive to high doses of diflubenzuron and presented similar responses of emergence inhibition, except for Euthyplociidae which proved to

be highly sensitive. The bioassays in the field revealed no significant differences between pre and post application samples in the 15-day period. Such data suggests rapid degradation of diflubenzuron in the environment when applied in places that are completely exposed to solar rays. However, the results obtained from the bioassays indicate that diflubenzuron is not selective and if applied in high doses presents toxicity, compromising the survival of other aquatic associated insect fauna with *Anopheles*.

Table II. Relative abundance (R.A %) of associated insect fauna with *Anopheles* sp. during the field experiment with diflubenzuron in clay pits, Iranduba, Amazonas.

Taxon	Family	Functional feeding	Pre	Post	n	R. A* (%)
Hemiptera	Belostomatidae	Predators	19	6	25	2
	Corixidae	Predators	20	217	237	21.5
	Gerridae	Predators	1	1	2	0.2
	Nepidae	Predators	1	0	1	0.05
	Notonectidae	Predators	14	29	43	4
Odonata	Coenagrionidae	Predators	32	23	55	5
	Libellulidae	Predators	50	70	120	11
	Aeshnidae	Predators	5	0	5	0.4
Ephemeroptera	Caenidae	Collectors	102	55	157	14
	Baetidae	Scrapers	2	10	12	1
	Leptophlebiidae	Collectors	1	0	1	0.05
Trichoptera	Hydroptilidae	Scrapers	28	18	46	4
Coleoptera	Hydrophilidae	Collectors	43	50	93	8.3
	Dytiscidae	Predators	4	1	5	0.4
	Gyrinidae	Predators	4	1	5	0.4
	Noteridae	Predators	2	0	2	0.2
Diptera	Chironomidae	Collectors	112	36	148	13.5
	Culicidae	Generalists	46	32	78	7
	Chaoboridae	Predators	3	32	35	3.5
	Ceratopogonidae	Predators	10	22	32	3.5
Total	20	4	499	603	1102	100

***R.A – Relative Abundance.**

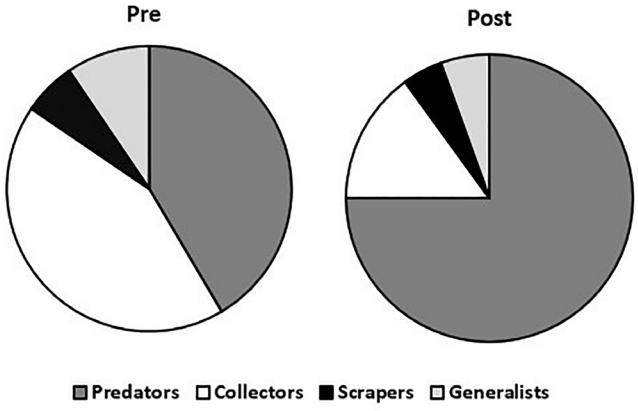

Figure 2. Functional trophic groups (%) found in the brick factory pits during the pre- and post-application of diflubenzuron.

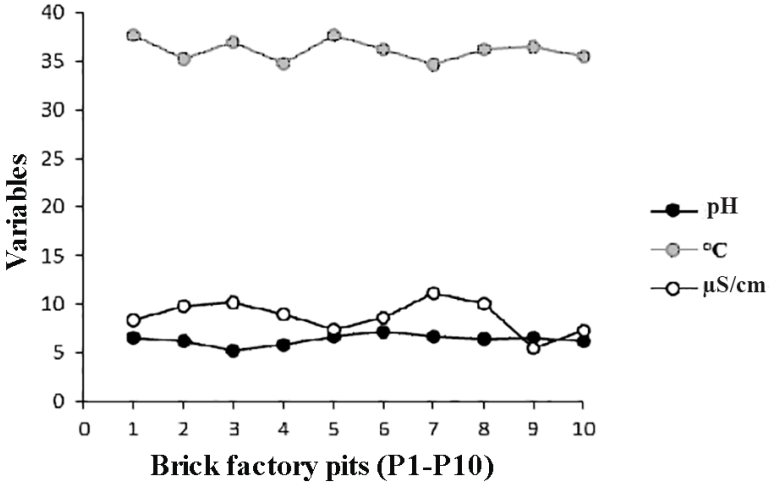

Figure 3. Physical and chemical parameters of pre-application in the clay pits (P1-P10), Km 2 at the AM 070, Iranduba, Amazonas.

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