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Short Communication

Anopheles darlingi and *Anopheles marajoara* (Diptera: Culicidae) susceptibility to pyrethroids in an endemic area of the Brazilian Amazon

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ABSTRACT

Introduction: This study aimed to evaluate the susceptibility of *Anopheles darlingi* Root (1926) and *Anopheles marajoara* Galvão & Damasceno (1942) to pyrethroids used by the National Malaria Control Program in Brazil. **Methods:** Mosquitoes from Amapá, Brazilian Amazon, were assessed for resistance to cypermethrin, deltamethrin, and alpha-cypermethrin. Insecticide-impregnated bottles were used as suggested by the CDC/Atlanta. **Results:** Diagnostic dose for *Anopheles darlingi* was 12.5µg/bottle during 30 min of exposure. Concentrations for *Anopheles marajoara* were 20µg/bottle of cypermethrin and deltamethrin and 12.5µg/bottle of alpha-cypermethrin. **Conclusions**: No resistance was recorded for *Anopheles darlingi*, but *Anopheles marajoara* requires attention.

Keywords: Insecticide resistance. Pyrethroids. Vector control.

The Amazon region of Brazil is considered the endemic area of malaria, with more than 99% of cases of malaria reported in this region[1]. Until the 1980s, vector control was performed with the indoor application of dichlorodiphenyltrichloroethane (DDT). However, in 1987, the use of this insecticide was banned in agriculture and, subsequently, in 1997, it was also banned in public health in Brazil[2]. With the prohibition of DDT use, synthetic pyrethroids have been used as the first choice of insecticides for vector control since the last decade. The main vector control activities in Brazil are ultra-low-volume spraying, thermal fog and, to a lesser degree, residual indoor spraying[3], by using several types of pyrethroids alternately, such as cypermethrin, lambda-cyhalothrin and, more recently, alpha-cypermethrin. Among the main problems associated with the pyrethroids used to control *Anopheles* mosquitoes are the short residual effect in indoor applications[4], especially under the extremely humid and hot conditions found in the Amazon region, and the possibility of selection for resistance. Although this class of insecticides shows rapid action, has high potency, and is not bioaccumulative, which could slow down the appearance of resistance, development of resistance has been recorded for several *Anopheles* species against pyrethroids mainly in African countries[5]. In addition, mutations in the voltage-gated sodium channel gene associated with pyrethroid resistance have been reported worldwide[6]. One of the main difficulties to conduct biological assays to assess insecticide resistance for *Anopheles* mosquitoes is the establishment of laboratory colonies. The main species responsible for malaria transmission in Brazil is *Anopheles darlingi* Root (1926)[1], although the importance of other species in local transmission has been recorded[7] [8]. Since the laboratory colonization of *A. darlingi* has not yet been possible, biological assays for this species need to be conducted using field populations and hence, tests need to be performed during the period of the year when these species are abundant. Thus, unlike *Aedes aegypti* Linnaeus (1762)[9], at present, there is no laboratory population that can be used as a susceptibility standard for *A. darlingi*. In 1998, Centers for Disease Control and Prevention (CDC) researchers developed the impregnated bottle technique[10] as an alternative to the World Health Organization's (WHO) impregnated paper technique. The efficiency of this new technology enabled the expansion of studies on *Anopheles* resistance, and populations of several mosquito species in Latin America have already been assessed[11] [12] [13] by using this technique. This study aimed to evaluate the susceptibility of *A. darlingi* and *Anopheles marajoara* Galvão & Damasceno (1942), the main species involved in malaria transmission in the

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State of Amapá, to pyrethroids (cypermethrin, deltamethrin, and alpha-cypermethrin) that are frequently used by the National Malaria Control Program as a baseline for the surveillance of pyrethroid susceptibility. The samples were collected from the Cities of Mazagão (Sítio Alcolumbre 0°08′45″ S; 51°18′01″ W) and Macapá (Ramal da Viúva 0°09′02″N; 51°18′01″ W), in the State of Amapá, Northern Brazil. *Anopheles* adults were collected using a Castro sucking tube by using animals to attract them (collection conducted in a corral), between 18.00 and 20.00h. The City of Macapá, the State capital, shows a high annual average temperature of 26.6ºC, which varies less than 3°C throughout the year. The mean annual precipitation is 2,570mm; the rainy season occurs between December and July (142-407mm), and the dry season extends from August to November (35-98mm)[14]. Mosquitoes were briefly anesthetized with ethyl acetate for identification and, subsequently, kept resting for 12h for recovery. Although molecular tests were not performed for species determination, previous studies have shown that *A. marajoara* is the only species of the *Anopheles albitarsis* complex present in this area[7]. Bottle tests were performed at the *Laboratório de Entomologia Médica*, *Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá* (IEPA). The CDC protocol was used to perform this bioassay[10] [15] with a few modifications. The insecticides used were 40% cypermethrin (WP; Fersol), deltamethrin TG (99% purity; Bayer), and alpha-cypermethrin TG (99.5% purity; Chem-Service). Briefly, four clean and sterilized 250-mL bottles were used per test. The bottles were coated with 8, 12.5, and 16µg of active ingredient (AI) of the insecticide plus 1mL of acetone (≥99.8% purity; Merck).

Additional concentrations were prepared in those cases where mosquitoes died immediately after application of 8µg AI or survived beyond 30 min in the concentration of 16µg AI. In all, 20 to 25 wild mosquitoes of unknown age were placed in each bottle. The number of dead mosquitoes, i.e., those that could not maintain their flight or land, was counted every 15 min until 120 min. Each assay consisted of three treated bottles and one control, and assays were repeated three times for each insecticide concentration. Data were plotted on a mortality graph according to exposure time. The diagnostic dose was determined by considering the minimum concentration necessary to kill 100% of mosquitoes during 30 min of exposure[11] [13] [15]. Resistance bioassays with the impregnated bottle method were performed for *A. darlingi* by using cypermethrin in the City of Santana in 2005 and by using deltamethrin in the City of Mazagão in 2006. Diagnostic doses were estimated to be 7µg and 8µg, respectively **(Figures 1A** and **1B)**. Bioassays for *A. marajoara* were conducted with cypermethrin in the City of Santana in 2005 and with deltamethrin and alpha-cypermethrin in the City of Mazagão in 2006 and 2010, respectively. Bioassays showed a variation among tests and insecticides, with an established diagnostic dose of 16µg for cypermethrin and 20µg for deltamethrin during the 30 min of exposure **(Figures 2A** and **2B)**. In 2010, the diagnostic dose was estimated to be 12.5µg for alpha-cypermethrin/bottle **(Figure 2C)**. In the present study, *A. darlingi* populations showed 100% mortality during the 30min of exposure when exposed to 7µg or 8µg of cypermethrin and deltamethrin, respectively. These values are lower than those reported previously in Latin America[12] [13] and those of 12.5µg/bottle established as a parameter of diagnostic dose by the CDC for *Anopheles* mosquitoes in general[15]. Since there is no standard strain for testing susceptibility in this species, these reported data were used as a reference, and hence, the populations of the evaluated area were considered to be susceptible to cypermethrin and deltamethrin. However, in order to prevent very small values from being used as a reference, which could erroneously label certain populations as resistant, we suggest that such values should not be used widely as a diagnostic dose. Instead, it would be preferable to raise the cut-off point for resistance in Brazil, as has been recommended by Fonseca-González et al.[12], who used a concentration of 12.5µg of cypermethrin and deltamethrin/bottle for 30 min of exposure as the diagnostic dose for *A. darlingi*. However, the values obtained in this study can be used as the baseline to observe a possible change in the susceptibility of local populations. Thus, from the baseline established in this study, recognizing changes in the pattern of mortality of this species in evaluations might become possible over time in this area. Besides *A. darlingi,* diagnostic dose information obtained using the CDC bioassays is available for only *Anopheles nuneztovari* Gabaldon (1940) and *Anopheles albimanus* Wiedemann (1820). In this study, we also recorded the diagnostic doses of cypermethrin, deltamethrin, and alpha-cypermethrin for *A. marajoara*.

These diagnostic doses, varying between 12.5µg and 20µg/bottle, were higher than those reported for *A. darlingi* for all insecticides and locations evaluated. However, whether these doses can be indicative of resistance is not known, because the diagnostic dose for *A. marajoara* has not been established, and this species might have biological properties that enable it to have different tolerance levels compared to those of *A. darlingi*. Therefore, this finding deserves attention once the doses of several pyrethroids evaluated thus far for the species of the genus *Anopheles* reached a maximum level of 12.5µg/bottle[15], which has only been observed for alpha-cypermethrin in this study. Although the diagnostic dose in 2010 was lower than that established in 2006, and the use of insecticides in public health was not so intense in this location, the difference could be primarily associated with the variation in response to the type of pyrethroid, rather than the change in the level of species tolerance to a certain insecticide. Thus, the concentrations of 20µg of deltamethrin and cypermethrin and 12.5µg of alpha-cypermethrin/bottle are suggested as diagnostic doses for *A. marajoara* or, more broadly, for the species of the *Anopheles albitarsis* complex. We strongly recommend that more bioassays should be conducted in other places of the Amazon to establish the local baseline levels and to provide further evidence about the anopheline response to pyrethroids, considering the absence of a reference strain.

Thus far, the resistance to pyrethroids frequently used by the National Malaria Control Program has not been observed in *A. darlingi* in Brazil, but diagnostic doses estimated for *A. marajoara* indicate that this species requires attention.

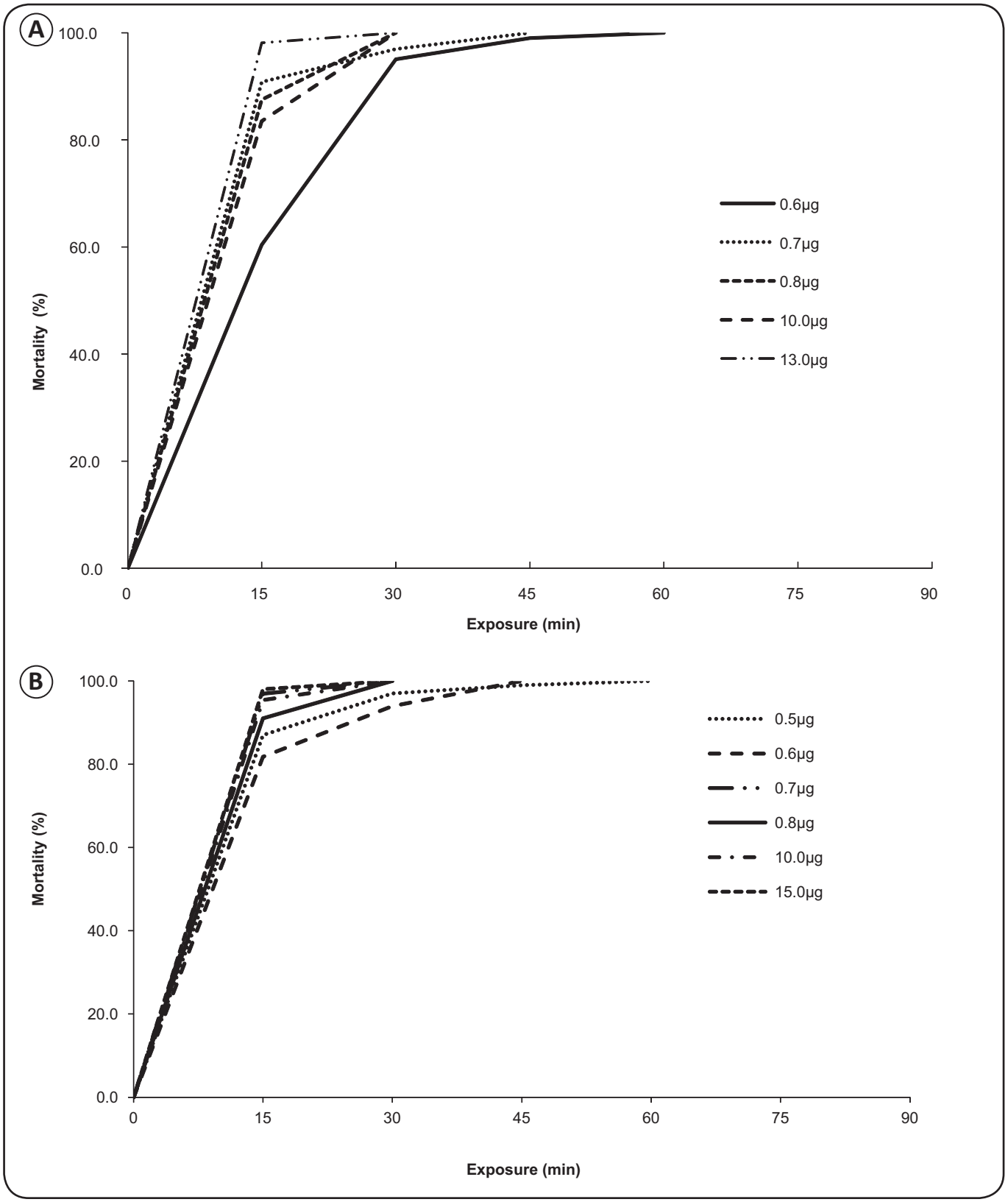

FIGURE 1 - Mortality of *Anopheles darlingi* exposed to bottles impregnated with pyrethroids: (A) Santana City: cypermethrin, 2005 and (B) Mazagão City: deltamethrin, 2006, in the State of Amapá, Brazil.

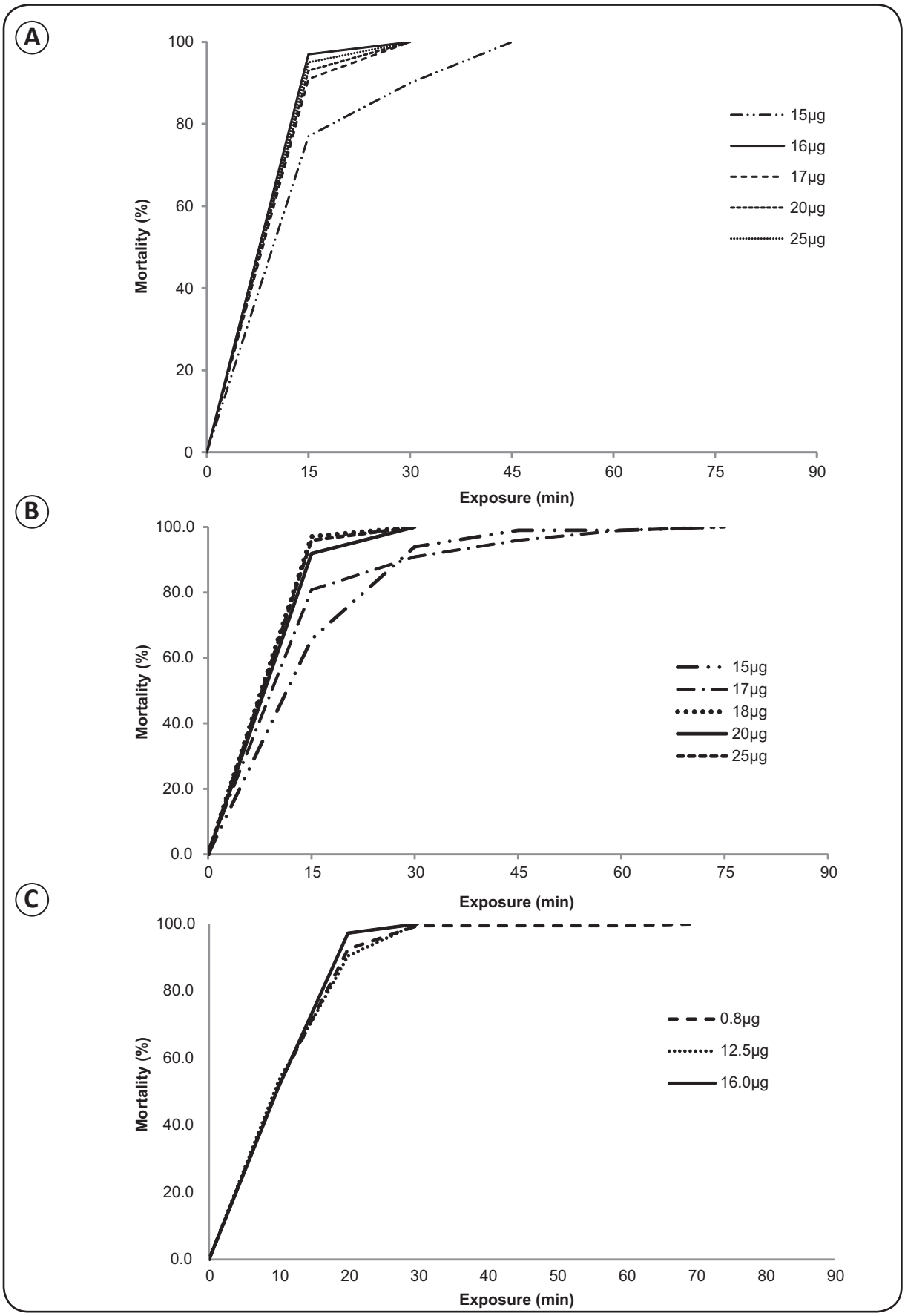

FIGURE 2 - Mortality of *Anopheles marajoara* exposed to bottles impregnated with pyrethroids. (A) Santana City: cypermethrin, 2005; (B) Mazagão City: deltamethrin, 2006; (C) Mazagão City: alpha-cypermethrin, 2010 in the State of Amapá, Brazil.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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