Assessment of the Susceptibility Status of Aedes aegypti (Diptera: Culicidae) Populations to Pyriproxyfen and Malathion in a National Wide Monitoring of Insecticide Resistance in Brazil, 2017-2018.

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Abstract

**Background:** In Brazil chemical control has been used since 1985. For the effectiveness of chemical control, it is essential to monitor the vector susceptibility to insecticides. This study aimed to describe bioassays standardization and to determine the susceptibility profile of *Ae. aegypti* populations to the products in use malathion and pyriproxyfen on national scale between 2017 and 2018, and to discuss the observed results impact in arboviruses control.

**Methods:** The diagnostic-doses (DD) of pyriproxyfen and malathion were determined as the double of the inhibition of adult emergence (EI) and lethal doses for 99% of the Rockefeller reference strain, respectively. For the monitoring of natural populations, collections were performed in 132 Brazilian cities, through egg traps. Colonies were raised in the laboratory for one or two generations (F1 or F2) and submitted to susceptibility tests with larvae exposed to DD of pyriproxyfen (0.03 µg/L) and adults with the malathion DD herein obtained (20 µg), in addition to the World Health Organization (WHO) indicated DD (50 µg) in bottle assay. Dose-response bioassays with pyriproxyfen were performed with populations which did not achieved 98% EI in the DD assays.

**Results:** Alteration of susceptibility to pyriproxyfen was recorded in 6 (4.5%) *Ae. aegypti* populations, with Resistance Ratio RR<sub>95</sub> from 1.51 to 3.58. These populations were concentrated in Bahia and Ceará states. For malathion, 73 (55.3%) populations distributed all over the country were resistant when exposed to the local DD 20µg/bottle. On the other hand, no one population was resistant, and only 10 (7.6%) populations were considered with decreased susceptibility (mortality ratio between 90 and 98%) when the WHO DD (50 µg/bottle was used). These populations are in the 8 from 27 states.

**Conclusions:** We evidenced the feasibility of conducting an insecticide resistance monitoring action at a national wide scale, employing standardized and strongly coordinated sampling methods and laboratory bioassays. We for the first time identified Brazilian *Ae. aegypti* populations with decreased susceptibility to pyriproxyfen. Local DD for malathion was more sensitive than the WHO DD to early detect decrease in susceptibility.

**Background**

In recent decades, the incidence of *Aedes*-borne diseases such as dengue, Zika, chikungunya and yellow fever has increased immensely in the world [1]. The actions against the mosquito *Aedes (Steegomyia) aegypti* (Linnæus, 1762) are mainly based on chemical and mechanical controls to reduce infestation, in addition to social mobilization, environmental management, and legislation protection seeking to maintain environments free of larval breeding sites.

Controlling the insect in the immature phase (egg, larva, and pupa) is more feasible, since their development occurs in specific and restricted locations, unlike the adult phase, which may be dispersed in various environments. The most effective form of vector control is environmental management involving the mechanical removal of reservoirs; however, the most practiced action to block the transmission of arboviruses is the application of chemical insecticides, aiming to rapidly reduce the mosquito populations and interrupt the arboviruses transmission [2].

For the chemical control of *Ae. aegypti*, the Brazilian Ministry of Health (MoH) provides insecticides pre-qualified by the World Health Organization (WHO) to all states. This process ensures that the whole country employs products with trusted evaluations of environmental safety, toxicity, and effectiveness [3]. In addition, the MoH evaluates the compounds under local conditions prior to purchases. The application of larvicides by public agents is recommended in domestic reservoirs that cannot be covered or eliminated, every two months. In addition, cycles of insecticide spatial applications are recommended whenever there is arbovirus transmission in a given locality [4]. Thus, the public health actions to control *Ae. aegypti* in Brazilian consume an expressive amount of insecticides yearly, considering that an average of 4,136 Brazilian municipalities registered dengue cases in the years 2014 to 2017, for instance [5].

With the intensive and continuous deployment of the same active ingredients, the resistant individuals in a population are favorably selected, possibly reaching levels where the efficacy of the insecticide is compromised. A rational chemical control strategy should be based on detailed knowledge about the vector territorial distribution, the susceptibility compounds of distinct classes and the mechanisms involved in selection of resistance in order to reduce the levels of vector infestation and consequent transmission of arboviruses [6].

In Brazil, insecticide resistance in *Ae. aegypti* was first recorded for the organophosphate (OP) larvicide temephos, in populations from Goiás and São Paulo states, in 1995 [7]. Few years later, a reduction in the persistence of temephos was detected in field studies, as well as a decrease in the susceptibility to the OP adulticides fenitrothion and malathion in several *Ae. aegypti* populations in the country [8]. In 2001, resistance to the pyrethroid (PY) adulticide cypermethrin was detected in populations from Rio de Janeiro state [9]. With this scenario, in 1999 the National Dengue Control Program (PNCD, Portuguese acronym) implemented the National Network for Monitoring the Resistance of *Aedes aegypti* to Insecticides (MoReNAa, Portuguese acronym), with the purpose of providing technical support to decisions for the management of *Ae. aegypti* chemical control. The MoReNAa Network carried out systematic insecticide resistance monitoring (IRM) of natural populations of *Ae. aegypti* from Brazil to insecticides used in governmental campaigns, from localities considered as priority or strategic for vector control interventions [10, 11].

Mosquito populations from about 80 cities, including those with the highest incidence of dengue and most populated, high mosquito infestation indexes and all state capitals, were evaluated every two years. Quantitative and qualitative bioassays for detection of larvae and adult resistance were performed according to WHO and Centers for Disease Control and Prevention (CDC) methodologies. For the identification of mechanisms of resistance, biochemical assays for quantification of enzymatic activity alterations and genotyping of kdr mutations were employed in order to investigate the molecular basis of insecticide resistance selection. The Network helped to support the technical decision about insecticide replacement until 2012, when the last monitoring round was carried out [10, 11]. Based on the increasingly detection of *Ae. aegypti* populations resistant to temephos, this compound was gradually replaced by insect growth regulators (IGR) since 2009 in the whole country, with adoption of the chitin synthesis inhibitor diflubenzuron, followed by novaluron [8].

The adoption of the IGR pyriproxyfen began in 2014 based on the intention of rotating insecticides with distinct mode of action. As a juvenile hormone analog, this product prolongs the immature stage for up to 20 days, inhibiting the development of imaginal characteristics. A complete metamorphosis is therefore compromised with mortality specially at the pupal stage or with the emergence of malformed adults [2]. There are few reports of resistance to IGR, likely
because their recent employment for Public Health purposes. Some alterations in the susceptibility to pyriproxyfen were observed in *Ae. aegypti* populations from Martinique (RR\textsubscript{50} 2.2, RR\textsubscript{95} 1.9), in 2007 [12], and *Ae. albopictus* from United States (RR\textsubscript{50} 1.8–2.4) [13]. Higher resistance however was observed in *Ae. aegypti* from Malaysia (RR\textsubscript{50} 6.1) [14] and from United States (RR\textsubscript{50} 38.7, RR\textsubscript{95} 81.5), in 2015 [15].

The OP malathion started being employed against adult mosquitoes in through ultra-low-volume (ULV) and residual spraying applications in Brazil in 1985. In 1989, it was replaced by fenitrothion for residual spraying, and continued to be used in ULV treatment in the following 10 years, when the OPs for adult control. After years without being used to control *Ae. aegypti* adults, malathion was again adopted with the introduction of IGRs for larval control throughout the country since 2009 [8]. OP’s are derived from phosphoric acid and its homologs and use the inhibition of the cholinesterase enzyme as an action mechanism [2]. Changes in the susceptibility of the *Ae. aegypti* mosquito to malathion have already been reported in countries in Caribbean and Latin America, including Brazil [16, 17].

This study was developed with the objectives of describe assay standardization and resistance monitoring of *Ae. aegypti* populations to insecticides used in public health on a national scale in Brazil between the years 2017 and 2018, as well as to discuss their results. We evidenced the feasibility of coordinating the collections and bioassays of more than 140 populations within one year, and with technical reports continuously presented to the MoH.

**Methods**

**Study populations**

Sampling points considered diverse areas in the Brazilian national territory, covering a large number of towns distant at shorter intervals, in urban conglomerates with high population density, as suggested by Chediak et al (2016) [18], and preferentially in sites previously evaluated during the 12-year period MoReNAA Network effort, as described by Valle et al. (2019) [8]. This proposal was adjusted also considering the operational capacity of the municipal sampling teams, in a way that 146 cities were selected for *Ae. aegypti* collections in the course of one year (Table 1). Field populations of *Ae. aegypti* were collected by the Endemic Control Agents of each town, using between 100 to 300 oviposition traps (ovitraps) in each city, according to their number of houses and following the methodology indicated by the MoReNAA Network in 2008 [19]. Sampling was carried out throughout the national territory.

The traps were installed in the grounds of houses evenly distributed with full coverage of the urban territory. As attractant to gravid females, we used a 0.04% yeast extract solution. In order to facilitate the preparation of this solution in the field, the agents were provided with a 50 mL conical tube containing 6 g of a commercial yeast extract (Arma Zen®). During the trap installation the tubes were filled with tap water to the mark of 50 mL and homogenized. With the aid of plastic Pasteur pipette, 1 mL of this solution was added to the trap, then filled with tap water to the mark of 300 mL. The traps were maintained in the households for 15 days, with one change of the paddles and the attractive solution at the end of the first week. The paddles containing the eggs were air-dried during 2 to 3 days prior to be sent to the laboratories.

The collection of samples occurred between August 2017 and December 2018, following a staggered schedule so as not to overload the laboratories. The field-collected samples were initially sent to a central entomology laboratory in their respective state, which then confirmed the correct registration of sampling in the origin sites and adequate storage of the paddles. The paddles were then shipped to Laboratório de Fisiologia e Controle de Artrópodes Vetores (LAFICAVE), Oswaldo Cruz Institute (IOC/Fiocruz), Rio de Janeiro/RJ, where arrival was registered, forms were stored and populations labeled with a code only known by the director of the study, in order to keep confidentiality of their origin. Half of the populations remained at LAFICAVE and the other half was sent to Laboratório de Entomologia Aplicada (LeNA), Superintendência de Controle de Endemias (SUCEN), Marília/SP. The sorting of *Ae. aegypti* specimens, maintenance of colonies and conduction of bioassays were performed by those two laboratories: LAFICAVE and LeNA.

**Mosquito rearing**

Paddles with eggs were submerged in dechlorinated water and hatched larvae were transferred to basins containing 1 L of dechlorinated water and 100 mg of fish food (TetraMin®, Tetra Marine Granules) added every three days. The resulting adult mosquitoes were identified to species and gender, sorting 500 females and 500 males *Ae. aegypti* to be kept in carton cages (33 × 24 × 8 cm), where a 10% sucrose solution was offered *ad libitum*. When the number of females were insufficient for producing a F1 generation (less than 100 females), new field collections were requested.

In order to produce eggs for the next generation, females were additionally fed on blood using guinea pig (*Cavia porcellus* - Linnaeus, 1758) after three days post-emergence (Fiocruz Ethics Committee on the Use of Animals authorizations LW-20/14 and L-004/2018). Alternatively, females were offered to feed on citrated rabbit blood through the membrane feeder Hemotek reservoir (Discovery Workshops, Accrington, UK), containing 6 mL of blood covered with a parafilm membrane, sealed with a rubber ring, under 37°C for 1 hour.

Mosquitoes from F1 generation were usually employed in the bioassays, however a F2 generation was required whenever the number of F1 generation to perform all larvae and adult assays was insufficient. For each population, a minimum of 960 and 1,760 larvae were required to four repetitions of dose-response and DD assays, respectively. In addition, 1,000 adult females were necessary to perform four repetitions of malathion susceptibility tests.

Insectaries were maintained under controlled temperature (26 ± 2°C) and humidity (70 ± 10%) following Fiocruz biosafety manual for vector insectaries and infectories [20]. About 50 male specimens of the parental generation were cryopreserved for the creation of a DNA bank for future genetic analyses. The Rockefeller [21] reference strain for insecticide susceptibility and vigor under laboratory conditions was employed for determination of diagnostic-doses (DD), and it was exposed in parallel, in each assay, as a quality control for the assay. For biological tests with adults and larvae, standardization was performed using this susceptible strain.
DD estimation in bioassays

Before the evaluation of susceptibility of *Ae. aegypti* field populations, it was estimated the DD for pyriproxyfen and malathion, respectively in larvae and adults, under our local conditions. It is worth noting that there is no reference from WHO for DD to pyriproxyfen. The locally established DDs were obtained by dose-response assays with the Rockefeller strain, a reference strain of susceptibility and vigor in the laboratory. The Rockefeller colony maintained in LEnA was used for the tests in both laboratories.

DD estimation for pyriproxyfen

Larval bioassays were conducted with the IGR pyriproxyfen analytical standard (Sigma Pestanal®), pre dissolved in acetone (Sigma Aldrich®) and further diluted in ethanol (Merck®). Following procedures described in the WHO Guidelines for larvicide bioassays with few modifications [22], third stage larvae (L3 stage) were submitted to a gradient of 13 product concentrations (0.0667 to 0.2337 µg/L), from which the percentage of the inhibition of adult emergence (EI) was evaluated at the end of 7 to 10 days, when all control larvae had emerged into adults. Four replicates, with 10 L3 larvae each, were prepared for each concentration, and an equal number of controls were prepared using only ethanol. The larvae were fed with 10 mg of fish food (TetraMin®, Tetra Marine Granules) on the first day and 5 mg on the third day after initial exposure of the larvae. The assays were read daily until the complete emergence of adults in the control group.

An assay would be discarded if the EI of the control group was > 10%, otherwise corrected by Abbott’s formula when there was some EI between 5% and 10% [22]. Four tests were performed at different times. When pupae started to arise, the cups were covered with a mesh to avoid eventual adult scaping. The mortality as well as the emergence of adults was recorded when all the specimens in control condition had emerged as adults. We considered as live adults those totally free of their exuviae and able to fly when gently touched. The EI were calculated using Probit (Polo-PC, LeOra Software, Berkeley, CA) and logistic regression analysis [23]. Finally, the DD of pyriproxyfen was determined as twice the dose that inhibited the emergence of adults in 99% (EI99) of Rockefeller larvae exposed to the compound.

DD estimation for malathion

To perform the bioassays, aliquots of OP stock solutions at a concentration of 3000 mg/L were prepared from malathion analytical standard (Sigma Pestanal®) and solvent acetone (Sigma Aldrich®) and stored in the freezer −80 °C. 250 mL glass bottles (Wheaton) were impregnated with 1 mL of malathion dissolved in acetone solution, in four concentrations (12, 15, 18 and 20 µg/bottle) prepared from the stock solution 24 h before the test. Two bottles per concentration and one control (impregnated with 1 mL of acetone only) were employed for each test, each bottle containing 25 females 3 to 5 days old.

Six tests with each dose were performed, in distinct days. Mosquitoes were exposed to the insecticide for up to 30 min, with mortality recorded every 10 min.

The dose that caused 100% mortality in 30 min, WHO recommended time, was considered the DD [22]. The DD tests with field populations consisted of 25 females 3 to 5 days old gently blown with a Castor aspirator inside the bottles: four bottles impregnated with the malathion DD and two controls containing only acetone. Addition tests were conducted with the WHO recommended DD (50 µg/bottle) [24]. Three independent assays were performed for each population and with both labs determined and WHO recommended DDs.

Evaluation of pyriproxyfen susceptibility in larvae of *Ae. aegypti* field populations

First screening with DD

Once the DD for pyriproxyfen was obtained, larvae of field population (16 replicates of 10 larvae, total of 160 larvae) were exposed to the IGR DD, in addition to 80 larvae (8 replicates of 10 larvae) as the negative control (ethanol only). In parallel, 80 Rockefeller larvae (8 replicas of 10 larvae) were also exposed to the DD, as an internal quality control of the assay. The IGRs solutions were prepared from pyriproxyfen analytical standard (Sigma Pestanal®) and solvent acetone (Sigma Aldrich®) and further diluted in ethanol (Merck®). Aliquots containing 15 µL of IGR at a concentration of 100,000 mg/L were prepared and stored in the freezer −80 °C. These aliquots were used to prepare 5 mL stock solutions at a concentration of 300 mg/L and stored in the refrigerator for 30 days. From these stock solutions, a new dilution was prepared in the same day of the tests, at a final concentration from which 1 mL would result in the desired DD in the 250 mL test cups. Each population was tested in four independents times. The EI result of the control group had to be between 5% and 10% [22].

Estimation of Resistance Ratio

Field populations not susceptible to pyriproxyfen (EI < 98%) in DD assays were submitted to a dose-response assay in order to quantify their levels of resistance, following the above procedures for the DD obtention. Larvae were exposed to a range of 10 concentrations (0.008–0.45 µg/L) in four replicates, with 10 L3 larvae each, four replicates of controls using only ethanol. Rockefeller was run in parallel, in 4 replicates, with larvae exposed to the DD only. Mortality and metamorphosis were recorded until the emergence of all adults in the control condition.

The inhibition of adult emergence concentrations 50% and 95% (EI50 and EI95) of each population were obtained by Probit analysis [25]. Resistance ratios were obtained by dividing the EI (50 and 95) of each population by the equivalent EI of the reference Rockefeller strain. Populations were classified as suggested by Mazzari and Georgiou [26] with low, moderate, or high resistance respectively for RR95 < 5, between 5.0 and 10.0, and > 10.0.

Evaluation of malathion susceptibility in adults
The *Ae. aegypti* field populations were tested using adult females, three to five days post emergence and not blood-fed, from F1 or F2 generations. Each test consisted of exposure of groups of 20 to 25 females per bottle, with four bottles impregnated with each DD (the herein evaluated and 50 µg/bottle) in addition to two bottles impregnated with acetone only, as a negative control. Rockefeller was run in parallel with two bottles impregnated with each DD. The mortality records were taken every 15 min, and mosquitoes that could not stand were considered dead. Mortality of the replicas of each DD was calculated at the diagnostic time (30 min) in each assay. The total of four bioassays were performed for each population, whereas the final result considered their mean mortality.

Diagnostic-dose and dose-response assays for both IGR and adulticide compounds were performed under the test-insectary conditions, with controlled temperature (26 ± 2°C) and humidity (60–80%).

**Insecticides, population and dengue cases data**

Details of the formulations and concentrations of insecticides distributed by MoH specifically for *Ae. aegypti* control in Brazil were requested to the agency through the Electronic System of the Citizen Information Service (Protocol 25820000829202031). Regarding pyriproxyfen and malathion, it was also requested the quantities distributed for each state, since the beginning of their use. Brazilian state population was obtained from the website of the Brazilian Institute of Geography and Statistics (IBGE, Portuguese acronym).

**Data analysis**

The percentage of the inhibition of adult emergence, lethal doses (LD), their respective confidence intervals (IC 95%) and the population slope were calculated by the software Polo-PC, employing a Probit analysis [25]. Resistance rations (RR) were obtained by the quotient between the LD of a population with Rockefeller's. Maps were constructed with the softwares QGIZ version 2.18.6 and GIMP version 2.10.14 [19, 23].

**Results**

In order to evaluate the susceptibility/resistance of *Ae. aegypti* from Brazil to the insecticides current employed in official national campaigns in the whole country, we selected 146 urban cities (Table 1, Fig. 1) based on a geographical representation proposal. We preferentially chose the State capitals, international borders and those cities with previous insecticide resistance data. From these localities, 140 (95.9%) were able to appropriately collect the eggs and send them to the laboratory. Eggs from 14 (9.6%) of these, however, did not hatch or the number of resulting larvae were insufficient for producing a F1 generation (less than 100 female), and therefore new collections were requested. Six localities sent back new samplings, in four localities even after second collection the female number remained low and F1 *Ae. aegypti* colonies were raised with less than 100 F0 females: Parintins (Amazonas State), Irecê (Bahia State), Quixadá (Ceará State) and Salgueiro (Pernambuco State). In the end, we evaluated 132 *Ae. aegypti* populations (94.3% of the initially planned point collections). The number of mosquitoes *Ae. aegypti* obtained by population varied from 48 to 2438 females and from 54 to 2563 males. For the records, *Ae. albopictus* was present in 59.8% (78/132) populations, rendering 1 to 419 females and 1 to 455 males.

Table 2 presents information regarding the geographical origins, number of total and positive paddles (paddles with eggs), total number of eggs, mean of eggs for positive paddles, total of resulting adults of both *Ae. aegypti* and *Ae. albopictus* species, together with the inhibition of adult emergence (EI) to the IGR larvicide and mortality to the organophosphate adulticide.

The dose-diagnostic (DD) obtained for pyriproxyfen was 0.015 µg/L (Table 3). Among 132 populations, six (4.5%) presented EI < 98%, thus indicating suggested resistance to the IGR pyriproxyfen. These six populations were all from Brazilian Northeastern cities: Itabuna, Brumado and Serrinha (Bahia State), Quixadá, Icó, and Juazeiro do Norte (Ceará State) (Table 2, Fig. 2). Resistance ratios (RR<sub>95</sub> and RR<sub>50</sub>) were small in these populations, ranging from 1.07 to 1.97 (RR<sub>50</sub>) or 1.51 to 3.58 (RR<sub>95</sub>) (Table 4), therefore classified as with low resistance.

The DD obtained for malathion under our laboratory conditions was 20 µg/bottle (Fig. 3), 2.5x lower than the WHO indicated DD (50 µg/bottle). In the 20 µg/bottle DD tests, 28 populations (21.4%) presented mortality above 98% (susceptible), 30 (22.9%) had mortality between 90 and 98% (suggested resistance) and 73 populations (55.7%) had mortality below 90% (confirmed resistance). On the other hand, when exposed to 50 µg/bottle, most of the populations (121, 92.4%) were considered susceptible, and the remaining (10, 7.6%) as with "suggested resistance", with mortality between 90 and 98%.”. As can be observed in the map of Fig. 4b, although the localities with populations where resistance to 20 µg/bottle malathion was suggested are spread all over the country, the north region concentrates 71.9% of these populations.

**Discussion**

Here we presented a national wide surveillance for evaluating the susceptibility of *Ae. aegypti* to the IGR pyriproxyfen and the OP malathion in Brazil, insecticides currently employed by the General Coordination of Arboviruses Surveillance - formerly PNCD. This monitoring was promoted by the Brazilian MoH and was the broadest evaluation ever carried out in a country of continental dimensions, resulting in the evaluation of populations from 132 cities in the time span of one year, in which a total of 137,280 larvae and 131,000 adults were tested. To the top of our knowledge, it is also globally the largest surveillance round recorded of insecticide resistance monitoring in *Ae. aegypti*.

We evidenced the feasibility of conducting an insecticide resistance monitoring action in a standardized and strongly coordinated manner, in a model that could be of assistance to the implementation of national monitoring plans in other countries. A systematic literature review covering insecticide resistance
data in *Ae. aegypti* field populations from Latin America showed that less than half of the countries in this continent have published some bioassay data between 2008 and 2018. Also, the number of populations representing each national surveillance was generally rather small [26].

The monitoring of susceptibility to temephos and deltamethrin carried out between 1999 and 2011 by the previous “National Network for Monitoring the Resistance of *Ae. aegypti* to Insecticides” generally used to evaluate between 25 and 74 populations, in every two years [16]. This time we were able to increase the number of tested populations due to at least three main factors.

Firstly, an increased funding for vector surveillance and control actions with the advent of Zika virus outbreak in 2015 and 2016, reserved the amount of around US$ 501,700 specifically for IR monitoring purposes. This financial resource was enough to supply all collection material to the municipalities, to temporarily hire laboratory technicians, to cover laboratory expenses with mosquito rearing and bioassays, to organize one workshop with participation of representatives from the 26 Brazilian States and to produce didactic material with instructions for collections. This awareness and training of at least two health agents of each State was crucial for a homogenous sampling, maintenance and registration of the paddles with eggs, and a correct shipment to the central laboratory, according to standardized procedures. This task was not trivial, especially in a country such as Brazil, whose geographic dimensions and organization complexity are enormous.

In addition to the presential meeting with State representatives, an instructional video was made available on the institutional webpages of the MoH and IOC/Fiocruz [27]. Overall, these aspects contributed for the success of egg collection (96% of the selected cities collected eggs appropriately), from which 94% of the samples were good enough to perform the bioassays.

The third aspect that made it possible to evaluate this high number of populations was that we employed diagnostic-dose tests for a screening of insecticide resistant populations. These assays require a smaller number of insects reared, space and time spent in colonies maintenance and tests execution, compared to dose-response tests. We are aware that in order to obtain a robust profile of a population, dose-response assays are more informative, since by performing a quantitative test it is possible to inform about the resistance ratios and the homogeneity of tested populations. However, as the last official data on IR monitoring occurred in 2013, and there was no record about pyriproxyfen and malathion carried out on a large scale throughout the country, it was preferred to obtain at least the qualitative status of susceptibility/resistance to the current used insecticides in a broader territorial distribution as possible. Population here classified as resistant to pyriproxyfen, were finely investigated with dose-response tests to assess larvicide resistance [24]. In the case of malathion, where two DDs were employed, at least ten populations were not considered as susceptible even when exposed to the higher dose. This suggests that these populations might present the higher levels of resistance among the 132 evaluated *Ae. aegypti* populations. In the Brazilian *Aedes* control program routine, the OP larvicide temephos was alternatively replaced by the benzo-phenyl urea (BPU) chitin synthesis inhibitors diflubenzuron (wettable powder 25%) and novaluron (emulsifiable concentrate 10%) between 2009 and 2014, after almost 30 years of use (starting in the 1980’s). In an attempt to reduce selective pressure on BPU, the Brazilian MoH introduced another class IGR, with a mode of action distinct from chitin synthesis inhibitors: the juvenile hormone analogue pyriproxyfen, applied at potable water reservoir tanks at 0.01 mg of active ingredient/liter [28]. The amount of pyriproxyfen and malathion distributed by the Brazilian MoH specifically for *Ae. aegypti* control between 2014 and 2019 are listed in supplementary file "Additional file 1: Table S1".

Out of all *Ae. aegypti* populations herein evaluated, 99.3% were classified as susceptible to the IGR pyriproxyfen. The six resistant populations were from the same geographic region (Northeast), in the States of Bahia (Itabuna, Brumado, Serrinha) and Ceará (Quixadá, Icó, Juazeiro do Norte), suggesting the emergence of resistance to pyriproxyfen, with a regionalized distribution. Interestingly some of these populations exhibited discrepant RR\(_{50}\) and RR\(_{95}\) values in some populations, suggesting a heterogeneous response within the population, as represented by their low slope values of their dose-response Probit analyses. These populations are likely experiencing an initial process of selection where only few individuals are resistant so far. The pyriproxyfen RR\(_{50}\) was low, when compared to those previously reported for temephos: Itabuna reported RR\(_{50}\) of 18.6 and 55.8 in 2004 and 2013, respectively; Serrinha had 254.9 in 2012 and Juazeiro do Norte showed RR\(_{50}\) of 10.4 in 2003 and 17.5 in 2004 [29, 3]. This regionalization should be related with differences in operational applications and quantity of insecticides used, but also with peculiarities in the genetic background of populations. Likewise, *Ae. aegypti* populations from the Northeast presented the highest levels of resistance to temephos in Brazil [8]. On the other hand, this same region presented the lowest levels of resistance to pyrethroids [8], accompanied by the lowest frequencies of *kdr* alleles [30].

Here we evidenced that the lowest concentration of malathion that killed 100% of Rockefeller females in 30 min was 20 μg/bottle, dose 2.5 times lower than that recommended by WHO in bottle assays (50 μg) [24]. We did not observe any populations resistant to malathion (mortalities less than 90%) when the WHO DD 50 μg/bottle was employed, while 73 populations (55.8% of the total evaluated) was classified as resistant under 20 μg/bottle exposition. The WHO-suggested DD is based on tests performed in reference laboratories and estimated from a variety of susceptible strains for resistance detection, seeking ease of testing and reliability. This DD should be considered as a guide that can be refined for the local situation whenever it is possible [31]. The local DD was more sensitive in early discriminating resistant individuals. This has an interesting approach in the sense of identifying decrease in susceptibility before it has reached levels that may mean loss of effectiveness of the insecticide in the field. The resistance monitoring program in Brazil seeks to detect changes in susceptibility early so that the product used can be changed in a timely manner. The early detection also permits management approaches in time that resistance is not so high that would be of difficult reversal.

The meaning of the laboratory observed resistance in correlation with effectiveness of the product under field conditions should be studied. Studies conducted two decades ago had already reported resistance of *Ae. aegypti* to malathion in Northeastern Brazilian populations, when OP was used to control both the larval phase (temephos) and the adult vector (malathion) [16].

The election of insecticides against *Ae. aegypti* in Brazil followed criteria established by the WHO, also indicating that change of the product should occur in places with a high RR (> 10.0) and with confirmed lack of efficacy in simulated field tests [10]. However, the substitution of an insecticide takes an average of two years to happen [2], since it depends on series of bureaucratic processes. Therefore, the time spent between the first detection of resistance in laboratory
bioassays and the effective change of the compound in the field was not effective for precluding insecticide resistance to spread. In order to avoid reduction in the effectiveness of the insecticides in the field, a most sensitive criterion for its replacement was adopted since 2006. Management has started to be indicated in localities where mosquito populations presented mortality rates below 70% in DD assays or with RR\textsubscript{50} > 3.0 [10]. Results obtained in São Paulo State were the basis of this arrangement, where simulated field trials with temephos and PY’s, demonstrated failures in the control of \textit{Ae. aegypti} in populations with RR > 5.0, and acceptable with RR < 3.0. PY’s were ineffective in simulated field trials against populations with mortality rates below 70% to the DD in laboratory bioassays [32].

Most of the \textit{Ae. aegypti} populations from Latin America tested for DDT showed resistance to this compound (86.7%). High frequencies of resistant populations were also observed for temephos and deltamethrin (75.7 and 33%, respectively). These patterns could be explained by the long and frequent use of these insecticides in the continent [26]. Considering that the pyriproxyfen larvicide has been used on a large scale in Brazil for more than six years, independently of the resistance status the necessity of discontinuity of its use is already evident, according to the insecticide rotation strategy adopted in this country.

Other larvicides allowed for use in water for human consumption with WHO pre-qualification are, in addition to the OP temephos, the IGRs novaluron and diflubenzuron, the biological \textit{Bacillus thuringiensis}, a formulation of \textit{B. thuringiensis} associated with \textit{B. sphaericus}, and spinosad [33]. It is also worth considering the possibility of the return of temephos use since it has not been applied for about 11 years, and there are indications of a reduction in resistance in some locations evaluated [8].

In relation to adulticides, the situation is alarming, since there is just one alternative to PY’s and to the OP malathion, the association of prallethrin with imidacloprid [33]. In the most recent national evaluation for PY’s (2011 and 2012) high RRs to deltamethrin were indicated throughout the country [7]. Also, in São Paulo, localities with higher numbers of dengue incidence were those with higher levels of resistance to PY’s, although these compounds were no longer being applied by governmental campaigns against \textit{Ae. aegypti}. This was correlated with the excessive use of insecticides by household, especially during arbovirus epidemic seasons and with PY’s application on other urban vectors, as it was the case in a locality where there was an intense campaign against vectors of \textit{Leishmania} [34]. Herein we showed resistance to malathion in most of the populations evaluated with the 20 µg/bottle DD. Therefore, the chemical control against \textit{Ae. aegypti} is crucially threatened in most of the country, as long as no other alternative compound is available.

Emerging resistance to all the main classes of neurotoxic insecticide (CA, OP, IGRs) has been detected in \textit{Ae. aegypti} from Americas, Africa, and Asia [29]. The occurrence of resistance to the IGR, most recently adopted class of insecticides, reinforces the importance of using integrated tools that can contribute to reducing the need for chemical vector control, modifying the determinants of arbovirus transmission in a sustainable manner, such as environmental management and education [35]. Consequently, lower use of chemical insecticides reduces the risk of factors associated such as ecological imbalance, outbreak of secondary pests, harmful effects to human health and to other non-target animals [36].

In line with the Vector Integrated Management Strategy, biological control acts on target species through the use of their natural predators or parasites, in the most target-specific manner [37]. The \textit{Bacillus thuringiensis} var \textit{israelensis} (BT) is for long been referred as a promising \textit{Ae. aegypti} larvicide alternative to neurotoxic compounds. However, the large-scale production of BT under formulations sufficiently persistent in environmental conditions, especially in containers exposed to sunlight, is an important limitation [38, 39]. This avoids BT to be a strategic option to be adopted in the routine of \textit{Ae. aegypti} control in Brazil at a national scale. Even if implemented, biological control must be usedrationally just as it is required for chemical insecticides. Despite the believed unlike emergence of resistance to BT due to its multiple modes of toxicity, some mechanisms of resistance to this compound were described in laboratory selected strains [29, 40].

Still seeking to reduce the need for chemical vector control, national and local campaigns of vector control have to reinforce measures to improve sanitary infrastructure and to strengthen community engagement in the destruction or correct treatment of possible larval breeding sites. In parallel, new alternative methods of vector surveillance and control endorsed by WHO should be implemented and monitored, considering regional peculiarities, as is the case of \textit{Wolbachia}. Sterile insect technique SIT, Release of insects with dominant lethality (RIDL), toxic sugar baits, autodissemination of IGRs, spacial repellents [1, 2, 41].

Finally, an alert should be made about the high frequency of populations with the presence of \textit{Ae. albopictus} mosquitoes (59.8%). Our collections were performed in the grounds of houses at urban territory, evidencing the high expansion of that species in the country, since its first record in 1986 in rural areas [42]. Further studies are recommended to better understand the role of \textit{Ae. albopictus} in the transmission of arboviruses in Brazil. In parallel, the monitoring of insecticide resistance of \textit{Ae. aegypti} should also consider \textit{Ae. albopictus} populations.

Conclusions

The challenge posed by the resistance of vectors to different active ingredients available for their chemical control reinforces the importance of implementing Integrated Management Strategies, which prioritize mechanical control and educational actions for the reduction of breeding sites [1, 2]. A well-structured system of insecticide resistance monitoring in mosquitoes is essential for a sustainable, integrated and effective plan based on chemical vector control strategies.

Here we describe the sampling and standardization activities of the monitoring tests and standardization and resistance monitoring of insecticide resistance in \textit{Ae. aegypti} from 132 Brazilian localities between 2017 and 2018 and discuss their results in the light of the acquired knowledge since the first monitoring round, back in 1999. We currently recommend the immediate substitution of pyriproxyfen to an alternative class of larvicide in order to preserve the efficacy in most of the territory, nevertheless not before testing alternative compounds in populations from distinct regions. In regard to the adulticides, there is currently only one recommended compound alternative to PY’s and the OP malathion.
Abbreviations

Ae. aegypti: Aedes aegypti; Ae. albopictus: Aedes albopictus; BPU: benzo-phenyl urea; Bti: Bacillus thurigiensis; CA: carbamate; CDC: Centers for Disease Control and Prevention; DD: diagnostic-dose; EI: inhibition of adult emergence; F1: first-generation; F2: second-generation; FIOCRUZ: Oswaldo Cruz Foundation; IGR: insect growth regulator; IOC: Oswaldo Cruz Institute; IR: insecticide resistance; LAFICAVE: Laboratory of Physiology and Arthropod Control Vectors; LD: lethal dose; LEnA: Laboratory of Applied Entomology; MoH: Ministry of Health; MoReNAa: National Network for Monitoring the Resistance of Aedes aegypti to Insecticides; PNCD: National Dengue Control Program; OP: organophosphate; PY: pyrethroid; RIDL: Release of insects with dominant lethality; RR: Resistance Ratio; SIT: Sterile insect technique; SUCEN: Endemic Control Superintendence; WHO: World Health Organization; WP: wettable powder.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Datasets required to reproduce analyzes and results presented in this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contribution

Conceptualization, funding acquisition: JBPL and KBC. Supervision: JBPL, MLGM and MTMA. Writing, original draft: KBC. Formal analysis, methodology: DFB. Project administration: JBPL and CMR. Quality management: CMR and DFB. Writing, review & editing: MTO, JBPL, MLGM, MTMA, AJM, DFB, CMR and LSD. All authors read and approved the final manuscript.

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Tables
Table 1 Brazilian towns participating in the 2017-2018 monitoring round of susceptibility to pyriproxyfen and malathion in *Aedes aegypti*

| Nº | Latitude  | Longitude  | Region | State | Town           | Nº | Latitude  | Longitude  | Region | State | Town           |
|----|-----------|------------|--------|-------|----------------|----|-----------|------------|--------|-------|----------------|
| 1  | -7.363    | -72.673    | N      | AC    | Cruzeiro do Sul| 74 | -13.535   | -48.224    | MW     | GO    | Minaçu         |
| 2  | -9.978    | -67.811    | N      | AC    | Rio Branco     | 75 | -14.088   | -46.362    | MW     | GO    | Posse          |
| 3  | -11.016   | -68.748    | N      | AC    | Brasília       | 76 | -16.769   | -47.607    | MW     | GO    | Cristalina      |
| 4  | -2.627    | -56.736    | N      | AM    | Parintins      | 77 | -16.673   | -49.256    | MW     | GO    | Goiânia        |
| 5  | -0.136    | -67.084    | N      | AM    | São Gabriel da | 78 | -16.440   | -51.120    | MW     | GO    | Iporã          |
|    |           |            |        |       | Cachoeira      |    |           |            |        |       |                |
| 6  | -7.512    | -63.027    | N      | AM    | Humaitá        | 79 | -17.886   | -51.721    | MW     | GO    | Jataí          |
| 7  | -4.232    | -69.946    | N      | AM    | Tabatinga      | 80 | -17.735   | -49.110    | MW     | GO    | Morrinhos      |
| 8  | -4.084    | -63.142    | N      | AM    | Coari          | 81 | -19.006   | -57.649    | MW     | MS    | Corumbá        |
| 9  | -3.135    | -60.023    | N      | AM    | Manaus         | 82 | -22.227   | -54.811    | MW     | MS    | Dourados       |
| 10 | 0.039     | -51.057    | N      | AP    | Macapá         | 83 | -20.789   | -51.710    | MW     | MS    | Três Lagoas    |
| 11 | 3.851     | -51.831    | N      | AP    | Olapoque       | 84 | -18.508   | -54.758    | MW     | MS    | Coxim          |
| 12 | 2.497     | -50.945    | N      | AP    | Calçoenne      | 85 | -22.486   | -55.711    | MW     | MS    | Ponta Porã     |
| 13 | -2.436    | -54.719    | N      | PA    | Santarém       | 86 | -20.458   | -54.616    | MW     | MS    | Campo Grande   |
| 14 | -7.102    | -49.943    | N      | PA    | Xinguara       | 87 | -15.570   | -56.073    | MW     | MT    | Cuiabá         |
| 15 | -1.460    | -48.488    | N      | PA    | Belém          | 88 | -16.470   | -54.634    | MW     | MT    | Rondonópolis   |
| 16 | -1.691    | -50.481    | N      | PA    | Breves         | 89 | -10.641   | -51.571    | MW     | MT    | Confresa       |
| 17 | -5.353    | -49.142    | N      | PA    | Marabá         | 90 | -9.872    | -56.922    | MW     | MT    | Alta Floresta  |
| 18 | -3.206    | -52.214    | N      | PA    | Altamira       | 91 | -14.049   | -52.159    | MW     | MT    | Água Boa       |
| 19 | -4.264    | -55.990    | N      | PA    | Itaituba       | 92 | -15.230   | -59.338    | MW     | MT    | Pontes e Lacerda |
| 20 | -3.767    | -49.667    | N      | PA    | Tucuruí        | 93 | -11.422   | -58.762    | MW     | MT    | Juina          |
| 21 | -8.028    | -50.030    | N      | PA    | Redenção       | 94 | -15.889   | -52.260    | MW     | MT    | Barra do Garças |
| 22 | -11.434   | -61.443    | N      | RO    | Cacoal         | 95 | -11.858   | -55.501    | MW     | MT    | Sinop          |
| 23 | -10.436   | -62.476    | N      | RO    | Jaru           | 96 | -20.850   | -41.112    | SE     | ES    | Cachoeiro do Itapemirim |
| 24 | -8.769    | -63.831    | N      | RO    | Porto Velho    | 97 | -20.320   | -40.322    | SE     | ES    | Vitória        |
| 25 | -10.774   | -65.324    | N      | RO    | Guajará-Mirim  | 98 | -18.713   | -40.402    | SE     | ES    | Nova Venécia   |
| 26 | -12.741   | -60.139    | N      | RO    | Vilhena        | 99 | -19.823   | -40.276    | SE     | ES    | Aracruz        |
| 27 | 0.937     | -60.425    | N      | RR    | Rorainópolis   | 100| -23.009   | -44.320    | SE     | RJ    | Angra dos Reis |
| 28 | 2.817     | -60.671    | N      | RR    | Boa Vista      | 101| -21.752   | -41.330    | SE     | RJ    | Campos dos Goytacazes |
| 29 | -11.625   | -46.820    | N      | TO    | Dianópolis     | 102| -22.510   | -44.094    | SE     | RJ    | Volta Redonda  |
| 30 | -10.163   | -48.351    | N      | TO    | Palmas         | 103| -22.877   | -43.228    | SE     | RJ    | Rio de Janeiro |
| 31 | -11.730   | -49.071    | N      | TO    | Gurupi         | 104| -19.938   | -43.926    | SE     | MG    | Belo Horizonte |
| 32 | -7.191    | -48.209    | N      | TO    | Araguaína      | 105| -18.853   | -41.947    | SE     | MG    | Governador Valadares |
| 33 | -9.661    | -35.702    | NE     | AL    | Macéio         | 106| -21.761   | -43.349    | SE     | MG    | Juiz de Fora  |
| 34 | -9.756    | -36.657    | NE     | AL    | Arapiraca      | 107| -16.723   | -43.865    | SE     | MG    | Montes Claros |
| 35 | -9.385    | -37.999    | NE     | AL    | Delmiro Gouveia| 108| -19.714   | -47.984    | SE     | MG    | Uberaba        |
| 36 | -11.303   | -41.859    | NE     | BA    | Irecê          | 109| -17.863   | -41.510    | SE     | MG    | Teófilo Otoni |
| 37 | -13.015   | -38.848    | NE     | BA    | Salvador       | 110| -19.525   | -42.624    | SE     | MG    | Coronel Fabriciano |
| 38 | -17.538   | -39.745    | NE     | BA    | Teixeira de Freitas | 111| -21.557   | -45.432    | SE     | MG    | Varginha      |
| 39 | -14.789   | -39.273    | NE     | BA    | Itabuna        | 112| -18.593   | -46.516    | SE     | MG    | Patos de Minas |
| 40 | -14.205   | -41.667    | NE     | BA    | Brumado        | 113| -21.185   | -47.805    | SE     | SP    | Ribeirão Preto |
| 41 | -11.660   | -39.008    | NE     | BA    | Serrinha       | 114| -22.123   | -51.387    | SE     | SP    | Presidente Prudente |
| 42 | -3.724 | -38.590 | NE | CE | Fortaleza | 115 | -23.499 | -47.458 | SE | SP | Sorocaba |
| 43 | -3.688 | -40.349 | NE | CE | Sobral | 116 | -20.813 | -49.381 | SE | SP | São José do Rio Preto |
| 44 | -5.177 | -40.668 | NE | CE | Crateús | 117 | -23.807 | -45.403 | SE | SP | São Sebastião |
| 45 | -4.964 | -39.012 | NE | CE | Quixadá | 118 | -23.567 | -46.570 | SE | SP | São Paulo |
| 46 | -6.403 | -38.863 | NE | CE | Icó | 119 | -25.542 | -54.587 | S | PR | Foz do Iguaçu |
| 47 | -7.211 | -39.317 | NE | CE | Juazeiro do Norte | 120 | -23.312 | -51.163 | S | PR | Londrina |
| 48 | -6.763 | -38.230 | NE | PB | Sousa | 121 | -23.082 | -52.462 | S | PR | Paranavaí |
| 49 | -7.149 | -34.873 | NE | PB | João Pessoa | 122 | -23.422 | -51.940 | S | PR | Maringá |
| 50 | -7.221 | -35.884 | NE | PB | Campina Grande | 123 | -26.078 | -53.056 | S | PR | Francisco Beltrão |
| 51 | -7.037 | -35.634 | NE | PB | Alagoa Grande | 124 | -27.867 | -54.478 | S | RS | Santa Rosa |
| 52 | -8.063 | -34.889 | NE | PE | Recife | 125 | -29.946 | -50.991 | S | RS | Gravatá |
| 53 | -8.071 | -39.121 | NE | PE | Salgueiro | 126 | -28.262 | -52.407 | S | RS | Passo Fundo |
| 54 | -8.889 | -36.493 | NE | PE | Garanhuns | 127 | -29.686 | -53.809 | S | RS | Santa Maria |
| 55 | -9.397 | -40.500 | NE | PE | Petrolina | 128 | -30.383 | -56.454 | S | RS | Quaraí |
| 56 | -8.679 | -35.588 | NE | PE | Palmares | 129 | -26.726 | -53.519 | S | SC | São Miguel do Oeste |
| 57 | -7.578 | -40.502 | NE | PE | Araripina | 130 | -26.875 | -52.405 | S | SC | Xanxerê |
| 58 | -7.955 | -36.204 | NE | PE | Santa Cruz do Capibaribe | 131 | -26.907 | -48.657 | S | SC | Itajaí |
| 59 | -6.770 | -43.021 | NE | PI | Floriano | 132 | -27.107 | -52.617 | S | SC | Chapecó |
| 60 | -5.086 | -42.805 | NE | PI | Teresina | 133 | -10.943 | -69.563 | N | AC | Assis Brasil |
| 61 | -2.903 | -41.778 | NE | PI | Paraíba | 134 | -9.065 | -68.656 | N | AC | Sena Madureira |
| 62 | -7.081 | -41.469 | NE | PI | Picos | 135 | 0.777 | -51.947 | N | AP | Pedra Branca do Amapari |
| 63 | -9.015 | -42.692 | NE | PI | São Raimundo Nonato | 136 | -0.856 | -52.539 | N | AP | Laranjal do Jari |
| 64 | -5.751 | -35.252 | NE | RN | Natal | 137 | -9.372 | -37.245 | NE | AL | Santana do Ipanema |
| 65 | -6.115 | -38.203 | NE | RN | Pau dos Ferros | 138 | -12.145 | -45.004 | NE | BA | Barreiras |
| 66 | -6.586 | -36.775 | NE | RN | Jardim do Seridó | 139 | -4.567 | -37.773 | NE | CE | Aracati |
| 67 | -5.194 | -37.357 | NE | RN | Mossoró | 140 | -4.232 | -44.782 | NE | MA | Bacabal |
| 68 | -2.532 | -44.298 | NE | MA | São Luís | 141 | -7.531 | -46.039 | NE | MA | Balsas |
| 69 | -10.907 | -37.048 | NE | SE | Aracaju | 142 | -5.508 | -45.239 | NE | MA | Barra do Corda |
| 70 | -10.216 | -37.420 | NE | SE | Nossa Senhora da Glória | 143 | -5.527 | -47.480 | NE | MA | Imperatriz |
| 71 | -10.686 | -37.427 | NE | SE | Itabiania | 144 | -22.286 | -42.533 | SE | RJ | Nova Friburgo |
| 72 | -10.915 | -37.666 | NE | SE | Lagarto | 145 | -17.220 | -46.875 | SE | MG | Paracatu |
| 73 | -15.794 | -47.888 | MW | DF | Brasília | 146 | -27.588 | -48.548 | S | SC | Florianópolis |

Notes: Underlined: State capitals. Regions acronyms: N: North, NE: Northeast, CW: Mid-West, SE: South-East, S: South. States acronyms: AC: Acre, AM: Amazonas, AP: Amapá, PA: Pará, RO: Rondônia, RR: Roraima, TO: Tocantins, AL: Alagoas, BA: Bahia, CE: Ceará, PB: Paraíba, PE: Pernambuco, PI: Piauí, RN: Rio Grande do Norte, MA: Maranhão, SE: Sergipe, DF: Distrito Federal, GO: Goiás, MS: Mato Grosso do Sul, ES: Espírito Santo, RJ: Rio de Janeiro, MG: Minas Gerais, SP: São Paulo, PR: Paraná, RS: Rio Grande do Sul, SC: Santa Catarina.
| Number | Region | State | Town                  | total | positive | total | paddles (mean for pp) | Ae. aegypti | Ae. albopictus | Insecticide | Pyriproxifen (Ae. aegypti larvae) |
|-------|--------|-------|-----------------------|-------|----------|-------|-----------------------|-------------|----------------|-------------|---------------------------------|
| 1     | N      | AC    | Cruziero do Sul       | 196   | 72       | 5281  | 73.3                  | 601         | 793           | 0           | 0.94                                           |
| 2     | N      | AC    | Rio Branco            | 294   | 188      | 15747 | 83.8                  | 2377        | 2533          | 0           | 1.61                                           |
| 3     | N      | AC    | Brasílêia             | 100   | 43       | 2912  | 67.7                  | 734         | 814           | 0           | 0.31                                           |
| 4     | N      | AM    | Parintins             | 196   | 39       | 2709  | 69.5                  | 90          | 54            | 91          | 0.94                                           |
| 5     | N      | AM    | São Gabriel da Cachoeira | 200  | 46       | 4680  | 101.7                 | 423         | 383           | 0           | 3.25                                           |
| 6     | N      | AM    | Humaitá               | 200   | 67       | 1933  | 28.9                  | 696         | 690           | 0           | 1.88                                           |
| 7     | N      | AM    | Tabatinga             | 172   | 50       | 3217  | 64.3                  | 472         | 504           | 0           | 0.00                                           |
| 8     | N      | AM    | Coari                 | 196   | 70       | Wi     | Wi                    | 253         | 216           | 0           | 0.63                                           |
| 9     | N      | AM    | Manaus                | 512   | 207      | 10092 | 48.8                  | 1021        | 187           | 98          | 1.50                                           |
| 10    | N      | AP    | Macapá                | 265   | 79       | 2571  | 32.5                  | 296         | 209           | 0           | 0.31                                           |
| 11    | N      | AP    | Oiapoque              | 200   | 28       | 928   | 33.1                  | Wi          | Wi            | Wi          | 2.81                                           |
| 12    | N      | AP    | Calçoene              | 74    | 14       | 634   | 45.3                  | 207         | 178           | 0           | 1.56                                           |
| 13    | N      | PA    | Santarém              | 302   | 87       | 3804  | 43.7                  | 362         | 382           | 102         | 78                                             |
| 14    | N      | PA    | Xinguara              | 202   | 35       | 3744  | 107.0                 | 515         | 501           | 0           | 0.94                                           |
| 15    | N      | PA    | Belém                 | 600   | 361      | 19844 | 55.0                  | 1751        | 1787          | 419         | 342                                           |
| 16    | N      | PA    | Breves                | 202   | 26       | 2645  | 101.7                 | 516         | 512           | 4           | 7                                              |
| 17    | N      | PA    | Marabá                | 300   | 96       | 7394  | 77.0                  | 503         | 500           | 1           | 2.75                                           |
| 18    | N      | PA    | Altamira              | 304   | 103      | 6893  | 66.9                  | 526         | 503           | 4           | 28                                             |
| 19    | N      | PA    | Itaituba              | 200   | 102      | 9813  | 96.2                  | 426         | 392           | 416         | 280                                           |
| 20    | N      | PA    | Tucuruí               | 198   | 93       | 7384  | 79.4                  | 504         | 501           | 219         | 158                                           |
| 21    | N      | PA    | Redenção              | 200   | 29       | 2571  | 88.7                  | 384         | 321           | 1           | 1                                              |
| 22    | N      | RO    | Cacoal                | 196   | 52       | 1521  | 29.3                  | 329         | 414           | 0           | 8                                              |
| 23    | N      | RO    | Jaru                  | 200   | 85       | 7.81  | 91.9                  | 1843        | 1607          | 141         | 72                                             |
| 24    | N      | RO    | Porto Velho           | 300   | 116      | 6269  | 54.0                  | 1222        | 1042          | 257         | 167                                           |
| 25    | N      | RO    | Guajará-Mirim         | 194   | 58       | 2575  | 44.4                  | 1248        | 1374          | 0           | 0.31                                           |
| 26    | N      | RO    | Vilhena               | 200   | 79       | 4513  | 57.1                  | 1457        | 1583          | 0           | 0.00                                           |
| 27    | N      | RR    | Rorainópolis          | 39    | 39       | 2124  | 54.5                  | 352         | 198           | 0           | 0.62                                           |
| 28    | N      | RR    | Boa Vista             | 300   | 166      | 13007 | 78.4                  | 2293        | 2428          | 1           | 6                                              |
| 29    | N      | TO    | Dianópolis            | 204   | 31       | 902   | 29.1                  | 206         | 249           | 0           | 0                                              |
| 30    | N      | TO    | Palmas                | 288   | 92       | 7152  | 77.7                  | 578         | 262           | 12          | 32                                             |
| 31    | N      | TO    | Gurupi                | 208   | 35       | 1054  | 30.1                  | 240         | 251           | 0           | 0.63                                           |
| 32    | N      | TO    | Araguaína             | 344   | 129      | 5893  | 45.7                  | 501         | 500           | 1           | 1                                              |
| 33    | NE    | AL    | Maceló                | 386   | 102      | 6212  | 60.9                  | 496         | 395           | 41          | 20                                              |
| 34    | NE    | AL    | Arapiraca             | 296   | 92       | 7382  | 80.2                  | 1128        | 1007          | 0           | 0                                              |
| 35    | NE    | AL    | Delmiro Gouveia       | 184   | 87       | 3287  | 37.8                  | 523         | 309           | 0           | 5.00                                           |
| 36    | NE    | BA    | Itacé                 | 210   | 23       | 396   | 17.2                  | 48          | 59            | 0           | 0.63                                           |
| 37 | NE | BA | Salvador | 878 | 327 | 21715 | 84.7 | 2264 | 2349 | 140 | 173 | 0.31 | 100.0 | NN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | NE | BA | Teixeira de Freitas | 220 | 83 | 4299 | 51.8 | 503 | 502 | 0 | 0 | 3.44 | 98.8 | NN |
| 39 | NE | BA | Itabuna | 349 | 155 | 9828 | 63.4 | 505 | 606 | 0 | 2 | 0.94 | 96.5 | NN |
| 40 | NE | BA | Brumado | 220 | 90 | 3904 | 43.4 | 289 | 322 | 1 | 1 | 1.56 | 91.6 | NN |
| 41 | NE | BA | Serrinha | 204 | 99 | 4656 | 47.0 | 500 | 500 | 0 | 0 | 0.63 | 85.8 | NN |
| 42 | NE | CE | Fortaleza | 696 | 269 | 18059 | 67.1 | 1491 | 1829 | 80 | 92 | 1.94 | 100.0 | NN |
| 43 | NE | CE | Sobral | 300 | 97 | 6864 | 70.8 | 872 | 927 | 0 | 0 | 1.88 | 99.8 | NN |
| 44 | NE | CE | Crateús | 100 | WI | 4624 | 871 | 1011 | 0 | 0 | 2.25 | 99.3 | NN |
| 45 | NE | CE | Quixadá | 192 | 34 | 2527 | 74.3 | 76 | 64 | 0 | 0 | 3.75 | 97.7 | NN |
| 46 | NE | CE | Icó | 200 | 131 | 9283 | 70.9 | 1919 | 1997 | 27 | 0 | 3.43 | 96.1 | NN |
| 47 | NE | CE | Juazeiro do Norte | 300 | 138 | 24587 | 178.2 | 502 | 500 | 0 | 1 | 1.56 | 95.3 | NN |
| 48 | NE | PB | Sousa | 200 | 63 | 1889 | 29.9 | 405 | 426 | 0 | 0 | 3.44 | 100.0 | NN |
| 49 | NE | PB | João Pessoa | 388 | 239 | 12014 | 50.3 | 1756 | 1816 | 34 | 31 | 0.63 | 100.0 | NN |
| 50 | NE | PB | Campina Grande | 300 | 91 | 3945 | 43.4 | 1007 | 1013 | 0 | 0 | 1.25 | 98.6 | NN |
| 51 | NE | PB | Alagoa Grande | 200 | 88 | 2733 | 31.1 | 510 | 508 | 0 | 0 | 0.63 | 98.1 | NN |
| 52 | NE | PE | Recife | 891 | 455 | 30800 | 66.1 | 731 | 730 | 87 | 68 | 0.00 | 100.0 | NN |
| 53 | NE | PE | Salgueiro | 224 | 18 | 413 | 22.9 | 86 | 127 | 0 | 0 | 0.31 | 100.0 | NN |
| 54 | NE | PE | Garanhuns | 219 | 47 | 1064 | 22.6 | 274 | 297 | 0 | 0 | 0.94 | 100.0 | NN |
| 55 | NE | PE | Petrolina | 300 | 29 | 544 | 18.8 | 126 | 138 | 0 | 0 | 0.62 | 100.0 | IS |
| 56 | NE | PE | Palmares | 198 | 90 | 6715 | 74.6 | 962 | 877 | 102 | 71 | 0.31 | 99.8 | NN |
| 57 | NE | PE | Araripina | WI | 107 | 5235 | 48.9 | 881 | 834 | 0 | 0 | 1.88 | 99.8 | NN |
| 58 | NE | PE | Santa Cruz do Capibaribe | 303 | 144 | 10095 | 70.1 | 511 | 566 | 0 | 0 | 2.19 | 98.9 | NN |
| 59 | NE | PI | Floriano | 190 | 56 | 1722 | 20.9 | 757 | 736 | 54 | 29 | 2.75 | 100.0 | NN |
| 60 | NE | PI | Tenesse | 414 | 125 | 5505 | 44.0 | 915 | 1034 | 360 | 273 | 2.00 | 99.8 | NN |
| 61 | NE | PI | Parnaíba | 251 | 190 | 14090 | 78.3 | 1950 | 2191 | 77 | 63 | 0.25 | 99.6 | NN |
| 62 | NE | PI | Picos | 100 | 29 | 1587 | 54.7 | 307 | 299 | 0 | 0 | 6.87 | 98.4 | 98.3 |
| 63 | NE | PI | São Raimundo Nonato | 100 | 23 | 462 | 20.1 | 165 | 191 | 0 | 0 | 2.58 | 98.3 | NN |
| 64 | NE | RN | Natal | 400 | 277 | 18294 | 66.0 | 1761 | 1847 | 144 | 188 | 0.00 | 100.0 | NN |
| 65 | NE | RN | Pau dos Ferros | 238 | 45 | 2660 | 59.1 | 806 | 854 | 0 | 0 | 0.83 | 100.0 | NN |
| 66 | NE | RN | Jardim do Seridó | 100 | 62 | 4592 | 74.1 | 507 | 507 | 0 | 3 | 3.44 | 100.0 | NN |
| 67 | NE | RN | Mossoró | 298 | 205 | 16114 | 78.6 | 2012 | 1858 | 0 | 0 | 1.00 | 99.9 | NN |
| 68 | NE | MA | São Luís | 406 | 154 | 8925 | 58.0 | 1882 | 2148 | 152 | 102 | 1.56 | 100.0 | NN |
| 69 | NE | SE | Aracaju | 416 | 196 | 15406 | 78.6 | 2438 | 2563 | 32 | 41 | 0.31 | 100.0 | NN |
| 70 | NE | SE | Nossa Senhora da Glória | 214 | 84 | 7944 | 94.6 | 500 | 502 | 0 | 7 | 3.75 | 99.7 | NN |
| 71 | NE | SE | Itabaiana | 324 | 139 | 6187 | 44.5 | 504 | 503 | 0 | 2 | 1.25 | 98.4 | NN |
| 72 | NE | SE | Lagarto | 328 | 192 | 15021 | 78.2 | 508 | 500 | 0 | 2 | 4.00 | 98.2 | NN |
| 73 | MW | DF | Brasília | 291 | 35 | 1778 | 50.8 | 454 | 526 | 6 | 8 | 1.25 | 100.0 | NN |
| 74 | MW | GO | Minaçu | 100 | 33 | 955 | 28.9 | 174 | 86 | 215 | 178 | 2.19 | 100.0 | NN |
| 75 | MW | GO | Posse | 200 | 81 | 3693 | 45.6 | 564 | 535 | 237 | 203 | 1.25 | 100.0 | NN |
| 76 | MW | GO | Cristalina | WI | 98 | 5308 | 54.2 | 1003 | 930 | 0 | 0 | 0.31 | 99.8 | NN 
| 77 | MW | GO | Goiânia | 604 | 222 | 12933 | 58.3 | 2211 | 2129 | 84 | 60 | 3.44 | 99.4 | NN 
| 78 | MW | GO | Iporá | 200 | 133 | 10943 | 82.3 | 508 | 509 | 0 | 8 | 0.50 | 99.1 | NN 
| 79 | MW | GO | Jataí | 214 | 121 | 9349 | 43.7 | 513 | 502 | 0 | 0 | 0.75 | 98.3 | NN 
| 80 | MW | GO | Morrinhos | WI | 98 | 8672 | 88.5 | 1375 | 593 | 1 | 0 | 0.94 | 98.1 | NN 
| 81 | MW | MS | Corumbá | 200 | 70 | 3163 | 45.2 | 802 | 1099 | 0 | 0 | 0.00 | 100.0 | NN 
| 82 | MW | MS | Dourados | 300 | 126 | 7404 | 58.8 | 1921 | 2104 | 6 | 7 | 0.00 | 100.0 | NN 
| 83 | MW | MS | Três Lagoas | 274 | 80 | 4961 | 62.0 | 919 | 962 | 12 | 13 | 0.63 | 100.0 | NN 
| 84 | MW | MS | Coxim | 188 | 43 | 1257 | 29.2 | 172 | 165 | 15 | 30 | 3.13 | 100.0 | NN 
| 85 | MW | MS | Ponta Porã | 189 | 46 | 1979 | 43.0 | 455 | 453 | 0 | 0 | 4.69 | 100.0 | NN 
| 86 | MW | MS | Campo Grande | 408 | 67 | 2988 | 44.6 | 663 | 611 | 0 | 0 | 0.31 | 99.1 | NN 
| 87 | MW | MT | Cuiabá | 394 | 28 | 2075 | 74.1 | 2399 | 2369 | 62 | 88 | 0.31 | 100.0 | NN 
| 88 | MW | MT | Rondonópolis | 900 | 158 | 8213 | 52.0 | 1207 | 1300 | 23 | 13 | 0.63 | 100.0 | NN 
| 89 | MW | MT | Confresa | 108 | 69 | 7673 | 111.2 | 1581 | 1715 | 103 | 121 | 2.19 | 100.0 | NN 
| 90 | MW | MT | Alta Floresta | 118 | 56 | 4670 | 83.4 | 1394 | 1411 | 246 | 170 | 2.18 | 100.0 | NN 
| 91 | MW | MT | Água Boa | 202 | WI | 3566 | WI | 518 | 510 | 3 | 7 | 1.25 | 99.8 | NN 
| 92 | MW | MT | Pontes e Lacerda | 208 | WI | 1536 | WI | 534 | 544 | 0 | 0 | 1.88 | 99.8 | NN 
| 93 | MW | MT | Juina | 132 | 93 | 6773 | 72.8 | 735 | 1006 | 0 | 0 | 1.25 | 99.1 | NN 
| 94 | MW | MT | Barra do Garças | 200 | 101 | 5987 | 59.3 | 503 | 503 | 7 | 34 | 1.88 | 98.7 | NN 
| 95 | MW | MT | Sinop | 150 | 17 | 523 | 30.8 | 102 | 85 | 2 | 0 | 0.94 | 98.7 | NN 
| 96 | SE | ES | Cachoeiro do Tapajós | 286 | 163 | 9949 | 61.3 | 1846 | 1925 | 248 | 293 | 1.88 | 100.0 | NN 
| 97 | SE | ES | Vitória | 448 | 233 | 20108 | 86.3 | 278 | 291 | 9 | 4 | 3.00 | 99.5 | NN 
| 98 | SE | ES | Nova Venécia | 192 | 93 | 6833 | 73.5 | 506 | 503 | 17 | 39 | 3.44 | 99.4 | NN 
| 99 | SE | ES | Aracruz | 202 | WI | 9243 | WI | 500 | 531 | 2 | 13 | 1.24 | 98.1 | NN 
| 100 | SE | RJ | Angra dos Reis | 323 | 107 | 3433 | 32.1 | 425 | 391 | 119 | 118 | 1.25 | 100.0 | NN 
| 101 | SE | RJ | Campos dos Goytacazes | 330 | 119 | 5693 | 47.8 | 1386 | 1242 | 14 | 8 | 0.00 | 100.0 | NN 
| 102 | SE | RJ | Volta Redonda | 296 | 183 | 16123 | 88.1 | 2140 | 2235 | 344 | 455 | 4.38 | 100.0 | NN 
| 103 | SE | RJ | Rio de Janeiro | 612 | 306 | 18861 | 61.6 | 2399 | 2260 | 90 | 82 | 1.75 | 100.0 | NN 
| 104 | SE | MG | Belo Horizonte | 1.766 | 935 | 63893 | 68.3 | 2360 | 2175 | 93 | 96 | 1.25 | 100.0 | NN 
| 105 | SE | MG | Governador Valadares | 288 | 230 | 13853 | 60.2 | 1731 | 1916 | 95 | 114 | 2.50 | 100.0 | NN 
| 106 | SE | MG | Juiz de Fora | 404 | 37 | 1005 | 27.2 | 218 | 244 | 46 | 20 | 0.00 | 100.0 | NN 
| 107 | SE | MG | Montes Claros | 396 | 68 | 1422 | 20.9 | 131 | 136 | 0 | 0 | 0.94 | 100.0 | NN 
| 108 | SE | MG | Uberaba | 94 | 53 | 1902 | 35.9 | 273 | 289 | 0 | 0 | 0.31 | 100.0 | NN 
| 109 | SE | MG | Teófilo Otoni | 296 | 110 | 3275 | 29.8 | 502 | 502 | 55 | 45 | 4.38 | 100.0 | NN 
| 110 | SE | MG | Coronel Fabriciano | 264 | WI | 3245 | WI | 107 | 103 | 0 | 0 | 1.25 | 99.2 | NN 
| 111 | SE | MG | Varginha | 292 | 39 | 758 | 19.4 | 210 | 191 | 6 | 3 | 4.38 | 98.4 | NN 
| 112 | SE | MG | Patos de Minas | 297 | WI | 2981 | WI | 510 | 504 | 10 | 2 | 0.94 | 98.1 | NN 

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Table 3 Dose-response bioassay to determine the pyriproxyfen diagnostic dose for *Aedes aegypti*, Rockefeller strain

| EI50 (µg/L)\(^a\) | CI50 (µg/L)\(^b\) | EI99 (µg/L)\(^a\) | CI99 (µg/L)\(^b\) | Slope |
|-------------------|-------------------|-------------------|-------------------|-------|
| 0.06205           | 0.06012 - 0.06394 | 0.15589           | 0.14655 - 0.16733 | 5.8164 |

Notes: \(\text{EI}50\) and \(\text{EI}99\): pyriproxyfen concentrations needed to inhibition of 50% and 99% adults emergence, respectively. \(\text{CI}\): confidential intervals.
Table 4 Dose-response bioassays with *Aedes aegypti* populations from Brazil resistant to pyriproxyfen, 2017 - 2018

| Region  | State | Population/ City | EI₅₀ (µg/L)² (CI) | EI₉₅ (µg/L)² (CI) | RR₅₀ᵇ | RR₉₅ᵇ | Slope | Resistance level | Notes |
|---------|-------|-----------------|------------------|------------------|--------|--------|-------|------------------|-------|
|         |       | Rockefeller     | 0.0621 (0.0620-0.0639) | 0.1190 (0.1137-0.1253) | 1.00   | 1.00   | 5.81  | -                |       |
| Northeast | Bahia | Serrinha        | 0.1207 (0.0312-0.4665) | 0.4257 (0.1711-1.0595) | 1.95   | 3.58   | 3.00  | Low             |       |
|         |       | Itabuna         | 0.1223 (0.0942-0.1588) | 0.4056 (0.2776-0.5927) | 1.97   | 3.41   | 3.16  | Low             |       |
|         |       | Brumado         | 0.0666 (0.0510-0.0871) | 0.3160 (0.2699-0.3699) | 1.07   | 2.66   | 2.43  | Low             |       |
| Ceará   |       | Juazeiro do Norte | 0.0835 (0.0498-0.1399) | 0.2495 (0.1884-0.3304) | 1.35   | 2.10   | 3.46  | Low             |       |
|         |       | Quixadá         | 0.0900 (0.0800-0.0900) | 0.2200 (0.2000-0.2400) | 1.45   | 1.85   | 4.31  | Low             |       |
|         |       | Icó             | 0.0700 (0.0600-0.0800) | 0.1800 (0.1500-0.2200) | 1.13   | 1.51   | 4.25  | Low             |       |

Notes: ¹EI₅₀ and EI₉₅: pyriproxyfen concentrations needed for inhibition of adult emergence of 50% and 95% of larvae, respectively. CI: confidence intervals. ²RR₅₀ and RR₉₅: resistance ratios. ³Resistance level: RR₉₅<5.0: low; RR₉₅ between 5.0 and 10.0: moderate; RR₉₅>10.0: high (Mazzari & Georghiou 1995)

Figures

Figure 1

Brazilian map showing the municipalities participating in the 2017-2018 monitoring round of susceptibility to pyriproxyfen and malathion in *Aedes aegypti*. The numbers in red represent State capitals. The continuous lines in Brazilian territory indicate the different States of the country.
Figure 2

Brazil's map displaying the results of the resistance evaluation to the IGR pyriproxyfen in Aedes aegypti populations, 2017-2018. Green circles or orange diamonds represent localities from which populations were susceptible or had suggested resistance (IE<98%), respectively. The states of Bahia (BA) and Ceará (CE) were highlighted and the municipalities with suggested resistance populations were shown.

Figure 3

Determination of the diagnostic-dose (DD) of malathion in Aedes aegypti, Rockefeller strain. A - Mortality over time of exposure to bottles impregnated with different doses. B - Three additional independent trials only with DD set to 20 µg/mL, showing 100% mortality in 30 minutes. The red arrow highlights the time of 30 minutes.
Figure 4

Brazil's map displaying the results of the resistance evaluation to the organophosphate malathion in Aedes aegypti populations, 2017-2018. Diagnostic-dose tests employed the (a) 20 µg/bottle or (b) 50 µg/bottle dose. Green circles, orange diamonds or red triangles represent localities from which populations were considered susceptible, with suggested resistance or with confirmed resistance, respectively.

Supplementary Files

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- TabS1.xlsx