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ORIGINAL RESEARCH ARTICLE

# Be quick or be dead: high temperatures reduce *Aedes aegypti* (Diptera: Culicidae) larval development time and pyriproxyfen larvicide efficiency in laboratory conditions

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## Abstract

Several studies have assessed the efficiency of insect growth regulators to control *Aedes aegypti* under laboratory conditions. However, empirical evidence evaluating how insect growth regulators respond to different temperatures is scarce. In this paper, we evaluate whether temperature may influence the efficiency of pyriproxyfen. To do so, we analyzed the effect of three temperatures (20, 25 and 30 °C) combined with five pyriproxyfen concentrations (0.0001, 0.001, 0.01, 0.1 and 1 mg $L^{-1}$) on *Ae. aegypti* larval control. Each experiment had five replicates containing 250 mL of the test solution and 20 larvae according to WHO protocol. Tests were conducted until all mosquitoes had either fully emerged from the control beakers or died. The outcomes showed that, as we increased the temperature, not only did the larval developmental time decrease (from 23.5 to 10.7 days) but also the concentration of larvicide required for 50% ($EC_{50}$ of 0.0002 and 0.0050 mg $L^{-1}$ for 20 and 30 °C, respectively) and 95% efficiency increased ($EC_{50}$ of 0.009 and 0.013 mg $L^{-1}$ for 20 and 30 °C, respectively). We highlight how temperature changes produce different results from larvicide applications ($p < 0.05$) and provide data that allow scientists and the government to evaluate field populations' responses and integrate the use of larvicides more appropriately. We suggest that the effects of environmental factors on larvicide applications should be tested for field mosquito populations so that different temperature conditions can be integrated with the surveillance programs of *Aedes aegypti*.

**Keywords** Endocrine disruptors · Juvenile hormone analogues · *Aedes aegypti* · Insecticides · Culicidae

## Introduction

The mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) still represents a remarkable challenge to public health due to its ability to transmit arboviruses, such as dengue, zika, chikungunya, and yellow fever viruses, especially in tropical regions (Kuno 2010; Mayer et al. 2017). Although there is currently an available and accessible vaccine as a preventive measure against the yellow fever virus, there are no specific treatments for the other diseases related to this mosquito (Kang et al. 2017). Therefore, public health efforts rely on mosquito control, which can achieve local success but might not be enough to prevent outbreaks (Wilke et al. 2019a, 2019b).

Controlling *Ae. aegypti* populations by the elimination of potential breeding sites is a priority for public health agencies, and the use of larvicides is a supplementary strategy, recommended for application in containers that cannot be destroyed, such as vases, buckets, pots, planters, ponds, containers, tarps and water fountains (Ramasamy and Surendran 2012; Wilke et al. 2018). Health authorities often intensify the application of insecticides in response to critical periods of outbreak threats (Zaim and Guillet 2002). Over the years, this practice could select for resistant strains and lead to less effective population control, thus failing to prevent arbovirus outbreaks (Macoris et al. 2018).

In Brazil, according to Zaim and Guillet (2002), more than 4000 tons of temephos was applied every year between 1996 and 2000 to control *Ae. aegypti*. In addition, several studies have detected populations resistant to this compound in several regions of Brazil, as early as 1998 (Macoris et al. 1999; Fontoura

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et al. 2012; Chediak et al. 2016; Dos Santos et al. 2017). Fonseca et al. (2019) emphazized that it is particularly important to manage the susceptibility of *Ae. aegypti* to new larvicides, in order to obtain an early diagnosis of resistant populations and to ensure the efficacy of these products in controlling vectors. Along with resistance monitoring, the Brazilian Secretary of Health Surveillance deliberated the rotational application method for larvicides with the utilization of a single compound for no longer than 4 years, in order to prevent and reduce such population resistance (SVS 2012). The need for new, effective, and safe insecticides designed for mosquito control has led to the development of pyriproxyfen, an insect growth regulator that prevents adult emergence during the final larval stage, causing death as a consequence of the development interruption (Schaefer and Mulligan 1991). To date, the Brazilian Ministry of Health (BMH) recommends the application of pyriproxyfen for larvae control (SVS 2012). Pyriproxyfen is a juvenile hormone analogue that acts as growth regulator and it is the active ingredient in Sumilarv® 0.5G - a granular insecticide for controlling mosquito populations.

Previous studies conducted in laboratory conditions evaluating this larvicide have shown high efficiency in *Ae. aegypti* control (Vythilingam et al. 2005; De Resende and Gama 2006; Lau et al. 2015; Maoz et al. 2017). However, these studies did not consider the fluctuations of any environmental conditions, which is common for laboratory testing protocols, but can fail to detect variations of efficiency in response to these changes. Temperature is an environmental feature of great importance because it is related to the life-history traits of mosquitoes and to the extrinsic incubation period (Tjaden et al. 2013).

Thermal conditions directly affect larval development time, adult body size and, population dynamics (Carrington et al. 2013; Simoy et al. 2015; Grech et al. 2015; Zeller and Koella 2016). According to Sullivan and Goh (2008), there is evidence that water temperature is positively correlated with a decrease in pyriproxyfen persistence in the aquatic environment, but the influence of temperature on its efficiency as a larvicide has not been established (Moura et al. 2020). In addition, the increasing temperature due to current climate change potentially influences mosquito population dynamics. Taking into account the complexity of the climate changing effects for different regions, there is a great concern because of the potential dispersion of mosquitoes to new locations. As an example, northern European regions has potential to exhibit warmer mean temperatures throughout the next years (Ryan et al. 2019; Kramer et al. 2020). Warmer winters will facilitate the survival of this species year-round and extensive colonization, while warmer summers mean reduced time of larval development (Wilke et al. 2019a, 2019b).

Considering the importance of efficient larvicide application and management to surveillance programs, we evaluated the combined effect of different temperatures and pyriproxyfen concentrations on *Ae. aegypti* control.

## Materials and methods

To investigate the role of thermal conditions on the response of *Ae. aegypti* to pyriproxyfen, we assessed the combined effects of temperature and larvicide concentration on early life-history larval development under controlled laboratory conditions.

### Larval rearing

We obtained *Ae. aegypti* eggs, established from a 1996 collection of the Rockefeller strain, from ASR (Analytical and Scientific Research Laboratory). The eggs were stored at room temperature (26 °C ± 2) and relative humidity of 70% (± 5). To induce egg hatching, we added 10 mg $L^{-1}$ of ground dog food to 250 mL of tap water 24 h before submerging the eggs according to Ponnusamy et al. (2011). Prior to the start of the bioassays, we reared the larvae in plastic vessels inside an incubator (Eletrolab®, Model EL212/4LED) under a light-dark cycle of 12:12 h at temperatures corresponding to those used during the bioassays (20 °C, 25 °C, or 30 °C) in order to avoid thermal stress upon initiation of the trial. We fed the larvae with 10 mg $L^{-1}$ of ground dog food every 2 days until the larvae reached late third or fourth instar.

We have chosen the thermal conditions based on the temperature recommended by the World Health Organization (WHO) for pyriproxyfen laboratory trials (25 °C) (WHO 2005; De Resende and Gama 2006; Seccacini et al. 2014; Marcombe et al. 2018). Moreover, we selected 30 °C and 20 °C for testing because these temperatures are representative in most Brazilian regions during warm seasons (spring and summer) and cold seasons (autumn and winter), respectively (Brazilian Meteorological Institute 2019).

### Insecticide

We used Sumilarv® 0.5G, provided by Epidemiological Surveillance of Araraquara (State of Sao Paulo, Brazil), for the bioassays. Sumilarv® 0.5G is produced by Sumitomo Chemical (Tokyo, Japan) as a granular formulation with a slow release in aqueous solution, and contains 0.5% active ingredient (weight:weight).

### Pyriproxyfen solution preparation

We prepared a stock solution dissolving 0.5 g of Sumilarv® 0.5G (CAS # 95737–68-1), macerated to the consistency of powder, with further agitation in tap water with magnetic stirrer (Gehaka AA-250®), following the methodology proposed by Sihuincha et al. (2005). We used this stock solution to derive five final concentrations of 0.0001, 0.001, 0.01, 0.1 and 1 mg $L^{-1}$ that were chosen based on the recommended concentration by the World Health Organization (WHO)

protocol ( 2005) and by the Brazilian Health Ministry (2014), 0.01 mg $L^{-1}$.

## Bioassays

We used 100 late third instar larvae separated into 5 batches of 20 individuals in beakers with 250 mL of pyriproxyfen solution according to the WHO protocol (2005). We provided 2.5 mg of ground dog food as a nutritional source at the beginning of the test and fed the larvae every 2 days until the end of the experiment. All beakers were covered with netting to prevent emerged adults from escaping. Simultaneously, we conducted control experiments with the same larval density in tap water and food source only. The containers were placed inside an incubator set to the temperature being tested (20 °C, 25 °C or 30 °C).

Daily, we recorded the number of larval or pupal mortality, removing them from the containers with a disposable pipette, in order to prevent possible interference by organic matter loads. When all mosquitoes from the control experiment either emerged or died, the experiment was considered finalized, and the running time was registered. We repeated the experiments four more times, beginning on different days.

## Data analysis

The inhibition of adult emergence was considered with counts of dead larvae and pupae, registered daily for each replicate. We calculated the concentration that inhibits the emergence of 50% and 95% of the larvae population ($EI_{50}$ and $EI_{95}$, respectively) for each temperature using the software Statistica®. To verify the normality of data, we used the Shapiro-Wilk test. We analyzed whether there was a significant difference between the mean running time of the tests with different temperatures by applying ANOVA and Tukey's post hoc tests.

We then evaluated whether different temperatures and larvicide concentrations resulted in different mean adult emergence inhibition, applying an ANOVA two-way test using PAST software (Hammer et al. 2001). All statistical tests were applied considering a 95% confidence interval.

# Results and discussion

We found that when we increased the temperature by ten degrees Celsius from 20 to 30 °C, the larval developmental period decreased by 12.8 days (Table 1), showing significant decrease in time ($p < 0.05$ in the Tukey test). This finding corroborates with that of Sukiato et al. (2019), who observed that increasing temperatures can decrease developmental times for *Ae. aegypti*. Experimental bioassays followed the same pattern of development time as that observed for larvae in the control experiments. We observed that at the end of the tests, larvae had died or survived, emerging successfully as adults, with notable exceptions in which larvae remained immature and alive inside the beakers.

**Table 1** *Aedes aegpti* immature development time under three thermal conditions

| Temperature (°C) | Larval development (days) | Standard Error |
|---|---|---|
| $20^{ab}$ | 23.5 | 0.81 |
| $25^{ab}$ | 15.2 | 1.25 |
| $30^{b}$ | 10.7 | 1.7 |

Letters are indicating which pairs are significantly different

Faster larval development at a higher temperature is coherent with more abundant population as consequence of an accelerated life cycle. In this scenario, more quickly emergence of mosquitoes is related with smaller adults (Briegel 1990; Moura et al. 2020). In turn, smaller *Ae. aegypti* females have higher dengue virus infections and dissemination rates (Alto et al. 2008a, 2008b). Differences in both temperature and concentration resulted in significantly different mean emergence inhibition values, with a decrease in pyriproxyfen efficiency at high temperatures and an increase in efficiency at high concentrations ($p < 0.05$) (Table 2). Tassou and Schulz (2012), testing the same larvicide on *Chironomus riparius* larvae, also found differences according to temperature, but unlike our results, *C. riparius* larvae exhibited high sensitivity to pyriproxyfen at high temperatures. These contrasting results may be explained by variations in species sensibility to chemical compounds, which is also shown by exposure to metals. *Chironomus riparius* is 16 times more sensitive to copper than *Ae. aegypti* ($LC_{50} = 2.09$ mg $L^{-1}$ and 33 mg $L^{-1}$ for *C. riparius* and *Ae. aegypti*, respectively) (Rayms-Keller et al. 1998; Béchard et al. 2008).

We found that the two highest concentrations of 0.1 and 1 mg $L^{-1}$ completely blocked emergence of adult mosquitoes at temperatures of 20 and 25 °C, except in 30 °C, in all replicates. Concentrations of 0.001 mg $L^{-1}$ and 0.01 mg $L^{-1}$

**Table 2** Tukey's post hoc test results for both temperature and pyriproxyfen factors ($\alpha = 0.05$)

| Tukey's post hoc - Factor temperature | | | |
|---|---|---|---|
| | 20 °C | 25 °C | 30 °C |
| 20 °C | | * | **** |
| 25 °C | *** | | *** |
| 30 °C | *** | *** | |
| Tukey's post hoc - Factor concentration | | | |
| | 0.0001 mg $L^{-1}$ | 0.001 mg $L^{-1}$ | 0.01 mg $L^{-1}$ |
| 0.0001 mg $L^{-1}$ | | 0 | 0 |
| 0.001 mg $L^{-1}$ | 0 | | **** |
| 0.01 mg $L^{-1}$ | 0 | **** | |

* = $p$ values <0.05; *** = $p$ values <0.005; **** = $p$ values <0.00005

**Table 3** Mean *Aedes aegypti* adult emergence inhibition for each combination of temperature and pyriproxyfen concentration

| Temperature (°C) | Pyriproxyfen concentration (mg $L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.0001 | 0.001 | 0.01 | 0.1 | 1 |
| 20 | 1% | 63% | 79% | 100% | 100% | 100% |
| 25 | 2% | 34% | 79% | 96% | 100% | 100% |
| 30 | 1% | 35% | 69% | 70% | 97% | 100% |

showed higher efficiencies at adult emergence inhibition for all three temperature ($p < 0.05$). Table 3 below shows the mean percentage of adult emergence inhibition for each combination of temperature and pyriproxyfen concentration.

For 50% adult emergence inhibition, higher doses were required as the temperature increased (Table 4). For each temperature tested, the concentrations needed to inhibit 50% of adult emergence were significantly lower than those recommended by the WHO (0.01 mg $L^{-1}$).

Temperature is a factor of interference for pyriproxyfen efficiency. Darriet and Corbel (2006), testing pyriproxyfen with the Rockefeller strain at 27 °C, found an $EI_{50}$ of 0.0001 mg $L^{-1}$, a concentration 50 times lower than the $EI_{50}$ at 30 °C found in our experiments. Similarly, Marcombe et al. (2018) tested pyriproxyfen on susceptible strains at 27 °C and found an $EI_{50}$ of 0.00008 mg $L^{-1}$, 62 times lower than our $EI_{50}$ at 30 °C.

Notwithstanding, the origin of the strain can strongly affect the recommended larvicide concentration when strains demonstrate a different susceptibility status for a chosen insecticide class. A sylvatic population is genetically more diverse than a laboratory strain and is submitted to evolutive pressure from natural conditions (Craig and Hickey 1966; Kuno 2010). As an example, Lau et al. (2015) collected *Ae. aegypti* from field populations in Malaysia, and testing them under laboratory standard conditions, found an $EI_{50}$ of 0.02 mg $L^{-1}$, a concentration four times higher than the $EI_{50}$ found by our experiments at 30 °C.

The concentration required to inhibit 95% of the adult emergence of the tested population was high in all three temperatures assessed, as shown in Table 5. As the temperature increased, we observed an increase in the concentration needed to achieve the same efficiency. Pyriproxyfen is a potent compound for *Ae. aegypti* population control that does not require high doses to achieve its effect, unlike other insecticide classes (Seccacini et al. 2008). Even at high temperatures, close to the thermal optimum of 28 °C for this species (Simoy et al. 2015), the concentration required for 95% efficiency did not exceed the concentration recommended by the WHO (2005).

**Table 4** Emergence inhibition of 50% of the population of *Aedes aegypti* at three different temperatures

| Temperature (°C) | $EI_{50}$ (mg $L^{-1}$) | Standard Error | Lower (mg $L^{-1}$) | Upper (mg $L^{-1}$) |
|---|---|---|---|---|
| 20 | 0.0002 | 0.000009 | 0.00002 | 0.0003 |
| 25 | 0.0021 | 0.000023 | 0.00139 | 0.0031 |
| 30 | 0.0050 | 0.00056 | 0.00498 | 0.0051 |

**Table 5** Emergence inhibition for 95% of the population at three different temperatures

| Temperature (°C) | $EI_{95}$ (mg $L^{-1}$) | Standard Error | Lower (mg $L^{-1}$) | Upper (mg $L^{-1}$) |
|---|---|---|---|---|
| 20 | 0.009 | 0.0059 | 0.008 | 0.009 |
| 25 | 0.008 | 0.0021 | 0.007 | 0.010 |
| 30 | 0.013 | 0.0044 | 0.009 | 0.021 |

Pyriproxyfen is a potent compound for *Ae. aegypti* population control that does not require high doses to achieve its effect, unlike other insecticide classes (Seccacini et al. 2008). Even at high temperatures, close to the thermal optimum for this species (28 °C) (Simoy et al. 2015), the concentration required for 95% efficiency did not exceed the concentration recommended by the WHO (2005).

## Conclusion

The temperature influences both the exposure time and the efficiency of pyriproxyfen in mosquito control. Higher temperatures of 30 °C not only reduced the larval developmental times, but also required higher doses of this insect growth regulator to attain the efficiency in population control. For a 5 °C increase from 25 to 30 °C, the mean concentration capable to inhibit the emergence of 95% of the mosquito population must be 1.5-fold increased. As the time for full larval development is reduced, so as the period of contact with the larvicide in the aquatic environment. As Kliot and Ghanim (2012) stated, coping with the toxicity of insecticides can require resource allocation and can be costly to the organism. Less contact time with the larvicide reflect a reduction in fitness costs in order to survive the compound, which is related to our findings of higher larvae survival in 30 °C comparing to the two lowered temperatures. Even though the concentration did not exceed the WHO recommended dose, it is important to note that the experiments reported here were performed using the Rockefeller strain of *Ae. aegypti*. Our experiments with a susceptible reference strain provide a reference for sensibility values when testing field populations, turning possible the comparison to evaluate resistance to the larvicide. Field

populations are exposed to different environmental conditions, so their susceptibility can vary from that of susceptible strains. In a climate-changing world, accounting for temperature variation and increase it is crucial to optimize pesticide applications. We recommend testing pyriproxyfen on field population strains of *Ae. aegypti* under varying field temperatures. The application of insecticides according to evidence-based knowledge can provide better results in controlling mosquito populations, saving financial resources, and contributes to protection of non-target species.

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**Authors' contributions** Lidia Moura: Conceptualization, methodology, investigation, data curation, validation, writing – original draft Barbara Lepretti de Nadai: investigation, data curation, writing – original draft Aline Christine Bernegossi: writing – original draft, data curation, validation, formal analysis Mayara Caroline Felipe: writing – review and editing, validation Gleyson Castro Borges: validation, formal analysis, writing – review and editing, validation Juliano José Corbi: Funding acquisition, project administration, supervision, resources, conceptualization, methodology, writing – review and editing.

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## Compliance with ethical standards

**Declaration of interests** The authors declare that they have no known competing financial interests or personal relationships that could Have appeared to influence the work reported in this paper.

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