Effect of pyriproxyfen in *Aedes aegypti* populations with different levels of susceptibility to the organophosphate temephos

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**Abstract**

Vector control with larvicides is an important component in dengue control programmes. In Brazil, the extensive use of temephos has led to the evolution of resistance in *Aedes aegypti* populations in many parts of the country. One of the strategies proposed for managing temephos resistance is the use of the insect-growth regulator – pyriproxyfen. This study evaluated the lethal concentration for this product in mosquito populations with different profiles of susceptibility to temephos and semi-field residual response to a commercial product. The results suggest the possibility of cross-resistance between temephos and pyriproxyfen.

**Keywords:** *Aedes aegypti*; Susceptibility to insecticides; Cross-resistance; Insect-growth regulators.

**Introduction**

It is estimated that about 975 million people around the world live in areas with dengue transmission risk[1]. In Brazil, all states are infested by *Aedes aegypti*[2], the main dengue vector in the tropical region[3].

The organophosphate temephos has been used as larvicide since the beginning of the 1980s for controlling *Aedes aegypti* in Brazil, and its use has been indicated by the National Program for Dengue Control (PNCD)[4]. The prolonged use of this larvicide has selected resistant populations in many parts of the world[5,6]. In Brazil, there are many reports on *Ae. aegypti* resistance to temephos in several states[7-15].

The first strategy for managing temephos resistance adopted by the Brazilian government was the substitution of larvicides by *Bacillus thuringiensis* var. *israelensis* (Bti)[16]. Under field conditions the use of biolarvicide presents the disadvantage of a shorter residual effect than the chemical one. Bti residual effect evaluated under field conditions varied 30–36 days[17] to 7–12 weeks[18,19]. In Brazil, a residual effect of

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larvicide lasted nearly 60 days for the control of *Ae. aegypti*, and this coincides with the frequency of visits of vector control teams in the context of PNCD\[^{4}\].

In order to manage the temephos resistance in *Ae. aegypti*, besides Bti-based larvicides, products that are classified as insect growth regulators (IGRs) have been pointed out as an alternative by the Ministry of Health in Brazil\[^{20}\]. The World Health Organization (WHO) recommends pyriproxyfen (IGR) technical grade ingredient for the control of *Ae. aegypti* population. Many studies indicated that pyriproxyfen will not adversely affect a non-target species when applied at rates usually <50 ppb in mosquito control programmes\[^{21}\]. Among IGRs, pyriproxyfen, which is a mimic of juvenile hormone, is a potent inhibitor of embryogenesis, metamorphosis and adult formation and shows a long residual effect\[^{22,23}\]. Although resistance to pyriproxyfen has not been reported in *Ae. aegypti* populations, it is important to evaluate its effect on insects that are resistant to organophosphates as they would be the target for the management of resistance to temephos.

The objectives of this study were to estimate lethal concentrations of pyriproxyfen and evaluate the residual effect of one commercial formulation of this product on *Ae. aegypti* populations with different susceptibility levels to the organophosphate temephos.

**Methods**

**Origin of *Ae. aegypti* populations**

Field populations were collected through ovitraps according to the sampling methodology used in the Brazilian network for the evaluation of resistance of *Ae. aegypti* to insecticides\[^{16}\]. *Ae. aegypti* populations were collected from two different regions of Brazil. From the south-
Effect of pyriproxyfen in Aedes aegypti populations with different levels of temephos resistance

east, four populations were collected from São Paulo state, cities of Araçatuba (AT), Bauru (BR), Marília (MA) and Santos (SA) and one from Paraná state, Maringa (MG). The second region, the north-east, was represented by three states: Salvador (SS) and Barreiras (BA) from Bahia state, Recife (RE) from Pernambuco state and Fortaleza (FO) from Ceará state. The geographical distribution of these cities is illustrated in the Figure. The eggs collected were used to rear laboratory colonies. The Rockefeller susceptible strain, provided by the Centers of Disease Control, Puerto Rico, was used for comparison.

Laboratory assays

Pyriproxyfen technical grade 98.5%, batch 2006018 [4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propylether] was evaluated for field tests. The stock solution (250 mg/L) was prepared in deionized water and stored at 4 °C. The work solution (2.5 mg/L) was prepared immediately before each test.

The effect of the insect growth regulator was evaluated by the estimation of concentrations that caused inhibition of adult emergence, according to the World Health Organization methodology[24]. This estimation was done through dose-response bioassays. Four Ae. aegypti populations were evaluated: Rockefeller, an insecticide-susceptible reference strain, and three field populations, on the second generation reared in laboratory (F2). The field populations used had been classified according to their temephos-resistance ratio (R.R.) which were calculated at the lethal concentration 95% (LC₉₅). The population Salvador (SS), highly resistant[25,26] (R.R. 11), the population Barreiras (BA), moderately resistant (R.R. 6.9) and the population Bauru (BR) with low level of resistance (R.R. 3.8) were compared to the susceptible Rockefeller strain. For each population, three bioassays were performed, each one with 720 third instar larvae exposed to eight pyriproxyfen concentrations. Eighty larvae were used per dose and for the control group, in four replicates with 20 larvae each. All larvae were fed every other day during observation period with 0.5 ml of solution made of 10% of fish food (Tetra Marine Granules®). Evaluations were done 48 hours after exposure and after each remaining 24 hours, through quantification of live and dead larvae, pupae and adult. The exposure lasted till the last individual died or emerged as adult. Bioassay data were pooled by doses and the percentage of inhibition of adult emergence at each dose was calculated dividing the percentage of adult emergence by the percentage of adult emergence at the control replicates[24]. After that, the estimation of doses that caused 50% and 95% of emergence inhibition (EI) was obtained by the software Polo-PC[27]. The EI doses obtained with Rockefeller strain were used for the calculation of resistance ratio of pyriproxyfen for field populations.

The comparison of populations was performed by analysing the overlapping of EI doses confident intervals.

Simulated field trial

The persistence of two products, Sumilarv® 0.5G (pyriproxyfen, SUMITOMO batch 5099X31) and Temefós Fersol 1G (Fersol Indústria e Comercio Ltda., Product batch 287SP0214000), was evaluated in two kinds of containers, i.e. glass vases and tyres, which are important breeding sites in Sao Paulo state. Glass vases are commonly used for maintaining

1 Technical material was provided by Sumitomo Chemical Co., Ltd. First, Tokyo, Japan
plants (cut or live plants) and contribute to around 30% of *Ae. aegypti* foci in many cities of Sao Paulo state[28].

The Rockefeller strain and six *Ae. aegypti* field populations with different susceptibility to temephos were used in the test. Among the field populations one was considered susceptible MA (R.R. 1.8), two with low level of resistance; MG, and AT (R.R. 2.7 and 2.6 respectively) and three moderately-resistant populations: SA, FO and RE (R.R. 4.6, 8.4 and 9.0 respectively).

For each population five breeding sites of each type were used, one for control and two for each product. Glass vases were filled with 4 l of water and tyres with 800 ml.

Pyriproxyfen at 0.05 ppm and 1 ppm temephos were applied as per the manufacturers’ instructions. All containers, vases and tyres, were kept in a shaded area in an open garage.

Insecticide application was performed at day “zero” and 30 third instar larvae were exposed one day later. New larvae were added at fifteen-day intervals in a period of two months. Mortality was recorded every 24 hours after exposure and daily until the emergence of adults. Pupae were transferred and observed daily to register adult emergence or death. The temperature and pH of treated containers were recorded once a week along with the test.

One-third of the water of each vase was replaced at 15-days interval, before a new exposition of larvae, in order to simulate the domestic situation. There was no water replacement in tyres. In this case the water volume was just filled to the original level before each new larvae exposure.

The control group was used for Abbott correction when the observed mortality was between 5% and 20%.

As pyriproxyfen – the candidate IGR act on different stages of mosquito development, the results were expressed as inhibition of adult emergence following the scheme presented by Pinzon et al.[29] where $\text{EI} = 1 - \left(\frac{\text{Ad}}{\text{Lexp}}\right)$, where $\text{Lexp} = \text{larae exposed and Ad = adults}$. The result was multiplied by 100 to express it as percentage of inhibition.

The effect of candidate IGR was evaluated through their capacity for providing at least 95% mortality along the time after treatment. As in Brazil, the cycle of visits for vector control proposed by the National Programme (PNCD)[4] is of 60 days, the expected effect of a larvicide should suit this period of time.

The results obtained in *Ae. aegypti* populations were compared after transformation of the mortality data into arcsin values. Data were pooled by group according to resistance status of populations being MG, MA and AT pooled at one group for presenting low level of resistance (R.R. below 4) and populations SA, RE and FO pooled in a second group for presenting moderate resistance to temephos (R.R. between 6 and below 10). Comparisons were made between the two groups and between each group and Rockefeller strain with Student t-test.

**Biochemical assays**

The activity of metabolic enzymes alpha and beta esterases, mixed function oxidases (MFO) and glutathione-S-transferase (GST) were evaluated in larvae according to the Centers of Disease Control protocol[30,31]. The enzymatic activity obtained by each larvae was corrected by the respective protein values. The results were analysed as proposed by Montella et al.[15], which is the standard method for analysis at the Brazilian Network for the evaluation of resistance of *Ae. aegypti* to insecticides[32]. The
enzyme activity of field populations was compared with the susceptible Rockefeller strain. The percentage of individuals with enzyme activity higher than the Rockefeller percentage 99 classifies that activity as “normal” when it is below 15%, “altered” when it is between 15% and 49%, and “highly altered” when it is more than 50%.

Results

Estimation of adult emergence inhibition concentrations

Pyriproxyfen exhibited a major effect on adult emergence with high mortality at pupal stage (97.2%) and very low mortality of larvae (2.8%). The estimated EI 50 and EI 95 of pyriproxyfen for the susceptible Rockefeller strain and the three field Ae. aegypti populations are presented in Table 1 where data of temephos lethal concentrations and resistance ratios of both products are compared.

By the analysis of confidence interval of EI concentrations, all the populations differed from the Rockefeller strain and also among each other. SS population presented the higher R.R. for both temephos and pyriproxyfen. Population BA, moderately resistant to temephos, had a lower R.R. for pyriproxyfen than population BR which had the smallest R.R. for temephos.

Enzyme activity

The enzyme activity was evaluated for all populations except the field RE and MFO enzyme for MA population. The number of

Table 1: Estimated adult emergence inhibition concentrations of pyriproxyfen and lethal concentrations of temephos (confidence interval). Resistance ratios based on Rockefeller concentrations.

<table>
<thead>
<tr>
<th>Pyriproxyfen</th>
<th>Population</th>
<th>EI 50(ppb)</th>
<th>EI95(ppb)</th>
<th>R.R. 50</th>
<th>R.R. 95</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>3.37</td>
<td>6.44</td>
<td>6.5</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.10 – 3.60)</td>
<td>(5.90 – 7.20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.74</td>
<td>2.70</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.64 – 0.84)</td>
<td>(2.31 – 3.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BR</td>
<td>1.86</td>
<td>4.13</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.70 – 2.00)</td>
<td>(3.75 – 4.63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rockefeller</td>
<td>0.52</td>
<td>1.17</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.48 – 0.55)</td>
<td>(1.06 – 1.32)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temephos</th>
<th>Population</th>
<th>LC 50 (ppm)</th>
<th>LC 95 (ppm)</th>
<th>R.R. 50</th>
<th>R.R. 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>0.028</td>
<td>0.053</td>
<td>11.0</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.027 – 0.029)</td>
<td>(0.05 – 0.056)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>0.013</td>
<td>0.029</td>
<td>5.2</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.012 – 0.013)</td>
<td>(0.026 – 0.033)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>0.0051</td>
<td>0.0110</td>
<td>2.0</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.0048 – 0.0053)</td>
<td>(0.014 – 0.016)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rockefeller</td>
<td>0.0025</td>
<td>0.0042</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.0024 – 0.0026)</td>
<td>(0.004 – 0.0045)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ppb: parts per billion; ppm: parts per million.
### Table 2: Quantification of enzyme activity in Ae. aegypti populations

<table>
<thead>
<tr>
<th>Populations</th>
<th>Alpha esterase (nmoles/mg ptn/min)</th>
<th>Beta esterase (nmoles/mg ptn/min)</th>
<th>Glutathion-S-transferase (mmoles/mg ptn/min)</th>
<th>MFO (nmoles/mg ptn/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rockefeller</td>
<td>150</td>
<td>11.1</td>
<td>2.6</td>
<td>21.7</td>
</tr>
<tr>
<td>Field populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marília (MA)</td>
<td>120</td>
<td>11.5</td>
<td>1.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Bauru (BR)</td>
<td>117</td>
<td>16.1</td>
<td>2.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Maringá (MG)</td>
<td>119</td>
<td>17.9</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>Barreiras (BA)</td>
<td>146</td>
<td>19.4</td>
<td>4.4</td>
<td>30.1</td>
</tr>
<tr>
<td>Araçatuba (AT)</td>
<td>150</td>
<td>19.5</td>
<td>3.7</td>
<td>26.7</td>
</tr>
<tr>
<td>Santos (SA)</td>
<td>147</td>
<td>24.7</td>
<td>16.5</td>
<td>67.3</td>
</tr>
<tr>
<td>Fortaleza (FO)</td>
<td>150</td>
<td>21.0</td>
<td>3.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Salvador (SS)</td>
<td>150</td>
<td>27.1</td>
<td>3.3</td>
<td>93.3</td>
</tr>
</tbody>
</table>

- **n**: number of larvae assayed; **med**: median; **sdev**: standard deviation; **p99**: percentage 99
- **p% > p99**: percentage of individuals with enzyme activity higher than Rockefeller percentage 99 activity
- Not determined

**Legend**:
- Normal enzyme activity
- Altered enzyme activity
- Very altered enzyme activity
lakes assayed, median activity of enzymes, standard deviation and percentage of individuals with activity higher than the percentage 99 of Rockefeller strain are given in Table 2. A higher activity of all four metabolic enzymes was observed on population SS, followed by SA and FO, which were characterized as resistant to temephos. The enzyme GST was altered in all field populations but at a higher level of alteration in SS and SA. Populations BA and MG, with respectively moderate and low level of resistance to temephos, presented normal activity of MFO and alteration on beta esterase and GST. Populations with the lowest R.R. to temephos, MA and BR, presented normal activity for all enzymes, except for GST with the smallest percentage of individuals with alteration on that enzyme activity (15.7% and 18.0% respectively).

**Residual effect of commercial products**

The adult emergence of all non-treated containers was higher than 90%. Temephos induced mortality at larval stage while pyriproxyfen treatment affected mainly the pupal stage. Larval mortality with pyriproxyfen was 2.3% on average. Exceptions were observed only in MA and AT populations, with larval mortality higher than 10.8%.

The residual effect of both products varied according to the containers. Both treatments presented a shorter residual effect in tyres.

Treatment of glass vases with temephos (Table 3) resulted in 100% inhibition of adult emergence during the whole test period, except for the FO population where inhibition ended in 92%. In tyres the effect of temephos decreased over time, especially for populations FO (13 days), RE and SA (29 days), which had the higher RRs for temephos. The populations MA and Rockefeller were the only ones to arrest 100% inhibition of adult emergence for 44 days.

On the vases treated with pyriproxyfen (Table 4), 100% of inhibition of adult emergence was observed at the 58-days period

**Table 3: Adult emergence inhibition observed in vases and tyres treated with temephos (Temefós Fersol 1G)**

<table>
<thead>
<tr>
<th>Days*</th>
<th>Rockefeller</th>
<th>MA</th>
<th>AT</th>
<th>MG</th>
<th>SA</th>
<th>FO</th>
<th>RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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<td>100</td>
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<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>44</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>58</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Tyres</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>95</td>
<td>84</td>
</tr>
<tr>
<td>44</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>78</td>
<td>30</td>
<td>51</td>
</tr>
<tr>
<td>58</td>
<td>58</td>
<td>84</td>
<td>9</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* Days after treatment
Table 4: Percentage of adult emergence inhibition observed in vases and tyres treated with pyriproxyfen (Sumilarv® 0.5 G)

<table>
<thead>
<tr>
<th>Days*</th>
<th>Vases</th>
<th>Aedes aegypti populations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rockefeller</td>
<td>MA</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>100</td>
<td>100</td>
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<td>29</td>
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<td>98</td>
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<td>98</td>
<td>98</td>
</tr>
<tr>
<td>44</td>
<td>85</td>
<td>91</td>
</tr>
<tr>
<td>58</td>
<td>61</td>
<td>43</td>
</tr>
</tbody>
</table>

* Days after treatment

only for Rockefeller and the field population MA. The shorter effect was observed in the SA population (29 days), and in all the other populations inhibition of adult emergence was observed to be higher than 95% for 44 days. In tyres, only Rockefeller showed inhibition during the whole test period. While AT presented a shift in inhibition (86% to 91%) during the observation period, MG presented the longer effect among field populations (44 days) followed by MA and RE (29 days). Again, the populations FO and SA presented the shorter residual effect, i.e. 1 and 13 days respectively.

The mortality data were converted into arcsin values and analysed by Student-t test to compare the response of groups of populations. The analysis of data from treatment in vases showed that while temephos treatment did not cause significant difference between the two groups of populations, treatment with pyriproxifen presented a significantly lower effect on the moderately resistant population groups when compared to the Rockefeller strain (p=0.01), and a significantly shorter effect when the results of all populations were compared with the treatment results with temephos (p=0.002).

The treatment with temephos in tyres showed a significantly shorter residual effect only on the group of moderately resistant populations (p=0.02), while treatment with pyriproxifen presented significant difference between the two groups of populations (p=0.02) and between each group and Rockefeller strain (p<0.05).

Discussion

The pyriproxifen doses that caused adult emergence inhibition estimated in this study were higher than the lethal concentrations observed by Hatakoshi et al. [33] (L.C. 50 of 0.023 ppb; Itoh et al. [34] (L.C. 50 of 0.056 ppb) and Henrick [35] (L.C. 50 of 0.0039 ppb). Our estimations are closer to the lethal concentrations described by Estrada and Mulla [36] (L.C. 50 of 0.33 ppb and L.C. 95 of 2.6 ppb).
Population SS, which presents the highest R.R. to temephos, also presented the highest R.R. to pyriproxyfen. No field population evaluated in this study had been previously exposed to pyriproxyfen, so they had not been under selection for resistance to this IGR.

Although there is no recorded evidence of pyriproxyfen resistance to Ae. aegypti, the possibility of cross-resistance between conventional insecticides and IGRs has been reported for other insects like Tribolium castaneum\textsuperscript{37} and houseflies\textsuperscript{38}. Oxidase activity seems to be involved on resistance to juvenile hormone in houseflies\textsuperscript{39-42}. In the present study, differences on pyriproxyfen IE concentrations among susceptible and resistant organophosphate populations were also observed, suggesting the possibility of cross-resistance between the IGR and temephos.

Braga et al.\textsuperscript{43} discuss the possibility of cross-resistance between temephos and methoprene, another juvenile hormone analogue, in Ae. aegypti populations that presented high esterase and monoxigenase activity. The metabolism of endogenous juvenile hormones is associated to both classes of enzymes in other insects\textsuperscript{40-43}.

The role of the studied metabolic enzymes on the observed resistance to temephos is not easy to define since all four enzymes were highly altered on resistant populations (SS, FO and SA). It is possible that the multiple metabolic alterations are responsible for temephos resistance and also for the higher pyriproxyfen observed on population SS. Nevertheless, the alteration on beta esterases observed for populations BA and MG, less susceptible than BR and MA, which had normal activity, also indicate a possible role of this class of enzymes. The enzyme GST might also play an important role on temephos resistance as it is highly altered on resistant populations, especially on population SS.

Although esterases have been previously related to temephos resistance in Ae. aegypti\textsuperscript{13,44-46} this class of enzyme is not the sole enzyme to be more active in resistant populations. GST was also characterized in Cuba\textsuperscript{44}, Braga et al.\textsuperscript{43} relates alterations not only in esterases activity but also in MFO and GST enzymes on temephos-resistant Brazilian Ae. aegypti populations, making it difficult to ascribe temephos resistance to only one class of enzymes. The same could be said for pyriproxyfen, as strain SS presented the higher R.R. and a high activity of all enzymes, although MFO presented the higher alteration on that population (75.6% of individuals) and the role of this enzyme on IGR resistance is well-documented in literature\textsuperscript{47-50}.

Data from the simulated field trial test in vases indicate that the commercial product pyriproxyfen showed a significant shorter effect on adult emergence when compared with temephos (p=0.02). Temephos-treated vases exhibited 100% inhibition compatible with the 60-day treatment cycle proposed by the PNCD\textsuperscript{4} while treatment with pyriproxyfen was effective for this period only for Rockefeller and MA (temephos-susceptible population).

On tyres, pyriproxyfen treatment promoted a 100% inhibition of adult emergence on temephos-susceptible population Rockefeller. For the three resistant populations, temephos lasted more for two of them (SA and FO) and less for RE. The duration of effect of inhibition of adult emergence above 95% in both products for tyres is not compatible with the cycle of treatments as they lasted half of the expected period in many populations.

The lack of temephos effectiveness for 60 days against the susceptible Rockefeller strain contrasts with previous studies\textsuperscript{51} and raises suspicion on the quality of commercial products.
Melo-Santos et al.[52] tested pyriproxyfen in water-storage cement boxes and plastic buckets at the same concentration 0.05 ppm with water replaced three times a week, and found a complete inhibition of adult emergence of 160 days at shaded area and 46 days at sunlight exposure. Resende[53], with pyriproxyfen trials found total inhibition of adult emergence of 90 days at 0.05 ppm dose and 45 days at 0.01 ppm dose for the Rockefeller strain in cement boxes and glass vases; but in plastic buckets it was 30 days. Vythilingam[54] testing an Ae. aegypti population from Malaysia, resistant to temephos, observed pyriproxyfen complete inhibition of adult emergence of 160 days at 0.02 ppm even with water reposition every fifteen days in earthen jars and plastic tubs and 100% EI was obtained for 10 weeks in earthen jars where water was replaced daily.

The variation found in literature for the time of complete inhibition of adult emergence of products might be explained by the difference in the surface of containers used in tests and also by the variation in climate conditions. Also, bigger volumes of water tend to promote a more stable situation for larvicide action. This might also play a role in persistence effect.

The choice of containers tested in this study was based on their distinct surface of absorption, aiming to reach the best and worst availability of larvicides, respectively, for glass vases and tyres. We do believe that the treatment of those kinds of recipients should not be encouraged. More sustainable actions should be encouraged. In this respect, community participation has shown very satisfactory results in controlling Ae. aegypti foci in plant-related containers[28].

**Conclusions**

The development of resistance to temephos in many Ae. aegypti populations makes the change for alternative larvicides for dengue control programmes most desirable.

Commercial products based on the IGR pyriproxyfen are one of the alternatives listed by the Brazilian Health Ministry for substituting temephos.

A temephos-resistant Ae. aegypti population showed higher pyriproxyfen adult inhibition concentrations than susceptible ones. Besides, in semi-field assays, pyriproxyfen exhibited a lower residual effect against populations characterized as temephos-resistant, suggesting interference of temephos resistance with pyriproxyfen action. The mechanism by which this interference acts is not clear since MFOs, cited on literature as involved, are not the only class of enzymes altered in temephos-resistant populations.

Data presented here should be considered to make further evaluations and studies about this IGR action as well as on the choice of this product for use in management strategies.

**References**

Effect of pyriproxyfen in Aedes aegypti populations with different levels of temephos resistance


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