The Role of Gut Bacterial Cytochrome-P450 of Mosquito Larvae in Degradation of Temephos Insecticide

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Abstract

The present experimental work was generated to test the hypothesis that if there is a role of larval-related bacteria in protecting the host larvae from larvicides via bacterial-cytochrome-P450-based degradation. Here, a group of 50 larvae of Aedes albopictus was subjected to temephos at 0.5 mg/land ketoconazole, an inhibitor of the bacterial cytochrome P450, at200mg/50 liter(TKG), 50 larvae received ketoconazole only at 200mg/50 liter (KG), and 50 larvae that played as a control group (CG) were only subjected to the larvicide. The grouping was made in a triplicate for each group. The larvae were monitored for livability every day until the end of the experiment that lasted for 2 days. The results indicated 88% and 100% mortalities in the TKG larvae in the first and the second day respectively of the experiment. However, partial deaths were seen in the CG larvae as 30 (60%) and 20 (100%) at day 1 and 2 respectively. Moreover, 10% of the larvae died in the KG when ketoconazole was used. TKG revealed significant ($p$<0.05) increases in the mortalities more than that in the CG and the KG. Day 2 of the experiment showed 100% mortalities in both TKG and CG larvae. This experiment provides valuable information that larval-
related bacteria act as potential protectors against the killing activities of larvicides via the degradation activity of the bacterial cytochrome P450.

Keywords: Bacteria, bacterial cytochrome P450, larvicide, mosquitos


Introduction

Mosquitos as arthropods are considered as one of the worst health-destroying problems in the human past, present, and probably future. These creatures transmit wide ranges of life-threatening diseases such as dengue virus, malaria, Zika virus, and lymphatic filariasis. Since a very long time of many types of research, scientists have worked to control those diseases via the use of certain medicines, but no full-effective drugs are found. The work on the control of these diseases also targets the disease vectors via the use of insecticides. However, the results face many limitations such as insecticide resistance and environmental contamination concerns (1,2,3 and 4). The resistance issue acts as a huge obstacle that faces the control process of mosquitos and or their larvae. Many steps that tried to stop the development of this resistance showed drawbacks over time. This resistance could be maintained via the detoxifying effects of certain enzymes that belong to mosquitos such as cytochrome-P450-related enzymes (5-9). These enzymes degrade certain chemicals that belong to groups of insecticides such as deltamethrin and permethrin\(^\text{10,11}\). Bacteria that live along with or inside mosquitos could be considered as important factors for mosquito viability because bacteria also have types of P450 enzymes. These enzymes play important roles in degrading toxins and chemical poisons in the environment (12-16, 17). Previously, the P450 was detected to be present in the gut bacteria of mosquito larvae after the use of a certain chemical encouraging changes in the gut bacteria from non-P450-bearing bacteria to P450-bearing bacteria\(^\text{18}\), and this triggered the current work hypothesis that bacteria may protect larvae via bacterial-P450 degradation of larvicides.
Materials and methods

Experimental design

The investigation raising conditions and techniques were adapted from (18). The instar larvae were collected from field mosquitos. Here, a group of 50 larvae of *Aedes albopictus* were subjected to temephos at 0.5 mg/l and ketoconazole, an inhibitor of the bacterial cytochrome P450, at 200 mg/50 liter (TKG), 50 larvae received ketoconazole only at 200 mg/50 liter (KG), and 50 larvae that played as a control group (CG) were only subjected to the larvicide. Each group was performed in a triplicate. The concentrations of the chemicals used in this experiment were prepared following (19, 20). The chemicals were pre-applied to autoclaved-distilled water that was used to rear the larvae in Petri-dishes. New Petri-dishes contained fresh CADW was used to transfer the larvae in every day during the experiment period. Ten larvae in each Petri-dish were reared. The larvae were monitored for livability every day until the end of the experiment that lasted for 2 days.

Statistical analysis

Data were processed using Prism Software v7.0 (Graphpad Inc., USA). Mean±SEM was used to reveal groups at $P<0.05$ significance.

Results

The results indicated 88% and 100% mortalities in the TKG larvae in the first and the second day respectively of the experiment. However, partial deaths were seen in the CG larvae as 30 (60%) and 20 (100%) at day 1 and 2 respectively. Moreover, 10% of the larvae died in the KG when ketoconazole was used. Table 1 shows some detailed numbers of the current experiment. TKG revealed significant ($p<0.0001$) increases in the mortalities more than that in the CG and the KG, (figure 1-A). Day 2 of the experiment showed 100% mortalities in both TKG and CG larvae, (figure 1-B).
Table 1: Shows detailed numbers of the current experiment

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Triplicates</th>
<th>Mortalities in groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TKG</td>
</tr>
<tr>
<td>Day 1</td>
<td>1</td>
<td>44 (88%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43 (86%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>45 (90%)</td>
</tr>
<tr>
<td>Day 2</td>
<td>1</td>
<td>6 (100%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7 (100%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

Figure 1-A. The difference (Day-1) between TKG, CG, and KG. TKG revealed significant ($p < 0.0001$) increases in the mortalities more than that in the CG and KG. B. The difference (Day-2) between TKG, CG, and KG. TKG revealed no significant ($p > 0.05$) differences in the mortalities when compared to that in the CG.
Discussion

Our results revealed significant larvicide activities of temephos on the first day of the experiment when it was supported by the use of ketoconazole. This effect was significantly less in the CG when temephos was used alone. Ketoconazole inhibits the microbial P450 (21), so when it was used in the TKG, it might have deactivated the P450-enzymatic system that was supposed to degradetemephos (22,23). According to this, temephos might have not been detoxified by the ketoconazole-suppressed P450, and that is why it was highly effective in the killing of the larvae in the TKG. For deep knowledge, the recommended dose for the use of temephos in the drinking water is 1mg/l (20), however, we used it at 0.5mg/l. Even though the dose was as low as 0.5mg/l, temephos was so effective when supported by ketoconazole. The mortalities seen in the KG larvae could be normal as a small percentage may die in Lab conditions.

The current data suggest P450 as an excellent target to deactivate the detoxifying effects of bacteria that use the P450 enzymatic system and potentiate the killing activities of larvicides.

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Conflicts of interest Statement:
The authors declare that there is no conflict of interests regarding the publication of this article. This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media.

Informed Consent Statement: Informed consent has been obtained from all individuals included in this study.

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