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Chapter VI

Schematic representation of mosquitocidal activity of isolated compounds against *Cx. quinquefasciatus* and *Ae. aegypti*

**Streptomyces sp. CFR 16**
- Ignaciomycin

**Streptomyces collinus BG 4**
- Phenyl acetic acid

**Nonomuraea pusilla VAS 16**
- Phthalic acid

**Larvicidal activity of isolated compounds**

**Ovicidal activity of isolated compounds against *Ae. aegypti***
- 0.5 ppm
- 1.0 ppm
- 1.5 ppm
- 2.5 ppm

**Ovicidal activity of isolated compounds against *Cx. quinquefasciatus***
- 0.5 ppm
- 1.0 ppm
- 1.5 ppm
- 2.5 ppm
CHAPTER VI

LARVICIDAL AND OVICIDAL EFFECTS OF ISOLATED COMPOUNDS AGAINST

CULEX QUINQUEFASCIATUS AND AEDES AEGYPTI

INTRODUCTION

Mosquitoes are the principal vectors of many diseases affecting human beings and animals. They are haematophagous insects in the group of arthropods. Members of the family Culicidae in the order Diptera are the major vectors of chikungunya, dengue, filariasis, malaria, Japanese encephalitis and yellow fever; they transmit several other pathogens. They continue to have devastating impact on human being (James 1992; Service 1983). Mosquitoes also cause allergic responses in humans including local skin and systemic reactions such as angioedma (Peng et al., 1999). Currently, mosquito borne diseases not only cause high levels of morbidity and mortality, but also inflict great economic pact, including loss in commercial and labor output, particularly, in tropical and subtropical countries; however, no part of the world is free from these diseases (Fradin 2002; Harrus et al., 2005; Rascalou et al., 2012).

Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population (Ghosh et al., 2012). Over 2.5 billion people over 40% of the world’s population are now at risk from dengue. WHO currently estimates that there may be 50-100 million dengue infections worldwide every year. In 2013, 2.35 million cases of dengue were reported in the Americas alone, of which 37,687 cases were severe dengue (WHO, 2013).

Culex quinquefasciatus Say is a member of the Culex complex and is one of the main widespread subspecies found in pan and subtropical Americas, the neotropics, Afrotropics
Chapter VI

(White, 1975), Indo-malayan, Australasian (Lee et al., 1989), East Asia, UK and parts of Middle East. *Cx. quinquefasciatus* is commonly known as southern house mosquito and acts as a primary vector of lymphatic filariasis with worldwide distribution. *Cx. quinquefasciatus* is a worldwide vector of bancroftian filariasis in the tropical and subtropical countries. Filariasis is caused by *Wuchereria bancrofti* (Cobbald), a helminth that lives in the lymph glands and vessels that provokes edemas by lymph obstruction (Mukhtar et al., 2003). Lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection and approximately 400 million people are at risk of contracting filariasis worldwide by *Cx. quinquefasciatus* resulting in the annual economic loss of 1.5 billion dollars (WHO 2002). In India alone 25 million people harbor microfilaria and 19 million people suffer from filarial disease manifestations (NICD, 1990).

*Aedes aegypti* (L.) is generally known as a vector of arbovirus responsible for major diseases like dengue fever, haemorrhagic fever and chikungunya (Harrington et al., 2005; Kannathasan et al., 2011); it is endemic to Southeast Asia, the Pacific island area, Africa and Americas (Akram and Ahmed, 2005). The gradual increase and abundance of *Ae. aegypti* and the unexpected outbreaks of dengue disease in India has illustrated the devastating impact of dengue viruses on human beings. During 2012, a total of 5376 dengue cases and 39 deaths were detected in the state of Tamil Nadu, southern India (Kannan, 2012). It is necessary to prevent the transmission of dengue virus and improve public health by controlling the primary vector *Ae. aegypti*.

A large amount of the insecticides available in the markets are synthetic chemical products with prohibitively high cost. Repeated use of synthetic insecticides has created a number of ecological problems, such as the development of resistant insect strains, ecological
imbalance, elimination of non target organisms in the environment and harm to mammals (Anyaele and Amusan, 2003). In recent years, many researchers have been looking for new biological insecticides. However, only a few components and formulations of biological origin are available commercially in the world (Cetin et al., 2004). Different organisms, such as plants and microbes, have been used for the development of new products (Mehlhorn et al., 2005). Hence there is a constant need for developing biologically active plant materials and microbes as larvicides, which are expected to reduce the hazards in humans and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products are generally preferred because of their less harmful nature to non target organisms and due to their innate biodegradability. Accordingly, the present study was undertaken to study the activity of isolated microbial compounds viz., phthalic acid, phenyl acetic acid and ignaciomycin from actinomycetes as biocontrol agents for ecofriently mosquito management against two vector mosquitoes namely Culex quinquefasciatus Say and Aedes aegypti L.

MATERIALS AND METHODS

Actinomycetes and Insects selected for the present investigation

For the present study phthalic acid from Nonomuraea pusilla VAS 16, phenyl acetic acid from Streptomyces collinus BG 4 and ignaciomycin from Streptomyces sp. CFR 16 were chosen to assess the effect of these compounds on Cx. quinquefasciatus and Ae. aegypti belonging to the family Culicidae of the order Diptera and to check their non target effect on Gambusia affinis.

Test Compound

Isolation of actinomycetes, crude extraction and isolation of compound have already been explained in previous chapters. Compounds such as phthalic acid, phenyl acetic acid and ignaciomycin were isolated from strains VAS 16, BG 4 and CFR 16, respectively.
Insect rearing

Two mosquito species namely *Cx. quinquefasciatus* and *Ae. aegypti* were continuously cultured in the laboratory for more than 10 generations. Eggs and larvae of two mosquito species were obtained from the laboratory culture and used for experiments. The rearing and experimental conditions in the laboratory were: 27-28°C temperature, 70-75% relative humidity and $11 \pm 1$ h photo period. Larval stages were reared on powdered commercial dog biscuits and yeasts (1:3). Adults were fed on wet raisins and 10% sucrose solution. Female mosquitoes were periodically blood fed on restrained albino rats principally for egg production.

Larvicidal bioassay

Larvicidal activity was evaluated using a modified method prescribed by the World Health Organization (2005). Five replicates with ten early third instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* were introduced into each test containers. Concentrations of compounds were 0.5, 1.0, 1.5 and 2.0 ppm. The volume in each container was increased to 250ml using tap water. The test materials were dissolved in DMSO. DMSO was used as negative control, and azadirachtin (40.86% purity) and temephos were used as positive controls. Mortality was registered after 24 h exposure period. The moribund and dead larvae were collected and larval mortality was calculated for each concentration. Larvae were considered dead when they failed to move to the surface of the medium when provoked with a needle. Percent mortality was calculated using the formula (1), and corrections for mortality when necessary were done using Abbott’s (1925) formula (2).

\[
\text{Percentage of Mortality} = \frac{\text{No. of Dead larvae}}{\text{No. of Larvae introduced}} \times 100
\]

\[
\text{1}
\]

1
Corrected percentage of mortality: \[
\frac{(1 - \frac{n_{in\ T\ after\ treatment}}{n_{in\ C\ after\ treatment}} \times 100)}{\times 100}
\]

where \(n\) is the number of larvae, \(T\) is the treated and \(C\) is the control. The corrected percentage mortality value for each concentration was considered to estimate \(LC_{50}\) and \(LC_{90}\) values using US EPA probit analysis software (version 1.5).

**Ovicidal assay**

Ovicidal activity was studied following the methods of Elango et al., (2009) and Reegan et al., 2014. Five replicates with ten freshly laid eggs of \(Cx.\ quinquiescissatus\) and \(Ae.\ aegypti\) were separately exposed to four different concentrations, namely 0.5 ppm, 1.0 ppm, 1.5 ppm, and 2 ppm, prepared using DMSO. Each concentration was replicated five times. Control (DMSO in water) was maintained separately and egg mortality was observed under the microscope. Azadirachtin and temephos were used as positive controls for comparison with five replications. The percent ovicidal activity was assessed at 120 h post-treatment using the following formula (3).

\[
\text{Percent ovicidal activity} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100
\]

**Effect of ignaciomycin on non-target organism**

The effects of most active compound (Ignaciomycin) were assayed as per the method of Maheswaran and Ignacimuthu (2012) against a non target organism. \(Gambusia affinis\) (predatory fish) was collected from pond of Fishery Research Institute, Chetpet, Chennai, India. Then the predators were exposed to different test concentrations of 1.0, 5.0 and 10 ppm with ten replicates. For each replication one predator was used in order to avoid cannibalism. Azadirachtin was used as positive control with ten replicates along with ten controls. The mortality of predators and other abnormalities were observed after 24 h exposure. Then the
exposed predators were transferred to normal water and the post treatment effect of ignaciomycin was observed continuously for 15 days.

RESULTS AND DISCUSSION

Development of resistance to commercial insecticides by mosquitoes has stimulated the search for new control strategies and there is a pressing need to search for new bio control agents from natural sources. As a result, terrestrial actinomycetes have motivated the revaluation of new sources of chemically diverse bioactive compounds. As part of an ongoing natural product research program, we have been screening microbial extracts and compounds for insecticidal activity (Isaka et al., 1999, 2000, 2001). Microbial control of insect vector populations can be highly effective and generally has advantages over chemical control because many are host specific and safe for non target organisms. Actinomycetes are Gram positive bacteria with a percentage of guanine cytosine higher than 55%, and most of them produce mycelia. They are particularly interesting due to their capacity to produce secondary metabolites with diverse chemical structures which are environmentally safe vector and pest managing agents (Valanarasu et al., 2008).

Larvicidal bioassay of ignaciomycin

In the present study, the ignaciomycin from Streptomyces sp. CFR 16 exhibited the lowest LC$_{50}$ value of 1.71 ppm with the fiducial limits of 1.47 ppm (lower) and 2.13 ppm (upper) and the LC$_{90}$ value of 4.39 ppm with the fiducial limits of 3.15 ppm (lower) and 8.36 ppm (upper) against the third instar larvae of Cx. quinquefasciatus. It also exhibited the LC$_{50}$ value of 1.82 ppm with the fiducial limits of 1.55 ppm (lower) and 2.36 ppm (upper) and the LC$_{90}$ value of 4.89 ppm with the fiducial limits of 3.38 ppm (lower) and 10.35 ppm (upper) against the third-instar larvae of Ae. aegypti. At 2 ppm concentration of CFR 16 showed 100% larvicidal activity
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against *Cx. quinquefasciatus* and *Ae. aegypti* in 24 h. The chi-square values were significant at *p* \(< 0.05\) level. These results were comparable to azadirachtin and Temephos, which recorded LC\(_{50}\) and LC\(_{90}\) values of 0.28, 0.34 and 0.55, 1.04, and 0.65, 0.92 and 1.62, 1.82 ppm against the third instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* respectively (Table 6.1 and 6.2). This result is also comparable to earlier reports of Govindarajan et al., 2005 and Govindarajan et al., 2006a who reported that the culture filtrates of five different soil fungi showed larvicidal activity against the larvae of *Cx. quinquefasciatus*. (2S,5R,6R)-2-hydroxy-3,5,6-trimethyloctan-4-one, a novel larvicidal and acaricidal produced by *Streptomyces* sp. showed significant activities against blood sucking parasites at 82.82 ppm; 0.957 against *Cx. quinquefasciatus*, 69.65 ppm; 0.906 against *An. subpictus* and 94.49 ppm; 0.982 against *R. microplus* (Deepika et al., 2012). The solvent control DMSO showed no mortality against the tested larvae.

**Ovicidal activity**

Exposure of freshly laid eggs to ignaciomycin has been found to cause higher mortality rates. The percentage of egg hatchability of *Cx. quinquefasciatus* and *Ae. aegypti* with ignaciomycin of *Streptomyces* sp. CFR 16 are presented in Tables 6.3 and 6.4. It showed 62 \(\pm 14.83\)% mortality at 2 ppm concentration against *Cx. quinquefasciatus* and 60 \(\pm 8.94\)% against *Ae. aegypti*. In control experiments, 100% hatchability was obtained. Recent studies have indicated that spinosad, a mixture of two tetracyclic macrolide compounds produced during the fermentation of a soil actinomycete, may be suitable for controlling a number of medically important mosquito species, including the dengue vector, *Ae. aegypti* (Antonio et al., 2009). The positive control azadirachtin and temephos recorded ovicidal activity of 68.0 \(\pm 4.0\)%, 32.0 \(\pm 2.82\)% and 60.8 \(\pm 1.78\)%, 22.8 \(\pm 2.19\)% at 2 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Similarly, the percentages of egg hatchability of
C. tritaeniorhynchus and C. gelidus with marine actinobacterial crude extracts of three Saccharomonospora spp. (LK-1), Streptomyces roseiscleroticus (LK-2), and Streptomyces gedanensis (LK-3) strains showed moderate to high larvicidal effects after 24 h of exposure at 1,000 ppm. Crude extracts of Saccharomonospora spp. and Streptomyces gedanensis showed no hatchability at 1,000 ppm against C. tritaeniorhynchus and C. gelidus, respectively (Karthik et al., 2011).

**Larvicidal bioassay of Phenyl acetic acid**

The third larvicidal activity of phenyl acetic acid from Streptomyces collinus BG 4 showed good activity against Cx. quinquefasciatus and Ae. aegypti. The LC$_{50}$ values of 2.07 and 2.22 ppm; LC$_{90}$ values of 4.87 and 6.33 ppm against Cx. quinquefasciatus and Ae. aegypti were recorded. Further, significant chi-square values were recorded in all the treatments. Azadirachtin and temephos were used as positive controls (Table 6.1 and 6.2). No mortality was observed in the control group. Our results were in agreement with the previous reports of Mohankumar Thenmozhi et al., (2013), Masahiko Isaka et al., (2002), Kumar Saurav et al., (2013).

**Ovicidal activity**

The mean percent of egg hatchability of Cx. quinquefasciatus and Ae. aegypti were tested at different concentrations of phenyl acetic acid, and the results are listed in Tables 6.3 and 6.4. It showed 50.3 ± 7.45% mortality at 2 ppm against Cx. quinquefasciatus and 42.0 ± 7.11% against Ae. aegypti. The percent hatchability was inversely proportional to the concentration of compound and directly proportional to the eggs. Azadirachtin and temephos were used as positive controls. Similarly, in the first time report of Luz et al., (2007), 21 hyphomycete fungi species screened for ovicidal activity against A. aegypti. A clear ovicidal activity with low values of hatching (1.3-40%) was observed after 25 days of incubation with Isaria farinosa,
Paecilomyces carneus, Paecilomyces marquandii, Isaria fumosorosea, Metarhizium anisopliae, Penicillium sp., Paecilomyces lilacinus, Beauveria bassian, and Evlachovaea kintrischica. More than 63% of eggs hatched after 25 days exposures to 11 other fungi species deemed as ineffective.

**Larvicidal bioassay of phthalic acid**

The bioassay of phthalic acid from Nonomuraea pusilla VAS 16 revealed that the effective LC$_{50}$ concentrations to kill the larvae were 4.27 and 4.67 ppm with the fiducial limits of 2.70, 2.80 ppm (lower) and 25.49, 691.67 ppm (upper) for Cx. quinquefasciatus and Ae. aegypti, respectively. The LC$_{90}$ values were 14.90 and 11.90 ppm with the fiducial limits of 5.98, 4.74 ppm (lower) and 631.57, 135137.35 ppm (upper) for Cx. quinquefasciatus and Ae. aegypti, respectively. The chi-square values were significant at p<0.05 level (Table 6.1 and 6.2). The control showed no mortality against the tested larvae. Azadirachtin and temephos were used as positive controls. Several earlier studies reported the larvicidal activity of several novel natural products isolated from actinomycetes (Murugesan et al., 2009, Vijayakumar et al., 2010, Reegan et al., 2014).

**Ovicidal activity**

The result of egg hatching inhibition by phthalic acid, at the dosage of 0.5, 1.0, 1.5, and 2 ppm showed 0%, 2.0 ± 1.3%, 5.0 ± 0.44% and 11.0 ± 1.34% against Cx. quinquefasciatus; 0%, 3.5 ± 5.47%, 5.25 ± 4.90%, and 10.3 ± 4.05% against Ae. aegypti, respectively and the results are presented in Tables 6.3 and 6.4. Azadirachtin and temephos were used as positive controls. Several earlier studies reported the ovicidal activity of several novel natural products isolated from actinomycetes (Antonio et al., 2009, Karthik et al., 2011).
**Effect of ignaciomycin on non-target organisms**

The effect of isolated compound (Ignaciomycin) against non-target organism *G. affinis* (Predatory fish) was evaluated. Survival and swimming activity of the tested mosquito predator *G. affinis* was normal as in control during the exposure to various doses of ignaciomycin. The compound did not cause any acute and chronic toxicity in the fish up to 10 ppm level.

**Conclusion**

Microbes could be alternative sources for mosquitocides as they constitute potential sources of bioactive molecules and are generally free from harmful effects. Regular use of natural derivatives in mosquito control instead of synthetic insecticides could reduce environmental degradation. Results of this research indicate that the potential ignaciomycin derived from *Streptomyces* sp. constitutes a good source of bioactive molecules and is generally free from harmful effects. This study clearly showed that the compound phenyl acetic acid showed moderate activity and phthalic acid showed weak activity when compared to ignaciomycin against the tested mosquito species. The isolated compound did not show any toxic effect against non target organism *G. affinis* (predatory fish). These results are very promising to formulate a potent and affordable natural product to control dreadful disease transmitting and nuisance crating human vector mosquitoes.
Table: 6.1 Lethal concentration (in ppm) of compounds isolated from *Nonomuraea pusilla VAS 16*, *Streptomyces collinus* BG 4, *Streptomyces* sp. CFR16 against the third-instar larvae of *Culex quinquefasciatus*

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Treatment</th>
<th>LC$_{50}$ (ppm)</th>
<th>95% confidence limit</th>
<th>LC$_{90}$ (ppm)</th>
<th>95% confidence limit</th>
<th>Intercept ± SE</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>Phthalic acid</td>
<td>4.27</td>
<td>2.70 to 25.49</td>
<td>14.90</td>
<td>5.98 to 631.57</td>
<td>3.5 ± 0.1</td>
<td>0.5*</td>
</tr>
<tr>
<td></td>
<td>Phenyl acetic acid</td>
<td>2.07</td>
<td>1.76 to 2.76</td>
<td>4.87</td>
<td>3.40 to 10.53</td>
<td>3.9 ± 0.1</td>
<td>4.8*</td>
</tr>
<tr>
<td></td>
<td>Ignaciomycin</td>
<td>1.71</td>
<td>1.47 to 2.13</td>
<td>4.39</td>
<td>3.15 to 8.36</td>
<td>4.26 ± 0.1</td>
<td>5.5*</td>
</tr>
<tr>
<td>Positive control</td>
<td>Azadirachtin</td>
<td>0.28</td>
<td>0.12 to 0.37</td>
<td>0.55</td>
<td>0.46 to 0.66</td>
<td>7.4 ± 0.3</td>
<td>0.1*</td>
</tr>
<tr>
<td></td>
<td>Temephos</td>
<td>0.65</td>
<td>0.56 to 0.73</td>
<td>1.62</td>
<td>1.42 to 1.93</td>
<td>5.5 ± 0.1</td>
<td>1.7*</td>
</tr>
</tbody>
</table>
Table: 6.2 Lethal concentration (in ppm) of compounds isolated from *Nonomuraea pusilla* VAS 16, *Streptomyces collinus* BG4, *Streptomyces* sp. CFR16 against the third-instar larvae of *Aedes aegypti*

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Treatment</th>
<th>LC$_{50}$ (ppm)</th>
<th>95% confidence limit</th>
<th>LC$_{90}$ (ppm)</th>
<th>95% confidence limit</th>
<th>Intercept ± SE</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LL</td>
<td>UL</td>
<td>LL</td>
<td>UL</td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Phthalic acid</td>
<td>4.67</td>
<td>2.80</td>
<td>691.67</td>
<td>11.90</td>
<td>4.74</td>
<td>135137.35</td>
</tr>
<tr>
<td></td>
<td>Phenyl acetic acid</td>
<td>2.22</td>
<td>1.81</td>
<td>3.30</td>
<td>6.33</td>
<td>3.97</td>
<td>18.35</td>
</tr>
<tr>
<td></td>
<td>Ignaciomycin</td>
<td>1.82</td>
<td>1.55</td>
<td>2.364</td>
<td>4.89</td>
<td>3.38</td>
<td>10.35</td>
</tr>
<tr>
<td>Positive control</td>
<td>Azadirachtin</td>
<td>0.34</td>
<td>0.22</td>
<td>0.43</td>
<td>1.04</td>
<td>0.90</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>Temephos</td>
<td>0.92</td>
<td>0.11</td>
<td>1.66</td>
<td>1.82</td>
<td>1.17</td>
<td>646.1</td>
</tr>
</tbody>
</table>
Table: 6.3 Percent ovicidal activity of compounds isolated from *Nonomuraca pusilla* VAS 16, *Streptomyces collinus* BG4, *Streptomyces* sp. CFR16 against *Culex quinquefasciatus* eggs

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Compounds</th>
<th>Concentration (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phthalic acid</td>
<td>0 ± 0</td>
<td>2.0 ± 4.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenyl acetic acid</td>
<td>6.0 ± 5.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.0 ± 5.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ignaciomycin</td>
<td>22 ± 4.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32 ± 8.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Positive control</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azadirachtin</td>
<td>15.2 ± 1.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.0 ± 5.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Temephos</td>
<td>8.0 ± 2.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.8 ± 4.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table: 6.4 Percent ovicidal activity of compounds isolated from *Nonomuraea pusilla* VAS 16, *Streptomyces collinus* BG4, *Streptomyces* sp. CFR16 against *Aedes aegypti* eggs

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Compounds</th>
<th>Concentration (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>Phthalic acid</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Phenyl acetic acid</td>
<td>4.0 ± 5.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ignaciomycin</td>
<td>24 ± 11.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive control</td>
<td>Azadirachtin</td>
<td>7.2 ± 1.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Temephos</td>
<td>5.6 ± 2.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>