ISOLATION OF MOSQUITOCIDAL BACTERIA FROM SOIL SAMPLES TO CONTROL MOSQUITO VECTORS

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ABSTRACT

In the present study, an attempt was made to screen and isolate potent and ecofriendly mosquitocidal bacteria from different soils collected from Union Territory of Puducherry, India. From a total of 140 soil samples, 16 isolates showed mosquitocidal activity. Out of these 16 isolates, two bacteria isolated from red soil of Kalapet and Kanagachettikulam villages of Union Territory of Puducherry, India with code no. PYKAL-31A and PYKC-33C were found to be most potent. Extensive bioassay was carried out with these two bacterial strains against larvae of Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. The LC₅₀ and LC₉₀ values of PYKAL-31A against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi were 0.007, 0.011, 0.015 and 0.015, 0.021 and 0.029, respectively and that of PYKC-33C were 0.009, 0.014, 0.013 and 0.015, 0.026, 0.023, respectively.

Key words: Bioassays, red soil, mosquito larvae, Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti, Bacillus thuringiensis israelensis, Bacillus sphaericus, dengue, biological control, mosquito control

Insects serve as carriers of the etiological agents of a number of harmful and dangerous diseases thereby causing an impact on global health (Ferguson, 2018). Recent zika virus epidemic issues demonstrated the emerging and re-emerging illnesses, especially due to arboviruses, which continue to be a global health threat. Many diseases, including malaria, dengue, chikungunya and yellow fever are mostly spread by mosquitoes. Chemical pesticides are mainly used to manage pests and the vectors. However, the repetitive use of these harms the environment, human health and non-target organisms (Ahsan and Shimizu, 2021). Additionally, insect pests become highly resistant to many pesticides as a result of their improper and overuse (Devine and Furlong, 2007). Microbial insecticides have attracted the attention of researchers due to the safety and lesser negative impact on environment (Ahsan and Shimizu, 2021). Bacillus spp. are the common biocontrol agents used in commercial mosquitocidal formulations (Nicholson, 2002). An entomopathogenic bacteria, Bacillus thuringiensis israelensis (Bti) de Barjac (de Barjac, 1978). Lysinibacillus sphaericus (L. sphaericus) Neide strains were later found to be highly toxic against Culicidae larvae (Kellen et al., 1965); L. sphaericus and Bti exhibit high and specific larvicidal activity against the vectors, Aedes, Culex and Anopheles (Lacey, 2007). However, mosquito species have developed resistance (Yuan et al., 2000). In this study, an attempt was made to screen and isolate new mosquitocidal bacteria from different soil types collected from Union Territory of Puducherry, India.

MATERIALS AND METHODS

Field survey was carried out in 2021 for collection of soil types like red, loamy and clay soils from various agricultural fields of Kalapet, Kodathur, Pandachozanallur, Kanagachettikulam, Kanuvapettai, Uruvaiyaru and Suthukeny villages, Union Territory of Puducherry, India. Soil samples were collected at three strata i.e., surface, 5 cm and 10 cm depth and stored in cold room (4°C) until further use. These were screened for mosquitocidal bacteria using serial dilution method.
One g of each soil sample was weighed and serially diluted (10⁻³) (Poopathi et al., 2014). Using spread plate method 100 μl of the dilution was spread on nutrient yeast extract salt medium (NYSM) and incubated for 24 hr at room temperature. Single isolated bacterial colony was picked and inoculated into 10 ml of NYSM broth and incubated at room temperature in Orbitek shaker (Scigenics Biotech LT4676, India) at 250 rpm for 72 hr. Preliminary toxicity assays of the bacterial culture were conducted against the laboratory reared late third instar larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. The larvae were treated with doses of 1 and 10 μl bacterial culture (72 hr bacterial culture). After 24 hr, the bioassay results were recorded. The bacterial isolates showing the highest mortality were selected for further studies. The purity of the positive bacterial isolates was further checked through quadrant spreading and subsequently preserved in glycerol (50%) and stored at -30°C until further use. Detailed toxicity assay was carried out using the late 3rd instar larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (WHO, 2005). Disposable wax coated 150 ml capacity paper cups were taken and 100 ml of tap water was added. Twenty-five larvae were introduced into the cups and left for acclimatization. Then the bacterial stock solution was prepared using lyophilized powder at the concentration of 5 mg/ 10 ml of water using mechanical homogenizer (Remi RQT 124A, India). Seven dosages were fixed and toxicity assay was carried out. Four replicates were maintained for each dose with appropriate controls. The dead larvae from each cup were recorded after 24 hr. Through probit analysis (SPSS 16.0 version) the lethal concentrations (LC50 and LC90) were determined.

**RESULTS AND DISCUSSION**

Totally 140 soil samples were collected including red, clay loam, clay, sandy and loamy soil. These were screened for mosquitocidal bacteria, using serial dilution method (Poopathi et al., 2014; Hemaladkshmi et al., 2023). Bacterial colonies were isolated and cultured till 72 hr in NYSM broth. Preliminary bioassay was carried out for all the isolated bacterial strains against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Sixteen bacterial strains revealed mosquitocidal activity. The majority of mosquitocidal bacterial strains were derived from red soil. Among the sixteen strains, seven were obtained in Kalapet village from red soil, two in Kodathur village from loamy soil, two in Kanagachettikulam village from red soil, and one each from Pandachozanallur village (clay soil), Kanuvapettai village (clay loam), Uruvaiyar village (clay loam), and Suthukeny village (loamy soil). Additionally, the mosquitocidal bacteria *B. thuringiensis israelensis* (VEVP-60) was isolated from red soil in Velampattu village, Vellore, Tamil Nadu (Hemaladkshmi et al., 2023). Eight isolates were from red soil in Tenkasi and Tirunelveli districts, Tamil Nadu (Abhisubesh et al., 2023).

Gram and spore staining were done for these isolates which showed that all were gram positive bacteria exhibiting subterminal sporulation. Among these, two bacterial strains isolated from red soil of Kalapet and Kanagachettikulam village of Puducherry, with sample code PYKAL-31A and PYKC-33C were found to show more toxic potency. Hence, PYKAL-31A and PYKC-33C strains were further analyzed in order to use them for vector control. Detailed toxicity assay was carried out against laboratory reared mosquito species, *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*. The result revealed their mosquitocidal activity against *Ae. aegypti* followed by *Cx. quinquefasciatus* and *An. stephensi*. The lethal concentration (LC50 and LC90) was determined for these two bacterial strains (Table 1). The lethal concentration (LC50 and LC90) of PYKAL-31A for *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* are 0.0075, 0.011, 0.015 and 0.015, 0.015, 0.015 and 0.015, 0.026, 0.023 (mg/ l) respectively and for PYKC-33C are 0.009, 0.014, 0.013 and 0.015, 0.026, 0.023 (mg/ l) respectively. For both these strains, *Ae. aegypti* was highly susceptible followed by *Cx. quinquefasciatus* and *An. stephensi* (*Ae. aegypti>* *Cx. quinquefasciatus>* *An. stephensi)*. Previous studies show that *Bt* strain isolated from Brazil (TOD651), India (BU55) was effective in controlling larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* (Roy et al., 2021; Alves et al., 2023). *Bacillus cereus* isolated from fresh water fish *Clarias batrachus* also showed promising activity against the larvae of *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. stephensi* (Manikandan et al., 2023). According to da Costa Fernandes et al. (2022), thirty *Bacillus thuringiensis* strains that were isolated from soil samples in Maranhao, Brazil, demonstrated high toxicity to *A. aegypti*. Eleven novel *Bt* isolates with strong efficacy against *Ae. aegypti*, *An. stephensi*, and *Cx. pipiens* larvae in their third instar have been isolated from Pakistani soil (Fatima et al., 2023). The isolates, the new isolates PYKAL-31A and PYKC-33C isolated from the red soil in the present study were superior in controlling mosquito larvae; *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* and *An. stephensi*. Further studies
on the identification of the bacterial strain, mechanism of synthesis of endotoxin(s), formulations for the field evaluations are in progress. Studies also required to be carried out and examine if there is any adverse effect against the non-target aquatic organisms.

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AUTHOR CONTRIBUTION STATEMENT

KG has prepared the manuscript, collected literatures, compilation, performed the analysis, interpretation of data from the experiments pertaining to laboratory and field work. BB, SM, VA, KA, SM, JL and PH have contributed in literature collection, toxicity bioassay, compilation, reference work. AM and KV have contributed in interpreting the background work. SP has given the background ideas, conception of the studies, reviewing the literatures, revision and modification of the MS. KG has written the manuscript. All authors approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES


Table 1. Toxicity values (LC$_{50}$ and LC$_{90}$) of PYKAL-31A and PYKC-33C

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Mosquito larvae (late third larvae)</th>
<th>Intercept</th>
<th>Slope</th>
<th>LC$_{50}$ (mg/l)</th>
<th>LC$_{90}$ (mg/l)</th>
<th>Chi-square</th>
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<tr>
<td></td>
<td>*LC-Lethal Concentration, UCL-Upper Confidence Limit, LCL-Lower Confidence Limit</td>
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<tr>
<td>PYKAL-31A</td>
<td>Aedes aegypti</td>
<td>-1.281</td>
<td>0.008</td>
<td>0.0075 (0.007-0.008)</td>
<td>0.015 (0.014-0.016)</td>
<td>117</td>
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<tr>
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<td>Culex quinquefasciatus</td>
<td>-1.391</td>
<td>0.006</td>
<td>0.011 (0.01-0.011)</td>
<td>0.021 (0.02-0.022)</td>
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<td>Anopheles stephensi</td>
<td>-1.456</td>
<td>0.005</td>
<td>0.015 (0.014-0.017)</td>
<td>0.029 (0.002-0.03)</td>
<td>119</td>
</tr>
<tr>
<td>PYKC-33C</td>
<td>Aedes aegypti</td>
<td>-1.794</td>
<td>0.010</td>
<td>0.0093 (0.008-0.01)</td>
<td>0.015 (0.01-0.017)</td>
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<td>Culex quinquefasciatus</td>
<td>-1.612</td>
<td>0.005</td>
<td>0.014 (0.014-0.015)</td>
<td>0.026 (0.026-0.028)</td>
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<td>Anopheles stephensi</td>
<td>-1.638</td>
<td>0.006</td>
<td>0.013 (0.012-0.014)</td>
<td>0.023 (0.02-0.024)</td>
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of *Bacillus thuringiensis* to control vector borne diseases against mosquito fauna. Saudi Journal of Biological Sciences 30(4): 103610.


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