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Development of mosquito control: Evaluate the larvicidal activity of biosurfactant against *culex* sp.

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Abstract
The larvicidal activity of biosurfactants refers to the ability of these naturally occurring surface-active molecules to kill or inhibit the growth and development of insect larvae. Biosurfactants, synthesised by microorganisms such as bacteria, yeast, and fungi, have garnered significant interest for their potential application in pest management. This interest stems from their environmentally friendly characteristics and minimal harm to humans and animals. Here are some key points regarding the larvicidal activity of biosurfactants. Biosurfactants can be applied in various ways, including as sprays, foams, or in water bodies where mosquito larvae breed. The choice of application method depends on the specific insect and environmental conditions. The present study showed that biosurfactants had larvicidal activity. Early-stage larvae of Culex sp. were collected from unused ponds on Parangipettai, and a previously developed biosurfactant was used. Test solution range: 50mg/ml. The larvicidal bioassay was conducted following the recommendations the World Health Organisation set forth. The findings of the study indicated that biosurfactants demonstrated larvicidal action. The biosurfactant had the most excellent effectiveness, resulting in a death rate of 91.29 ±1.30% when applied at the highest concentration tested, and 400 ppm. The findings of the bioassays demonstrated that the results varied depending on the concentration. The results indicate that using biosurfactants effectively manages the population of *Culex* sp. larvae.

Keywords: Larvicidal activity, *Culex* sp., biosurfactant, biological control

Introduction

The transmission of arbovirus infections such as dengue and chikungunya occurs through the bite of the diurnal mosquito species *Aedes aegypti*. *Culex quinquefasiatus* serves as the vector for the transmission of lymphatic filariasis. Zika is a newly identified viral infection in which the mosquito species *Ae. aegypti* is widely recognized as the primary vector. However, evidence suggests other mosquito species, such as *Cx. quinquefasciatus*, may also transmit the virus (Benelli and Beier, 2017; Benelli and Romano, 2017; Guedes *et al*., 2017; Benelli and Mehlhorn, 2016) [3, 4, 5, 7].

Vector management plays a crucial role in the worldwide approach to treating diseases transmitted by mosquitoes, with insecticide application as a fundamental element within this strategy. Larvicides are substances that effectively eliminate mosquitoes during their juvenile stages, preventing their development into adult mosquitoes that feed on blood. Larvae exhibit a strong affinity for their respective habitats, facilitating more efficient control measures through larvicides. The primary method for controlling larvae is the use of synthetic chemical control agents. The repetitive application of chemical insecticides has proven beneficial, but it has also led to resistant mosquitoes and environmental contamination (Liu, 2015; Lawler *et al*., 2017) [11, 12]. Additionally, the limited performance of biocontrol programmes targeting Culicidae has prompted the exploration of alternative insecticides (Benelli, 2015) [2]. Plant-based treatments are emerging as a viable substitute for synthetic chemical agents in pest management and control.

Vector control plays a crucial role in the comprehensive approach to managing diseases transmitted by mosquitoes, and the administration of insecticides is a fundamental element within this strategy. Larvicides effectively eliminate mosquitoes during their juvenile stages, preventing their transition into adult mosquitoes that feed on blood. Larvae exhibit a strong affinity for their respective habitats, facilitating more efficient control measures through the application of larvicides. The primary method of larval control predominantly involves using synthetic chemical control agents. The repetitive use of chemical pesticides has proven beneficial, but it has also led to the development of mosquito populations resistant to these chemicals.

Additionally, this practice has resulted in environmental contamination, as evidenced by studies conducted by Liu (2015) [12] and Lawler *et al*. 2003). Despite efforts to manage mosquito populations using biocontrol programmes targeting the Culicidae family, the outcomes have been limited, as highlighted by Benelli (2015) [2]. Plant-derived products are emerging as a viable substitute for synthetic chemical agents in pest management and control.

Various surfactants, including anionic, cationic, amphoteric, and non-ionic, are employed in numerous pesticide-producing businesses (Mulqueen, 2003) [14]. Surfactants are extensively employed in the manufacture of insecticides. It is crucial to acknowledge that excessive use of surfactants in pesticide formulations can lead to their accumulation in soil, adversely affecting plant texture, colour, and growth. According to Blackwell (2000) [6], leaching hazardous pesticides from soil to groundwater is a significant concern.

Pesticide residues have been observed to have long-term persistence within the soil and the ability to disperse through air and water channels. According to Street (1969) [20], these contaminants persist on the external surfaces of fruits and vegetables.

Furthermore, it has been acknowledged that synthetic surfactants possess significant potential as organic contaminants in soil (Hari *et al*., 2022 and Petrovic & Barcelo, 2004) [16]. In light of the detrimental impact of pesticides and the surfactants commonly used, exploring environmentally benign biosurfactants as a substitute for these harmful surfactants within the pesticide industry is imperative. This substitution would mitigate pollution concerns (Hopkinson *et al*., 1997). Surfactants find several applications within the realms of agriculture and agrochemical industries. Nevertheless, the utilization of biosurfactants, which possess enhanced environmental friendliness, is hardly observed. The precise function of surfactants for improving the efficacy of other systems as biocontrol agents remains unexplored and requires further exploration. These investigations were mosquito control with environmentally friendly alternatives.

Materials and methods

Test insect culture

The early-stage larvae of Culex sp. were gathered from the dormant ponds in Parangipettai, India. The larvae were cultivated in glass troughs, where they were provided with a meal consisting of a mixture of yeast and dog biscuits in a ratio of 3:1. The larvicidal bioassay employed fourth-instar larvae.

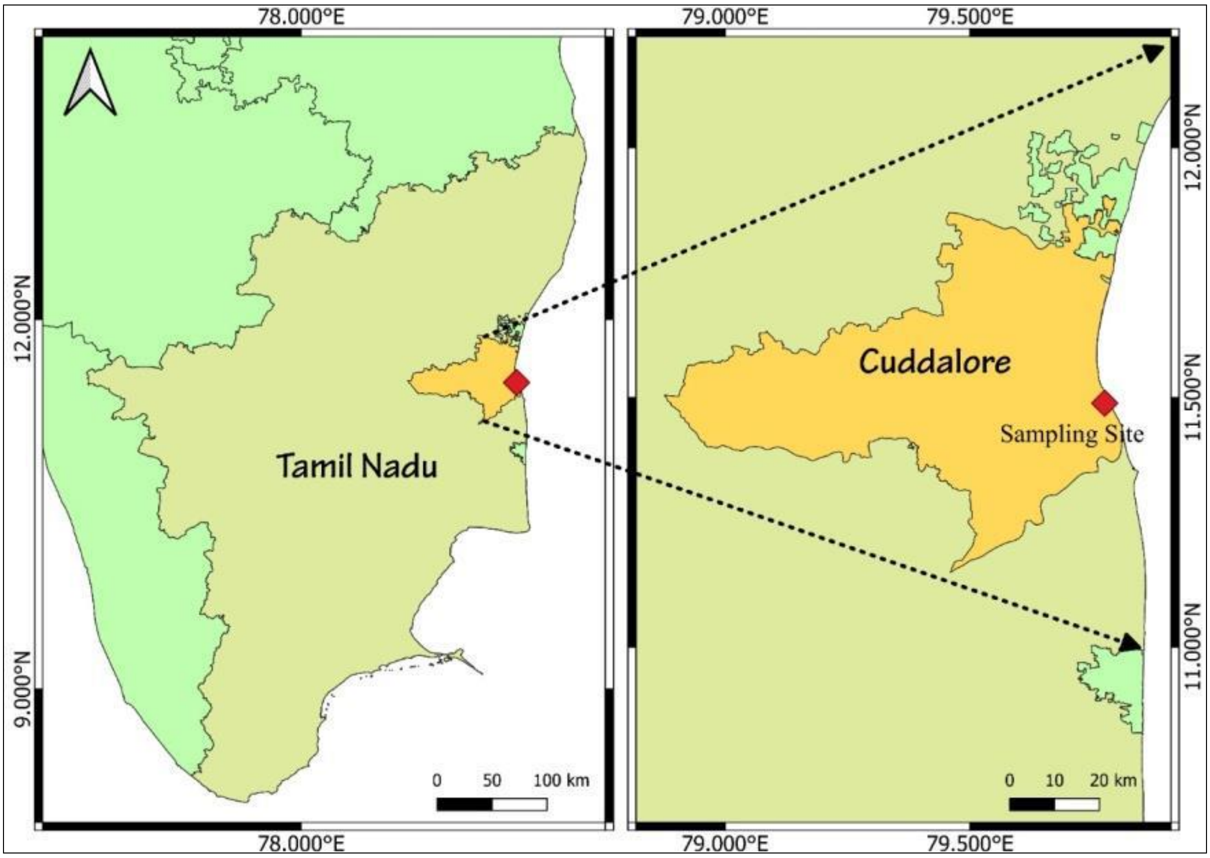

Fig 1: Showing mosquito larva collection place

Production of biosurfactant

Previously, biosurfactants were developed to create a cost-effective medium formulation by utilizing agricultural waste as substrates for the manufacture of biosurfactants. Basha *et al*.'s study from 2022 says that the Plackett-Burman experimental design was used to find the best components for statistical screening, while the Response Surface Methodology (RSM) was used to find the best substrate concentrations. When fish scales (Bocha and Rohu) were used instead of commercially available sucrose, peptone, yeast extract, and malt extract, BS production increased by 2.8-fold, and the final concentration was found to be 944 mg L-1. This study is the first time that the simultaneous use of Bocha and Rohu fish scales for biosurfactant (BS) synthesis by *Bacillus tequilensis* KM15 has been looked into. According to Basha *et al*. (2022) [1].

Test solution preparation

A quantity of 50 mg of biosurfactant was introduced into 1000 ml of distilled water in a separate manner, preparing a stock solution with a concentration of 1000 ppm. A series of five distinct concentrations (25, 50, 100, 200, and 400 ppm) were created for the test solutions using the dilution procedure.

Larvicidal bioassay

The larvicidal bioassay adhered to the protocols outlined by the World Health Organisation (WHO, 2005). The larvae were distributed into three groups, each consisting of 25 individuals. Each group was placed in a separate 100-mL test solution within a 250-mL test cup. The quantification of deceased larvae was documented following 6, 12, and 24 hours of exposure, and the percentage mortality was calculated based on the average of three replicates using the formula

Mortality % = (Number of dead larvae / Total larvae population) × 100.

Results and discussion

The findings of the current investigation are presented in Table: 1 and Figure: 3. the biosurfactant has demonstrated efficacy in combating the fourth-instar larvae of *Culex* sp. At the highest quantity tested, which was 400 ppm, the use of biosurfactant resulted in a mortality rate of 91.29 ±1.30. In contrast, a concentration of 200 ppm reached a mortality rate of 50% Amurta *et al.*, 2011 observed a larvicidal activity of 100% at a biosurfactant concentration of 50 mg/ml. The study's findings un equivocally demonstrated the efficacy of biosurfactants in combating the fourth instar larvae of *Culex* sp., with a notable increase in mortality observed at concentrations of 200 ppm and beyond. Kirthi *et al*. (2011) documented that silver nanoparticles synthesized using plant-mediated methods have gained significant traction in recent years. This approach has garnered considerable attention within the scientific community due to its notable efficacy in targeting certain organisms, such as Culex, Anopheles, Aedes mosquitoes, and other pests. However, it is essential to acknowledge that these nanoparticles exhibit a high level of toxicity towards the organisms, as mentioned earlier.

Fig 2: Showing 4th instar larvae of *Culex* sp.

The findings of in this study are consistent with those of prior research. In their study, Nishan and Subramaniam (2015) [15] looked at how well methanolic extracts from *Azadirachta indica* and *Murraya koenigii* killed larvae independently and when used together. The study's findings revealed that *A. indica* showed significant larvicidal activity, resulting in a mortality rate of 96.30% at a dose of 3.75 mg/ml. In comparison, biosurfactants exhibited a slightly lower mortality rate of 91.29% at the identical concentration. The synthesis of the two excerpts demonstrated an additional impact. In their study found that Biosurfactant exhibited more larvicidal effectiveness against the larvae of *culex* sp.

Table 1: Mean mortality rates ± SD of Biosurfactant against the 4th instar larvae of *Culex* sp.

Concentration in ppm	Biosurfactant
0	0
25	36.55±1.60
50	49.41±2.30
100	63.50±1.71
200	72.31±1.81
400	91.20±1.30

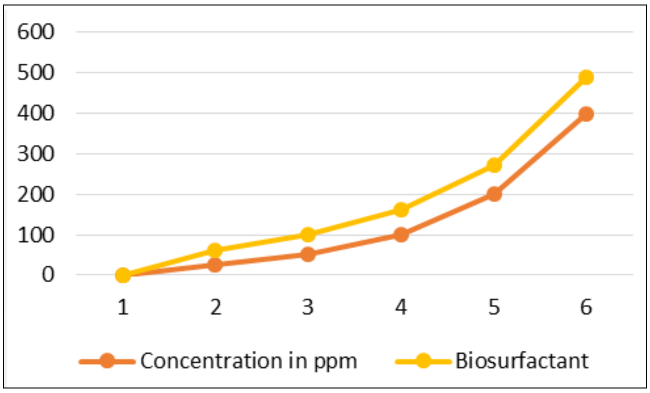

Fig 3: Showing 4th instar larvae of *Culex* sp.

Conclusion

The larvicidal effectiveness of biosurfactants can vary depending on the type of biosurfactant, concentration, and the targeted insect species. Research is ongoing to optimize their efficacy, and biosurfactants hold promise as eco-friendly alternatives to chemical larvicides for controlling insect larvae. Their effectiveness and safety depend on various factors, and further research and development are needed to harness their full potential in pest management strategies.

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