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A NEW AQUEOUS FORMULATION FROM INDIGENOUSLY ISOLATED *BACILLUS THURINGIENSIS ISRAELENSIS* VCRC B646 FOR MOSQUITO CONTROL

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ABSTRACT

In the present study, in order to control mosquito vectors in the field, a new aqueous formulation was developed using an indigenously isolated bacterial strain of *Bacillus thuringiensis israelensis* VCRC B646. The composition of formulation was, *Bti* lyophilized powder (5%), sodium benzoate (0.15%), sodium alginate (2.5%), Congo red (0.03%), citric acid (0.15%), glycerol (6%), molasses (8%) and polyethylene glycol (3%) in 100ml of water. Laboratory bioassay was carried out using this formulation against the late third instar of mosquito larvae. The result showed that the LC_{50} and LC_{90} values against *Aedes aegypti, Culex quinquefasciatus,* and *Anopheles stephensi* were 0.0082 and 0.016 mg/ l, 0.0084 and 0.0172 mg/ l, 0.0139 and 0.0294 mg/ l, respectively. Results from simulated field trial showed that the efficacy of *Bti* formulation at LC_{90} level was highly significant up to six weeks (42 days). Thereafter, the formulation efficacy was started declining. No side effect was observed against non-target aquatic organisms. It was concluded from this study that the formulation developed from newly isolated strain if *B. thuringiensis israelensis* VCRC B646 was very useful on the control of mosquito vectors.

Key words: *Bacillus thuringiensis israelensis*, aqueous formulation, *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi*, simulated field trial, residual activity, lethal concentrations, biopesticide, bioassay, non-target organisms.

Mosquitoes, as insect vectors play an important role in transmitting various diseases, including filariasis, malaria, dengue, west Nile fever Japanese encephalitis, Zika etc. Transmission involves major mosquito species belonging to the genera of *Anopheles, Aedes*, and *Culex* in the order Diptera, specifically family Culicidae (Anoopkumar and Aneesh, 2022). Effective mosquito vector management, is the primary strategy for controlling and preventing infectious diseases. Numerous strategies are currently being implemented to eradicate mosquito populations with the goal of minimizing the incidence of vector-borne diseases. Chemical based control and biological control play pivotal roles in mosquito-vector control (Dahmana and Mediannikov, 2020). Nevertheless, the chemical control approach has yielded negative repercussion on both the ecosystem and human health, coupled with the accelerated development of resistance to insecticides. Microbial agents serve as environmental friendly substitutes for chemical insecticides, addressing mosquito resistance while exhibiting target specificity. *Bacillus thuringiensis* serovar *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*), are notable among bacterial biocontrol agents. Both are spore-forming, gram-positive bacteria widely employed as biological agents to effectively manage mosquito population at larval stages (Poopathi et al., 2014; Dahmana et al., 2020; Abhisubesh et al., 2023). Recent findings indicate a growing resistance of mosquitoes to these biocontrol agents. Consequently, there is an urgent need to explore and identify new biological larvicidal agents that not only exhibit higher efficacy but also maintain target specificity to mosquitoes.

It is a well-known fact, that agriculture and forests are vital for the global economy, environment, and society. Given increasing concerns about the negative impacts of chemical insecticides, there is a notable shift towards biopesticides, motivated by the necessity to reduce environmental harm while efficiently addressing pest challenges in agriculture and forestry. (Fenibo et al., 2022). Several biopesticides have been employed to manage insects that pose a threat to both forests and agricultural crops. Biopesticides based on *Bacillus thuringiensis (Bt)* are particularly significant, representing nearly 97% of the global biopesticide

market (Sujayanand et al., 2021). *Bt* is a spore-forming, gram-positive bacterium known for its insecticidal properties that selectively affect certain insect orders, specifically Lepidoptera, Diptera and Coleoptera. In the present study, an attempt was made to develop a new aqueous formulation from indigenously isolated strain of *Bacillus thuringiensis israelensis* VCRC B646 and examined for its toxic efficacy against mosquito larvae under simulated field conditions, a method of evaluating residual efficacy of *Bti* under stimulated conditions.

MATERIALS AND METHODS

Soil samples were collected from experimental site of Kanchipuram district of Tamil Nadu. Bacterial strains were isolated based on colony morphology (Abhisubesh et al., 2023). The isolates were inoculated into 10 ml nutrient broth and incubated for 72 hours at room temperature under the shaker at 250 rpm. Screening was done earlier to segregate the potential mosquitocidal bacteria. The purity of the potential isolate was tested through quadrant streaking. The positive isolate, namely *B. thuringiensis israelensis* VCRC B646 was cultured for 48 hours and dry biomass was collected (Manikandan et al., 2023). The *B. thuringiensis israelensis* VCRC B646 aqueous suspension formulation (5%) was developed following the principles of suspension formulation. The formulation components were as follows: *Bti* powder served as the active ingredient (5%); sodium alginate (2.5%) as the suspender ingredient to prevent settling; citric acid (0.15%) as the dispersant to prevent agglomeration; a non-ionic surfactant, polyethylene glycol (3%) for wetting and spreading; sodium benzoate (0.15%) as a preservative; glycerol (6%) acted as a humectant to maintain moisture levels, and molasses (8%) as a viscosity modifier and water constituted the carrier liquid, making up the final volume to 100 ml (Johnson et al., 2020). The formulation developed through weighing and sequentially combining these components in the specified order, followed by stirring and homogenization to ensure even distribution. The resulting product was stored at 4°C to maintain stability until further use.

Toxicity bioassays carried out both in the laboratory and simulated field with new formulation on late third instar larvae of laboratory-reared three major mosquito species of *Cx. quinquefasciatus, An. stephensi,* and *Ae. aegypti*. Homogeneous stock solution was prepared from the new formulation and conducted bioassay in the laboratory against late 3rd instar larvae of *Ae. aegypti, Cu. quinquefasciatus,* and *An. stephensi* and calculated LC_{50} and LC_{90} values were calculated through probit regression analysis using SPSS 16.0 software (Poopathi et al., 2014; Hemaladkshmi et al., 2023). Bioassays were also conducted with non-target aquatic organisms, including damselfly larvae, water bugs, back swimmers, tadpoles, water striders, and snails collected from the paddy fields. Four different doses were applied based on LC_{90} values (1, 10, 20, and 30 folds). The mortality was recorded after 24 and 48 hr (Dettner, 2019).

The simulated field study was carried out using cement tanks (75 l) featuring specific dimensions (outer surface diameter: 60 cm, height: 47.5 cm), which were strategically placed under a shaded roof to simulate field conditions. A batch of 50 laboratory reared (obtained from Unit of Mosquito Rearing and Colonization, Vector Control Research Centre, Puducherry) late 3rd instar larvae of the mosquito species were released into each tank containing 50 l of water. After 2 to 3 hr acclimation period for the larvae, each tank was treated with pre-defined dosages. A minimum of four replicates of each dosage along with four controls were kept. The tanks were covered with nylon mesh screen to prevent other mosquitoes or insects from laying eggs and to protect anything falling into the tank. For the assessment of residual activity, a fresh batch of laboratory-reared late 3rd instar larvae were transferred in every week into each tank, accompanied by the addition of food, following WHO guidelines (2005). The percentage mortality was recorded till 7th week. This methodological experiment ensures the systematic evaluation of treatment effects on mosquito larvae under controlled conditions.

RESULTS AND DISCUSSION

In the present study, a new aqueous formulation from indigenously isolated bacterial strain of *B. thuringiensis israelensis* VCRC B646 was examined to preserve the bacterial toxin for longer duration in the field environment. The new aqueous formulation (5%) was basically developed with *Bti* VCRC B646 lyophilized powder as active ingredient. Sodium benzoate (0.15%) was used as preservatives for prolonged efficacy, whereas previous studies used 3 to 6% of sodium benzoate as stabilizer to preserve toxicity (El-bendary and Moharam, 2019). Additionally, sodium alginate (2.5%) used as stabilizer and thickening agent to ensure controlled release of toxin (Karim et al., 2022). Citric acid with slight acidity reduces oxidation and improves shelf life (Alhaj Alali et al., 2023). The integration of various UV screens into formulations, as demonstrated

in previous studies (Nagaraju and Mohan, 2021), entails introducing a range of compounds, such as, congo red (Shapiro et al., 1989), molasses (Ndao et al., 2020) and alginate (Rodrigues et al., 2016). The primary aim of integrating these compounds is to enhance the UV protection properties of the formulation, with the potential to influence its stability and performance in field applications.

The findings in the present study, focused that the formulated *Bti* VCRC B646 exhibited consistent effectiveness against *Culex* and *Aedes* mosquito larvae over the duration of six-week in the simulated field environment. It showed the LC_{50} and LC_{90} values for *Ae. aegypti*, *Cu. quinquefasciatus* and *An. stephensi* were 0.0082 and 0.016 mg/ l, 0.0084 and 0.0172 mg/l, 0.0139 and 0.0294 mg/ l respectively in laboratory (Table 1). The larvicidal activity of the developed formulation lasted over a period of 6 weeks, using different fold of LC_{90} dosages (1, 2, 4, 6 and 8 folds) for three mosquito species: *Cx. quinquefasciatus, An. stephensi,* and *Ae. aegypti*. The formulation was observed to be effective for *Cx. quinquefasciatus* and *Ae. aegypti* throughout the six weeks. *An. stephensi* exhibited a decline in susceptibility over time, particularly at lower dosages. Notably, during the initial weeks (1st and 2nd week), for all dosages of each species, 100% larval mortality was achieved. These findings found in conformity with previous research indicating that the *Bti* formulation retains 100% efficacy for up to 14 days (Uragayala et al., 2018).

In 3rd week, *Cx. quinquefasciatus* and *Ae. aegypti,* sustained high mortality rates, while *An. stephensi* exhibited a slight decline. In 4th week, efficacy continued for *Cx. quinquefasciatus* and *Ae. aegypti,* but *An. stephensi* displayed further reduced susceptibility at lower doses. By the 5th week, the formulation further illustrated effective control for *Cx. quinquefasciatus* and *Ae. aegypti,* whereas, *An. stephensi* mortality sharply decreased. In 6th week, it maintained moderate to high mortality for *Cx. quinquefasciatus* and *Ae. aegypti,* while *An. stephensi* remained less effective. Therefore, *Bti* B646 preserved the toxicity in the field environment with no significant variations in the toxicity levels (LC_{50} and LC_{90}). Even though, there was a notable decline in susceptibility for *Anopheles,* particularly at lower dosages. During the initial two weeks, the formulation demonstrated high efficacy, with 100% larval mortality observed for all dosages for each mosquito species. *An. stephensi* in higher doses-maintained toxicity up to 5 weeks of 50% mortality (Fig. 1). The observed persistence of toxicity indicates the high potency of *Bti* VCRC B646 formulation in the field environment. The observed trends in susceptibility over the six weeks suggest that the effectiveness of the formulation may vary among different mosquito species and dosage levels (Mani et al., 2018).

The results were promising and coordinating with previous studies, indicating the consistent efficacy of *Bti* in reducing larval density in field. Vectobac GR, a new formulation of the bacterial larvicide *Bti* AM65-52,

Table 1. Efficacy of *Bti* formulation against mosquito larvae

Sample	Larval species	LC_{50} (mg/ L) (UCL-LCL)	LC_{90} (mg/ L) (UCL-LCL)	Slope	Intercept	Chi-square
Bacillus thuringiensis israelensis (VCRC B646)	*Aedes aegypti*	0.0084 (0.0080-0.0088)	0.0172 (0.0165-0.0181)	0.007	-1.224	58.517
	Culex quinquefasciatus	0.0082 (0.0079-0.0086)	0.0160 (0.0153-0.0167)	0.008	-1.375	58.493
	Anopheles stephensi	0.0139 (0.0129-0.0149)	0.0294 (0.0280-0.0310)	0.007	-1.224	117.684

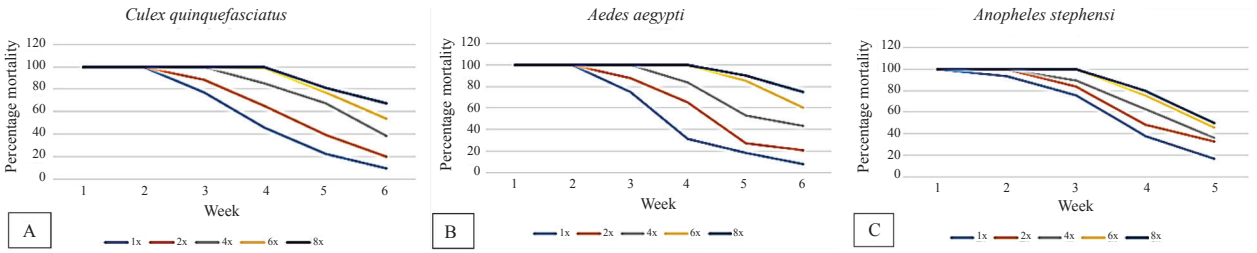

Fig. 1. Residual efficacy of *Bti* VCRC B646 formulation against mosquito larvae

was evaluated in simulated field conditions and natural habitats in Benin. The study reported efficacy for only 2 to 3 days against larvae and up to 10 days against pupae in the simulated field condition (Djenontin et al., 2014: Johnson et al., 2020). All the ingredients used were safe to the environment and categorized under GRAS (Generally Regarded as Safe) as per United States Food and Drug Administration (FDA). The differential susceptibility observed among three species, particularly *An. stephensi* requires further investigation into the factors influencing mosquito larvae to the availability of toxins in the field environment (Manikandan et al., 2023). Additionally, a long-term assessment could help to elucidate the longer-term dynamics of the formulated *Bti* VCRC B646.

There are various types of formulations of *Bti* which have been developed to control mosquito larvae. Formulation plays an important role in determining final efficacy and stability of the biocontrol agent. The efficacy of newly isolated strain of *B. thuringiensis israelensis* in fields can be significantly compromised by diverse environmental factors, such as, UV radiation, precipitation, pH, foliage, moisture and temperature, leading to a decline in both its toxicity and persistence. Diverse formulations are designed with the aim of enhancing the efficacy and durability of the active ingredient when applied in the field (Prummongkol et al., 2019). The stability of the active ingredient is maintained, and its susceptibility to degradation in the environment is balanced through the incorporation of stabilizers, adjuvants, or carriers, protectants and other supplements (Mani et al., 2018). These components, either in solid or liquid form, contribute in ensuring that the active ingredient maintains its effectiveness over an extended period, thereby improving its performance in the intended application. It is expected that, the development of bio-pesticide formulation from *Bti* in the form of aqueous suspension improves the versatility of application, ensuring comprehensive coverage in various types of fields and rapid absorption in the aquatic environment. Further it will reduce the inhalation hazard and ensures a homogenous mixing of the active ingredient.

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AUTHOR CONTRIBUTION STSTEMENT

AV has prepared the manuscript, collected literatures, compilation, performed the analysis, interpretation of data from the experiments pertaining to laboratory and field work. SM have contributed in collection of literatures, framing the manuscript, grammatical corrections. AK data interpretation and data tabulation, KG aided in the DNA extraction and data compilation. HP assisted in molecular works, BB contributed in microscopic studies, JL contributed in finding suitable journals, formatting references, checking for plagiarism, cleaning glassware. AM have contributed in interpreting the background work. SP provided the background ideas, conceptualized the studies, reviewed the literature, and contributed to the revision and modification of the manuscript. AV authored the manuscript. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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