South Asian Journal of Research in Microbiology

10(2): 17-21, 2021; Article no.SAJRM.70886
ISSN: 2582-1989

Isolation and Use of *Bacillus thuringiensis* for the Production of Bio-Insecticide in Control of Mosquito Larvae

A. A. Ibiene[1], S. D. Lawson[2*], S. O. Enyinnaya[2], F. E. Amos[1], L. Nnodim[3] and G. A. Uzah[1]

[1]Department of Microbiology, Faculty of Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Rivers State, Nigeria.
[3]Department of Microbiology, Faculty of Sciences, Rivers State University, Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2021/v10i230225
Editor(s):
(1) Dr. Chamari Hettiarachchi, University of Colombo, Sri Lanka.
Reviewers:
(1) Dan Răzvan Popoviciu, "Ovidius" University, Romania.
(2) Poornachandra Rao K, University of Mysore, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/70886

Original Research Article

Received 26 May 2021
Accepted 02 August 2021
Published 04 August 2021

ABSTRACT

Bacillus thuringiensis is a widely studied bacterium and it is known for its use in pest management. It is selectively active on pests and less likely to cause resistance; hence it is considered a suitable replacement to chemical pesticides. The study assessed the potential of *Bacillus thuringiensis* in controlling mosquito larvae. *Bacillus thuringiensis* isolates selected were tested against secondary stage larvae of mosquito. Thirty-six larvae (6 each) were transferred into each test tubes (7 x 9) cm with 30ml sterile distilled water. The stock suspension of cultures of *Bacillus thuringiensis* in broth was diluted to 10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 in sterile water, following the McFarland standard method for microbial load count. The test tubes were kept at room temperature, larval mortality was observed over time within 24hrs. The results showed that all mosquito larvae died at the 10^7 and 10^6 dilutions but at dilutions 10^5, 10^4 and 10^3 though affecting mosquito larvae, it was highly

**Corresponding author: Email: stephensonlawson@yahoo.com;*

dependent on time because there was a decrease in concentration. The study showed that *B. thuringiensis* is safe for use in aquatic environments, including drinking-water reservoirs, for the control of mosquito, black fly and nuisance insect larvae. The products should contain the ICPs and be free from other microorganisms and biologically active metabolites.

Keywords: Bacillus thuringiensis; bioassay; mosquitoes; pest control.

1. INTRODUCTION

Mosquitoes are vectors responsible for transmission of parasites threatening and debilitating human health. Approximately 30 of the 400 known species of mosquitoes are capable of transmitting parasite (malaria parasites) to human. The three most important groups of mosquito vectors are *Anopheles* spp*., Culex* spp*. and Aedes* spp. Diseases vectored by mosquitoes include; malaria, filariasis, yellow fever, dengue fever, Chikungunya virus, Rift valley virus and West Nile virus [1]. Malaria parasite presents the highest health risk to human. According to the World Health Organization, 214 million people worldwide were infected with malaria in 2015, and 438,000 died [2]. With the health care risk that mosquitoes pose to humans, various approaches like use of chemical insecticides to controlling mosquitos' population have been adapted. Biological insecticides on the other hand are valuable because their toxicity to non-target animals and human is extremely low and is a crucial part of integrated pest management [3,4]. There is a large number of microorganisms such as bacteria, fungi, protozoa and mycoplasma known to kill mosquitoes [5,6] These are called "microbial insecticides". The microbial insecticides are non-hazardous, non-phytotoxic and selective in their action. Interestingly, *Bacillus thuringiensis* is an important insect pathogen which is highly toxic to mosquito larvae and related dipterans [2,7]. *Bacillus thuringiensis* is a widely studied bacterium and it is known for its use in pest management. It is selectively active on pests and less likely to cause resistance; hence it is considered a suitable replacement to chemical pesticides. *Bacillus thuringiensis* is a widely distributed bacterium, which can be isolated from soils, litters and dead insects [3,4,8]. It is an aerobic, spore forming bacterium which produces several toxins (such as alpha, beta, gamma and delta endotoxins) that are pathogenic to larva of Lepidoptera [1,9,10]. Beta-exotoxin contains adenin, ribose, glucose whereas delta endotoxins are composed of a glycoprotein subunit and these toxins have insecticidal properties [3,11,12]. Structural formula of beta exotoxin of B. thuringiensis has been found a strong antagonist to be used as a biological agent [11,12]. This study assessed the potential of Bacillus thuringiensis in controlling mosquito larvae.

2. METHODS

2.1 Isolation and Identification of *Bacillus thuringiensis* from Soil Samples

Using the method as described by Becker et al. [8], 1g of soil samples - was suspended in 10 mL nutrient broth medium supplemented with 0.25g sodium acetate in a conical flask (125 mL). Mixture was shaken for 4hrs on a rotary shaker (Model Kama). Thereafter, it was heated for 5 minutes in a water bath of 80 ^{0}C to kill vegetative cells and non-spore forming bacteria. 0.5 mL was then plated out and poured on a nutrient agar plates without sodium acetate in a spread plate method and was allowed for 48hours at room temperature of 28 ^{0}C. Colonies were sub-cultured on nutrient agar plates by streaking, and incubated at 37 ^{0}C for 24hours. As colonies appeared to have similar morphology as *B. thuringiensis*, characterization was then performed by conducting Gram reaction test and some biochemical tests for further identification of *Bacillus thuringiensis*.

2.2 Breeding of Mosquito Larva

Water containers were left to stand in an open space at ambient temperature for 14 days. This was done to facilitate laying of eggs by mosquito. The water containers were monitored daily to observe emergence of the larva. The larvae of the female mosquito were then harvested using sieve and placed on a moistened cotton wool to prevent them from dying before use. Species of mosquito larva were used as target insects. The mosquito larvas were maintained in insectaria with the methods described by Walker et al. [13].

2.3 Bioassay

In primary screening, all of the *Bacillus thuringiensis* isolates selected were tested

against secondary stage larva of mosquito. Thirty-six larvae (6 each) were transferred into each test tubes (7 x 9) cm with 30ml sterile distilled water. The stock suspension of cultures of Bacillus thuringiensis in broth was diluted to 107, 106, 105, 104, 103 and 102 in sterile water, following the McFarland standard method for microbial load count. The test tubes were kept at room temperature, larval mortality was observed over time within 24 hrs. After treatment, those isolates that caused mortality, the same were noted and kept for further experiment and (LD50) lethal dose concentration values of spores and crystal complex were determined by using the method described above for each isolate.

2.4 Statistical Analysis

Data obtained were subjected to statistical analysis and diversity index at each observation were calculated. The differences in abundance of each organism being observed were compared using two ways analysis of variance (ANOVA); statistical package for the social science (SPSS) version 25.0, which was calculated – microbial load over time for each of the isolates obtained from the different samples.

3. RESULTS

The findings of the study shows that *B. thuringiensis* is a spore forming Gram positive bacterium which requires Low Dose (LD50) 50% concentration obtained from dilution 10^4 to kill or inhibit the growth of mosquito larva as stated in the tables in the overleaf. With the different isolates (A1, B1, B2 and C1) for bioactivity, tables of dead mosquito larva were deduced. From these tables, it was obtained that the entire mosquito larva died at the 10^7 and 10^6 dilution but at dilutions 10^5, 10^4 and 10^3 though susceptible to the mosquito larva, it was highly dependent on time because there was a decrease in concentration. The isolates with the highest killing power were isolate A1 and B2. It was observed that these isolates were able to kill 100% larva even at dilution 10^4. For the other two isolates (B1 and C1) the death of the entire mosquito larva was observed at dilution 10^7 and 10^6. Even though there was dead at other dilution, it was time dependent. As the dilution progresses, the concentration was reduced and some of the mosquito larvae were able to survive the toxicity of the isolate as show in Figs. 1 - 3.

4. DISCUSSION

The current study monitored the abundance of *B. thuringiensis* in soil samples and its effectiveness on mosquito larva considering the dilution/concentration factor and time. Although widely investigated, the detailed mechanism of toxin in insect midgut is still controversial [13–15]. Larva control strategy serves to extend the useful life of insecticides by reducing selection pressure for resistance development and the strategy is equally effective in controlling both indoor and outdoor biting of mosquitoes [15]. The obtained result thus corroborated with previous findings of other studies; indicating a high level of abundance of *B. thuringiensis* in soil and high rate of toxicity to mosquito larvae when applied in a recommended rate [7,13,15,16]. The findings of the current study thus agree with those of previous studies indicating a high level of effectiveness when used for mosquito control. However, in most cases, treatments were either overdosed or the adverse effects were due to other factors such as turbidity or methodological errors. Comparison of the activity of the different isolates (A1, B2 to B1, C1) though indicated their efficacy against the mosquito larvae, there was a significant difference in the rate of toxicity to larva. A possible explanation to this could either

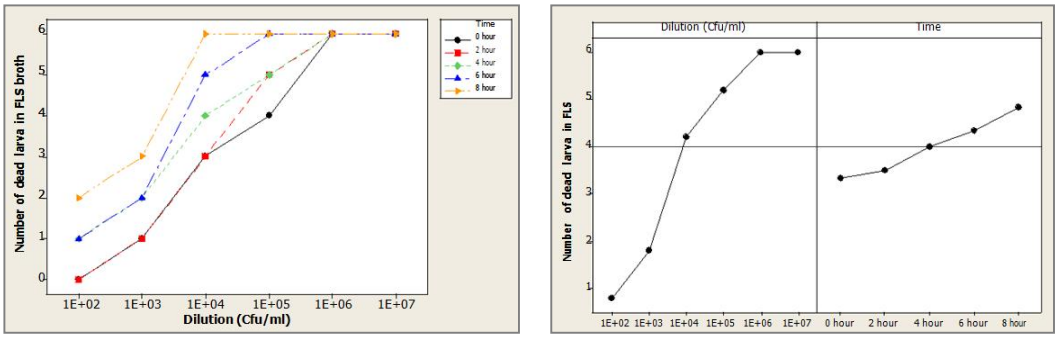

Fig. 1. Effectiveness of FLS Bioassay

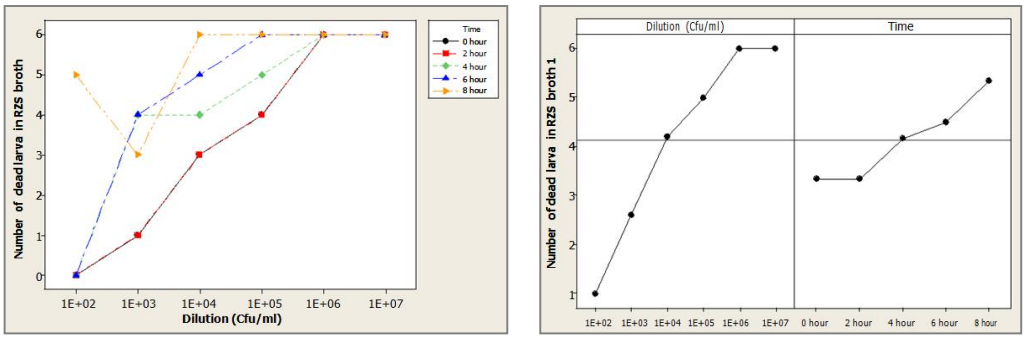

Fig. 2. Effectiveness of RZS Bioassay

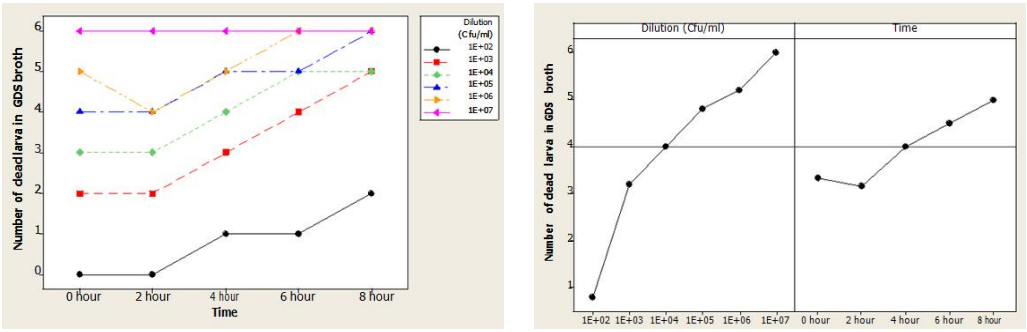

Fig. 3. Effectiveness of GDS bioassay

be that those two isolates-B1, C1 did not release enough toxins to have acted effectively against larva or that those isolates were subspecies of *B. thuringiensis* which are highly toxic against other insect order such as Lepidoptera, coleopteran, rhabditida, hemiptera, hymenoptera, gastropoda and the human - cancer cells, of which the mosquito belongs to the insect order of dipteral. Insecticide resistance and behavioral adaptations of malaria vector thus, calls for novel control methods that prevent or delay evolution of traits [2,10,11,16]. In this respect, the importance of microbial larvicides and their potential for inclusion to integrated vector management (IVM) strategies cannot be overemphasized. With their high level of safety to the environment, they preserve organisms that not only provide ecosystem services (food web and pollination) but also regulate mosquito proliferation through predation and competition.

5. CONCLUSION

The result obtained in this study clearly demonstrates the efficiency of the *Bacillus thuringiensis* in controlling mosquito larva. The increasing emergence/resurgent of mosquito-borne diseases such as malaria, yellow fever, dengue fever, zika virus, cancer cells, which threatens human life, can be controlled using bio insecticides. The study shows that *Bacillus thuringiensis* is selectively active on target organism and has less effect on humans making it a potential suitable replacement for chemical (synthetic) insecticides.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Benelli G, Mehlhorn H. Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. Parasitology Research. 2016;115:1747–1754.
2. Brévault T, et al. Potential shortfall of pyramided transgenic cotton for insect resistance management. Proc. Natl. Acad. Sci. U. S. A. 2013;110:5806–5811.
3. Ceballos G, Ehrlich PR, Dirzo R. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc. Natl. Acad. Sci. U. S. A. 2017;114:E6089–E6096.

4. Ben-Dov E. *Bacillus thuringiensis* subsp. Israelensis and Its dipteran-specific toxins. Toxins. 2014;6:1222–1243.
5. Coetzee M, Koekemoer LL. Molecular systematics and insecticide resistance in the major African malaria vector anopheles funestus. Annual Review of Entomology. 2013;58:393–412.
6. Ceballos G, et al. Accelerated modern human-induced species losses: Entering the sixth mass extinction. Sci. Adv. 2015;1.
7. Allgeier S, Friedrich A, Brühl CA. Mosquito control based on Bacillus thuringiensis israelensis (Bti) interrupts artificial wetland food chains. Sci. Total Environ. 2019;686:1173– 1184.
8. Becker N, Ludwig M, Su T. Lack of resistance in aedes vexans field populations after 36 years of bacillus thuringiensis subsp. israelensis applications in the upper rhine valley, GERMANY. J. Am. Mosq. Control Assoc. 2018;34:154–157.
9. Duguma D, et al. Microbial communities and nutrient dynamics in experimental microcosms are altered after the application of a high dose of Bti. J. Appl. Ecol. 2015;52:763– 773.
10. Brodman R, Dorton R. The effectiveness of pond-breeding salamanders as agents of larval mosquito control. J. Freshw. Ecol. 2006;21:467–474.
11. Benelli G. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitology Research. 2015;114:3201–3212.
12. Brown MD, Carter J, Thomas D, Purdie DM, Kay BH. Pulse-exposure effects of selected insecticides to juvenile Australian crimson-spotted rainbowfish (*Melanotaenia duboulayi*). J. Econ. Entomol. 2002;95:294–298.
13. Walker T, Jeffries CL, Mansfield KL, Johnson N. Mosquito cell lines: History, isolation, availability and application to assess the threat of arboviral transmission in the United Kingdom. Parasites and Vectors. 2014;7:382.
14. Lagadic L, Roucaute M, Caquet T. Bti sprays do not adversely affect non-target aquatic invertebrates in French Atlantic coastal wetlands. J. Appl. Ecol. 2014;51:102–113.
15. Derua YA, et al. Microbial larvicides for mosquito control: Impact of long lasting formulations of Bacillus thuringiensis var. israelensis and Bacillus sphaericus on non-target organisms in western Kenya highlands. Ecol. Evol. 2018;8:7563–7573.
16. Brühl CA, et al. Environmental and socioeconomic effects of mosquito control in Europe using the biocide Bacillus thuringiensis subsp. israelensis (Bti). Science of the Total Environment. 2020;724:137800.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/70886