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Original Article

In vitro assessment of the Larvicidal activity of *Bacillus thuringiensis israelensis* (Vectobac 12AS formulation) on Anopheles mosquito larvae

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ABSTRACT

This research aimed to evaluate the larvicidal activity of the lower doses of commercially synthetic *Bacillus thuringiensis israelensis* (Vectobac 12 Aqueous Solution (12AS)) against the fourth instar larva. One hundred and fifty blood-fed female anopheles mosquitoes were collected from different resting sites from Abuja Quarters in June 2022 using an aspirator and allowed to breed until the first instar larva appeared. The larvae were monitored and fed with 10% yeast until the third instar emerged. 240 healthy third instar larvae were selected and grouped into three treatments containing sixty (60) larvae each and replicated three times. The first, second and third treatments were respectively treated with 0.84, 0.42 and 0.21ml/l of Vectobac 12AS. Each treatment has a control containing twenty (20) larvae. Larval mortality was determined using a glass rod at an interval of 15 minutes for 24 hours. ANOVA was used to statistically analysed differences in the larval mortality between the treatment and probit analysis was used to determine the lethal concentration (LC) and the lethal time (LT). Mortality of 1(6.7%) and 3(5.0%) were observed in the first treatment (0.84ml/l) after 15 and 30minute of exposure respectively. The highest mortality of 60(100%) was observed in all the treatments after 24 hours of exposure. Statistically, there was no significant difference *(F=0.081, P> 0.05)*. 2.35 ml/l, 5.54 ml/l and 8.15 ml/l was determined to be LC_{50}, LC_{90} and LC_{99} respectively and LT_{50}, LT_{90} and LT_{99} were found to be, 1809.29min and 2451.34min respectively. Conclusively vectobac 12AS has demonstrated a high level of efficacy as it revealed 100% larval mortality even at a lower recommended dose. Further research should be carried out to study the impact of other biological and environmental factors on the efficacy of vectobac 12AS.

1. Introduction

Several mosquito species serve as vectors for quite a number of parasitic diseases like malaria, dengue, yellow fever, Zika, and chikungunya [1, 2], and they also serve as a serious nuisance due to their biting habit [3]. Effective prevention and control of vector-borne diseases heavily depend on three main cardinal points which include early diagnosis, chemotherapy and Vector control [4].

Vector control, specifically remains the most generally effective measure to prevent malaria parasite transmission and other vector-borne diseases and therefore is one of

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the four basic technical elements of the Global Malaria Control Strategy. Most of the available vector preventive and control strategies heavily depend on the use of expensive synthetic chemicals which makes the vector to develop resistant to the insecticide [5, 6] thereby making the control measure/strategies less effective [7]. This undesirable side effect of these synthetic insecticides and their highlight effects which include action on non-target organisms coupled with their persistence in the environment which in most cases translated into bio-accumulation in the food chain makes them undesirable to be used as vector control measures. Presently Biological control is one of the popular and most promising strategies for reducing mosquito vector populations [8]. As such biological control, specifically, bio-larvicides which are effective and eco-friendly serves as the prepared alternative in vector control and management [9].

Larviciding is one of the vector control strategies that involve the regular application of synthetic insecticide or biological control agent (bio-larvicide) on to the breeding site of the vector in order to or kill or suppress the larval population [10, 11]. One of the advantages of larviciding is the massive and easy control of the larval stage of the vector as they are usually concentrated in the breeding site, relatively immobile and occupy minimal habitat area compared with the adult, which has several means of escaping the action of insecticide or any intervention technique. One of the most active and effective bio-larvicidal control agents is *Bacillus thuringiensis israelensis* [12].

Bacillus thuringiensis is a Gram-positive, rod-shaped bacterium [13-16] and is a spore-forming bacterium that produces some highly toxic crystalline inclusions during the sporulation phase, which is highly toxic to mosquitoes but safe to the environment [17], and it has no effect on the non-target organism (Selective in action) [18]. This bacterium was first isolated in 1977 by Goldberg and Margalit, from dead *Culex pipiens* larvae and is the most widely used biological control agent against the larval stage of mosquitos and other dipterans of medical importance [19].

The specific toxicity of *Bacillus thuringiensis israelensis* (Bti) against larval mosquitoes and black flies has quickly led to its commercial development and registration for use in aquatic environments [20]. Therefore currently there are several commercially prepared formulations of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* [21] which include soluble granules (vectobac WG) and watery form (vectobac12AS) which are normally used in the field as larvicidal for mosquito vector control. These formulations tend to produce desirable and effective results in various field trials at various selected concentrations. For example, out of the 77 bacterial strains isolated from the Amazonian microenvironment, 19 strains demonstrated a very good larvicidal activity against *Aedes aegypti* which produced 50% mortality at a concentration of 5mg/mL [22]. Never the less, the effectiveness of bti is seriously affected by so many factors such as; sinking to the bottom of the body of water in the case of granules formulations, adsorption onto silt particles and organic matter, consumption by other organisms to which it is nontoxic, and inactivation by sunlight [23], in addition to some environmental factors. In most cases, these factors besides affecting its effectiveness also interfere with recommended dosage concentration of the formulated bti. Therefore this research aimed to determine the least effective lethal dose and the larvicidal activity of synthetic *B. thuringiensis isrealensis* (Vectobac 12AS) under laboratory conditions.

2. Materials and Methods

2.1. Study area

The research was carried out in Gombe local government area, Gombe State. The local government is located within the sub – Sudan climatic zone between latitude 12^0 8'and $10^0$24'N longitude 11^0 22' and 11^0 24'E, with a total population of 250,000 (National Population Census, 2006) and a covered land area of 52.434square kilometres (Figure 1).

2.2. Mosquito collection and Breeding

Female blood-fed mosquitoes were collected from the nearest available resting site using Aspirators. The standard method and protocol of collecting indoor resting

mosquitos were adopted as explained by previous research [24].The collected mosquito was placed in collecting cups and transported to the insectary of biological sciences of Gombe state university. In the insectary, the mosquitoes were released into cages, reared and fed with a 10% sugar solution. Eggcups were placed in each cage when it was observed that the mosquitoes were gravid. Filter papers were placed on the cups containing 30ml of water in order to keep the filter paper always moist. The eggs were collected from the filter paper the following morning and transferred into three containers (45×20cm) containing distilled water (unchlorinated). No food was provided to the containers until the first instar appeared, then they were fed with yeast (10%) and Sieving was conducted once the water was dirty. The larvae were monitored until the third instar larvae developed, usually six days after the emergence of the first instar, which was the time they were ready for the test.

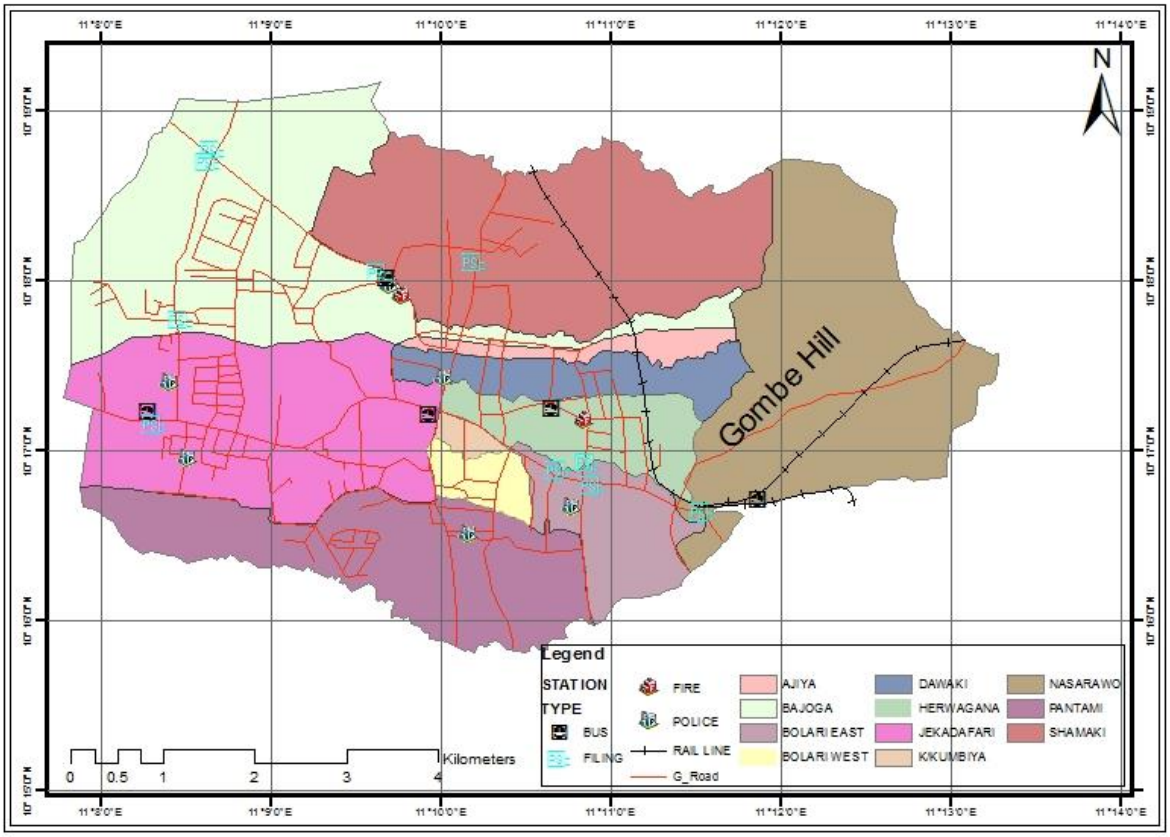

Fig. 1. Map ofGombe local government area (Source: GIS Laboratory, Geography Department, Gombe State University)

2.3. Vectobac (Bti) working solution

Three different concentrations of Vectobac 12AS were made based on the manufacturer's dose as standard. The concentrations were ½, ¼ and 1/6 of the recommended dose (1.67ml).

2.4. Experimentation

All laboratory activities were carried out at the Biological Sciences Department Laboratory, Gombe State University. Two hundred and forty (240) healthy third instar larvae were selected and grouped into three treatments containing sixty (60) larvae each and replicated three times. The first, second, and third treatments were respectively treated with 0.84, 0.42 and 0.21ml/l of Vectobac 12AS, and the control was treated with 0% Vectobac12AS.

2.5. Mortality determination

A glass rod was used to determine whether the larvae were dead or not after one hour. The rod was dipped into the container and brought close to suspected dead larvae (which

usually lie flat on the water surface), for the larvae that were still alive will respond rapidly by either bending or moving away from the rods. The wrinkle movement confirms the status of the larvae. In a situation where the mortality rate in the control exceeds 10%, Abbot's formula will be used to correct the mortality in the treatment.

P=Po-Pc/100-Pc×100
Where Po = Observed mortality, Pc = Control mortality.

2.6. Efficacy determination

Probit analysis was used to determine the effectiveness of vectobac12AS by determining the least effective doses and $LC_{50,}$ $LC_{90,}$ LT_{50}, and $LT_{90.}$

2.7. Data analysis

All data generated for the research were entered into SPSS Software version 16.0. ANOVA will be used to determine any significant difference in the mortality of mosquito larvae with respect to all variables. All tests were done at 0.05 significant levels.

3. Result

In all the treatments there was no larval mortality at the first 1 hour except in the first treatment (0.84ml/l), where 1(1.67%) was observed. Similarly, at 30min mortality of 3(5.0%) was observed at the first concentration, but for the second concentration (0.42 ml/l) and third (0.28ml/l) treatment and the control, there was no mortality at 45min. larval mortality of 4(6.67%), 8(13.33%) and 2(3.33%) were recorded at 0.48, 0.42 and 0.21 ml/l respectively. At 60min, mortality of 7(11.67%), 12(20.0%) and 3(5.0%) were recorded in the first, second and third treatments respectively. A hundred percent (100%) mortality was observed in all the treatments after 24 hours of exposure except in the control, where there was no mortality observed as shown in Table 1 below. Statistically ANOVA result revealed that there was no significant difference in the larval mortality with regards to the different concentrations used (F=0.081, P> 0.05), but there was a statistically significant difference in the larval mortality for the time of larval exposure to the different concentrations of Vectobac 12AS (F=11.031, P>0.005). 2.35 ml/l, 5.54 ml/l and 8.15ml/l was determined to be LC_{50}, LC_{90} and LC_{99} respectively and LT_{50}, LT_{90} and LT_{99} were found to be, 1809.29min and 2451.34min respectively as shown in table 2 below.

Table 1. Anopheles Larval mortality at different times and concentrations of the Vectobac12AS.

Concentration/ Treatment	no. of larvae	Mortality after				
		15min	30min	45min	60min	24hr
0.84 ml/l	60	01(1.67%)	3(5%)	4(6.67%)	7(11.67%)	60(100%)
0.42 ml/l	60	0(0%)	0(0%)	8(13.33%)	12(20%)	60(100%)
0.21 ml/l	60	0(0%)	0(0%)	2(3.33%)	3(5%)	60(100%)
0.0 ml/l (Control)	60	0(0%)	0(0%)	00(0.00%)	00(0%)	2(3.33%)

Table 2. Lethal concentration (LC) and Lethal time (LT) values of Vectobac 12AS

LC	Value(ml/l)	LT	Value(min)
LC_{50}	2.35	LT_{50}	1021.75
LC_{90}	5.54	LT_{90}	1809.29
LC_{99}	8.15	LT_{99}	2451.34

LC= Lethal Concentration, LT= Lethal Time

4. Discussion

The introduction of the new concept and approach toward vectors and vector-borne diseases prevention through integrated vector management has completely changed the old narrative of vector control, which heavily depends on the use of synthetic chemicals that have a serious negative effect on the environment. For some diseases like chikungunya and Zika that lack prophylactic drugs, vector control and management is the

only ultimate target for preventing the diseases. Various formulations of *Bacillus thuringiensis israelensis* (Vectobac 12AS, Vectobac WG) are effective in controlling the larval stage of mosquitoes in so many parts of the world [25, 26]. In the present study, the larvicidal activity of a commercially synthetic formulation of *Bacillus thuringiensis israelensis* (Vectobac 12AS) was evaluated at different selected concentrations (0.84, 0.42, 0.21ml/l) throughout 24hrs.

From the findings of this study, it is revealed that the larvicidal activity of Vectobac 12AS is dose-dependent which in turn also depend on the actual time of the larval exposure to the Vectobac 12AS, as all selected concentration showed maximum (100%) larval mortality after 24hr of exposure. This finding is similar to the findings of previous research [27] who also reported 100% larval mortality of *Anopheles coluzzii* exposed as third instars to *Bacillus thuringiensis israelensis* for 48 h increased with increasing Bti concentrations. This high percentage of mortality could be attributed to the high level of susceptibility of Anopheles mosquitos to different formulations of the Bti. On the other hand, a low level of larval mortalities was observed in all the treatments as the concentrations of Vectobac 12AS decreases. This finding is in agreement with the findings of previous research [28] who also reported that most of these *Bacillus thuringiensis israelensis* extracts showed some toxic activity toward mosquito larvae in high concentrations, but their activity decreased as the dilution increased. This could be attributed to the fact that, much higher concentrations of *Bti* formulation are required in order to induce mortality in Anopheline larvae than in any other larval mosquito specie.

Lethal concentration (LC) and lethal time (LT) are two main factors and determinants under which the efficacy of any given chemical can be assessed, as they provide statistical or theoretical insight into the concentration and the time required to kill some specific number of the target organisms. In addition, both LC and LT values are very useful in the actual field application *(in-vitro).*

5. Conclusion

Vectobac 12AS Proved to be a very effective biological control agent as it produced 100% mortality even at a concentration lower than the recommended dose. The effectiveness of the vectobac 12AS is directly correlated with the exposure time and is also dose-dependent. In addition, considering the LC_{99} and LT_{99} values obtained, Vectobac 12AS can lead to maximum larval mortality within a very short period.

Conflict of Interest

The authors hereby declare that they have no conflict of interest.

Author's contributions

All authors equally participated in designing experiment analysis and interpretation of data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors have read and approved the final manuscript for publication.

Availability of data and material

The authors have embedded all data in the manuscript.

Informed Consent

The authors declare not used any patients in this research.

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