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In vitro assessment of the larvicidal activity of lower doses of *Bacillus thuringiensis israelensis* (Vectobac water dispensable granules) formulations on Culex mosquito larvae

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Abstract
Biological control serves as the most effective and environmentally friendly strategy for controlling several mosquito specie. Different biological control agents in different forms and formulations are widely used. Therefore the aim of this research was to assess the larvicidal activity of the lower doses of commercially synthetic *Bacillus thuringiensis israelensis* (Vectobac WDG) against the third instar larva of Culex mosquito. One hundred and fifty blood-fed female anopheles mosquitoes were collected from different resting sites 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 2.5, 1.25 and 0.833g of Vectobac WDG. Each treatment has a control containing twenty (20) larvae. Larval mortality was determined using a glass rod at an interval of 15 minutes for a period of 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). A mortality of 1(6.7%) was observed in the first treatment (2.5g) after 15 and 30 minutes of exposure. The highest mortality of 60(100%) was observed in all the treatments after 24 hours of exposure, except in the first treatment where 59(98.33%) was observed. Statistically, there was no significant difference *(*F=11.031, P> 0.05). 20.87, 120.57 and 201.84g were determined to be LC_{50}, LC_{90} and LC_{99} respectively and LT_{50}, LT_{90} and LT_{99} were found to be, while 622.93, 1847.33, and 2845.53minute respectively. In conclusion, Vectobac WDG 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 WDG.

Keywords: Biological control, *Bacillus thuringiensis*, Vectobac WDG, Culex mosquito, lower dose

1. Introduction
Biological control is one of the vector control strategies which depends on the use of natural enemies of a given vector in order to suppress their population below the level at which they can cause any damage, injury and or transmit infectious agent of several parasitic diseases (Prasad *et al*., 2012) [27]. This is becoming a widely acceptable technique nowadays as a promising strategy for reducing mosquito vector populations (Aneha *et al*., 2022) [4]. Before the implementation of Integrated Vector Management of which biological control is one of the major component, vector control especially of different species of mosquito which serves as vectors to so many deadly parasitic diseases affecting human beings like Malaria, Dengue, Yellow fever, Zika, and Chikungunya (Soares-da-silva *et al*., 2017) [28] mainly depends on the use of synthetic insecticides which include Pyrethroids, Organochlorine, Organophosphate, and Carbamate compounds (Hassanali & Lwande, 2022) [17] in form of Indoor Residual Spraying (IRS) and also the use of Insecticide Treated Nets (ITNs) (Dambach, 2018) [10]. In the last decades, these control strategies are normally adopted against the adult stage of the mosquito and have significantly decrease mosquito borne diseases, especially malaria

transmission in many areas of sub-Saharan Africa. (Tesfahuneygn & Gebreegziabher, 2019) [29]. However larval stages are also controlled with the use of different insecticide formulations of different classes of chemicals in form of larviciding, which target the larval stage of the mosquito in their active breeding site. Larviciding for long has demonstrated a very good promising results in the control of larval stage of different species of mosquito including Anopheles mosquito larvae, thereby drastically reducing the rate of malaria transmission and even eliminating malaria vectors and disease transmission generally in some countries (Nkondjio *et al*., 2021) [24]. For that, larviciding has been recommended by World Health Organisation so as to supplement malaria elimination efforts along with IRS and Long Lasting Insecticide Nets (Ingabire *et al*., 2017) [19]. Some of the advantages of larviciding are, the larvae are easily accessible, concentrated in confined area with limited ability of escaping the action of any formulation (Dambach *et al*., 2020) [11].

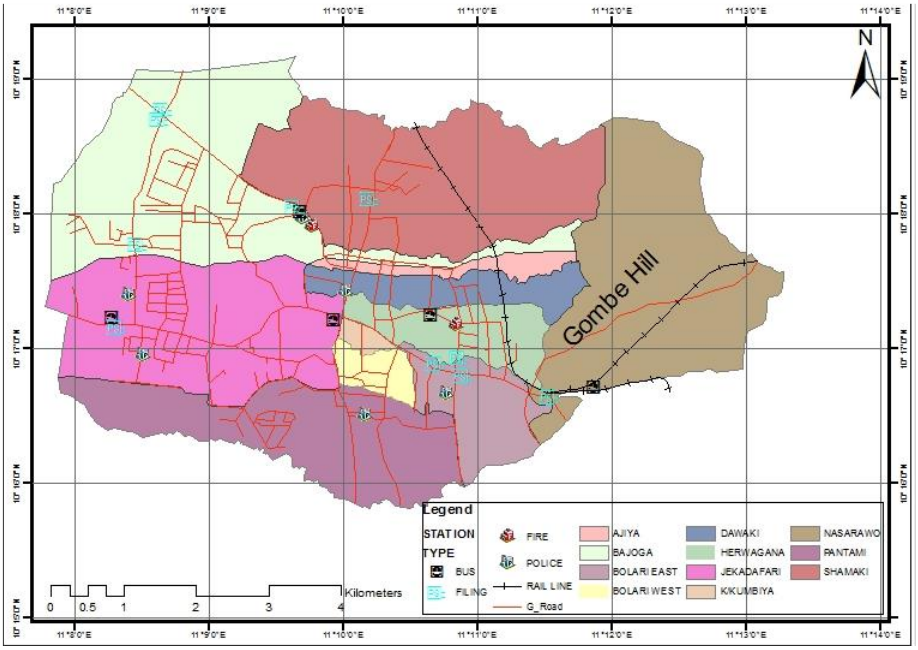
However, several studies have documented that, mosquitoes have adopted various strategies of avoiding contact with insecticide treated surfaces, these strategies include; change in the biting time, outdoor biting and switching to animal host instead of human host in order to escape the action of insecticide (Bras, 2022; Hakizimana *et al*., 2022) [6, 16]. In addition, most of the available insecticide that are used either as larvicidal formulations or IRS in case of the adult are associated with high material and operational costs due to the need for frequent habitat re-treatment at some regular interval for example on weekly or monthly basis, as well as logistical issues in the field (Zhou *et al*., 2016) [32]. Also most of the insecticides are non-selective, as such they pose disastrous effect on non-target organisms and the environment (Milugo *et al*., 2021) [22], thus causing ecological and environmental imbalance. Above all, one shortcomings and major drawback of these synthetic insecticides is the continuous fading away of their effectiveness mainly due to development of resistance either by the larval or adult stage of the mosquito (Chansang *et al*., 2020) [9], thus these coupled with environmental impact of the synthetic insecticide necessitate the use of microbes as biological control agent (Poulin *et al*., 2022) [26].

Some strains of bacteria, for example *Bacillus thuringiensis israelensis (Bti)* are widely used as microbial agent of biological control (Chandre *et al*., 2014; Gowelo *et al*., 2020) [8, 15], as they are considered as the most powerful environmental-friendly biological alternative component used in integrated programs to control disease vectors (Elleuch *et al*., 2015) [14]. *Bacillus thuringiensis israelensis* is among the 10 formulations recommended by World Health Organisation Pesticide Evaluation Scheme (WHOPES) to be used as microbial agent for mosquito larval control (Chandre *et al*., 2014) [8]. *Bti* a gram-positive, spore-forming entomopathogenic bacterium first isolated in 1977. As a bio- logical control agent, it has demonstrated high efficacy against target organisms, primarily mosquito and black fly larvae (Boyce *et al*., 2013) [5]. During sporulation, *Bti* produces a spherical, parasporal inclusion that contains larvicidal proteins which are active against the target organisms (Wirth, 2010) [31]. Presently, *Bacillus thuringiensis israelensis* is usually synthesise and prepared in different formulations with different brand name, for example aqueous (Vectobac 12S) and granules (Vectobac WDG) with also different level of toxicity and recommended doses for field application. Therefore the aim of this paper was to evaluate the larvicidal activity of *Bacillus thuringiensis israelensis* (Vectobac WDG) at lower concentration below the recommended dose.

2. Materials and Methods

2.1. Study area

The research was carried out in Gombe Local Government Area, Gombe State, Nigeria. 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 covered land area of 52.434square kilometres (Figure 1).



Source: GIS laboratory, geography department, Gombe state University

Fig 1: Map of Gombe local government area

2.2. Mosquito collection and Breeding

Female blood-fed mosquitoes were collected from the nearest available resting site using Aspirators. Standard method and protocol of collecting indoor resting mosquitos was adopted as explained by (Ndiath *et al*., 2011) [23]. The collected mosquito were placed in collecting cups and transported to the insectary unit of biological sciences of Gombe State University. In the insectary, the mosquitoes were released into cages, reared and fed with 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 500ml 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.

2.3. Vectobac WDG (Bti) working doses

Three different concentrations of Vectobac WG were made base on base on manufacturer's dose as standard. The doses were ½, ¼ and1/6 of the recommended dose (5.0g), these doses 2.5, 1.25 and 0.833g gram respectively.

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 2.5, 1.25 and 0.833g of Vectobac WG, and the control was treated with 0g Vectobac WDG.

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 would be used to correct the mortality in the treatment.

$$P=Po-Pc/100-Pc\times100$$

Where Po = Observed mortality, Pc = Control mortality

2.6. Efficacy determination

Probit analysis was used to determine the effectiveness of vectobac WG 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. Results

Table 1 shows the result of Culex larval mortality after 24hours of larval exposure to different doses of Vectobac WG. The result revealed that, in the first treatment (2.5g), a larval mortality of 1(1.67%) was recorded after 15minute of exposure to the vectobac WDG. At 30, 45 and 60minute exposure time, larval mortality of 9(15.0%), 21(20.0%), and 34(56.0%) were respectively recorded. In this treatment a larval mortality of 59(98.33%) was observed after 24hours. 100% larval mortality was observed in the second treatment (1.25g) after 24hours of larval exposure. On the other hand, larval mortality of 1(1.67%), 7(11.67%), 12(20.0%) and 23(38.33%) were observed after 15, 30, 45 and 60minute of larval exposure to the Vectobac WG respectively. Larval mortality of 2(3.33%), 7(11.67%), 40(66.67%) and 46(76.67%) were respectively reported after an exposure period of 15, 30, 45, and 60minute in the third treatment (0.833g). Statistically ANOVA result revealed that, there was no significant difference in the larval mortality with regards to the different concentrations used ($F=1.545$, $P> 0.05$), and also with the time of larval exposure to the different concentrations of Vectobac WDG ($F=11.031$, $P>0.005$). LC_{50}, LC_{90} and LC_{99} were found to be 20.87, 120.57 and 201.84g of Vectobac WDG respectively, while 622.93, 1847.33, and 2845.53minutes were found to be LT_{50}, LT_{90} and LT_{99} respectively as shown in table 2 below.

Table 1: Culex Larval mortality at different doses of Vectobac WG and time of exposure

Dose (g)/Treatment	no. of larvae			Mortality after		
		15min	30min	45min	60min	24hr
2.5g	60	1(1.67%)	9(15.0%)	12(20.00%)	34(59.67%)	59(98.33%)
1.25g	60	1(1.67%)	7(11.67%)	12(20.00%)	23(38.33%)	60(100%)
0.833g	60	2(3.33%)	7(11.67%)	40(66.67%)	46(76.67%)	60(100%)
0.0g(Control)	60	0(0%)	0(0%)	00(0.00%)	00(0%)	00(0.00%)

Table 2: Lethal concentration (LC) and Lethal time (LT) values of Vectobac WG

LC	Value(g)	LT	Value(min)
LC_{50}	20.87	LT_{50}	622.93
LC_{90}	120.57	LT_{90}	1847.33
LC_{99}	20184	LT_{99}	28451.53

Discussion

The discovery of Bti and other safe agents of biological control against several vectors of parasitic disease has completely changed the old narratives of fear of resistant development by vectors and negative effect on non-target organisms and the environment (Almeida *et al*., 2020; Poopathi & Abidha, 2010) [3, 25]. Synergistic effects of the multiple crystal proteins present in Bti-based products coupled with the different modes action of the bti, make it difficult for the culex larvae to develop resistance (Valtierra-de-Luis *et al*., 2020) [3]. Therefore, in the

present study larvicidal effectiveness of lower doses of Vectobac WDG, a synthetic form of Bti was determined. Almost 100% larval mortality was recorded at all selected doses after 24hours of larval exposure, this clearly demonstrated a very good high efficacy. The good efficacy of the lower doses of Vectobac WDG demonstrated in this study could be attributed to the fact that, this agent of biological control (Bti) has not been widely used for larviciding in the study area, though it has been extensively used in so many part of the world (Katak *et al*., 2021) [20] as such most of the Culex larvae in the study area were never exposed to the Vectobac WDG, and for this, therefore maximum larval mortality was highly expected. One factor that could be attributed to this mortality is the inability of larvae to avoid or escape the action of the Vectobac WG (Derua *et al*., 2018) [12]. This has also clearly demonstrated the fact that, biolarvicides specifically bti are highly effective (Ahmed *et al*., 2016) [2] in the control mosquito larvae, even at lower doses (Edmond *et al*., 2022) [13], thus serves as another additional advantage over conventional insecticides.

In all the three treatments (2.5, 1.25 and 0.833g) larval mortality was highly correlated with increase with both increase in concentration and time of exposure. This is similar to the findings (Marin *et al*., 2020) [21] who also reported increase in larval mortality with in the concentration of the biological control agent (*Bacillus thuringiensis*). Exposure time plays a vital role in larval mortality, as the larvae has to locate and ingest the Vectobac WDG Particles which is completely time dependent and in addition, in order to release the basic activity after ingestion, the insecticidal crystals must be digested and released the active toxins which finally binds to the mid-gut receptors (Helena *et al*., 2021) [18]. This leads to pore formation in the mid-cell membrane and subsequently dead of the larvae. An increase in larval mortality was observed though with a decrease in the concentration of Vectobac WDG. This could be basically attributed to the ample time taken by the lower doses in the larval mid-gut to produce the maximum amount of the active toxins. Therefore, this clearly demonstrated that the efficacy of Bti as the biological control agent depends on two major factors, these are concentration and exposure time of the larvae to the Vectobac (Bti-formulations), but exposure time is the main determinant.

Theoretical lethal concentration (Lc) and Lethal time (LT) recorded in this study showed was highly appreciated as it suggested a very lower concentration below the recommended dose especially in LC_{50}, LC_{90}. For the LT_{50}, LT_{90} and LT_{99}, the theoretical (Statistical) time reported was justified, as the toxic crystals of *Bacillus thuringiensis* formulations can be present in the environment from weeks up to years after a treatment, depending on the environment (Brühl *et al*., 2020) [7].

Conclusion

Vectobac WDG 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 WDG is directly correlated with the exposure time and is also dose-dependent, in addition the exposure time is the main determinant. Considering the LC_{99} and LT_{99} values obtained, Vectobac WDG can lead to maximum larval mortality within a very short period of time.

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