ASSESSMENT OF LARVICIDAL ACTIVITIES OF *BACILLUS* SPECIES ISOLATED FROM SOIL AGAINST THE MOSQUITO *AEDES AEGYPTIA* (DIPTERA: CULICIDAE) IN SOKOTO, NORTHWESTERN NIGERIA

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ABSTRACT

Three *Bacillus* species (*B. cereus, B. sphaericus* and *B. thuringiensis) known to have* potentials for mosquito larvicidal properties were tested against *Aedes* mosquito larvae. The *Bacillus* species showed pronounced larvicidal activities with *B. cereus* having 60% mortality, *B. sphaericus* 84% and *B. thuringiensis* 56% against the mosquito tested. The lethal concentrations (LC_{50}) determined were 0.033, 0.033 and 0.029 for *B. cereus, B. sphaericus* and *B. thuringiensis* respectively. *Aedes* mosquitoes were more sensitive to *B. thuringiensis* than to other *Bacillus* species with determined potency of 827 ITU/mg. These results compared favourably with that of the standard *B. thuringiensis* from Paris, France, which recorded 100% mortality against the mosquito. There was no significant difference ($P > 0.05$) between the local isolates but variation exists ($P < 0.05$) when compared with the standard *B. thuringiensis* powder. Post experimental analysis using the Least Significant Difference test (LSD = 16.84) showed that the standard strain stands out different from the other three isolates. These findings strengthen the earlier reports that *B. thuringiensis* is a good control agent for *Aedes* mosquito. There is, however, the need to continue further search for *Bacillus* species with larvicidal potentials.

Keywords: *Bacillus* Species, Larvicidal Activity, Soil, Sokoto, *Aedes* Mosquitio.

INTRODUCTION

The use of bacteria to control insect had started from the agricultural sector and have now been extended to the public health (Maurice and Pearce, 1987; Manga, 2004). *Bacillus* species particularly *B. thuringiensis* and *B. sphaericus* were considered as pathogenic agents against vectors of human diseases like the mosquitoes and *Simulium* species (Lacey and Undeen, 1986; Manga and Galadima, 2003; Manga *et al*., 2008; 2011). Some of the diseases caused by these and other vectors are malaria, yellow fever, filariasis, onchocerciasis and leishmaniasis; which are known to affect between 500 million to a billion people each year (Daniel and Molta, 1989; Krogstad *et al*., 1990). One of the mosquitoes involved in the disease transmission is the *Aedes* species which transmits the yellow fever virus and parasites of certain filariasis. The aim of the present work was to evaluate larvicidal potentials of three *Bacillus* species (*B. cereus, B. sphaericus* and *B. thuringiensis)* against *Aedes* mosquito larvae. This was with the view to providing information that will be useful in formulating control strategies against the disease for which these mosquitoes are vectors.

MATERIALS AND METHODS

Three locally isolated *Bacillus* species (*B. cereus, B. sphaericus* and *B. thuringiensis)* from the soil in Sokoto, Northwestern Nigeria, and reported to have larvicidal potentials (Manga, 2004; Manga *et al*., 2008; 2011) were used in the present study. The activities of these *Bacillus* species were compared with a known standard (*Bacillus thuringiensis*) which was obtained from Paris, France in the form of a water dispersible powder. It was assigned a potency level of 1500 ITU/mg. The test was carried out according to WHO (1987) and Anonymous (1997).

Source of Larvae

The adult *Aedes* mosquitoes were collected from around Sokoto metropolis using insect hand nets and collection cage. The insects were separated using aspirator and identified according to Chandler and Reed (1961) and placed in a rearing cage. The caged mosquitoes were fed with 4% sucrose solution and grass infusion of the *Pennisetum* species to serve as food and for egg laying. Adult female mosquitoes were blood-fed by introducing a skinned pigeon into the cage and left overnight twice weekly. Observation was made daily for oviposition. Laid eggs were transferred to larval rearing pan flooded with water. Hatched larvae were allowed to reach the L4 stage (Dyers law) before use.

Preparation of Various Concentrations of Standard *Bacillus*

Fifty milligrams (50 mg) of the dry powder of the standard *Bacillus* was weighed and placed in universal bottle containing 10ml sterile distilled water (5000mg/1000ml) and agitated. From this mixture, a stock solution was made in a universal bottle by adding 0.1ml of the mixture to 9.9ml of sterile distilled water followed by agitation for one minute. This dilution was a hundred-fold dilution and the concentration of the stock solution became 50 mg/l.

From the stock solution (50 mg/l), subsequent dilutions were prepared by taking the following volumes using micropipettes: 15, 30, 60, 90, 100 and 120 microlitres and adding to six beakers each containing 150 ml of water. The concentration of *Bacillus* suspension in each beaker then became 0.05, 0.1, 0.2, 0.3, 0.33 and 0.4 mg/l respectively. This was to ensure that at least there were two mortality rates on either side of the LC_{50} within the range of 10 to 90% mortality. All concentrations were prepared in duplicates. A control beaker was set up having only water and larvae with no bacteria included.

Preparation of Various Concentrations of the Test *Bacillus* Species

The bacterial powder was obtained by scraping a pure culture of *Bacillus* with a clean razor blade. The blade containing the culture paste was then air-dried. This was followed by grinding using a sterilized glass rod. The methods used for the preparation of various concentrations of the isolates were the same with that of the standard.

Testing for Larvicidal Activity

To each set of beakers containing the concentrations of the *Bacillus thuringiensis,* 25 fourth-instar larvae of the mosquito were added using a dropping pipette. The larvae exposed to *Bacillus* concentrations were allowed to stand for 48 hours at 37^0C after which the number of dead larvae in each beaker was counted and the LC_{50} and LC_{90} were obtained from a graph of mortality against concentrations. Similar procedures were repeated for all the *Bacillus* species with larvicidal activities. Potency levels in international toxicity unit/milligram were determined using the formula below:

$$\text{Potency} = \frac{\text{Titre of standard} \times LC_{50} \text{ of standard}}{Lc_{50} \text{ of isolate}}$$

Statistical Analysis

One-way Analysis of Variance and Least Significance Difference (LSD) were employed to determine the level of significance or otherwise of the results.

RESULTS AND DISCUSSION

The larvicidal activities of the *Bacillus* isolates tested against the mosquito species belonging to the genus *Aedes* are presented in Table 1. The time taken to record most of the activities was 24 hours. Larvicidal activities were found to increase as the concentration increased. *Bacillus cereus* had the larvicidal activity of 60% while *Bacillus sphaericus* and *Bacillus thuringiensis* had 84% and 56% respectively. All control tests set up for each isolate against each concentration of the *Bacillus* did not show mortality after 24 hours. The LC_{50} values of 0.033, 0.033 and 0.029 were obtained for *B. cereus, B. sphaericus* and *B. thuringiensis* respectively. The results of larvicidal tests against larvae of *Aedes* treated with the standard powder of *B. thuringiensis* at the varying concentrations are presented in Table 2. The time taken to achieve maximum larval mortality was three hours. However, the first 15 minutes did not show larval mortality. Larval mortality started after 30 minutes of exposure through the third hour. The larval mortality increased as the concentration increased. The highest percentage mortality (100%) was obtained at 100 μg/l concentration followed by 96% each at 60 and 120 μg/l. The lethal concentration was determined from the graph of mortality against the concentrations. The LC_{50} value of 0.031 was obtained while the LC_{90} was 0.0166. Statistical analysis of the results of the three *Bacillus* isolates showed no significant difference in larvicidal activities at 5% confidence limit ($P > 0.05$). But when the results were compared against the standard strain, significant difference exists at 5% confidence limit, hence the post experimental analysis was carried out using the least significant difference test (LSD=16.84). The standard strain stood out different from the local isolates. The standard isolates when tested against the *Aedes mosquitoes* showed a gradual increase in activity as the concentration increased. This means that as toxicity increased, the lethal effect also increased. At the lower concentrations (15 and 30μg/l), the toxin and/or spores were not

sufficient to attack the larvae appreciably. This is because it is known that toxins or spores have to be ingested by the larvae so that the crystals can be released inside the host mid gut, which being highly alkaline can dissolve the crystals (Davidson, 1982; Lacey and Undeen, 1986). Pronounced activity was noticed between the concentrations of 60 and 120 μg/l. *Bacillus cereus* encountered in this research is not an uncommon finding because the current *B. thuringiensis* in use today as a model for inter-laboratory comparison was initially thought to be *B. cereus*. The only differentiating factor between it and *B. thuringiensis* was that the latter possesses a parasporal body and specific larvicidal activity (Delaporte and Beguin, 1955; Heimpel and Angus, 1959). However, the larvicidal activity may not necessarily be connected with the parasporal body because it has been detected that larvicidal activity, where parasporal body are absent, could be due to toxins located in the cell wall or cytoplasm (Myers and Yousten, 1980; Davidson, 1982). *B. sphaericus* in comparison to the *B. cereus* kills its host by means of a cell-bound toxin and also that both the vegetative and sporulating cells including the spores are toxic. Comparing *B. thuringiensis* with the two organisms, no larvicidal activity has been reported where there was no parasporal body (Charnley, 1991). In conclusion, the local *Bacillus* species compared favorably with the standard strain. However, there is the need to continue further search for more *Bacillus* strains and other bacteria. In addition, there is the need for specification so that a particular *Bacillus* species can be directed against a particular mosquito species.

Table 1: Mortality of *Aedes* Larvae Treated with Various Concentrations of *Bacillus* Species Isolated from Soil in Sokoto, Northwestern Nigeria

Isolates	**Mortality (%) (Average of three replicates)**					
	Concentration (μg/l)					
	15	30	60	90	100	120
B. cereus	1(4)	0(0)	4(16)	1(4)	10(40)	15(60)
B. sphaericus	0(0)	1(4)	1(4)	10(40)	15(60)	21(84)
B. thuringiensis	0(0)	0(0)	7(28)	12(60)	17(68)	14(56)

Table 2: Mortality of *Aedes* Species Larvae Treated with Various Concentrations of Standard Powder of *B. Thuringiensis*

Time (minutes)	Mortality (%)					
	Concentration (μg/l)					
	15	30	60	90	100	120
15	0	0	0	0	0	0
30	0	3	2	3	4	0
60	4	5	14	12	8	11
120	6	9	7	8	9	11
180	2	2	1	0	3	3
Total	12	19	24	23	25	25
Mortality	48	76	96	92	100	100

Table 3**:** Potency Levels of the Bacterial Isolates Tested

Isolates	**Average LC_{50}**	**Standard *B.thuringiensis* LC_{50}**	**Potency (ITU/mg)**
B. cereus	0.033	0.016	800
B. sphaericus	0.033	0.016	800
B. thuringiensis	0.029	0.016	827

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