Journal of Scientific Research & Reports
7(2): 117-128, 2015; Article no.JSRR.2015.193
ISSN: 2320-0227

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Physiological Changes in *Clarias gariepinus* Induced with *Bacillus* Species Used as Biological Agent in Aquatic Environment

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Authors' contributions

This work was carried out in collaboration between both authors. Author FOO designed the study managed the literature searches and wrote the first draft of the manuscript. Author FCA wrote the concept of the article, also managed the literature searches and the experimental process. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2015/8711
Editor(s):
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(5) Anonymous, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1126&id=22&aid=9054

Original Research Article

Received 26th December 2013
Accepted 20th May 2014
Published 30th April 2015

ABSTRACT

In order to study the potential use of *Bacillus subtilis* and *B. cereus* as biological control agents of mosquitoes larvae in aqueous environments as happens with *B. thuringiensis* var. *israelensis*, fish model was used hence treatments with these bacterial species will be in aquatic environment. The aim of the study was to evaluate any possible physiological alterations and toxicity to aquatic animals (catfish by inoculation with bacillus species). In this view, dissolved oxygen (DO), chemical oxygen demand (COD), pH of the experimental water culture was estimated. Effects of the *Bacillus* species were also monitored in fish haematology and histopathology of gill and liver tissues. Results showed that there was no significant variation ($P<0.05$) in the water quality parameters with catfish

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inoculated with *B. thuringiensis* and *B. subtilis* in comparison with the inoculation with the *B. cereus*. In the haematological parameters, values obtained from catfish inoculated with *B. thuringiensis* and *B. subtilis* treatments were related to values of the control group. Changes in body weight of *B. cereus* treated fish were reflected in the liver tissue histopathology with severe hepatic and damages to gill rakes and filaments. Summarily, the safety of *B. subtilis* as a biological control agent in an aquatic environment is guaranteed but *B. cereus* as an alternative to the existing biological control agent lack valuable safety records as it can cause harm in aquatic animals and human environments.

Keywords: Bacillus species; biocontrol; aquatic animal; toxicity; Nigeria.

1. INTRODUCTION

The use of chemicals in control of mosquitoes appears to have many disadvantages such as its harmful to non target populations as well as the environment. It also causes resistant to mosquitoes which may make their control more difficult in the future [1].

Microbial control agents are particularly suitable components for integrated control programmes [2]. They can be used in isolation or mixed with other larvicides which are formed over the water surface; any larvae which have not been killed by the microbial control agent can be controlled by the surface film technique. The basic requirement for the successful use of microbial control agents was the development of effective formulations suited to the biology and habitats of the target organisms. Today, microbial control agents are widely available commercially and can be obtained in various formulations, including wettable powders, liquid formulations, granules, floating briquettes, pellets and tablets [1,3,4]. Because several microbial insecticides are pest-specific, the potential market for these products may be limited. Their development, registration and production costs cannot be spread over a wide range of pest control sales; consequently, some products are not widely available or are relatively expensive. The limitations or any known disadvantages of microbial control do not prevent the successful use of microbial insecticides. According to Wirth et al. [5], understanding how these limitations affect specific microorganisms will help users to choose effective products and take necessary steps to achieve successful results. By understanding the larval feeding behaviour, knowing which habitat they thrive in, having experience with routine treatments and using the appropriate dosages of the biological control agents, both ecological and economical advantages over chemical insecticides will be possible. Vector management and reduction of malaria may be achieved through larvicides. Larviciding by treating breeding sites can be achieved either by the use of microbes to cause toxin in mosquito larvae when feeding on them (biological control) or using chemicals to kill mosquito larvae.

The main advantages for using bacteria species over chemical conventional insecticides are its cost-effectiveness [6], target specificity, ease of production [7], amenability to formulation [8], safety to non-target organisms and mammals [9].

When attempting to describe the influence that a pathogen has on its hosts, two of the most fundamental issues are how much of the pathogen is required to cause the effect in question, and how much time elapses after the host is exposed to the pathogen before the effect takes place. In considering lethal entomopathogens such as *Bacillus thuringiensis* var *israelensis* (*Bti*), the effect that is of interest is usually the death of the host. This is true for studies in biological control because maximizing the death of the host is the usual aim of an investigation.

About two hundred microbial products for insect control are currently on the market, 50% of them are based on *Bacillus* entomopathogenic bacterial. These microbial agents are the most widely used around the world for Integrated Pest Management (IPM), especially to control the leptidopterans and coleopterans species [2]. Effective control of aquatic larvae such as those of mosquitoes and black flies, have been achieved using *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* [10,11].

The production and formulation of *Bti* have been improved to attain the desired effect of larvae control in a variety of breeding sites. *Bacillus thuringiensis* var. *israelensis* is a natural pathogen of some “pests and its insecticidal proteins produced are extremely” toxic to certain

pests, but cause little or no harm to people, wildlife, and most beneficial insects [12]. *Bacillus cereus* is closely related to *B. thuringiensis*, but can be distinguished from *B. thuringiensis* as it cannot produce parasporal bodies [13]. *Bacillus subtilis* unlike other bacillus species is spore forming pupicidal bacteria whose metabolites can kill both the larvae and pupal stages of mosquitoes [14]. Hence some *Bacillus* species have been successfully used as biological control agent for mosquito in an aquatic environment, the aim of the study is to source for the most promising species among *Bacillus cereus* and *Bacillus subtilis* in relation to *Bacillus thuringiensis* var. *israelensis* to be used as biological control of mosquito in their breeding environment with low or no harm to aquatic animals and possibly to humans using fish model.

2. MATERIALS AND METHODS

2.1 Toxic Activity for Aquatic Organism (*Clarias gariepinus*)

Potential toxic activity for aquatic organism was carried out according to Das and Mukherjee [15]. Juveniles of catfish (*Clarias gariepinus*) with mean body weights of between 52.32±0.22g to 53.03±0.39g were used as a model aquatic organism for assessing the bio-safety of the bacteria species. They were obtained from the Department of Fishery and Aquaculture Technology, Federal University of Technology, Akure. The fish were kept in a glass aquarium containing 30 L of water with constant aeration. The water temperature was maintained at 25± 2.0℃. The fish in the aquarium was fed with commercial fish meal twice daily for one week to acclimatize. Thereafter, the fish was divided into four groups of four each having three replicates for each group and a control for *B. thuringiensis* var. *israelensis, B. subtilis* and *B. cereus* treatments. Chemical oxygen demand (COD), dissolved oxygen (DO) and pH were monitored in the various tanks for each treatment. The fish were starved for 24 h before injected subcutaneously with 100 µl of 10^6 cfu/mL cell doses daily with the *Bacillus* species for seven days. Fish was observed shortly after each dosing, and thereafter were observed twice daily for the period of one week infection for a general behavioral signs of toxicity and possibly mortality. The behavioral signs of toxicity observed included changes in the skin, rate of food consumption, agility and change in body weight. The tested fish was conducted in compliance with NIH Guide for Care and Use of Laboratory Animals. Fish was anaestizing with fume of chloroform at the end of experiment and sampled for weight gain; blood was collected immediately for hematological analysis and vital organs namely gill and liver was collected for histopathological analysis.

2.2 Bacterial Isolates

Bacillus subtilis and *B. cereus* strains were isolated from decaying cockroach (*Periplaneta americana*) collected from a refuse dump site in Akure North Local Goverrment Area of Ondo State, Nigeria and were identified based on their cultural characteristics, morphological, physiollogical and biochemical tests as described in Bergey's Manual of Determinative Bacteriology [16,17] and Bergey's Manual of Systematic Bacteriology [18]. The determined cultural characteristics of bacteria were colour, surface type, edge and elevation of the resultant colonies. The physiological tests carried out were Gram staining, catalase, spore staining, coagulase, oxidase and motility, while the biochemical tests performed include carborhydrate utilization of sucrose, maltose, glucose, arabinose, mannitol, lactose, sorbitol and fructose, starch hydrolysis, catalase, nitrate reduction, utilization of citrate, indole production, oxidative fermentation, methyl red and voges proskauar. The ability of the bacterial isolates to grow at temperatures of, 30℃, 50℃ and 60℃ and in 2%, 5%, 7%, and 10% NaCl media. *B. thuringiensis* var. *israeliensis* (HD522) was obtained in the invertebrate pathology and microbial pest control laboratory, School of Molecular and Cell Biology, University of Witwaterstrand, Johannesburg, South Africa, and strains were maintained on Nutrient Yeast Salt Medium (NYSM) [glucose 5 g, peptone 5 g NaCl 5 g, beef extract 3 g, yeast extract 5 g, $MgCl_2$ 203 mg, $MnCl_2$ 10 mg and $CaCl_2$ 103 mg.

2.3 Bacterial Growth Conditions

For fish inoculation, the bacterial was grown aerobically in NYSM broth and incubated for 24h at 37℃. Cells were harvested from the broth culture by centrifugation, washed severally with distilled sterile water and used for the inoculation.

2.4 Collection of Blood

Blood was collected from fishes by cardiac puncture with the aid of disposable syringe and

needle into heparrinized vials. The blood samples were taken to the laboratory for immediate haematological analysis

2.5 Haematological Parameters

The hematological parameters studied included haemoglobin and total red blood cells count (RBC), white blood cells count (WBC), differential count of leucocytes such as neutrophil (%), lymphocyte (%), monocyte (%) and packed cell volume (PVC) were enumerated.

2.6 Packed Cell Volume (PCV)

PCV was estimated by spinning 75% of each blood sample in a haematocrit micro centrifuge at 1200 rpm for five minutes and the value was read on the heamatocrit reader as a percentage of the total blood volume using the equation:

$$\text{PCV (\%)} = \frac{\text{Height of packed cell column}}{\text{Height of whole blood column}}$$

2.7 White Blood Cell (WBC)

Ethylenediamine tetra acetic acid (EDTA)-treated whole blood at a 1:20 ratio with blood cell diluting fluid (made up of 3.8 bg sodium citrate, 0.21 g neutral formalin and 0.5 g brilliant cresol blue and 100 mL distilled water). The diluted sample was then mixed and loaded into the counting chamber. The WBC in the chamber was counted leaving out the edges of the chamber and the total WBC was determined using the equation.

$$\text{WBC } (10^3/\text{mm-}^3) = \frac{N \times DF \times 10^6}{A \times D}$$

Where A = the area counted, N = number of cells, DF = Dilution factor, D = depth of chamber.

2.8 Red Blood Cell

This was determined using the Neabeauer counting chamber, dilution pipette, dilution fluid (formol-citrate prepared by mixing 10 mL of formalin with 1 L of trisodium citrate solution) and counting the cells under the compound microscope with a hand tally.

$$\text{Red cell count } (10^6/\text{mm}^-3) = \frac{N \times DF \times 10^6}{A \times D}$$

Where A = the area of chamber counted, N = number of cell, DF = Dilution factor D = depth of chamber.

2.8.1 Haemoglobin (Hb)

Blood samples (0.02 mL) were mixed gently for one minute and drawn using a 0.02 mL micro-pipette and expelled into a tube containing 4 ml of Drabkin's solution. The tube was stopped, mixed and left to stand for 5 minutes to allow full colour development. A standard was prepared using a blood sample of known haemoglobin concentration. Using plain Drakin's solution both the sample and standard blood dilution were then read on the colorimeter (Corning colorimeter, 253) at 550 nm. The haemoglobin concentration in the blood sample was calculated using the formula:

Hb concentration (%) =

$$\frac{\text{Reading of test} \times \text{standard Hb concentration}}{\text{Reading of standard}}$$

2.8.2 Histopathology

The internal organs namely gill and liver of Catfish were preserved in 10% formalin solution, after which they were processed for histopathological studies [19,20]. The tissues were cut into small sizes of about 3 cm and were dehydrated in different grades of alcohol from 50% through 100%. Thereafter, the tissues were cleared in xylene for 2 hours and impregnated in molten wax. The impregnated tissues were embedded in paraffin wax, allowed to solidify, marked out with sharp knife and mounted on wooden block for sectioning. The tissues were sectioned with microtome at 5 µm. The sectioned tissues were spread out in a water bath regulated at 45 ^{o}C and picked with slides previously rubbed with egg albumin. The sectioned tissues were dewaxed and hydrated in alcohol grades from 100 – 50%, stained with haematoxylin and eosin [21], the excessive stain was washed with 70% ethanol, clear in xylene, and mouned in Canada balsam.

2.8.3 Determination of the water culture quality

Water quality parameters such as, chemical oxygen demand (COD), dissolved oxygen (DO) and pH were determined as described below.

2.8.4 Determination of chemical oxygen demand (COD)

The water culture samples were stirred very well and 250 mL was taken into a clean 500 mL bottle. These bottles were placed in the water bath at 27℃ for 4 hours. A 10 mL of 1:4 H_2SO_4:H_2O mixture followed by an accurate measured volume of N/80 $KMnO_4$ was added to each bottle and then mixed by gentle rotation. A blank determination using only the reagents and double distilled water was also carried together with water samples. After 4 hours of incubation, bottles were cooled to room temperature and a few crystals of potassium iodide were added. Titration was carried out in the bottles with N/80 thiosulphate; thiosulphate used will exactly be the amount of $KMnO_4$ left in the bottle. The titre value subtracted from the amount of $KMnO_4$ used in titration should give the amount of $KMnO_4$ consumed in the oxidation of chemical substances present in the sample [22].

2.8.5 Estimation of dissolved oxygen

Dissolved Oxygen (DO) in water was estimated by titration using Winkler method as described by Michael [23]. The method was based on the fact that Sodium hydroxide reacts with Manganese sulphate to give a white precipitate of Manganese hydroxide which was oxidized by oxygen to brown colour of manganese oxhydrate. Water samples were collected in 250 mL reagent bottles without air trap and corked. One mL of Winkler reagents A (manganese sulphate) and B (potassium hydroxide + potassium iodide) were added subsequently tilting the bottle side ways to allow the reagents to mix thoroughly in the bottle.

A white to brownish colour precipitate was formed after the addition of reagents which is proportional to the amount of O_2 present. To estimated O_2 level, 1 mL of H_2SO_4 was added after the flocculent precipitate was introduced into a conical flask placed on a white surface. 0.0125N sodium thiosulphate was run into the conical flasks from the burette till the brown colour of the sample became pale yellow. After this, 5 drops of starch solution were added and mixed properly. The sample turned blue and titration continued quickly until the solution turned colourless.

2.8.6 pH determination

The pH of the effluent was measured using a pH meter (Knick, 766 Calimatic Model) according to AOAC [24]. At constant temperature, a pH change produces a corresponding change in the electrical property of the solution. This change was read by the electrode [23]. The sensitive end of the instrument was dipped into the sample for about 30-40 seconds and the pH read off the scale after the reading had stabilized.

2.9 Statistical Analysis

Statistical tools employed include Analysis of Variance (ANOVA) to test for significance among means: Duncan Multiple range Test to separate significant means and probit analysis to determine the LC_{50} that will kill 50% of test population.

3. RESULTS

There was no significant variation ($P< 0.05$) in water quality parameters for each system throughout the experimental period. Table 1 shows the mean values of water quality parameters of different *Bacillus* spp for the duration of the experiment. The DO decreased significantly from 5.63±0.18 to 5.01±0.15 mg/L and pH from 7.32±0.00 to 6.83±0.00. The COD increased significantly from 17.26±0.91 – 24.20±0.44 mg/L. Tables 2 show the haematological parameters of catfish treated with *B. cereus*, *B. subtilis* and *B. thuringiensis* var. *isrealensis* HD522. In the *B. cereus* treated fish, there was a general significant decrease in the haematological parameters when compared with the control. The PCV and RBC values of catfish fed with *B. subtilis* (29% and 1.92 x $l0^6$ mm^{-3}) and *B. thuringiensis* var. *isrealensis* HD522 (29% and 2.02 x l 0^6 mm^{-3}) showed slight decrease when compared with the control group (PCV 33% and RBC - 2.30 x 10^6 mm^{-3}). A significant decrease in the values of their WBC counts (10^7 x 50 mm^{-3} and 10^6 x 50 mm^{-3}, respectively) when compared with the control group (108 x 50 mm^{-3}) was also recorded. Table 3 shows the results of weights of catfish before and after the treatments. Catfish treated with *Bacillus cereus* decreased in body weight by 3.53%. Fish in the control experiment and those treated with *B. subtilis* and *B. thuringiensis* gained weight by 0.91%, 1.35% and 3.36% respectively. Observation of the Catfish infected with *B. cereus* revealed significant damages in the liver and gills when compared with the control group (Fig. 1D). While there was no observable pathological changes in gills of catfish fed with *B. thuringiensis* var. *isrealensis* (Fig. 1A) and *B.*

subtilis (Fig. 1B), The catfish treated with *B. cereus* (Fig. 1C) showed gill rakes and filaments heavily affected (degenerated). The liver of catfish treated with *B. cereus* inflamed (Fig. 2B) while those treated with *B. thuringiensis* var. *isrealensis* (Fig. 2A) and *B. subtilis* (Fig. 2C) did not show pathological defects when compared with the control (Fig. 2D).

4. DISCUSSION

Very few reports exist on the mosquitocidal activity exhibited by strains of *B. subtilis* [15], unlike *B. thuringiensis* var. *israelensis* and *B. sphaericus,* whose spore crystal complex is known to be larvicidal for worldwide application as mosquitocidal agent. The cyclic lipopeptides (CLPs) present in the culture supernatant of *B. subtilis* have been reported to be responsible for mosquito larvicidal property [15]. Despite this information of some *Bacillus* species as biological control of mosquito larvae and pupa, no result has been published on their suitable use in an aquatic environment. Though *B. cereus* has been investigated for biological control agent [25], its hazards on aquatic animals and human environment will not approve its use hence other harmless *B.* species have been isolated and successfully used. Hence haematological parameters can provide information on physiological response of fish due to the close association of the circulatory system with the external environment, their employment could serve as a guide to what harm may ensue when microbes are employed for use in an aquatic environment. Exposure of *Clarias gariepinus* to *B. cereus* might have resulted in an anemic condition as interpreted by results obtained from haematological parameters analyzed. However, same exposure of the fish to other test bacillus species could not manifest physiological changes in the fish because of their low toxicity. Studies have shown that when water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters [26].

Omoregie *et al.* [27] who worked on the effect of plant derived pesticides on fish had reported that sub-lethal concentrations of toxicants in aquatic environment often resulted in several physiological dysfunctions instead of outright mortality of fish. Haematological parameters such as PCV, Hb, RBC and WBC decreased in the group treated with *B. cereus* when compared to the control group. Similar reductions in haematological indices have been reported by various workers [27,28,29]. The reductions in packed cell volume, haemoglobin and .red blood count suggested haemodilution, indicating a probable anemic condition in the fish. Anemia is a blood condition involving an abnormal reduction in the number of red blood cells (erythrocytes) or in their haemoglobin content.

Haematocrit (packed cell volume) is the measurement of the percentage of red blood cells in whole blood. Anemia may arise from reduced production of red blood cells, which may result from deficiency in nutrients or hormones, or from disease or other conditions and excessive destruction of red blood cells [22]. Present result agrees with findings observed on studies in the effect of electroplating effluent on haematological parameters of *Oreochromis mossambicus* [30].

Table 1. Values of water quality parameters of cultural medium for catfish treated with three *Bacillus* species

	Control	***Bacillus thuringiensis***	***Bacillus subtilis***	***Bacillus cereus***
Temperature (℃)	23.99±0.29a (23.75 – 24.23)	23.99±0.29a (23.75 – 24.23)	24.01±0.32a (23.75 – 24.28)	24.04±0.33a (23.76 – 24.31)
pH	7.32±0.00a (7.26 – 7.37)	7.11±0.00b (7.08 – 7.15)	7.03±0.00c (7.00 – 7.06)	6.83±0.00d (6.76 – 6.91)
Dissolved oxygen (DO) (mg/L)	5.63±0.18a (5.48 – 5.77)	5.35±0.00b (5.27 – 5.43)	5.23±0.18b (5.08 – 5.37)	5.01±0.15c (4.89 – 5.13)
Chemical oxygen demand (COD) (mg/L)	17.26±0.91c (16.50 – 18.03)	23.20±0.55b (22.74 – 23.65)	23.74±0.69ab (23.24 – 24.23)	24.20±0.44a (23.83 – 24.56)

Values in parenthesis = range; number of observations = 4; values in the same row with different letters are significantly different from each other ($p<0.05$), according to Duncan's test

Table 2. Haematological parameter of catfish treated with *Bacillus* species

Test organisms	PCV	RBC x 10^6 mm^{-3}	WBC x 50 mm^{-3}	Hemoglobin (%)	Lymphocyte (%)	Eosinophill (%)	Neutrophil (%)	Monocyte (%)	Basophil (%)
Bacillus cereus	22.0±1.00c	1.22±0.02c	102±0.57c	7.6±0.10c	57.6±1.52a	0.67±0.57c	25.6±0.57a	10.6±0.57d	2.6±0.50a
Bacillus subtilis	29.0±1.00a	1.92±0.02ab	107±1.00b	9.4±0.10b	56.0±1.00ab	1.0±0.00b	27.0±1.00a	12.0±1.00c	2.3±0.57a
Bacillus thuringiensis Var *isrealensis* (HD522)	29.0±1.00a	2.02±0.02a	106±1.15b	9.3±0.10b	58.7±0.57a	1.0±0.00b	22.0±1.00b	15.6±0.57b	1.67±0.57ab
Control	33.0±1.00a	2.30±0.10a	108±2.90a	11.2±0.15a	57.0±1.00a	2.5±1.00a	25.0±1.00a	25.0±1.00a	2.0±0.00a

Each value represents the mean±standard deviation; PCV= Packed cell volume; RBC= Red blood cell; WBC= White blood cell; values in the same column with different letters are significantly different from each other (p<0.05), according to Duncan's test

Table 3. Effect of *Bacillus* species on cat fish bodyweight

Tested organisms	Initial fish weight (g)	Final fish weight (g)
Bacillus thuringiensis var. *israelensis*	52.8 ± 0.15a	54.15 ± 0.58c
	(52.80-52.99)	(54.25-54.82)
Bacillus subtilis	53.03 ±0.39a	53.94 ± 0.61b
	(52.85-53.14)	(53.66-53.87
Bacillus cereus	52.73 ± 0.22a	49.20 ± 0.26a
	(52.73-53.12)	(49.41-49.35
Control	52.32 ± 0.22a	55.68 ± 0.41d
	(52.32-52.68)	(55.11-56.04)

Mean±standard error; values in parenthesis = range,; number of observations = 4; values in the same column with different letters are significantly different from each other (p < 0.05), according to Duncan's test.

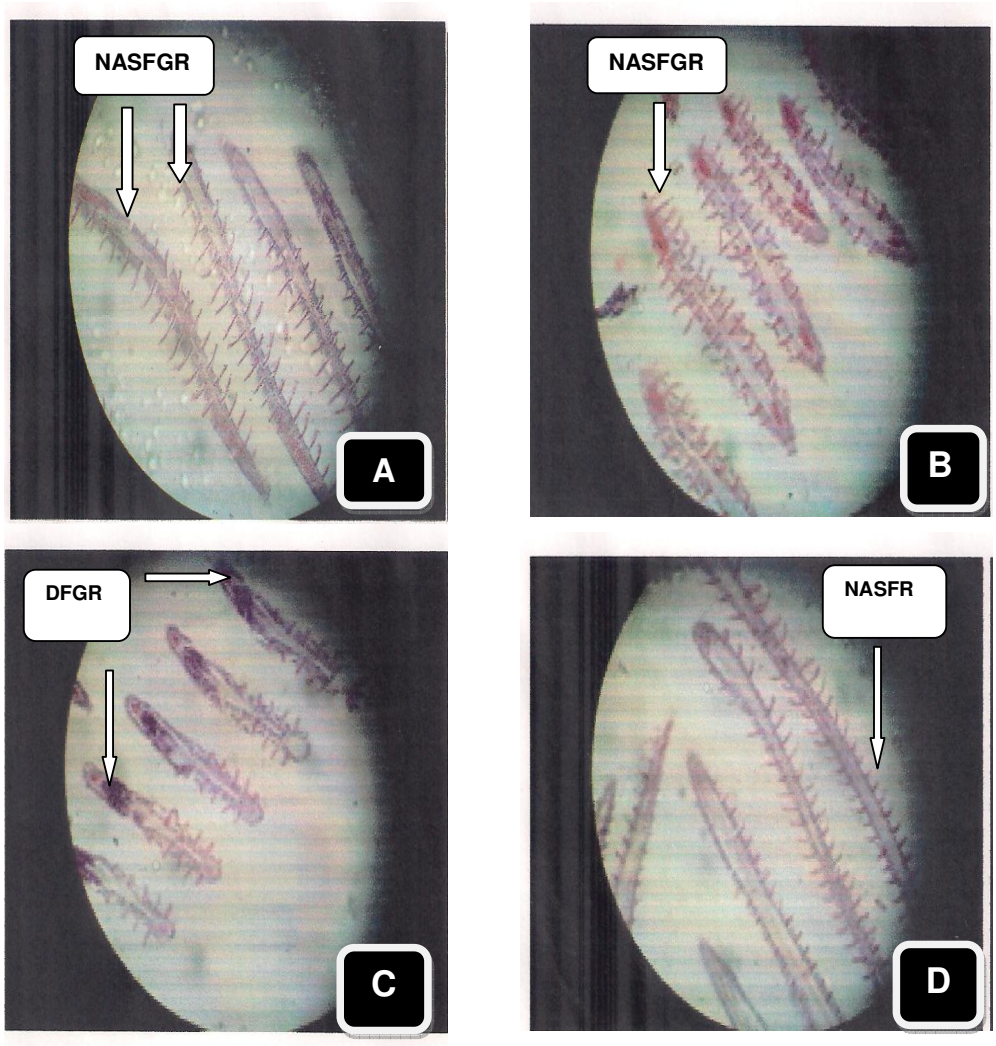

Fig. 1. (A) Photomicrograph of histopathological section of catfish gills treated with *Bacillus thuringensis* showing normal architectural structure of filaments and gill rakes (NASFGR), (B) Photomicrograph of histopathological section of catfish gills treated with *B. subtilis* showing Normal architectural structure of filaments and gill rakes (NASFGR), (C) Photomicrograph of histopathological section of catfish gills treated with *B. cereus* showing degraded filaments and gill rakes (DFGR) in the various, and (D) Photomicrograph of histopathological section of control catfish gill showing the normal architectural structure of filaments and gill rakes (NASFGR)

Haemoglobin is a protein in the red blood cells and it is the most prevalent of the special blood pigments that transport O_2 from the lungs to the body cells, where it picks up carbon dioxide for transport back to the lungs to be expired. Distinct decrease in the level of haemoglobin observed suggests a haemodilution mechanism being operational. Haemodilution has been interpreted as a mechanism that reduces the concentration of an irritating factor in the circulatory system [31].

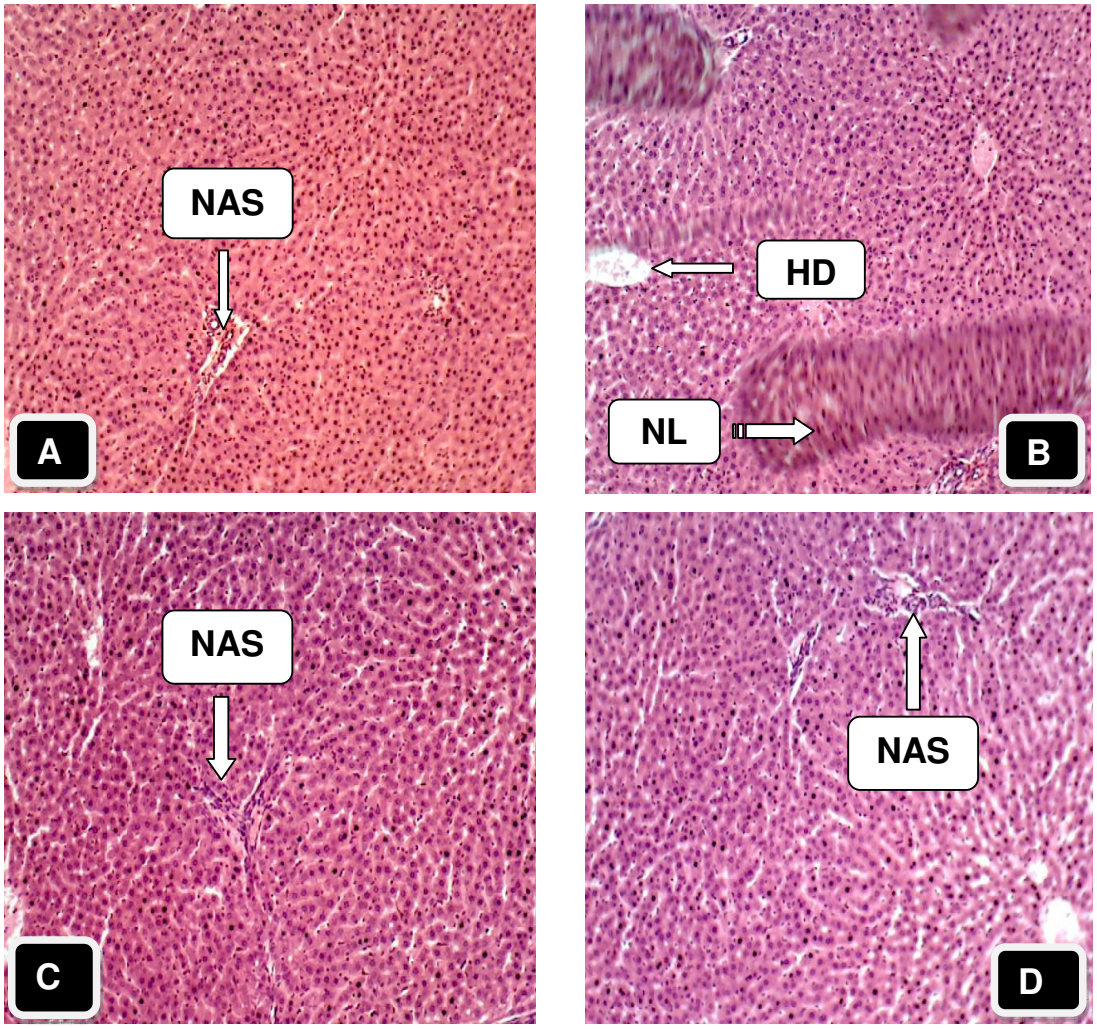

Fig. 2. (A) Photomicrograph of histopathological section of catfish liver treated with *Bacillus thuringensis* showing normal architectural structure (NAS), (B) Photomicrograph of histopathological section of catfish liver treated with *B. cereus* showing hepatocellular damage (HD) and necrotic lesions (NL), (C) Photomicrograph of histopathological section of catfish liver treated with *B. subtilis* showing normal architectural structure (NAS and (D) Photomicrograph of histopathological section of control catfish liver showing normal architectural structure (NAS)

Red blood count determines the number of red blood cells in one cubic meter of blood. Red blood cells are the means by which O_2 is carried to the various parts of the body. Thus shortage in red blood cells would therefore lead to less O_2 supply by the red cells. Hypoxia is a state of acute O_2 deficiency. It produces a variety of reactions in the body which includes mild intoxication and stimulation of the nervous system, followed by progressive loss of attention and judgment until unconsciousness occurs. Respiration and pulse rate increase, and the systemic O_2 content was also reduced. Prolonged lack of O_2 may cause damage to the brain [22].

Decreases in red blood count observed in catfish could be attributed to destruction and or impaired synthesis. The major portion of toxin in the system was sequestered in the red blood cells and a relatively small proportion was transported via the plasma to target organs. Similar trends in red blood count alongside packed cell volume and haemoglobin in fishes

exposed to various toxicants (carbon tetrachloride, DDT) persisting till the end of the experiment have been observed by other workers [28,32,30,33].

White blood count is part of the immune system of the body and expresses the number or white blood count in one microliter or whole blood. Part of the functions of the white blood cell is to distribute anti-bodies in immune response. Effluent induced toxicity on WBC might have resulted in fewer white blood cells being available for the transport of anti-bodies in addition to reduced anti-bodies (Gamma) globulins present. Lower WBC in fishes has also been reported by [30,33] caused by toxicants

Good water quality management is an important aspect for the understanding of this study. Poor water quality often induces emaciation, gill filament ulceration, gill opercula malformation [34] and high mortality [35]. To ascertain it is not poor water quality that may cause harm to the employed fish but the introduced species of *Bacillus*, some water quality parameters were investigated to serve as clew that any observed physiological or pathological effect in the fish is resulting from the injected species of *Bacillus*.

Water pH (6.8) in the tank of *B. cereus* treated fish was significantly different from other *Bacillus* species treatments whose pH were similar and not significantly different from control experiment. pH is a measure of the intensity of acidity or alkalinity and the concentration of proton in water. It is widely known that pH has no direct adverse effect on health but high pH induces the formation of tri-halomethanes which are toxic [36]. Also pH affects the dissolved oxygen level in water and metabolic rates of aquatic organisms which become more susceptible to pollution. A change in stream water pH can also affect aquatic life indirectly by altering other aspects of water chemistry such as low pH levels which can increase the solubility of certain heavy metals that can contribute to low immunity of the fish thus more prone to infection. During the study, a significant increase ($P<0.05$) in COD and significant decrease ($P<0.05$) in DO in *B. cereus* treatment than *B. subtilis* and control groups was observed. Increase in COD could be as the negative effect *B. cereus* caused in the fish, making them fall sick thus their less activity in feeding habit. The decay of fish pellets and the high metabolic activities from fish could result to accumulation or presence of large organic materials in the water culture while decrease in DO could be as a result of eutrophication and decrease in bacterial population with fish wastes and available nutrient from pellets unconsumed by fish. The high activities of fish in the *B. subtilis* and control treatments resulted to the reduction in COD which of course resulted to less turbidity of the culture water than the *B. cereus* treatment. Similar results were reported by [37,38].

The liver of catfish treated with *B. cereus* had slightly vacuolated cells showing evidence of' fatty degeneration. This was similar to the histopathological examination of kidneys, gill and liver of fish exposed to cassava effluent as conducted by Wade *et al.* [39]. Necrosis of some portions of the liver tissue which were observed during the study could probably have resulted from the excessive effort for fish to get rid of *B. cereus* toxicant for its body during the process of detoxification by the liver. In addition, Wade et al. [39] stated that inability of fish to regenerate new liver cells could also lead to necrosis.

5. CONCLUSION

Observations of the catfish infected with *B. cereus* revealed some relevant damages in the liver, gill rakes and filaments. Inflammations were also observed in the liver of catfish infected with *B. cereus*. This organism could indirectly enter into the food chain in an ecosystem or be in contact with certain animals or human being when used as a biocide. So, if its safety cannot be assured, it may cause harmful to humans and therefore it is not suitable as a biological control agent for mosquito in an aquatic environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al-Akel AS, Suliman EM. Biological control agent for mosquito larvae: Review on the killifish, *Aphanius dispar dispar* Rüppel, 1829. African Journal of Biotechnology. 2011;10(44):8683-8688.
2. De Araujo AP, de Melo-Santos M.AV, de Oliveiralos, Rios EM, Regis L. Evaluation of an experimental product based on *Bacillus thuringiensis serovar. Israelensis* against *Aedes aegyti* larvae

(Diptera:Culicidae). Biological Control. 2008;41:339-347.
3. Armengol G, Hernandez J, Velez JG, Orduz S. Long-lasting 3. effects of a *Bacillus thuringiensis* serovar. *israelensis* experimental tablet formulation for *Aedes aegypti* (Diptera: Culicidae) control. J. Econ Entomol. 2006;99:1590-5.
4. Medeiros F, Santos MA, Regis L, Rios EM, Rolim NPJ. Development of a *Bacillus sphaericus* tablet formulation and its evaluation as a larvicide in the biological control of *Culex quinquefasciatus*. Mem Inst Oswaldo Cruz. 2005;100:431-4.
5. Wirth MC, Jainnino JA, Federici BA, Walto WE. Evolution of resistace towards *Bacillus sphaericus* or a mixture of *Bacillus sphaericus* + Cyt1A from *Bacillus thuringiensis*, in the mosquito, Culex quinquefasciatus (Diptera: Culicidae). Journal of Invertabrates Pathology. 2005;88:154-162.
6. Balaraman K, Hoti SL. Comparative cost of mosquito control with larvicidal Bacilli and insecticides. Indian Journal of Malariology. 1987;24:131-134.
7. Balaraman K. Mass production and storage of microbial agents for use in mosquito control. Indian Journal of Medical Research. 1980;72:222-226.
8. Prabakaran G, Hoti, SL, Manonmani AM, Balaraman K. Coconut water as a cheap source for the production of endotoxion of *Bacillus thuringiensis var. israelensis*, a mosquito control agent. Acta Tropica. 2008;105:35-38.
9. Mulla MS, Darwazeh HA, Yousten AA. Larvicidal efficacy of bacillus thuringiensis serotype H-14 against stagnant water mosquitoes and its effects on non-target organisms. Environmental Entomology. 1982;11:788-795.
10. Regis L, Silva-Filha MH, Nielsen-LeRoux C, Charles JF. Bacteriological larvicides of dipteran disease vectors. Trends in Parasitology. 2001;17:377-380.
11. Manonmani AM, Geetha I, Bhuvaneswari S. Enhanced production of mosquitocidal cyclic lipopeptide from *Bacillus subtilis* subsp. *Subtilis*. Indian J. Med. Res. 2011;134:476-482.
12. Tabashnik BE, Liu YB, Malvar T, Heckel DH, Masson L, Ferr J. Insect resistance to *Bacillus thuringiensis*: uniform or diverse? Philosophical Transactions of the Royal Society B: Biological Sciences. 1998;353: 1751-1756.
13. Yuan Z, Hansen BM, Andrup L, Eilenberge J. Detection of Enterotoxin Genes in Mosquito-Larvicidal *Bacillus* Species. Current Microbiology. 2002;45:221-225.
14. Geetha I, Prabakaran G, Paily KP, Manonmani AM Balaraman K. Characterisation of three mosquitocidal *Bacillus* strains isolated from mangrove forest. Biol. Control. 2007;42:34-40.
15. Das K, Mukherjee AK. Assessment of mosquitolarvicidal potency of cyclic lip peptides produced by *Bacillus subtilis* strains. Acta Tropica. 2006;97:168-173.
16. Holt JG, Krieg NR, Sneath PHA, Stanley JT Williams ST. Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore. 1994; 783.
17. Claus DC, A standard Gram-staning procedures. World Journal of Microbiology and Biotechnology. 1992;8:451-452.
18. Sneath PHA, Muir NS, Sharp ME, Holt JG. Bergey's Manual of Systemic bacteriology, Baltimore, Williams and Wilkins. 1986;2.
19. Hawk PK. Urine qualitative analysis. In Hawk's Physiological Chemistry, edited by Oser, B. L. 14th edition. Tata McGraw Hill Books Company Ltd, New Delhi, India. 1965;1032-1034.
20. Varly H, Gowenlock AH, Bell M, Prophyrins R. Haemoglobin and related compounds. In: Gowenlock, A. H., (editor). Practical Clinical Biochemistry, 5th edition. Williams Heinemann Medical Books Ltd., London. 1980;451.
21. Silva JM, Day SH, Nicoli-Griffith DA. Induction of cytochrome-P450 in cryopressed rats and human hepatocytes. Chem-Biology Interact. 1999;121:49-63.
22. Encyclopaedia Wikipedia, the free encyclopaedia, 11th ed. Encyclopaedia Britannica Inc., USA; 2001.
23. Michael P. Ecological methods for field and laboratory investigations. Tata 2nd edn. McGrawhill Publishing Company Ltd, New Dilhi. 1986;103-388.
24. AOAC. Official Methods Analysis: Association of Official Analytical Chemistry. 17th edn. Washington DC. 1990;12-98.
25. Omoya FO, Akinyosiye FA. Evaluation of the potency of some entomopathogenic bacteria isolated from insect cadavers on *anopheles arabiensisgiles* (order: deptera, family culicidae) mosquito larvae in Nigeria. African Journal of Microbiology Research. 2013;7(12):4877-4881.

26. Van Vuren JHJ. The effect of toxicants on the haematology of Labeo lumbratus (Teleosteic cyprinidae). Comparative Biochemistry and Physiology. 1986;83C: 155-159.
27. Omoregie E, Ufodike EBC, Kekle IR. Tissue chemistry of *O. niloticus* exposed to sub-lethal concentrations of Gammalin 20 and Actellic 25 EC. Journal of Aquatic Sciences. 1990;5:33-36.
28. Bhagwant S, Bhikajee M. Induction of hypochromic macrocytic anaemia in *Oreochromis hybrid* (Cichlidae). Exposed to 100mg/l (sub-lethal dose) of aluminium. Science and Technology- Research Journal. 2000;5:9-20.
29. Olaifa FE, Olaifa AK. Onwude TE. Lethal and sub-lethal effects od copper to the African catfish *C. gariepinus*) juveniles. Africal Jouyrnal of Biotechnology Research. 2004;17(2):65-70.
30. Navaraj PS, Kumaraguru AK. Effects of electroplating effluent on haematological parameters of *Oreochromis mossambicus*. Jour. Physiol. 2003;107:935-939.
31. Smith GL, Hatting J, Burger AP. Haematological assessment of the effects of the anaesthetic MS222 in natural and neutralized form in three fresh water fish species: interspecies differences. Jour. Fish Biol. 1979;15:633-643.
32. Agbon AO, Omoniyi IT, Teko AA. Acute toxicity of tobacco (*Nicotinia tubaccum*) leaf dust on *O. niloticus* and haematiligical changes resulting from sub-lethal exposure. Jour. Aquatic Science. 2002; 17(1):5-8.
33. Osuigwe DI, Obiekezie AA, Onuoha GC. Some haematological changes in hybrid catfish *Heterobranchus longitilis* and *C. gariepinus* fed with different levels of raw and boiled jack bean (*Canavalia ensiformis*) seed meal. African Journal of Biotechnology. 2003;4(9):1017-1021.
34. Jung JT. A survey of parasitic and bacterial diseases of aquarium fishes and studies on the resistance of fish pathogenic bacteria to some antimicrobics. Master's thesis. National Taiwan Oceanic University, Department of Aquaculture, Keelung. (In Chinese.); 1993.
35. Chen CC. Head-hole disease and relative syndromes in the aquarium cultural Cichlid fishes. Aqualife magazine. 1998;118:117–121.
36. Kumar A, Bisht BS, Talwar A, Chandel D. Physico-Chemical and Microbial Analysis of Ground Water from Different Regions of Doon Valley. International Journal of Applied Environmental Sciences. 2010; 5(3):433–440.
37. Aggarwal TR, Singh KN, Gupta AK. Impact of sewage containing domestic wastes and heavy metals on the chemistry of Varuna River. Polutl. Res. 2000;19(13):491- 494.
38. Nwabueze AA. Water quality and micro-organisms of leachate-contaminated pond. American Journal of Scientific and Industrial Rersearch. 2011;2(2):205-208.
39. Wade JN, Omoregie E, Ezenwaka I. Toxicity of cassava *Manihot esculentum* Crantz) effluent on the Nile tilapia (*Oreochomis nitolicus* (L) under laboratory condition. Journal of Aquatic Science. 2002;17(2): 89-94.

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