LEPCON-1, BAFOG-1 (S) AND ECOBAC-1 (EC), *Bacillus thuringiensis* BASED-PRODUCTS ARE NOT TOXIC AGAINST THE FRESHWATER FISH, *Tilapia nilotica*

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ABSTRACT

The aim of the ecotoxicity test was to investigate the effect of Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) against the freshwater fish, Tilapia nilotica. *The ecotoxicity test assessed the toxicity of these products by exposing* T. nilotica *to various concentrations of the sample. Media preparation and toxicity test was done according to the* Organisation for Economic Cooperation and Development (OECD) 203 Fish, Acute Toxicity Test (OECD) Guidelines for Testing of Chemicals *(1992). The results showed that the exposure of Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC), each product at 320 mg litre^{-1}, did not cause any mortality to* T. nilotica *after 96 hr of exposure. Therefore, these three products are considered non-toxic to* T. nilotica.

Keywords: fresh water fish, *Tilapia nilotica*, *Bacillus thuringiensis*, Lepcon-1, Bafog-1 (S), Ecobac-1 (EC).

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INTRODUCTION

Bacillus thuringiensis (Bt) is a gram-positive bacterium that produces protein crystals that are toxic to certain insect pests. The insecticidal activity of Bt is attributed to the parasporal crystals or proteinaceous inclusions during sporulation (Roh *et al*., 2007), known as delta endotoxins or insecticidal crystal proteins (ICP), which are toxic to the larval forms of the insect pests (Xavier *et al*., 2007). Several Cry proteins or δ-endotoxins have receptor proteins in the gut lining of a number of insects, including the bagworm, *Metisa plana* (Lepidoptera: Psychidae), an oil palm pest. The activated toxins bind to the gut receptors and cause osmotic lyses and death of the larvae of the bagworm (Siti Ramlah *et al*., 2009).

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Monitoring of aquatic pollution represents one of the major measured activities aimed at environmental protection. Usage of non-target organisms in environmental toxicology is needed to discover a wide range of toxic effects due to pesticides and other pollutants on different organisms (Amanchi and Hussain, 2008). *Tilapia nilotica* was used as a bio-assay organism due to its sensitivity to toxic effects. Furthermore, it was used as metal biological marker in toxicological studies in which it was substantiated with having the highest sensitivity to toxic effect (Patin, 1984). According to Rashed (2001), *T. nilotica* was found to be a good bio-assay indicator for water pollution for lead and cadmium and this fish can easily be found in the lake.

Prior to commercial field evaluation of the Bt products, an ecotoxicity study of the products is needed. Ecotoxicity refers to the toxic effects of chemicals on the living environment, which includes animals and aquatic organisms. Since most of the

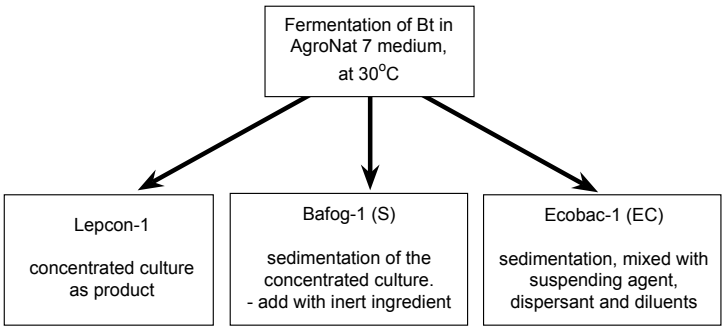

Figure 1. Schematic diagram showing the preparation of different Bacillus thuringiensis *(Bt) products.*

pollutants are found in water, and also for practical reasons such as ease of testing, aquatic organisms are used as the first indicator of environmental effects of chemicals (Razmah and Salmiah, 2002).

The Malaysian Palm Oil Board (MPOB) has conducted an intensive research on the development of Bt products to control bagworms (Najib *et al*., 2008; 2013; Mazmira *et al*., 2011). The indigenous MPOB Bt1 was identified and it was proven effective to control bagworms. MPOB Bt1 was successfully mass produced on the MPOB medium, AgroNat 1 that gave high yield of cells and crystal proteins (Siti Ramlah *et al*., 2005). Three different formulations of the product were developed and named as Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC). This study reports the ecotoxicity of these three products of MPOB Bt1 against the freshwater fish, *T. nilotica.*

MATERIALS AND METHODS

Test Samples

Preparation of Bt Products. Lepcon-1 is a flowable concentrate (FC), Bafog-1 (S) is a soluable concentrate and Ecobac-1 (EC) is an emulsifiable concentrate, consisting of indigenous *Bacillus thuringiensis* (MPOB Bt1). All three products were produced at the Microbial Technology and Engineering Centere (MICROTEC) at MPOB, Bangi, Selangor. The sample size of each Bt product used was 100 ml. Each treatment was done in two replications, including blank.

The Bt products were prepared after 48 hr of fermentation at a temperature of 30°C in AgroNat-7 laboratory prepared medium (patent No. PI2011000307). The fermentation was conducted in a bioreactor with a working volume of 300 litres. These Bt products were produced using the following method.

Test Method

Test organism. In this test, a local freshwater fish, *Tilapia nilotica*, was selected as the test species as it was found suitable in earlier studies (Razmah and Salmiah, 2002; Najib *et al*., 2011). It is a tropical fish and can be found in water bodies throughout Malaysia. The fish is available throughout the year, easy to rear, and convenient to be used as a test species. With this attributes, *T. nilotica* meets the suitability requirements for ecotoxicity test as stated in the *OECD Guidelines for Testing of Chemicals* (1992).

The freshwater fish were purchased from a local supplier in Rawang, Selangor, Malaysia. The fish, about 2-5 cm long, were acclimatised for at least 12 days in dechlorinated tap water of 50 to 250 mg $CaCO_3$ $litre^{-1}$ hardness. The fish were fed twice daily with commercial dry fish food until the day before the test commenced. During the test, no food was given.

Ecotoxicity test. Ecotoxicity tests of Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) were conducted by exposing *T. nilotica* to different concentrations of the three Bt products. Media preparation and toxicity test were conducted according to the *OECD Guidelines for Testing of Chemicals* (1992). The fish were held in the laboratory and preconditioned for at least seven days before they were used for testing. They were exposed to 12 hr to 16 hr photoperiod daily at a temperature of 25°C ± 1°C.

The test was carried out in two stages. The first stage was called the range-finding test. This test was conducted before the definitive test to enable the choice of the appropriate concentration range. The range-finding test involved a wide range of concentrations of these substances in logarithmic series, *e.g.* 0.1, 1.0, 10.0 and 100.0 mg $litre^{-1}$. It was a short-term test (24 hr) with five fish per concentration. For each Bt product with two replications, 40 fish were used (N=40) with four concentrations.

In the definitive test, the concentration that killed all the fish and the concentration that killed very few or no fish were used as the upper and lower concentration limits, respectively. The definitive test used at least five concentrations in a geometric series with a factor of 2. The number of fish used per Bt product was 20 (N=20) or two fish per concentration and with five concentrations. Conditions of exposure were similar to the pre-conditioning period. The fish were not fed during the test.

The treated fish were inspected after 24 hr, 48 hr, 72 hr and 96 hr. Fish were considered dead if there was no discernible movement and if touching of the caudal peduncle produced no reaction. Dead fish were removed and the percentage mortalities were recorded. The lethal concentration 50% (LC_{50}) values were calculated based on the concentration causing no mortality and 100% mortality after 96 hr. The toxicity of the tested Bt products was rated according to the rating scheme used by the United States Fish and Wildlife Services (2002) for aquatic toxicity as shown in *Table 1*.

RESULTS AND DISCUSSION

The results showed that Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) did not cause any mortality to the fresh water fish, *T. nilotica* even at the highest concentration of 320 mg litre^{-1} (*Table 2*). Based on the rating scheme used by US to categorise chemical toxicity to aquatic organisms, all the tested Bt products were rated as practically non-toxic.

In accordance with the *Short Guidance on the Threshold Approach for Acute Fish Toxicity* (2010), an acute fish test is performed according to the limit test (OECD TG 203) at the threshold concentration, (TC). If the TC is greater than 100 mg litre^{-1}, the test substance concentration should be 100 mg litre^{-1} in the limit test. Therefore, in this study, the TC was extended above the limit test (100 mg litre^{-1}) up to 320 mg litre^{-1} to seek for any mortality at the higher concentration. However, after 96 hr of treatment, zero mortality was recorded. The results confirmed that the three Bt products are safe and not toxic to fish.

It is well documented that biopesticides based on Bt are not toxic to other organisms such as oil palm pollinators, *Elaeidobius kamerunicus* and beneficial insects associated with *Cassia cobanensis* (Najib *et al*., 2009; 2012). A study by Travis and Maureen (1998) showed that the application or direct exposure of *Bti* did not cause any effect on fish, *Fundulus heteroclitus*. The acute toxicity test in the laboratory showed that *Bti* was less toxic to the mummichog, *F. heteroclitus*, as compared to common mosquito larvicidal pesticides such as temephos (LC_{50} mummichog = 980 mg litre^{-1} and LC_{50} temephos = 0.04 mg litre^{-1}).

On other species of fish, Travis and Maureen (1998), reported that *Bti* application caused no effect on mortality or weight change of caged rock bass fish, *Ambloplites rupestris*. In laboratory studies, application of *Bti* at 1.3-1.7 x 10^{10} cfu g^{-1} did not cause any effect on bluegill sunfish, *Lepomis macrochirus*, sheephead minnow, *Cyprinodon variegates* and rainbow trout, *Oncorhynchus mykiss*.

TABLE 1. RATING SCHEME USED BY THE UNITED STATES FISH AND WILDLIFE SERVICES FOR AQUATIC TOXICITY

Rating	LC_{50} (mg litre^{-1})
Super toxic	< 0.01
Extremely toxic	0.01 – 0.1
Highly toxic	0.1 – 1.0
Moderately toxic	1.0 – 10.0
Slightly toxic	10.0 – 100.0
Practically non-toxic	100.0 – 1000.0
Relatively harmless	> 1000.0

Source: Drozd (1991).

CONCLUSION

The ecotoxicity test conducted in this study concluded that Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) are non-toxic to freshwater fish, *T. nilotica*. After 96 hr, exposure at recommended rate (a dose that can start or cause fish mortality) of 80 mg litre^{-1} or even higher concentrations at 320 mg litre^{-1} did not cause any mortality. In conclusion, it was proven that Lepcon-1, Bafog-1 (S) and Ecobac-1 (EC) are non-hazardous biopesticides. Therefore, the application of these products for biological control of oil palm insect pests such as bagworms and nettle caterpillars contributes to cleaner and safer oil palm ecosystem.

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TABLE 2. THE MORTALITY OF *T. nilotica* AFTER EXPOSURE TO LEPCON-1, BAFOG-1 (S) AND ECOBAC-1 (EC) FOR 96 hr

Concentration (mg litre^{-1})	No. of dead fish*			% Mortality		
	Lepcon-1	Bafog-1	Ecobac-1	Lepcon-1	Bafog-1	Ecobac-1
0.0	0	0	0	0	0	0
40.0	0	0	0	0	0	0
80.0	0	0	0	0	0	0
160.0	0	0	0	0	0	0
320.0	0	0	0	0	0	0

Note: * Mean of two readings.

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