ECOTOXICITY OF *Bacillus thuringiensis*, TERAKIL-1® AND TERACON-1® AGAINST FRESHWATER FISH, *Tilapia nilotica*

MOHD NAJIB AHMAD*; SITI RAMLAH AHMAD ALI*; MOHAMED MAZMIRA MOHD MASRI*; RAZMAH GHAZALI* and MOHD BASRI WAHID*

ABSTRACT

Ecotoxicity test was conducted to study the effect of Terakil-1® and Teracon-1® against freshwater fish Tilapia nilotica *by exposing it to various concentrations of the sample. Terakil-1® and Teracon-1®, each led to 30% and 12.5% corrected mortality of freshwater fish,* Tilapia nilotica *at 128 mg litre^{-1} at 4 days after treatment (96 hr). The LC_{50} of two MPOB Bt1 products was 100-1000 mg litre^{-1} implying that Terakil-1® and Teracon-1® are practically non-toxic. Terakil-1® and Teracon-1® are target specific to palm insect pests such as bagworms and nettle caterpillars.*

Keywords: freshwater fish, *Tilapia nilotica*, *Bacillus thuringiensis* (Bt), Terakil-1®, Teracon-1®.

Date received: 4 March 2009; **Sent for revision:** 23 June 2010; **Received in final form:** 5 December 2010; **Accepted:** 19 April 2011.

INTRODUCTION

Bacillus thuringiensis (Bt) is a naturally-occurring soil bacterium that produces toxins which cause disease in insects. A number of insecticides are based on these toxins. Bt is considered ideal for pest management because of its specificity to pests and its lack of toxicity to humans or the natural enemies of many crop pests. There are different strains of Bt, each with specific toxicity to particular types of insects (Extoxnet, 1994).

Bacteria are primitive one-cell organisms, which belong to a group of organisms called prokaryotes. Prokaryotes are neither plants nor animals. Like certain members of the plant kingdom, such as ferns and mushrooms, Bt forms asexual reproductive cells, called spores, which enable it to survive in adverse conditions (NPTN, 2004). During the process of spore formation, Bt also produces unique crystalline bodies as a companion product. The spores and crystals of Bt must be eaten before they can act as stomach poisons in the target insects. Bt is therefore referred to as a stomach poison. Bt crystals dissolve in response to intestinal conditions of susceptible insect larvae. This paralyses the cells in the gut, interfering with normal digestion and triggering the insect to stop feeding on host plants. Bt spores can then invade other insect tissue, multiplying in the insect's body fluids, until the insect dies. Death can occur within a few hours to a few weeks of Bt application, depending on the insect species and the amount of Bt ingested (Espinasse *et al*., 2002).

No effect has been found on fish present during *Bacillus thuringiensis israelensis,* Bt application, nor in direct exposure experiments (Travis and Bruce, 2007). Bt was tested against several species of fish including *Gambusia affinis, Lucania parva, Gasterosteus wheatlandi, Lepomis macrochirus, Salvelinus fontinali*, *etc*. Bt is practically non-toxic to fish. Rainbow trout and bluegills exposed for 96 hr to Bt at concentrations of 560 and 1000 mg litre^{-1} did not show adverse effects. A small marine eel (*Anguilla anguilla*) was not negatively affected by exposure to 1000 to 2000 times the level of Bt expected during spray programmes. Field observations of populations of brook trout, common white suckers, and smallmouth bass did not reveal adverse effects one month after aerial application of Bt

* Malaysian Palm Oil Board,
P. O. Box 10620, 50720 Kuala Lumpur, Malaysia.
E-mail: mnajib@mpob.gov.my

formulation. However, shrimp and mussels may be affected adversely (Extoxnet, 1996).

MPOB has conducted research on the use of Bt to control bagworms (Siti Ramlah *et al.*, 2005). Initially, focus was given on strain isolation and characterisation, followed by propagation and formulation. Each Bt isolate produces mixtures of crytalliferous toxins during sporulation that varies in shape (Zelany *et al.,* 1995). Indigenous MPOB Bt1 contains Cry proteins effective for the control of bagworms. MPOB fermentation medium, AgroNat 1 was developed and gave high yield of cell and crystal proteins. As the bacteria sporulate, it produces crystalliferous toxins within the cell.

MATERIALS AND METHODS

Materials

Terakil-1®, a wettable powder and Teracon-1®, a protein concentrate of indigenous Bt, were MPOB Bt1 products generated at Microbial Engineering and Technology Centre (MICROTEC), MPOB and used as treatments for testing their toxicity against fish. The total amount of Terakil-1® and Teracon-1® used for the experiment was 100 g and 100 ml, respectively.

Acute Toxicity of Terakil-1® and Teracon-1® to Freshwater Fish, *Tilapia nilotica*

Ecotoxicity test assessed the toxicity of Terakil-1® and Teracon-1® by exposing the freshwater fish *Tilapia nilotica* to various concentrations of the sample. Media preparation and toxicity test were conducted according to OECD 203 (Organisation for Economic Cooperation and Development) Fish, Acute Toxicity Test Standard Method. Mortalities were recorded daily and the LC_{50} values were calculated based on the concentration causing no mortality and 100% mortality after 96 hr. The sample was stored in the cold room at 4°C until used.

Ecotoxicity Tests

The fish (*Tilapia nilotica*) were held in the laboratory for at least seven days before they were used for testing (pre-conditioning period). They were exposed to 12 to 16 hr photoperiod daily, temperature of 25°C ± 1°C and at least 80% oxygen concentration. Toxicity tests were performed using a local species of freshwater fish, *Tilapia nilotica*. The fish were standardised by taking those with length of 2-5 cm and maintained according to the method described in the test guidelines. They were acclimatized for at least 12 days in de-chlorinated tap water and fed twice daily with commercial dry fish food until one day before the start of tests. They were not fed during the bioassay.

Test solutions of the chosen concentrations were prepared by dilution of a stock solution of 5 g litre^{-1}. The tests were carried out in two stages. The first stage was called the range-finding test. This test was conducted before the definitive test (second stage) to enable the choice of the appropriate concentration range. The range-finding test involved a wide range of concentrations of substance in logarithmic series. It was a short-term test that took about 24 hr to complete. In the definitive test, the concentration of substance that killed all the fishes 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. Conditions of exposure were the same as the pre-conditioning period. The fish were not fed during testing and the oxygen concentrations in the test chambers were maintained at more than 60%.

The fish were inspected after 24, 48, 72 and 96 hr. Fish were considered dead if there was no visible movement and if touching of the caudal peduncle produces no reaction. Dead fish were removed and numbers of mortalities were recorded. Rating scheme used by the United States Fish and Wildlife Services for aquatic toxicity is shown in *Table 1.*

Data Analysis

The percentage and corrected mortality of freshwater fish, *Tilapia nilotica* were calculated for different days after treatment (DAT). The data on corrected mortality was analysed using ANOVA according to the general linear model procedure. The corrected mortality was compared using Least Significant Difference, LSD Pos Hoc Test at P = 0.05 using SPSS software.

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	100.0-1 000.0
Relatively harmless	>1 000.0

Source: US Fish and Wildlife Services.

TABLE 2. THE MORTALITY OF *T. nilotica* AFTER EXPOSURE TO TERAKIL-1® AND TERACON-1® AT 96 hr

Concentration (mg litre^{-1})	No. of dead fish*		% Mortality	
	Terakil-1®	Teracon-1®	Terakil-1®	Teracon-1®
0.0	1	2	10	20
64.0	2	2	20	20
128.0	3	3	30	30
256.0	4	5	40	50
512.0	7	7	70	70

Note: *mean of two readings.

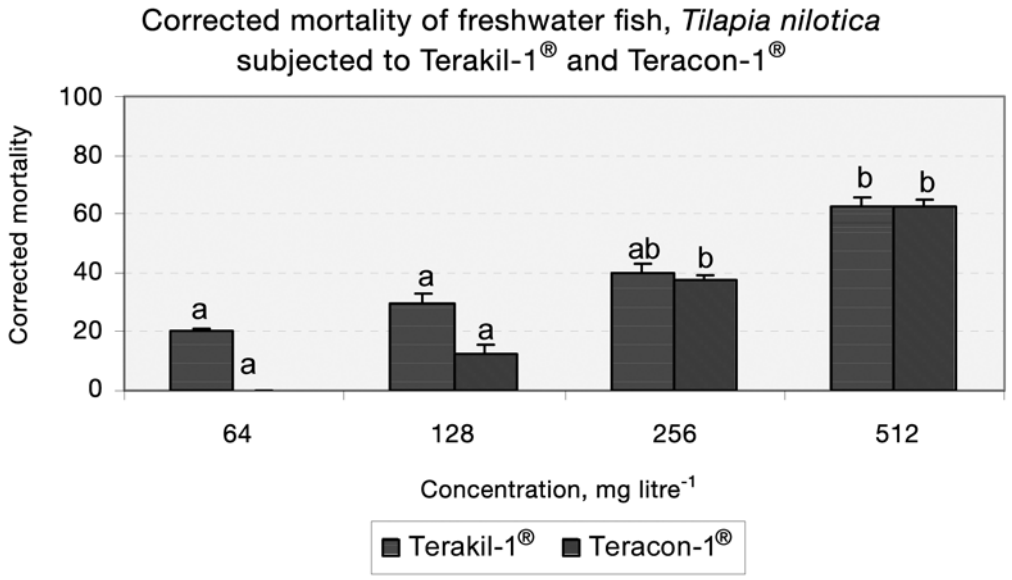

Figure 1. Corrected mortality of freshwater fish, Tilapia nilotica *after 96 hr of exposure to Terakil-1® and Teracon-1®. Note: bars with different groups with the same letters are not significantly different (P>0.05) when tested in one-way ANOVA using Least Significant Difference (LSD) analysis.*

RESULTS AND DISCUSSION

At 4 DAT or 96 hr of exposure to Terakil-1®, the corrected mortality of freshwater fish, *Tilapia nilotica* at dose of 128 mg litre^{-1} was not significantly different as compared to the lowest concentration, 64 mg litre^{-1} at P>0.05 when tested in one-way ANOVA using LSD analysis. As stated in *Table 1*, although Terakil-1® caused 62.5% corrected mortality of *T. nilotica* at the highest dosage, 512 mg litre^{-1}, it was still rated as practically non-toxic. Whereas, Terakil-1® caused 30% corrected mortality of *T. nilotica* at dose of 128 mg litre^{-1} at 4 DAT (*Figure 1*).

The toxicity of Terakil-1® and Teracon-1® is illustrated in *Table 2*. It was concluded that less than 40% of the fish died at 128.0 mg litre^{-1} of Teracon-1® and Terakil-1®. The LC_{50} of both Bt products was more than 100 mg litre^{-1}. Therefore, it can be regarded as safe in the aquatic environment.

According to Travis and Bruce (2007), no effect has been found on fish present during *Bti* application, nor in direct exposure experiments. The acute toxicity of *Bti* to the mummichog, *Fundulus heteroclitus*, in the laboratory was compared to other common mosquito larvicidal pesticides. The Bti was the least toxic, with a 96 h LC_{50} of 980 mg litre^{-1} compared to temephos, the most toxic, at 0.04 mg litre^{-1}.

Teracon-1® resulted in 12.5% corrected mortality of *T. nilotica* at 4 DAT at dose of 128 mg litre^{-1} (*Figure 1*). At 4 DAT or 96 hr of exposure to Teracon-1®, the corrected mortality of freshwater fish, *Tilapia nilotica* at dose of 128 mg litre^{-1} was not significantly different as compared to the lowest concentration, 64 mg litre^{-1} at P>0.05 when tested in one-way ANOVA using LSD analysis. As stated in *Table 1*, although Teracon-1® caused 70% mortality of *T. nilotica* at the highest dosage, 512 mg litre^{-1}, it was still rated as practically non-toxic. Teracon-1® at

dose of 128 mg litre^{-1}, 256 mg litre^{-1} and 512 mg litre^{-1} was significantly different at 4 DAT as compared to the control. However, these ranges of concentration which caused fish mortality were still rated as practically non-toxic, implying that Teracon-1® was considered as safe to aquatic organism.

Study by Ramle *et al.* (2004), reported that the fish mortality at high spore concentration of *Metarhizium* at 1000 mg litre^{-1} was only 25% and 2000 mg litre^{-1} was 40%. According to Genther and Middaugh (1995), when developing embryos of the inland silverside fish, *Menidia beryllina*, were exposed to spores of *M. anisopliae*, several adverse effects were observed in both embryos and newly-hatched larvae. This result proved that the spores of *Metarhizium* as compared to Bt can be considered as non-toxic to the fish.

Travis and Bruce (2007) reported that Bt strain *israelensis* application against blackflies in Michigan found no effect on mortality or weight change of caged rock bass (*Ambloplites rupestris*) or fish numbers and species composition. In laboratory studies, no effect of *Bti was* found on bluegill sunfish (*Lepomis macrochirus*), sheephead minnow (*Cyprinodon variegatus*) and rainbow trout (*Oncorhynchus mykiss*) when exposed to 1.3-1.7 × 10_{10} CFU g^{-1} of diet.

CONCLUSION

Through the ecotoxicity test, it was concluded that Terakil-1® and Teracon-1® are non-toxic to freshwater fish, *Tilapia nilotica*. At 4 DAT or 96 hr of exposure to all MPOB Bt1 products, the LC_{50} of the products was 100-1000 mg litre^{-1} implying that Terakil-1® and Teracon-1® are practically non-toxic. Terakil-1® and Teracon-1® are target specific to palm insect pests such as bagworms and nettle caterpillars and safe for aquatic animals like fish.

ACKNOWLEDGEMENT

The authors would like to thank to the Director-General of MPOB and Director of Biological Research, MPOB for permission to publish this article. The authors would also like to thank the Head of Plant Protection Unit, MPOB for editing the article and the Microbial Technology Group members for the production of the MPOB Bt1 products.

REFERENCES

ESPINASSE, S; GOHAR, M; CHAUFAUX, J; BUISSON, C; PERCHAT, S and SANCHIS, V (2002). Correspondence of high levels of beta-exotoxin I and the presence of *cry1B* in *Bacillus thuringiensis*. *Applied and Environmental Microbiology, 68(9)*: 4182-4186.

EXTOXNET (1994). *Bacillus thuringiensis*. Pesticide Information Profiles. Cornell University.

EXTOXNET (1996). *Bacillus thuringiensis*. Pesticide Information Profiles. Cornell University.

GENTHER, F J and MIDDAUGH, D P (1995). Non-target testing on an insect control fungus: effects of *Metarhizium anisopliae* on developing embros of the inland silverside fish, *Melidia betyllina*. *Dis. Aquatic Organisms, 22*: 163-171.

NPTN TECHNICAL FACT SHEET (2004). Bacillus thuringiensis *Technical Fact Sheet*. Oregon State University.

RAMLE, M; ZAINI, M A; RAZMAH, G; NORMAN, K and BASRI, M W (2004). The effects of *Metarhizium anisopliae* var. major on coleopteran insects under oil palm, and its toxicity to the mammalian and fish. Poster paper presented at the International Conference on Pests and Diseases of Importance to the Oil Palm Industry - Fostering Global Cooperation in Instituting Quarantine Shield. 18-19 May 2004. Kuala Lumpur.

SITI RAMLAH, A A; MOHD MAZMIRA, M M; MOHD NAJIB, A and MOHD BASRI, M W (2005). *Bacillus thuringiensis*, Teracon-1 (TI) for biological control of bagworms. *MPOB Information Series No. 263.*

TRAVIS, J and BRUCE, M D (2007). Declines in ravine-inhabiting Dusk Salamanders of the Southeastern US Coastal Plain. *Southeastern Naturalist, 6(1)*: 83-96.

TRAVIS, R G and MAUREEN, O (1998). Environmental and health impacts of *Bacillus thuringiensis israelensis. Report for the Ministry of Health*. Biocontrol & Biodiversity, Grasslands Division, AgResearch. p. 1-58.

ZELANY, B; STEPHAN, D and HAMACHER, J (1995). Irregular crystal formation in some isolates of *Bacillus thuringiensis*. *J. Invertebrate Pathology, 63*: 229-234.