EFFECT OF *Bacillus thuringiensis* BASED-PRODUCTS ON RATS

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ABSTRACT

Registration of pesticides with the National Pesticide Board must undergo laboratory testing for short-term and long-term health effects. Laboratory animals such as rats are purposely fed with high doses of the pesticide to cause toxic effects. The objectives of this study are to investigate the effect of Bacillus thuringiensis*-based Bafog-1 (S) and Ecobac-1 (EC) against* Sprague Dawley *rats, to assess the toxicity of these Bt products. The acute oral and acute dermal toxicity studies were conducted according to* OECD (Organisation for Economic Co-operation and Development) Guideline for Testing of Chemicals 425 (2001) and 402 (1987)*, respectively. The oral LD_{50} of Ecobac-1 (EC) and Bafog-1 (S) on female rats is more than 5000 mg kg^{-1}, when administered orally with dose of 175 mg kg^{-1}, respectively. Correspondingly, the LD_{50} for dermal administration of both Bt products is more than 2000 mg kg^{-1} for both male and female rats. There was no toxic symptom observed during the two weeks observation period in male and female rats. Post-mortem of the dermally treated animals at the end of the experiment did not show changes or adverse effect in the rats' internal organs, indicating these two products are non-toxic to rats. Bt as an environmental-friendly microbial insecticide is generally not toxic to human, domestic animals and vertebrates.*

Keywords: rat, *Sprague-Dawley*, *Bacillus thuringiensis*, acute oral, acute dermal, Bafog-1 (S), Ecobac-1 (EC).

Date received: 3 January 2014; **Sent for revision:** 22 July 2014; **Received in final form:** 3 November 2014; **Accepted:** 5 January 2015.

INTRODUCTION

Bacillus thuringiensis (Bt) is a spore-forming bacterial insect pathogen and has been extensively studied in various laboratories around the world for its potential as an effective biological control agent against various insect pests, including the bagworm (Lepidoptera: Psychidae) (Ramlah Ali and Mohd Basri, 1997; Ramlah Ali, 2000; Ramlah Ali and Mahadi, 2001). Bt is a gram-positive bacterium and produces crystalliferous proteins during sporulation that are toxic to certain insect pests (Roh *et al*., 2007; Xavier *et al*., 2007). The crystal proteins known as Cry proteins or δ-endotoxins, are highly toxic to a wide variety of insect pests. Several Cry proteins or δ-endotoxins have receptor proteins in the gut lining of insects, including the bagworm, *Metisa plana*, *Pteroma pendula* and *Mahasena corbetii* (Lepidoptera: Psychidae). The activated toxins bind to the gut receptors and cause osmotic lyses and death of the larvae of the bagworm (Ramlah Ali *et al*., 2009).

Broad-spectrum chemical insecticides have been widely used for controlling insect pests which affected food production and agricultural crops. The residues of these insecticides cause toxic effects on non-target organisms and produce numerous adverse effects on human beings (Meher *et al*., 2002). Furthermore, the uncontrolled usage of insecticides

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has led to the emergence of resistant pest variants. Therefore, during the last three decades, efforts are being made to develop microbial insecticides as biological control agents. Insect pathogenic bacilli such as *B. thuringiensis*, *Bacillus lentimorbus* and *Bacillus sphaericus* are among the widely used bacteria for controlling agricultural insect pests (Sutherland and Khoo, 1987).

Registration of pesticides with the National Pesticide Board must undergo laboratory testing for short-term and long-term health effects. Laboratory animals are purposely fed with high doses to cause toxic effects. These tests help scientists to evaluate the effect of these chemicals on human, domestic animals, and wildlife in cases of overexposure (NPIC, 2000).

Bt provides very low toxicity with no adverse effects to rats fed with Bt pesticide (NPIC, 2000). Measurement of acute toxicity is the lethal dose (LD_{50}) or lethal concentration (LC_{50}) which causes death in 50% of the treated animals with limited exposure. The LD_{50} is generally expressed as the dose in milligrammes (mg) of chemical per kilogramme (kg) of body weight. The LC_{50} is often expressed as mg of chemical per volume (L) of medium (air or water) exposed to the organism. Chemicals are considered highly toxic when the LD_{50} and LC_{50} are small and practically non-toxic when these values are large (*Table 1*). However, the LD_{50} and LC_{50} do not reflect any effects from long term exposure such as cancer, birth defects, or reproductive toxicity, that may occur at levels below those that cause death (NPIC, 2000). *Table 1* shows the summary of the toxicity category.

The Malaysian Palm Oil Board (MPOB) has conducted an intensive research on the development of Bt products to control bagworms. Two different formulations of products were developed and named as Bafog-1 (S) and Ecobac-1 (EC). This study reports the acute oral and acute dermal effect of MPOB Bt1 products, Bafog-1 (S) and Ecobac-1 (EC) against the *Sprague-Dawley* rats.

MATERIALS AND METHODS

Preparation of Bt Products

Bafog-1 (S) is a solution (S) concentrate and Ecobac-1 (EC) is an emulsified concentrate (EC), consisting of indigenous *Bacillus thuringiensis* (MPOB Bt1). Both products were produced at the Microbial Technology and Engineering Centre (MICROTEC), MPOB, Bangi, Selangor, Malaysia. The total amount of Bafog-1 (S) and Ecobac-1 (EC) used for the experiment was 10 ml kg^{-1} for oral administration and 5 ml kg^{-1} for dermal, respectively. Both test substances were suspended in 25% tragacanth mucilage.

The Bt products were prepared after 48 hr of fermentation using laboratory prepared medium, AgroNat-7 (patent No. PI2011000307) (Najib *et al*., 2014; Ramlah Ali *et al*., 2011). The fermentation was conducted in a bioreactor with a working volume of 300 litres and temperature of 30°C. Both Bt products were produced using the method shown in *Figure 1*.

Animals

Eight-week old *Sprague Dawley* rats obtained from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia (USM), Pulau Pinang, Malaysia, were quarantined for a week before use. Rats for oral and dermal toxicity studies were housed individually.

The rats were kept at room temperature with 12 hr light/dark cycle. Each rat had free access to water and feeding. The feed for the rats was obtained from Gold Coin Malaysia Sdn Bhd, Butterworth, Pulau Pinang, Malaysia.

Acute Oral Toxicity Study

The acute oral toxicity study was conducted using the *Organisation for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals 425* (2001).

Female rats (nulliparous and non-pregnant) were used for oral toxicity study. A female rat was fasted overnight and administered with a single oral dose of Ecobac-1 (EC) or Bafog-1 (S). The rat was observed closely for any symptom of toxic effects. The onset and duration of the toxic symptom observed were recorded. If the first rat died, a second rat would be administered with a lower dose of Ecobac-1 (EC) or Bafog-1 (S). If the first rat survived for more than 48 hr, the second female rat would be administered with a higher dose and observed as before. Similarly, if the second rat died, the third female rat would be administered with a lower dose of Ecobac-1 (EC) or Bafog-1 (S). If the second rat survived for more than 48 hr, the third female rat would be administered with a higher dose of Ecobac-1 (EC) or Bafog-1 (S). Treatment of an animal at the next higher dose was delayed until one was confident that the previously dosed animal would survive. The time interval for dosing may be adjusted. Administration of higher and lower doses of Ecobac-1 (EC) or Bafog-1 (S) to the rats was carried out until data on six rats were obtained. Observation on the rats that survived was conducted daily for 14 days before being sacrificed for necrosis.

Acute Dermal Toxicity Study

The acute dermal toxicity study was conducted using the *OECD Guideline for Testing of Chemicals 402* (1987).

The dorsal area of the trunk of two groups of five male rats and five female rats (nulliparous and non-pregnant) were shaved approximately 24 hr before the study. The test substance, either Ecobac-1 (EC) or Bafog-1 (S), was applied at the dose of 2.0 g kg^{-1} uniformly on the shaved part, covering about 10% of the body surface area. This area was then covered with semi-permeable film, such as opsite and held in contact to the skin with non-irritant tape. After 24 hr of exposure to the control or test substances, the tape and semi-permeable film were removed and the exposed skin of the treated area was cleaned with water (to remove the residual test substances). The dermal toxic symptoms of the dermally treated animals were closely monitored on the first day and daily for a period of 14 days.

In the limit test, if all the five animals of the same sex survive, the LD_{50} is considered higher than 2000 mg kg^{-1} for that particular sex. If compound-related mortality is produced, a detailed study is warranted. In the detailed study, at least five males and females are used for each dose level. At least, three dose levels are used and spaced appropriately to produce test group with a range of toxic effects and mortality rates.

Data Analysis

The LD_{50} value for oral administration of the test products was determined using the software AOT425 StatPgm program. Whereas, for dermal administration, the data obtained from the test was sufficient to produce a dose-response curve and where possible, permitted an acceptable determination of the LD_{50}. The LD_{50} value for dermal administration of the test substances, Ecobac-1 (EC) and Bafog-1 (S) was determined using probit analysis (Finney, 1952).

RESULTS AND DISCUSSION

Acute Oral

The result showed that Ecobac-1 (EC) and Bafog-1 (S) did not cause toxic symptom observed in the first female rats, when each product was administered orally with a dose of 175 mg kg^{-1} (*Tables 2* and *3*). Subsequently, the second female rats were administered each with a higher dose of 550 mg kg^{-1} of Ecobac-1 (EC) or Bafog-1 (S) for 48 hr. The second female rats also survived without any toxic symptom for 48 hr. The third female rats were administered with a higher dose of 1750 mg kg^{-1} of Ecobac-1 (EC) or Bafog-1 (S). Similarly, the third female rats also survived without showing any toxic symptom. As a consequences, 48 hr later, a higher dose of 5000 mg kg^{-1} of Ecobac-1 (EC) or Bafog-1 (S) were administered to the fourth female rats. The fourth female rats also survived without any toxic sign or symptom. Subsequently, the dose to the fifth and sixth female rats was not increased further because the toxic effect above this dose level is more towards the bulk effect of the volume administered rather than the inherent toxic effect of Ecobac-1 (EC) and Bafog-1 (S). Technically, it will be difficult to administer to a rat even by using syringe with oral needle because the solution/emulsion/suspension of the test compound will be too thick and cannot pass the oral needle. Even if it can, the volume will be very big which may fill the whole stomach with the test compound. This condition is referred to as 'bulk effect' or volume effect. It means that, the toxic effect observed in the animal is due to the stomach being too full causing gastrointestinal disturbance such as gastrointestinal block, constipation, *etc*. That is not the toxic effect of the test compound but the volume or bulk effect. According to the *Acute Oral Toxicity (OECD Guideline for Testing of Chemical 425* (2001), the maximum dose which can be given is 5000 mg kg^{-1}. Finally, the fifth and sixth female rats were given 5000 mg kg^{-1} of Ecobac-1 (EC) or Bafog-1 (S). All female rats survived during the 14 days observation without showing any toxic signs or symptoms. Previously the oral feeding of Bt *kurstaki* at various doses, 3 – 5 ml containing 2.5 x 10^7 spores ml^{-1} reported no mortality, or gross behavioral changes in rats, during the observation period at normal body weight (Meher *et al*., 2002).

All rats were subsequently sacrificed after being monitored for 14 days. There were no changes in the internal organs observed during necropsy in any of the Ecobac-1 (EC) and Bafog-1 (S) orally treated female rats. The results showed that the LD_{50} of orally administered Ecobac-1 (EC) and Bafog-1 (S) in female rats was higher than 5000 mg kg^{-1} (*Table 4*).

Acute Dermal

All the male and female rats dermally treated with 2000 mg kg^{-1} of Ecobac-1 (EC) or Bafog-1 (S) survived within the two weeks experiment (*Tables 5, 6, 7* and *8*). It was found that the LD_{50} for dermal administration for Ecobac-1 (EC) and Bafog-1 (S) was more than 2000 mg kg^{-1} in male and female rats (*Tables 9* and *10*). There was no toxic symptom observed during the two weeks observation period in male and female rats (*Tables 5, 6, 7* and *8*). Post-mortem of the dermally treated animals at the end of the experiment did not show changes or adverse effect in the rats' internal organs. The weight

TABLE 1. INFORMATION ON TOXICITY CATEGORY FROM DIFFERENT TYPES OF TOXICOLOGICAL TESTS AGAINST RATS

Type of tests	High toxicity (danger)	Moderate toxicity (warning)	Low toxicity (caution)	Very low toxicity (caution)
Oral LD_{50}	Less than 50 mg kg^{-1}	50 – 500 mg kg^{-1}	500 – 5000 mg kg^{-1}	Greater than 5000 mg kg^{-1}
Dermal LD_{50}	Less than 200 mg kg^{-1}	200 – 2000 mg kg^{-1}	2000 – 5000 mg kg^{-1}	Greater than 5000 mg kg^{-1}
Inhalation LC_{50}	Less than 0.05 mg $litre^{-1}$	0.05 – 0.5 mg $litre^{-1}$	0.5 – 2 mg $litre^{-1}$	Greater than 2 mg $litre^{-1}$
Eye effects	Corrosive	Irritation persisting for 7 days	Irritation reversible within 7 days	Minimal effects, gone within 24 hr
Skin effects	Corrosive	Severe irritation at 72 hr	Moderate irritation at 72 hr	Mild or slight irritation

Source: NPIC (2000).

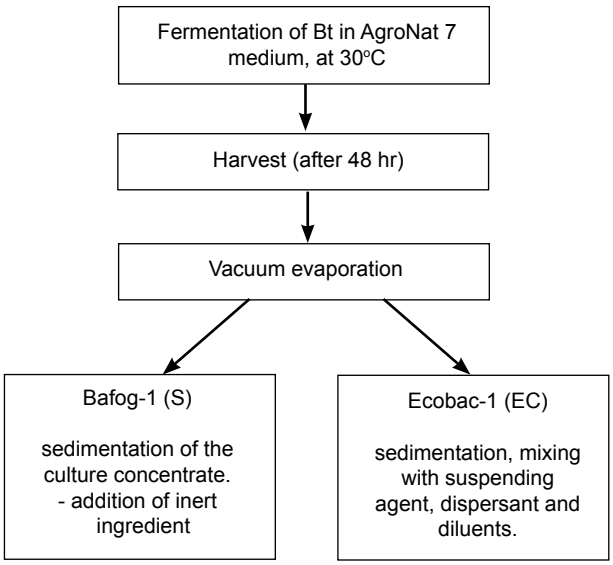

Figure 1. Schematic diagram showing the preparation of different Bt products (Najib et al.*, 2012; 2014).*

TABLE 2. THE EFFECT OF ORAL ADMINISTRATION OF DIFFERENT DOSES OF ECOBAC-1 (EC) ON BODY WEIGHT, TOXIC SYMPTOMS AND INTERNAL ORGANS OF FEMALE RATS

Rat No.	Dose (mg kg^{-1})	Weight of rat (g)				Onset of death (hr)	Toxic symptoms	Internal organs
		Week 0	Week 1	Week 2	At death			
1	175	199.2±3.0	213.3±2.0	224.2±2.0	-	-	Normal	All organs normal
2	550	185.4±2.1	205.4±1.6	220.3±4.7	-	-	Normal	All organs normal
3	1 750	183.6±4.2	195.8±3.4	206.4±3.3	-	-	Normal	All organs normal
4	5 000	183.1±1.8	195.2±2.6	215.9±5.1	-	-	Normal	All organs normal
5	5 000	194.8±1.1	215.3±2.2	230.2±3.5	-	-	Normal	All organs normal
6	5 000	191.0±2.6	206.1±3.3	221.4±3.3	-	-	Normal	All organs normal

Note: The values of mean body weight for six rats were analysed using Student's t test and found to be significantly increased for two consecutive weeks.

TABLE 3. THE EFFECT OF ORAL ADMINISTRATION OF DIFFERENT DOSES OF BAFOG-1 (S) ON BODY WEIGHT, TOXIC SYMPTOMS AND INTERNAL ORGANS OF FEMALE RATS

Rat No.	Dose (mg kg^{-1})	Weight of rat (g)				Onset of death (hr)	Toxic symptoms	Internal organs
		Week 0	Week 1	Week 2	At death			
1	175	179.1±2.2	183.0±2.7	200.6±3.2	-	-	Normal	All organs normal
2	550	182.5±2.6	205.6±1.3	213.5±4.7	-	-	Normal	All organs normal
3	1 750	181.4±1.9	185.8±4.4	191.2±2.3	-	-	Normal	All organs normal
4	5 000	175.5±2.8	178.9±2.8	193.3±4.7	-	-	Normal	All organs normal
5	5 000	182.0±3.1	190.1±3.2	192.7±2.5	-	-	Normal	All organs normal
6	5 000	183.9±2.7	192.0±2.8	200.0±3.8	-	-	Normal	All organs normal

Note: The values of mean body weight for six rats were analysed using Student's t test and found to be significantly increased for two consecutive weeks.

TABLE 4. THE EFFECT OF ORAL ADMINISTRATION OF ECOBAC-1 (EC) AND BAFOG-1 (S) ON MORTALITY OF FEMALE RATS

Test seq.	Animal ID	Dose (mg kg^{-1})	Mortality				Estimated LD_{50} (mg kg^{-1})	95% Confidence interval (mg kg^{-1})
			Short-term result (in 48 hr)		Long term result (in 14 days)			
			Ecobac-1 (EC)	Bafog-1 (S)	Ecobac-1 (EC)	Bafog-1 (S)		
1	1	175	0	0	0	0	> 5 000	-
2	2	550	0	0	0	0		-
3	3	1 750	0	0	0	0		-
4	4	5 000	0	0	0	0		-
5	5	5 000	0	0	0	0		-
6	6	5 000	0	0	0	0		-

Note: 0 - Survive. x - Death

TABLE 5. THE EFFECT OF DERMAL ADMINISTRATION OF ECOBAC-1 (EC) ON BODY WEIGHT, TOXIC SYMPTOMS AND INTERNAL ORGANS OF MALE RATS

Dose (mg kg^{-1})	Weight of rat (g)				Onset of death (hr)	Toxic symptoms	Internal organs
	Week 0	Week 1	Week 2	At death			
2 000	240.1±2.2	232.2±2.6	257.8±2.7	-	-	Normal	All organs normal
	225.8±2.6	240.3±2.5	264.7±2.2	-	-	Normal	All organs normal
	225.3±2.2	235.8±2.2	274.1±2.5	-	-	Normal	All organs normal
	240.2±2.3	255.0±2.6	270.3±3.2	-	-	Normal	All organs normal
	259.1±2.2	268.4±2.4	276.2±4.1	-	-	Normal	All organs normal
Control	262.3±2.2	274.6±2.6	286.3±2.6	-	-	Normal	All organs normal
	272.8±4.6	287.3±2.5	312.1±2.4	-	-	Normal	All organs normal
	272.3±2.4	284.1±2.5	298.4±2.6	-	-	Normal	All organs normal
	260.9±3.5	273.0±3.2	289.2±3.1	-	-	Normal	All organs normal
	259.2±2.7	270.3±2.9	285.3±2.2	-	-	Normal	All organs normal

Note: The values of mean body weight obtained before and after treatment were analysed using Student's t test and found to be significantly increased ($P<0.05$) for two consecutive weeks.

increased in the dermally treated rats was not significantly different (P>0.05), as compared to the control group every week (*Tables 5*, *6*, *7* and *8*).

There were no toxic symptoms when the male and female rats were dermally treated with 2000 mg kg^{-1} of Ecobac-1 (EC) or Bafog-1 (S). According to Meher *et al*. (2002), after dermal application of Bt *kurstaki* (B.t.k) to albino rabbits, it was reported that no mortality or deleterious effects were observed, either immediately or during 21 days observation period at dose of 1 ml containing 2.5 x 10^7 spores ml^{-1}. Therefore, the LD_{50} of dermally administered Ecobac-1 (EC) and Bafog-1 (S) was more than 2000 mg kg^{-1} in both male and female rats.

Based on the report by the Standing Committee on Biocidal Products (2010), the lethal oral dose level, LD_{50} of Bt subsp. *israelensis* in rats was determined to be greater than 5000 mg kg^{-1}. The lethal dermal

TABLE 6. THE EFFECT OF DERMAL ADMINISTRATION OF ECOBAC-1 (EC) ON BODY WEIGHT, TOXIC SYMPTOMS AND INTERNAL ORGANS OF FEMALE RATS

Dose (mg kg^{-1})	Weight of rat (g)				Onset of death (hr)	Toxic symptoms	Internal organs
	Week 0	Week 1	Week 2	At death			
2 000	210.1±2.2	222.1±2.6	232.8±2.7	-	-	Normal	All organs normal
	196.8±2.6	220.5±2.5	227.7±2.2	-	-	Normal	All organs normal
	198.3±2.2	216.8±2.2	230.1±2.5	-	-	Normal	All organs normal
	203.2±2.3	215.0±2.6	228.3±3.2	-	-	Normal	All organs normal
	196.1±2.2	205.4±2.4	218.2±4.1	-	-	Normal	All organs normal
Control	225.3±2.2	230.6±2.6	243.3±2.6	-	-	Normal	All organs normal
	214.8±4.6	229.3±2.5	236.1±2.4	-	-	Normal	All organs normal
	210.3±2.4	218.1±2.5	230.4±2.6	-	-	Normal	All organs normal
	214.9±3.5	226.0±3.2	240.2±3.1	-	-	Normal	All organs normal
	223.2±2.7	230.3±2.9	240.3±2.2	-	-	Normal	All organs normal

Note: The values of mean body weight obtained before and after treatment were analysed using Student's t test and found to be significantly increased (P<0.05) for two consecutive weeks.

TABLE 7. THE EFFECT OF DERMAL ADMINISTRATION OF BAFOG-1 (S) ON BODY WEIGHT, TOXIC SYMPTOMS AND INTERNAL ORGANS OF MALE RATS

Dose (mg kg^{-1})	Weight of rat (g)				Onset of death (hr)	Toxic symptoms	Internal organs
	Week 0	Week 1	Week 2	At death			
2 000	401.9±2.1	389.2±2.6	401.6±2.9	-	-	Normal	All organs normal
	418.7±2.8	403.0±2.4	407.1±2.6	-	-	Normal	All organs normal
	388.1±2.6	371.4±2.2	384.8±2.5	-	-	Normal	All organs normal
	383.2±2.3	401.2±2.4	406.0±3.3	-	-	Normal	All organs normal
	391.4±2.3	387.6±2.4	365.2±4.5	-	-	Normal	All organs normal
Control	250.9±3.0	278.9±2.6	293.1±2.6	-	-	Normal	All organs normal
	216.6±4.6	242.5±2.3	274.6±2.5	-	-	Normal	All organs normal
	240.3±2.8	276.3±2.6	284.7±2.6	-	-	Normal	All organs normal
	234.7±3.1	251.0±3.2	261.8±3.1	-	-	Normal	All organs normal
	262.8±2.6	276.1±2.1	296.1±2.1	-	-	Normal	All organs normal

Note: The values of mean body weight obtained before and after treatment were analysed using Student's t test and found to be significantly increased (P<0.05) for control group and not significantly increased (P<0.05) for Bafog-1 (S) treatment within two consecutive weeks.

TABLE 8. THE EFFECT OF DERMAL ADMINISTRATION OF BAFOG-1 (S) ON BODY WEIGHT, TOXIC SYMPTOMS AND INTERNAL ORGANS OF FEMALE RATS

Dose (mg kg^{-1})	Weight of rat (g)				Onset of death (hr)	Toxic symptoms	Internal organs
	Week 0	Week 1	Week 2	At death			
2 000	169.5±2.0	180.5±2.7	204.4±3.5	-	-	Normal	All organs normal
	194.0±2.7	200.2±2.1	216.0±2.8	-	-	Normal	All organs normal
	181.6±2.5	188.9±2.5	207.6±2.8	-	-	Normal	All organs normal
	181.5±2.8	179.6±2.8	195.0±3.5	-	-	Normal	All organs normal
	195.6±2.8	200.6±2.3	213.3±4.7	-	-	Normal	All organs normal
Control	238.2±3.5	236.0±2.5	248.7±2.9	-	-	Normal	All organs normal
	265.1±4.5	258.6±2.7	263.2±2.7	-	-	Normal	All organs normal
	274.5±2.7	272.6±2.9	275.2±2.9	-	-	Normal	All organs normal
	242.0±3.6	248.1±3.7	249.3±3.0	-	-	Normal	All organs normal
	261.8±2.7	248.9±2.7	233.5±2.5	-	-	Normal	All organs normal

Note: The values of mean body weight obtained before and after treatment were analysed using Student's t test and found to be not significantly increased ($P<0.05$) for control group and significantly increased ($P<0.05$) for Bafog-1 (S) treatment within two consecutive weeks.

TABLE 9. THE EFFECT OF DERMAL ADMINISTRATION OF ECOBAC-1 (EC) ON THE MORTALITY OF MALE AND FEMALE RATS

Sex	Dose (mg kg^{-1})	Mortality			LD_{50} (mg kg^{-1})	95% Confidence interval (mg kg^{-1})
		Deaths/No.	Onset of death (hr)			
			Mean	Std error		
Male	2 000	0/5	-	-	>2 000	-
	Control	0/5	-	-		
Female	2 000	0/5	-	-	>2 000	-
	Control	0/5	-	-		

TABLE 10. THE EFFECT OF DERMAL ADMINISTRATION OF BAFOG-1 (S) ON THE MORTALITY OF MALE AND FEMALE RATS

Sex	Dose (mg kg^{-1})	Mortality			LD_{50} (mg kg^{-1})	95% Confidence interval (mg kg^{-1})
		Deaths/No.	Onset of death (hr)			
			Mean	Std error		
Male	2 000	0/5	-	-	>2 000	-
	Control	0/5	-	-		
Female	2 000	0/5	-	-	>2 000	-
	Control	0/5	-	-		

dose level, LD_{50} of Bt subsp. *israelensis* in rabbits was found to be greater than 5000 mg kg^{-1}. The test Bt product, VectoBac produced no deaths, no clinical signs other than procedurally induced changes in behaviour on the day of exposure. Bodyweights were unaffected by treatment.

CONCLUSION

It is concluded that Bafog-1 (S) and Ecobac-1 (EC) are not toxic to the *Sprague-Dawley* rats. The oral LD_{50} of Ecobac-1 (EC) and Bafog-1 (S) on female rats was more than 5000 mg kg^{-1}. Whereas, the LD_{50} for dermal administration of both Bt products was more than 2000 mg kg^{-1} for both male and female rats. Therefore, Bt as an environmental-friendly microbial insecticide is not toxic to humans, domestic animals and vertebrates in general.

ACKNOWLEDGEMENT

The authors would like to thank to the Director-General and Director of Biological Research of MPOB for permission to publish this article. The authors would like to thank members of the School of Pharmaceutical, USM for conducting the experiment. Last but not least to the members of Microbial Bioprospecting and Bioprocessing Group who were involved in assisting in the production of the Bt products.

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