SAFETY ASSESSMENT OF *Bacillus thuringiensis*-BASED PRODUCTS USING EYE/OCULAR IRRITATION ON RABBITS

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ABSTRACT

To register with the National Pesticide Board, Malaysia, a pesticide must undergo laboratory testing for short-term and long-term health effects. Laboratory animals such as rabbits are tested with high doses of Bacillus thuringiensis *(Bt) products to assess the potential to produce lesions or reversal effects on eyes. The objective of this study was to investigate the effect of Bafog-1 (S) and Ecobac-1 (EC) against young New Zealand albino rabbit's eye or the potential of Bt products to cause eye or ocular irritation. The test was conducted according to the Guidelines of International Organisation for Standardisation 10993-10, Test for Irritation and Sensitisation. The results showed that Bafog-1 (S) is not an irritant to rabbits, whereas, Ecobac-1 (EC) produced irritation that was reversal after 120 hr of instillation. The Bt as an environmental-friendly microbial insecticide was generally non-toxic to human, domestic animals and vertebrates. Therefore, Bt is recommended to be used as a safe microbial insecticide for controlling bagworm in oil palm plantation.*

Keywords: *Bacillus thuringiensis*, Bafog-1 (S), Ecobac-1 (EC), eye irritation, pesticides, bagworm, oil palm.

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INTRODUCTION

Bacillus thuringiensis (Bt) is known as a spore-forming bacterial insect pathogen and it has been extensively studied in various laboratories worldwide for its potential as an effective biological control agent against almost all insect pests in the world, including the bagworm (Lepidoptera: Psychidae) (Ramlah Ali and Mohd Basri, 1997; Ramlah Ali, 2000; Ramlah Ali and Mahadi, 2001). The Bt as a gram-positive bacteria will produce crystalliferous proteins during sporulation which are toxic to specific insect pests (Roh *et al*., 2007; Xavier *et al*., 2007). These activated toxins, known as delta-endotoxins, bind to the gut receptors and cause osmotic lyses and death of the bagworm (Ramlah Ali *et al*., 2009). Nowadays, the broad-spectrum insecticides are widely used for controlling insect pests which affect food production and agricultural crops. The residues of these insecticides cause toxic effects on non-target organisms (Meher *et al*., 2002) and the uncontrolled usage of insecticides has led to the emergence of resistant pest variants. Eventually, in the last three decades, efforts are being made to develop microbial insecticides from insect pathogenic bacilli such as *B. thuringiensis*, *B. lentimorbus* and *B. sphaericus*

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as biological control agents. They are among the widely known and employed bacteria for controlling agricultural insect pests (Sutherland and Khoo, 1987).

Basically, the general requirement for registration of pesticides with the National Pesticide Board is to conduct laboratory testing for short-term and long-term health effects. For example, laboratory animals are fed with high doses of tested chemicals to assess the toxic effects and the results will help scientists to evaluate and identify the effect of these chemicals on humans, domestic animals and wildlife (National Pesticide Information Centre, 2000).

Toxic effects involving eyes or vision are very important side-effects of chemical or biological substances used in the industry, especially cosmetics. It has frequently evaluated the result of eye irritation test by using rabbits as well as the nature of the most crucial effects that can lead to serious eye damage (Ingrid *et al*., 2005). Eye irritation is defined as the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application (http://alttox.org). The Bt is found not to be an eye irritant on rabbits (Donghai *et al*., 2008). There is very slight irritation from inhalation in test animals which may be caused by the physical rather than the biological properties of the Bt formulation tested. The Bt has not been shown to have any chronic toxicity or any carcinogenic effects. There are also no indications that Bt causes reproductive effects or birth defects in mammals (Glare and O'Callaghan, 2000; Carrie, 1994).

The Malaysian Palm Oil Board (MPOB) has developed Bt products through an intensive research for controling bagworms (Najib *et al*., 2008; 2013; 2014; 2015; Mazmira *et al*., 2011). Two different formulations of products were developed, namely Bafog-1 (S) and Ecobac-1 (EC). This study reports the effect of MPOB Bt1 products, Bafog-1 (S) and Ecobac-1 (EC) against the young New Zealand albino rabbits eye/ocular irritation.

MATERIALS AND METHODS

Preparation of Bt Products

Bafog-1 (S) is a solution (S) and Ecobac-1 (EC) is an emulsified concentrate (EC), consisting of indigenous Bt (MPOB Bt1). Both products were produced at the Microbial Technology and Engineering Centre (MICROTEC), MPOB, Bangi, Selangor, Malaysia. The Bt products were prepared after 48 hr of fermentation using the laboratory prepared medium, AgroNat-7 (Patent No. PI2011000307) (Najib *et al*., 2014; 2015; 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 a vacuum evaporator and sedimentation technique as reported by Najib *et al*. (2012; 2014; 2015).

Animals

Six eight-week old young New Zealand albino rabbits obtained from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia (USM), Pulau Pinang, Malaysia, were acclimatised for five days before use. The weight of the rabbits used was 2.0 to 3.0 kg. The number of rabbits used per Bt product was 3 (n=3) or three rabbits per concentration and with a single concentration.

Treatments

Approximately 0.1 ml of the undiluted Bt products was instilled using a sterilised syringe into the lower conjunctival sac of the right eye with the left eye (untreated) serving as control (Donghai *et al*., 2008). The eyelid was held together for approximately 1 s after instillation of the Bt products. The original concentrations of Bafog-1 (S) solution and Ecobac-1 (EC) used were 7.4×10^{11} cfu ml^{-1} and 4.5×10^{11} cfu ml^{-1}, respectively.

Observation

Both eyes of the rabbits were examined at 1, 24, 48 and 72 hr after receiving a single instillation of Bt products. Extended observation was carried out if there was persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions or their reversal.

RESULTS AND DISCUSSION

Effect of Bafog-1 (S) on Ocular/eye Irritation of Rabbits

The result showed that Bafog-1 (S) was proven as non-irritant to the rabbit's eyes at the highest concentration of 7.4×10^{11} cfu ml^{-1} (*Table 1*). Bafog-1 (S) led to conjunctival redness at 1 and 24 hr after a single instillation of one eye for all tested rabbits. Whereas, for chemosis, the Bt product only caused swelling above normal condition at 1 hr after instillation for all tested rabbits.

Effect of Ecobac-1 (EC) on Ocular/eye Irritation of Rabbits

The results on eye irritation experiment indicated that Ecobac-1 (EC) produced irritant reversal after 120 hr of instillation at the highest concentration of 4.5×10^{11} cfu ml^{-1} (*Table 2*). The Ecobac-1 (EC)

TABLE 1. THE EFFECT OF BAFOG-1 (S) ON EYE/OCULAR IRRITATION OF RABBITS

Evaluation	Rabbit's No.	1		2		3	
	Sex	Female		Male		Male	
	Treatment (hr)	Test	Control	Test	Control	Test	Control
Cornea	1	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
Iris	1	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
Conjunctival redness	1	1	0	1	0	1	0
	24	1	0	1	0	1	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
Chemosis	1	1	0	1	0	1	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0

Note: Evaluation according to the number in *Table 1*.

Cornea:	**Iris:**	**Redness:**	**Chemosis:**
Normal - 0	Normal - 0	Normal - 0	Normal - 0
Diffused capacity - 1	Congestion - 1	Some vessel injected - 1	Any swelling above normal - 1
Iris slightly obscured - 2	Haemorrhages - 2	Crimson red - 2	Partial lid inversion - 2
Nacreous - 3	Cut off point = 0-2	Beefy red - 3	Lids half closed - 3
Iris not discernible - 4		Cut off point = 0-3	Lids more than half closed - 4
Cut off point = 0-4			Cut off point = 0-4

led to conjunctival redness at 1 hr until 96 hr of single instillation in all tested rabbits. Furthermore, chemosis in the Ecobac-1 (EC) resulted in half close of lids for all tested rabbits at 1 hr post-instillation. At 24 hr post-instillation, rabbit No. 2 led to partial lid inversion until 48 hr of the single instillation. However, at 72 hr after the single instillation, the eyes of all tested rabbits had slowly recovered from partial lid inversion to swelling above normal condition until 96 hr of the single instillation. Finally, after 120 hr of the single instillation, the eyes became normal or the Ecobac-1 (EC) produced irritant reversal after 120 hr of the single instillation.

Ecobac-1 (EC) is an emulsified concentrate produced and mixed with an inert ingredient during formulation. Paraffin liquid is included as one of the component in the formulation. According to the Material Safety Data Sheet (2004) and the National Pesticide Information Centre (2000), in potential health effects, eye irritation may occur with exposure to concentrated vapours or contact with the paraffin liquid. Based on this fact, Ecobac-1 (EC) contained paraffin liquid that may cause eye irritation to young New Zealand albino rabbits and the Bt was found not to be an eye irritant on test rabbits (Donghai *et al*., 2008). In other words, there was a very slight irritation from inhalation in test animals which may be caused by the additional of an inert ingredient during formulation rather than the biological properties of the Bt formulation tested (Glare and O'Callaghan, 2000; Carrie, 1994).

CONCLUSION

It is concluded that Bafog-1 (S) produced no eye irritation to rabbits and Ecobac-1 (EC) produced irritant reversal after 120 hr of instillation. Ecobac-1 (EC) with emulsifiable formulation requires safety precaution during handling and mixing. However, Bt as an environmental-friendly microbial insecticide is non-toxic to humans, domestic animals and aquatic fish.

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TABLE 2. THE EFFECT OF ECOBAC-1 (EC) ON EYE/OCULAR IRRITATION OF RABBITS

Evaluation	Rabbit's No.	1		2		3	
	Sex	Female		Male		Male	
	Treatment (hr)	Test	Control	Test	Control	Test	Control
Cornea	1	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
	96	0	0	0	0	0	0
	120	0	0	0	0	0	0
	144	0	0	0	0	0	0
Iris	1	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
	96	0	0	0	0	0	0
	120	0	0	0	0	0	0
	144	0	0	0	0	0	0
Conjunctival redness	1	1	0	1	0	1	0
	24	1	0	1	0	1	0
	48	1	0	1	0	1	0
	72	1	0	1	0	1	0
	96	1	0	1	0	1	0
	120	0	0	0	0	0	0
	144	0	0	0	0	0	0
Chemosis	1	3	0	3	0	3	0
	24	3	0	2	0	3	0
	48	1	0	2	0	2	0
	72	1	0	1	0	1	0
	96	1	0	1	0	1	0
	120	0	0	0	0	0	0
	144	0	0	0	0	0	0

Note: Evaluation according to the number in *Table 2*.

Cornea:	**Iris:**	**Redness:**	**Chemosis:**
Normal - 0	Normal - 0	Normal - 0	Normal - 0
Diffused capacity - 1	Congestion - 1	Some vessel injected - 1	Any swelling above normal - 1
Iris slightly obscured - 2	Haemorrhages - 2	Crimson red - 2	Partial lid inversion - 2
Nacreous - 3	Cut off point = 0-2	Beefy red - 3	Lids half closed - 3
Iris not discernible - 4		Cut off point = 0-3	Lids more than half closed - 4
Cut off point = 0-4			Cut off point = 0-4

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