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The Use of *Bacillus thuringiensis* var. *kurstaki* in Protecting Stored Bee Wax Combs and Wax Foundations against the Greater Wax Moth Larvae, *Galleria mellonella* L.

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ABSTRACT

Toxicological study using the commercial formulation Dipel-2X based on the entomopathogenic bacterium, *Bacillus thuringiensis* var. *Kurstaki* in the concentrations of 2, 4, 6, and 8 g/ 100g diet were carried out versus larvae (L_3) of the greater wax moth, *Galleria mellonella* L.. The calculated LC_{50} reached 4.784g/100g diet. The LC_{100} (9.568g/100g diet /or water) was used to treat the wax combs and foundation wax sheets by the spraying technique that followed by artificial infestation with eggs of the greater wax moth (ca 200 egg/comb or foundation sheet) and stored in boxes at 26-27 °C and 60-70% R.H. Weight of stored Dipel-2X-treated and -untreated bee wax material was estimated each 2 months for one year. Infestation was completely reduced (zero%) in *B.t.*var. *kurstaki* treated wax combs and foundation sheets, while untreated combs (control) lost 26, 60,90,98, and 100% of their wax content after 2, 4, 6, 8 and 10 months, respectively. Also, respective losses of 28, 66, 91, 98 and 100% were recorded for untreated wax foundation sheets at the same intervals.

Key words: *Galleria mellonella* , *Bacillus thuringiensis* var. *kurstaki*, toxicity, bee wax, microbial control.

INTRODUCTION

The greater wax moth, *Galleria mellonella* L. and the lesser wax moth, *Achroia grisella* (F.) are economic pests attacking bee wax. The former pest attacks wax combs whether in bee hives or in store, while the latter pest attacks it only in the store. Both pests cause economic losses for bee keeping industry (Ibrahim, 1980). Within 10-15 days, *G. mellonella* larvae could completely damage the combs in bee hives leaving them as frames covered with heavy layer of silk threads and faeces (Beck, 1960, and Abou Bakr and El-Shemy, 1991). Williams (1976) estimated losses caused by *G.mellonella* in the USA by 3 million dollars in 1973 and 4 million dollars in 1974, and Zhou *et al.* (1989) reported serious damage by this pest in Chinese honey bee (*Apis cerana*) hives, reducing honey yields by over 20%.

Control of the wax moth, *G. mellonella* depended on fumigation with chemical insecticides, *e.g.*, naphthalene, calcium cyanide, carbon dioxide, carbon tetrachloride, paradichlorobenzene, and ethylene dibromide (Grout, 1949; Cantwell *et al.*, 1972; and Morse, 1980). As hazards of chemical insecticides to human and environment became clear, the search for safe alternatives received great interest. The early known parasitoid natural enemies of *G. mellonella* were studied and experimented for controlling this pest, *e.g.*, *Bracon hebitor* (Say) (Awadallah *et al.*, 1985; and Tawfik *et al.*, 1985); and *Apanteles galleriae* Wilk. (El-Hemaesy, 1983; Al-Arnaooty, 1985, and Tawfik *et al.*, 1985).

The remarkable success of using the environmentally safe entomopathogenic spore forming bacterium *Bacillus thuringiensiss* Beliner in controlling certain lepidopteran pests drew the attention to use it against larvae of *G. mellonella* as specific lepidopteran bioinsecticide proved safe to bees, natural enemies, and mammals (Lautenschlager and Podwaite, 1980; Burges, 1980; El-Husseini, 1981; and Abou Bakr and EL-Shemy, 1991).

The present work aims to investigate the toxicity of *B. thuringiensis* (Dipel-2X) versus larvae (L_3) of *G. mellonella* to estimate the required concentration (LC_{100}) for protecting the stored wax combs and foundation sheets from infestation with this pest.

MATERIALS AND METHODS

Rearing the Greater Wax Moth, *G. mellonella*:

Larvae were reared on a semi-synthetic diet composed of 90 g wheat flour, 20 g corn flour, 10g milk powder, 10g dry yeast, 20 ml bee honey and 20 ml glycerin as described by Ibrahim *et al.* (1984). First, all dry components were mixed. Then, honey and glycerin were mixed and added gradually to the dry material and mixed well in the form of a delicate paste. The diet could be freezed till needed. A diet layer of 5-7 cm thick is placed in a 2-L glass container, on which the eggs of *G. mellonella* were placed. The containers were covered with plain paper fitted in place with 2 rubber bands. Rearing containers were incubated at 28-30°C associated with 60-70% relative humidity. More diet was added to the developing larvae as needed till pupation took place. Emerged adults were collected and kept in similar empty glass containers (egg laying cages) provided with a paper cone having lids to the outside of the glass container, and covered also with plain paper. Eggs were laid at base of the paper cone around the lid of the egg-laying container. Paper cones carrying the eggs were removed for egg collection, and replaced by new ones.

The Tested *Bacillus thruingiensis* :

The commercial formulation Dipel-2X (wattable powder) based on *B. thuringiensis* var. *kurstaki* (Abbott Laboratories. Illinois, Chicago, USA) was used in 5 concentrations (2, 4, 6, 8 and 10%) to treat the larvae of *G. mellonella* (L_3) by mixing into the experimental diet (w/w), from which the bee honey was excluded because of its known anti-bacterial effect. Daily mortality rates were recorded and the LC_{50} was calculated.

Control of *G. mellonella* in Stored Combs and Wax Foundation:

Ten extracted bee combs and 10 sheets of wax foundation were sprayed with Dipel-2X at the LC_{100} concentration (9.568 g/L) using 0.5L hand atomizer. Another 10 combs and 10 sheets of wax foundation were sprayed only with water and served as untreated control. Equal amount of *G. mellonella* eggs (ca 200 eggs) was placed on each comb or wax foundation placed horizontally on each other until hatching. After five days they were stored vertically in a wooden box at room temperature (25-27°C and 60-70% RH) for one year. Weight of each comb and wax foundation was recorded every 2 months, and rates of loss (%) were calculated.

RESULTS AND DISCUSSIONS

Toxicity of *B.t.* var. *kurstaki* to *G. mellonella:*

The daily-recorded mortality rates are presented in Table (1). Data showed that the concentration 2% caused a mortality value of 10% on the 7th day post treatment. Meanwhile, values of 40, 60, and 80% mortality were recorded on the 7th day for the concentrations 4, 6 and 8% compared to zero % among larvae of the control.

Table (1): Mortality (%) among larvae (L_3) of *G. mellonella* fed on diet treated with different concentrations (w/w) of Dipel-2X (*B.t.* var. *kurstaki*).

Concent-rations	Days after treatment 1	2	3	4	5	6	7
2%	0	0	2	3	7	8	10
4 %	0	3	12	20	28	36	40
6 %	4	10	21	37	42	51	60
8 %	8	19	30	39	52	76	80
Control	0	0	0	0	0	0	0

The LdP-Line Software (Licensed by Ehab Bakr) was used in processing the mortality values reached on the 7th day post treatment. Transformed values are shown in Table (2), Toxicity line of the transformed data is calculating the LC_{50} as 4.784 g of Dipel-2X100 g diet. Meanwhile, the lower and upper limits for the LC_{50} were 4.359 and 5.25 g Dipel-2X /100g diet. The toxicity line is associated with a slope of 3.434 ± 0.346 showing high reaction by increasing the concentration, *i.e.* high susceptibility to the tested bacteria.

The present toxicity results of Dipel-2X (*B.t. kurstaki*) against larvae (L_3) of *G. mellonella* agree with those reported by Szczepanik (1993). Using other *B.t.* varieties gave different LC_{50} values and caused high mortality rates when used at high concentrations, *e.g.*, *B.t.* var. *thuringiensis* of the commercial formulations BTB, Thuricide, Biospore and Biotrol or *B.t.* var. *aizawai* of the product Certan. However, all the six larval instars were found susceptible to the *B.t.* spore-endotoxin-complex (Herfs, 1964; Ali *et al.* 1973; Goodwin, 1985; Arraras *et al.*, 1986 and Mahmoud *et al.*, 1988). The varieties *galleriae* and *wuhanensis* were bioassayed by Li *et al.* (1987) versus larvae of *G. mellonella* on treated diet. In one of their experiments, they tested the effect of the endotoxin crystals and the spores of *B.t.* var. *aizawai*, and found that each alone is not toxic to larvae of *G. mellonella*; but the addition of few spores to the crystals induced high larval mortality. Connecting this result of Li *et al.* (1987) with the three categories of insects proposed by Krieg (1961) concerning gut microflora and susceptibility to *B.t.* spore-endotoxin-complex; larvae of the greater wax moth, *G. mellonella* would be placed in the category of high susceptible insects with no aggressive gut microflora, thus it needs both; the crystals (as protoxin protein) to prepare a pathway in midgut epithel and the *B.t.* spores to penetrate to the haemocoel for germination and vegetative reproduction causing death of the host larvae (septicemia).

Table (2): Transformed mortality values by the LdP-Line software computer program.

Rate/gm/ 100g diet	Observed responded %	Linear responded%	Log (Rate/conc)	Linear Probit
2	10	9.67871	0.301	3.699
4	40	39.4703	0.602	4.733
6	60	63.2018	0.778	5.337
8	80	77.8287	0.903	5.766

The reason that *G. mellonella* larvae have no aggressive gut microffora could be due to its monophagous feeding on wax combs, where the bee honey and propolese with their well known antimicrobial effect are important components of the larval natural diet. On the other hand, Li *et al.* (1987) demonstrated high mortality in larvae of the cabbage butterfly, *Pieris brassicae* using only the crystals of *B.t.*; thus larvae of this insect belong to the category of susceptible insects with aggressive gut microflora that invade the haemocoel, replicate, and cause death of the host.

Control of *G. mellonella* in Stored Combs and Wax Foundation:

The *B.t.*-treated combs showed no infestation with *G. mellonella* although they have been artificially heavily infested with the wax moth eggs after treatment. Meanwhile, the untreated combs showed increased losses in weight due to feeding of the wax moth larvae generation after generation in the store until the wax combs were completely lost leaving only the wooden frames with the fixing wire. After 2 months, the infested combs and wax foundation sheets lost between 25-30% of their wax, that increased to 80% after another 4 months and a complete loss (100%) was recorded after 8 months post infestation as seen in Table (3).

Table (3): Loss % of wax foundations (F) and extracted bee combs (C) each infested with 200 eggs of *G. mellonella* in relation to *B.t.* treatment.

Treated Material		Months after treatment					
		2	4	6	8	10	12
Treated	C	0	0	0	0	0	0
	F	0	0	0	0	0	0
Untreated	C	26	60	90	98	100%	
	F	28	66	91	98	100%	

The protection of wax combs using *B.t.* against *G. mellonella* for more than one year was reported by Burges (1977 and 1978), McKillup and Brown (1991), and Casanova-Ostos (1992). Calvert (1982) added that the *B.t.* treatment is safer than chemical fumigants and provides longer and continuous protection to the stored combs. Meanwhile, Goodwin (1985) used *B.t.* var. *aizawai* that protected the combs for 16 months as well as the wax foundation by dipping in suspension of *B.t.*, while Verma (1995) protected them for 13 months (100 g Dipel /L). Tutkum *et al.* (1987) mentioned that the damage in wax combs was reduced by 65.6, 50.8, 43.5 and 41.8% after treatment with Tarmih (*B.t. thuringiensis*), Certan (*B.t. aizawai*), Dipel and Thuricide-Hp(*B.t. kurstaki*), respectively On the other hand, treatment with *B.t.* in bee hives protected the wax combs for a shorter period of only 52 days (Moran-Rodriguez and Sandoval, 1991). More economical method for prolongation of the protection period inside bee hives up to 13 months was reported by Verma (1995) through dipping the wax foundation in a suspension of Dipel (100 g /L). In his article "Reduced Chemical Beekeeping", Wenning (2002) discussed a control strategy for controlling the wax moths including the use of *Trichogramma,* and *B. thuringiensis*, as well as open and cold storage systems.

Thus, according to results of the present study, spraying wax foundation and extracted bee wax combs with a suspension of Dipel-2X containing 100 g /L could be recommended for a safe and long protection period (more than one year) against infestation with *G. mellonella* in the store.

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