Effect of *Bacillus thuringiensis* var. *kurstaki* on Different Biological Parameters of the Greater Wax Moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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ABSTRACT

Comparing with the untreated (control) larvae, biological parameters in larvae fed on diet treated with Dipel 2X (LC$_{50}$) show a decreased larval weight from 1.34 to 0.46 g; consumed food from 10.95 to 3.15 g; produced faeces from 9.56 to 3.1g/20 larvae; weight of silk cocoon from 0.19 to 0.08 g/15 cocoon; pupal weight from 0.08 to 0.04 g/pupa; larval duration in survivals prolonged from 11.56 to 14.2 days/larvae; pupation % from 100 to 41.6%; adult emergence from 10 to 33.3%; adult deformations increased from zero to 30.3%, while the pupal period remained unchanged (7.5 days).

INTRODUCTION

The greater wax moth, *Galleria mellonella* L. is an economic pest attacking bee wax in bee hives or in store causing economic losses for bee keeping industry (Ibrahim, 1980). The remarkable success of using the environmentally safe entomopathogenic spore forming bacterium, *Bacillus thuringiensis* Beliner in controlling various lepidopteran pests as specific lepidopteran bioinsecticide proved safe to bees, natural enemies, and mammals (Lautenschlager and Podwaite, 1980; Burges, 1980; El-Husseini, 1981), drew the attention to use it against larvae of *G. mellonella* (Abou Bakr and EL-Shemy, 1991). Bioassay studies proved that all larval instars of this pest are highly susceptible to *B.thuringiensis* (Herfs, 1964; Ali *et al.* 1973; Goodwin, 1985; Arraras *et al.*, 1986 and Mahmoud *et al.*, 1988 and Szczepanik, 1993). Effect of this bacterium on the different biological parameters in *G. mellonella* was not studied; only Mahmoud *et al.* (1988) investigated larval mortality, pupal weight and pupal period. Thus, the present study deals with bioassay of Dipel 2X (*B. thuringiensis* var. *kurstaki*) versus larvae (L$_3$) and the effect of applied LC$_{50}$ on some biological parameters of *G. mellonella, i.e.*, weight of larvae, ingested food, faeces, cocoons, pupae, as well as duration of larval stage, pupation %, pupal period, adult emergence % and deformations in emerged adults.

MATERIALS AND METHODS

Larvae were reared on a semi-synthetic diet composed of 90 g wheat flour, 20 g corn flour, 10 g milk powder, 10 g dry yeast, 20 ml bee honey and 20 ml glycerin as described by Ibrahim *et al.* (1984). The commercial formulation Dipel 2X-wettable powder based on *B. thuringiensis* var. *kurstaki* (Abbott Laboratories. Illinois, Chicago, USA) was used to treat the larvae of *G. mellonella* (L$_3$) by mixing into the experimental diet, from which the bee honey was excluded because of its known anti-bacterial effect.

Bioassay test was carried out to estimate the LC$_{50}$. A broad spatula was used to mix the *B.t.* (Dipel 2X) with the diet on a glass plate before being offered to the larvae. Diet treated with different concentrations of Dipel 2 X (2, 4, 6, 8, and 10% w/w) was prepared.100 larvae (L$_3$ in 4 replicates each of 25 larvae) were allowed to feed on each of the treated diet for 24 hours, then transferred onto untreated diet free from the honey component, beside 100 larvae fed on untreated diet and served as control. Mortality was recorded daily for one week post treatment and the "LdP-Line Software computer program-Ehab Bakr" was used for calculating the LC$_{50}$ and LT$_{50}$. The tests were carried out under 25°C and 60-70% R.H.

The LC$_{50}$ was used in treating large numbers of *G. mellonella* (L$_3$). The larvae were left on the treated diet for 24 hours, thereafter they were fed on untreated diet for the next 6 days. 1100 larvae divided into 11 replicates each of 100 ones were used for the treatment with LC$_{50}$, to study the 11 selected biological parameters and a similar number for the control. The weight of larvae, ingested food, produced faeces, pupae, the silk cocoons, as well as duration of larval stage, pupal period, rates of pupation, adult emergence, and teratogenic effects or deformations in adult stage, were calculated and compared with those of the control larvae fed on the same diet (without honey) and kept under the same thermal and humid conditions (28-30°C and 60-70 R.H.).

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 7*th* day post treatment. Meanwhile, values of 40, 60, and 80% mortality were recorded on the 7*th* day for the concentrations 4, 6 and 8% compared to zero % among larvae of the control. Transformed values are shown in Table (1), and the toxicity line of the above transformed data is showed the LC$_{50}$ as 4.784 g of Dipel 2X /100 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 was associated with a slope of 3.434±0.346 showing high reaction by increasing the concentration, *i.e.* high susceptibility to the tested bacterium; taking into consideration that mortality % among larvae of the control remained at zero along the test period.

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

Table (1): Mortality % among larvae (L_3) of *G. mellonella* on the 7th day post treatment and transformed values by the LdP-Line software computer program.

Concentration /g/ 100g diet	% Mortality	Linear responded %	Log (Dose/conc.)	Linear Probit	LC_{50} /g/ 100g diet	Slope
2	10	9.67871	0.301	3.699	4.484	3.434
4	40	39.4703	0.602	4.733	(4.329-4.359)	±0.346
6	60	63.2018	0.778	5.337		
8	80	77.8287	0.903	5.766		

gave different LC_{50} values and caused high mortality 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 allowing the *B.t.* spores to penetrate to the haemocoel for germination and vegetative reproduction causing death of the host larvae (septicemia).

The reason that *G. mellonella* larvae have no aggressive gut microflora could be due to its monophagous feeding on wax combs, where the bee honey with its well known antimicrobial effect is an important component of the larval 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.

Effect on Biological Parameters
1. Larval Weight

Data presented in Table (2) showed a gradual decrease in weight of treated larvae(L_3) from 1.34±0.35 at the time of treatment (zero time) to 0.90, 0.78, 0.68, 0.46 and 0.46 g /20 larvae on the 2nd to 6th days post treatment, respectively. Meanwhile, larvae fed on untreated diet increased in weight from 1.34±0.35 to 1.63±0.25 g/20 larvae during the same period.

This biological parameter was not studied before in *G. mellonella*, but rarely in some other lepidopteran insects. Larvae of *Achaea janata* lost weight when ingested *B.t.* treated die (Srivastava, 1991), as well as larvae of *S.*

obliqua (Biswas et al., 1996). Also, feeding on transgenic plants expressing the insecticidal *B.t.* protein CryIA(c) from the variety *kurstaki* caused the same phenomenon as reported by Halcomb et al. (1996) in larvae of *H. virescens* fed on transgenic cotton (*G. hirsutum*), and by Adamczyk et al. (1998) for *S. frugiperda* fed on leaves of the transgenic cotton variety NuCotn 33B.

2. Consumed Food

As shown in Table (3), weight of ingested food treated with *B.t.* was 0.36±0.22 g/20 larvae one day post treatment, opposed to 2.23±0.3 g taken up by larvae of the control. The consumed food remained very low at the same value (0.36±0.26) by treated larvae and continued high in the control (2.43±0.63) on the second day of the test. Food consumption began to decrease in the control larvae as entering moulting changes and recorded values of 1.70±0.36, 1.73±0.4, and 1.43±0.30 g/20 larvae for the next successive 4 days. Meanwhile, food consumption by treated larvae (survived larvae) remained between 0.28±0.08 and 0.70±0.24 g/20 larvae in the same period (Table 3).

Food consumption in relation to treatment with *B.t.* was not studied before in *G. mellonella*. But the present results are in agreement with those obtained for other insect species. Decreased food consumption due to *B.t.* treatment was recorded in larvae of *Trichoplusia ni* (Gharib and Wyman, 1991), *Heliothis viressens* (Navon et al., 1992), *Limantia dispar* (Farrar and Ridgway, 1995),*Heliothis zea* (Jyoti et al., 1996), *Heliothis armigera* (Gupta et al., 1998), as well as in *Plodia interpunctella* and *Tribolium castaneum* (Abdel-Razek et al., 1999).

3. Faeces Production

According to the available literature, this biological parameter was not studied before neither in *G. mellonella*, nor in any other insect species treated with *B.t.* Table (4) showed that faeces produced by the healthy larvae (untreated control) reached 2.13±0.35 compared to 0.54±0.21 g/20 larvae in the treatment one day later, and remained in relatively similar values on the 2nd day of the test. Then, it decreased to 1.43±0. 1.41±0.4, 1.23±0.21 on the next successive days for larvae of the control compared to 0.72±0.12, 0.36±0.08, and 0.78±0.23 g/20 larvae in the treatment, respectively. Faeces production is certainly correlated to the amount of ingested food; and the results shown in Table (4) meet those presented in Table (3) for the amounts of consumed food for both healthy and diseased larvae of *G. mellonella*.

Table (2): Effect of *B.t* .var. *kurstaki* (LC$_{50}$) on larval weight (/g/20 larvae) in *G. mellonella*.

Days post-treatment	Treatment			Control		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Zero	1.30	1.40	1.34 ± 0.35	1.30	1.40	1.34 ± 0.35
1	0.90	1.10	1.40 ± 0.10	1.40	1.50	1.46 ± 0.07
2	0.80	1.00	0.90 ± 0.10	1.30	1.70	1.53 ± 0.06
3	0.70	0.90	0.78 ± 0.08	1.20	1.80	1.53 ± 0.21
4	0.60	0.80	0.68 ± 0.28	1.30	1.90	1.63 ± 0.30
5	0.50	0.80	0.46 ± 0.28	1.30	1.90	1.63 ± 0.25
6	0.40	0.60	0.46 ± 0.26	1.40	1.90	1.63 ± 0.25

Table (3): Effect of *B.t.* var. *kurstaki* (LC$_{50}$) on consumed food (/g /20 larvae) in *G. mellonella*.

Days post-treatment	Treatment			Control		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
1	0.10	0.60	0.36 ± 0.22	1.90	2.60	2.23 ± 0.34
2	0.00	0.70	0.36 ± 0.26	1.70	2.80	2.43 ± 0.63
3	0.70	0.90	0.75 ± 0.10	1.30	2.00	1.70 ± 0.36
4	0.20	0.40	0.28 ± 0.08	1.30	2.10	1.73 ± 0.40
5	0.30	1.30	0.70 ± 0.24	2.10	3.20	1.43 ± 0.30
6	0.40	1.00	0.70 ± 0.24	1.00	1.70	1.43 ± 0.30

Table (4): Effect of *B.t.*var.*kurstaki* (LC$_{50}$) on produced faeces (/g/20 larvae) in *G. mellonella*.

Days Post treat.	Treatment			Control		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
1	0.30	0.80	0.54 ± 0.21	1.80	2.50	2.13 ± 0.35
2	0.10	0.90	0.52 ± 0.28	1.50	2.50	2.13 ± 0.28
3	0.30	1.00	0.72 ± 0.12	1.10	1.60	1.43 ± 0.18
4	0.30	0.50	0.36 ± 0.08	1.03	1.70	1.41 ± 0.40
5	0.30	1.30	0.78 ± 0.23	1.90	2.90	1.23 ± 0.21
6	0.40	0.90	0.78 ± 0.23	1.00	1.40	1.23 ± 0.21

4. Duration of Survived Larval Stage

As shown in Table (5), the period needed for L$_3$ larvae of *G. mellonella* to complete the development of the larval stage ranged between 12 and 15 days with an average of 14.20 ± 0.06 days when fed on diet treated with *B.t.* var. *kurstaki* (LC$_{50}$). For larvae fed on untreated diet, this period lasted between 9 and 13 days with an average of 11.56±1.06 days.

The effect of *B.t.* on duration of *G. mellonella* larvae was not studied before, but the prolongation of this period in *B.t.* treated larvae of the wax moth agrees with those obtained for other insects by different authors. It was reported for *B.t.* treated larvae of *P. interpunctella* and *S. cereallella* (Salama *et al.,* 1991a), *S. obliqua* (Biswas *et al.,* 1996), the sunflower pest *C. hospes* (Barker, 1998), and *H. armigera* (Ajanta *et al.,* 1999). Also, larvae fed on *B.t.*-transgenic plants showed prolonged duration for the larval stage as reported for *H. zea* (Halcomb *et al.,* 1996). On the other hand, only Gharib and Wyman (1991) reported reduction of this period in larvae of *T. ni* intoxicated by *B.t. kurstaki*.

5. Weight of the Silk Cocoons

Survived larvae of *G. mellonella* after treatment with the sublethal concentration (LC$_{50}$) from *B.t.* var. *kurstaki* produced thin silk cocoons ranged between 0.07 and 0.11

with an average of 0.08±0.02 g/15 cocoons (Table 5). Meanwhile, larvae of the untreated control produced relatively heavy cocoons averaged 0.19±0.08 g/15 cocoons as shown in Table (5), where this value ranged between 0.16 and 0.22 g. In all the available literature concerning the effect of *B.t.* on many lepidopteran insects including *G. mellonella*, no study was carried out to show the late effect on the silk cocoons produced by the survived larvae. Thus, the present study is recording this result for the first time in *G. mellonella* and so far in lepidopteran species.

6. Pupal Weight

Data presented in Table (5) showed that pupae resulted from *B.t.* treated (LC$_{50}$) larvae of *G. mellonella* were less in weight than those resulted from the healthy (untreated control) larvae. The former ones weighed between 0.01 and 0.04 with an average of 0.02±0.008 g/pupa, compared to 0.07 and 0.10 with an average of 0.08±0.008 g /pupa for the latter group (untreated) and they differ greatly in size. The present results agree with those reported by Mahmoud *et al.* (1988) for *G. mellonella* treated with sublethal concentrations of *B.t.* var. *aizawai*. The same trend was recorded in pupae of *A. ipsilon* using *B.t.*var. *galleriae* (Salama and Sharaby, 1988) and in *S. oblique* (Biswas *et al.,* 1996); and *H.*

Table (5): Effect of *B.t.* var. *kurstaki* (LC$_{50}$) on some biological parameters in *G. mellonella* fed on treated diet.

Parameters	Test	Min.	Max.	Mean ± SE
Duration of survived larval stage/ day	T	12.00	15.00	14.20 ± 0.060
	C	9.00	13.00	11.56 ± 1.060
Weight of 15 cocoons /g	T	0.07	0.11	0.08 ± 0.020
	C	0.16	0.22	0.19 ± 0.080
Pupal weight/g	T	0.01	0.04	0.02 ± 0.008
	C	0.07	0.10	0.08 ± 0.008
Pupal period/day	T	7.00	8.00	7.50 ± 0.520
	C	7.00	8.00	7.50 ± 0.510

T = treatment, C = control

Table (6): Effect of *B.t.*var. *kurstaki* (LC$_{50}$) on larval mortality, pupation, adult emergence, and deformation % in *G. mellonella*.

Variants	Biological parameters			
	Larval endmortality %	Pupation %	Adult emergence %	Deformation in adults %
Treatment	55	41	33	30
Control	0	100	100	0

armigera (Ajanta *et al.*, 1999) using *B.t. kurstaki*. Similar results were obtained by feeding larvae on *B.t.* transgenic cotton plants as stated by Halcomb *et al.* (1996) for *H. zea* and by Adamczyk *et al.* (1998) for *S. frugiperda*.

7. Pupal Period

Results presented in Table (5) showed no difference in pupal period (7-8 days) between those of the treatment (7.5±0.52 days) and of the control (7.5±0.51 days) wax, while Mahmoud *et al.* (1988) stated a delayed pupal development due to *B.t. aizawai* in the same insect. On the other hand, Biswas *et al.* (1996) found that pupae of *S. obliqua* resulted from *B.t. kurstaki* treated larvae showed longer pupal period and a slight reduction in body length and weight. Also, feeding larvae on *B.t.* transgenic cotton plants showed no difference in pupal period of *H. zea* and *H. virescens* compared to feeding on the non-transgenic plants as stated by Halcomb *et al.* (1996). On the other hand, Adamczyk *et al.* (1998) found that transgenic cotton plants (variety Nu CO TN 33B) induced longer pupal period in *S. frugiperda* compared to those produced after feeding on the normal cotton variety DP 5415.

8. Larval Mortality

Larval mortality induced by the LC$_{50}$ reached 50% on the 7*th* day after treatment and recorded 55% at the end of the test (Table 6) compared to zero % among untreated larvae of *G. mellonella*. The resulted mortality varied proportional to the tested *B.t.* concentration as previously shown in Table (1), where it increased by increasing the concentration as in other tested lepidopteran species (El-Husseini, 1976, 1980, 1981; Ali *et al.*, 1973; Mahmoud *et al.*; 1988, Salama *et al.*, 1991b, and Keever, 1994).

9. Pupation Percent

Data presented in Table (6) showed that 41% of the survived larvae of *G. mellonella* reached the pupal stage. Meanwhile, all untreated larvae (control) reached pupal stage (100%). Decreased pupation rates for survived indi-

viduals (larvae) post treatment with *B.t.* were recorded in different lepidopteran insects, *e.g.*, in *Pandemis heparana* (El-Husseini, 1976 and El-Husseini and Sermann, 1977); in *Earias insulana* (El-Husseini and Afifi, 1980); and in *H. armigera* (Ajanta *et al.*, 1999).

10. Adult Emergence

Adult emergence in *G. mellonella* treated previously in larval stage (L$_3$) with *B.t.*var.*kurstaki* (LC$_{50}$) was significantly reduced to 33% compared to 100% in the untreated control (Table 6). El-Husseini (1976) and El-Husseini and Sermann (1977) observed similar results in *P. heparana*, Salama *et al.* (1991b) in *P. interpunctella* and *S. cerealella*, Keever (1994) in *E. kuhniella*, and Ajanta *et al.* (1999) in *H. armigera*. Also, feeding larvae of *H. zea* (Halcomb *et al.*, 1996) and *S. frugiperda* (Adamczyk *et al.*, 1998) on *B.t.*-transgenic cotton plants decreased adult emergence to less than 50% compared to the control.

11. Adult Deformation

Adults of *G. mellonella* emerged form pupae formed by larvae previously treated with *B.t.* (LC$_{50}$) showed morphological deformation rate of 30% as presented in Table (6). Meanwhile, all adults of the control were apparently normal. Deformed adults due to previous *B.t.* larval infection were reported by El-Husseini (1976) in *P. heparana* and by Salama and Sharaby (1988) in *A. ipsilon*. Moreover, other effects of *B.t.* on emerged adults like decreased female fecundity and fertility (egg-hatchability) were stated by Mahmoud *et al.* (1988) in *G. mellonella* and by Faruki and Khan (1993) in *C. cautella*. Meanwhile, Biswas *et al.* (1996) observed no great reduction in fecundity of *S. obliqua* previously treated in L$_1$ with *B.t. kurstaki*.

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