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EFFECTS OF LARVAL AGE ON SUSCEPTIBILITY OF COTTON LEAFWORM, *Spodoptera littoralis* (boisd.) (LEPIDOPTERA: NOCTUIDAE) TO *Bacillus thuringiensis* var. *kurstaki*

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ABSTRACT

Susceptibility of different instars larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* var. *kurstaki* was determined in the laboratory using a leaf dip bioassay. The concentration-mortality was used to estimate the relative susceptibility among the instars of S. *littoralis*. The results showed that there was no trend between successive instars, the second and fourth instars was the most and the least sensitive, respectively, and the third instar larvae was the least sensitive at second day. A sublethal concentration of *B. thuringiensis* var. *Kurstaki* was studied, the study proved adversely effect on the surviving larvae. Treated second instars had significantly weight less than the controls. Additionally, the pupal weight and adult emergence were significantly reduced. Moreover, reproductive potential and adult sex ratio of survivors was affected. Second instars of S. *littoralis* may be the main target to *B. thuringiensis* var. *Kurstaki* control.

INTRODUCTION

Resistance to insecticides is a worldwide threat to agriculture and public health (Georghiou 1990, Roush and Tabshnik 1990). This problem is exemplified by the cotton leafworm, *Spodoptera littoralis* (Boisd.), a major pest in cotton crops in Egypt (El-Gindy et al. 1980). Pyrethroids are considered to be the most effective insecticides against *S. littoralis*. In recent years, *S. littoralis* has developed resistance against pyrethroids and most chemical insecticides (El-Guindy 1999) and this has prompted development resistance management strategies including avoidance of pyrethiods in early season. *Bacillus thuringiensis Kurstaki* is included in current insecticide recommendations for control of *S. littoralis* on cotton early season (Recommendations of Ministry of Agriculture, Egypt).

Dipel 2X (*Bacillus thuringiensis* Berliner var *kurstaki*) is currently registered for control of cotton leafworm in Egypt. However, the relative susceptibility of different instars of *S. littoralis* to this formulation of *B. thuringiensis* var *kurstaki* is important for optimal timing of insecticide applications (Betana 2000). Previous research demonstrated that early instars of some lepidopterous species are more susceptible to *B. thuringiensis* than later instars (Afify & Merdan 1969, Altahtawy & Abaless 1973, Morries 1973, Hornby & Cadner 1987). In other species, later instars are more sensitive than early instars (Rock & Monroe 1983, James et al. 1993, Li et al. 1995). Under field conditions, some individuals may only exposure to sublethal concentration of Dipel 2X. The sublethal effects of *B.*

thuringiensis var *kurstaki* of the development of surviving *S. littoralis* larvae have not been determined.

The objectives of this work was to determination the relative susceptibility of different instars of *S. littoralis* to *B. thuringiensis* , and to determine the effects of sublethal concentration of *B. thuringiensis* on the development of *S. littoralis*.

MATERIALS AND METHODS

Insects. Rearing of the cotton leafworm was carried out at the Central Agriculture Pesticide Laboratory (CAPL) under room temperature (25±2°C with 65± 5% RH) and at a 12:12 h (light: dark) photoperiod, as described by El-Defrawi et al. (1964). The larvae were daily provided with clean and dry castor bean, *Ricinus communis* leaves. Larvae were daily transferred to clean and suitable containers. After pupation, the pupae were collected and kept in plastic containers placed in rearing cages (40 x 40 x 60 cm) with 10 % sugar solution on cotton pads. The cage was supplied with the leaves of *Nerium oleander* as ovipositional site. Newly emerged adult males and females were kept in pairs. The eggs were collected; upon hatching, larvae transferred to the leaves fresh castor bean. Leaves were replaced as needed.

Dipel 2X (potency 32,000 IU/mg, 6.4 %, produced by Abbott laboratories)

Susceptibility tests. The response of each instar to various concentrations of *B. thuringiensis* was determined by using a leaf dip bioassay technique. Dipel 2X was diluted with water to make series of six concentrations. Controls were treated with water alone. Leaf dip bioassays for each instar were done as follows. Leaves were immersed in each concentration of *B. thuringiensis* or water for 10 s. Dipped leaves were allowed to air dry and were then placed in plastic cups (10-cm diameter by 5-cm depth). Larvae of appropriate instars were transferred into the cups. Three to five replicates of approximately 100 instar larvae for each concentration were tested.

The first instar larvae chosen for bioassays were ≥ 24 h. old. Larvae of all other instars had molted 1 or 2 days previously. Larvae were allowed to feed on the treated leaves for 2 days before the leaves were replaced with fresh, untreated ones. Larvae in cups were maintained under room temperature (25±2°C with 65± 5% RH) and at a 12:12 h (light: dark) photoperiod. Larval mortality was recorded at 1-7 days after treatment. Abbott's formula (1925) was used to correct for control mortality. The cumulative mortality data at 5 d. were subjected to probit analysis using a probit program of POLO (LeOra, software 1987).

Sublethal effects: A sublethal concentration of *B. thuringiensis* may affect the development of survivors differently, depending on treated the larval instar. The second instar larvae were treated with sublethal concentrations of *B. thuringiensis* at 8, 4 and 2 ppm, which were expected to cause ≤ 50 % mortality. The leaves treatment with *B. thuringiensis* or water was the same as described for the susceptibility tests.

Second instar larvae were fed on castor bean leaves with Dipel 2X for 2 days thin fed fresh, untreated leaves. Survivors from treatment and control groups were observed daily and the mortality was recorded in larval, perpupae and pupal stages. Sex of pupae was determined, and they were weighted 3 days after pupation. Treatment effects of sublethal concentrations of *B. thuringiensis* on biotic potential i.e. larval weight to determined the percentage of reduction weight, pupal weight, adult emergence and adult sex ratio.

RESULTS AND DISCUSSION

Table (1) indicted that the response of different instars to range of concentrations of *B. thuringiensis* var *kurstaki*. The LC_{50} values of Dipel 2X treated 1st, 2nd, 3rd, 4th and 5th instar larvae showing 7.63, 4.05, 27.99, 65.06 and 26.32 ppm at 5 days from start exposure to *B. thuringiensis*, respectively. The relative susceptibly gave 1.9, 1.0, 6.9, 16.1 and 6.5 for 1st, 2nd, 3rd, 4th, and 5th larval instars after 5 days of application, respectively. However, the relative susceptibly at the second day gave 9.76, 5.82, 3.74, 1.67 and 1.00 for 2nd, 5th, 1st, 4th and 3rd larval instars, respectively. Examination of the tabulated data showed that, the effect is increased as a period of exposure increased. Slope increased instar larvae age increased up to 4th instar larvae, however, decreased with 5th instar larvae. LC_{50}s for second instars were significantly than others, and those for fourth instars were significantly higher than others, suggesting that second instar was the most sensitive in both 2 days and 5 days, but the third and fourth instars were least sensitive at 2 days and 5 days to *B. thuringiensis* var *kurstaki*, respectively. The differences among instars could be related to the physiological differences between the second and other instars to *B. thuringiensis*. Falcon (1971) reported that the pH and activity of digestive enzymes required to activate the delta endotoxin in the insect gut might differ among instars.

Table 1. Relative susceptibility of different instar larvae of *S. littoralis* to Dipel 2X (B.t.) after the second and fifth day from treatment.

Instar	2 days			5 days		
	LC_{50} (ppm) (% 95 FL)	Slope ± SE	Relative toxicity	LC_{50} (ppm) (% 95 FL)	Slope ± SE	Relative toxicity
1st	304.40 (115.25 -1254.90)	0.43 ± 0.13	3.74	7.63 (5.51- 9.84)	1.13 ± 0.12	8.53
2nd	116.94 (62.82 - 2283.15)	0.49 ± 0.18	9.76	4.05 (1.98 – 6.20)	1.32 ± 0.19	16.06
3rd	1140.76 (474.07 –1630.63)	0.85 ± 0.23	1.00	27.99 (23.73 – 32.28)	2.03 ± 0.20	2.32
4th	683.23 (374.46 – 4713.28)	1.28 ± 0.36	1.67	65.06 (54.50 – 75.84)	2.03 ± 0.22	1.00
5th	195.90 (124.08 - 234.07)	1.12 ± 0.32	5.82	26.32 (21.67 – 31.61)	1.49 ± 0.15	2.47

Table (2) shows that the second instar larvae which was fed on *B. thuringiensis*-treated leaves with 2, 4, and 8 ppm for 48 h. affected significantly on reducing the weight of surviving larvae, depending on

treatment concentrations.The higher concentration caused the higher reduction weight and vice versa. The reduction in larvae weights reached to the highest level at fourth day from exposure to *B. thuringiensis* than at 5 day. Table (3) indicated that *B. thuringiensis* caused considerable mortality on pupae stage developed from treated larvae. It is clearly evident that the percentage of survival pupation, at any of the tested concentrations, was significantly decreased than those in untreated control, pupation was decrease from 96% in control to 50, 63 and 73% at 8.0, 4.0 and 2.0 ppm for Dipel 2X, respectively. However, all tested concentrations caused significant decreasing in pupal weights (Table 3).

The effect of Dipel 2X on the adult emergence is shown in Table (3) data indicate that Dipel 2X significantly decreased the emergence of moths treated as larvae treatment. In terms of, the emergence percentages were 39, 57 and 68 at 8, 4 and 2 ppm, compared to that 95% in control. Also, there were differences between the sex ratio in treated individuals and this was pronounced with all tested concentrations.

Table 2. Reduction weight percentage for treated 2nd instar larvae of *S. littoralis* with Diple 2X through seven days.

Concentration (ppm)	% Reduction weight					
	1	2	3 (days)	4	5	7
64	8.69	56.86	43.56	56.10	47.74	27.13
32	8.69	45.36	45.96	48.06	40.70	25.62
16	4.34	21.57	38.87	46.34	29.40	19.54
8	4.34	11.76	12.20	34.28	21.11	14.25
4	3.96	8.88	11.22	25.09	19.10	4.20

Table 3. Effect of sublethal concentrations Dipel 2X on biotic potential of second instar larvae of *Spodoptera littoralis* (laboratory strain).

Conc.	% larval	% pupal	%	% adult	% accumulative	pupal weight (mg)			Generation period
(ppm)	mortality	mortality	pupation	emergence	mortality	male	female	sex ratio [a]	(day)
2	22.0	10.0	73.0	68.0	32.0	283	313	0.79 : 1	35
4	27.0	16.0	63.0	57.0	43.0	260	271	0.67 :1	36
8	36.0	25.0	50.0	39.0	61.0	253	251	0.54 : 1	36
Control	3.0	1.0	96.0	95.0	5.0	302	317	0.95 : 1	33

Concerning on the effects of the tested insecticides on the developmental period is shown in Table (3). Data indicate that, Dipel 2X significantly prolonged the developmental period from 33 d in control to 36 days in treatment the for second instar larvae of *S. littoralis*. This finding is in

agreement with that obtained by Moreau and Bauce (2003) who mentioned that the developmental time increased linearly with B.t. formulation concentration. The results proved that exposure of second instar larvae to Dipel 2X significantly caused reduction in pupal size and adult emergence as compared with untreated larvae.

Table (4) shows the effects of Dipel 2X on the reproductive potential of treated second instar larvae of *S. littoralis*. Data clearly indicated that, Dipel 2X decreased the average number of eggs laid by females. Accordingly, the hatchability with Dipel 2X treatment with 8.0, 4.0 and 2.0 ppm showed 30.0, 47.92 and 56.77 %, respectively. The percentages of sterility treated with Dipel 2X formulation (B.t.) were 67.80, 48.56 and 39.06 % at the tested concentrations, respectively. Similar results were reported by Hornby and Gardener (1987) in which fecundity of *S. frugiperda*, *H. zea* and *T.ni* moths developing from larvae exposed to B.t toxin was lower than fecundity of moths developing from larvae fed on untreated control. Finally, surviving larvae might have reduced their feeding rate after ingesting *B. thuringiensis*, and partial starvation might have contributed to reduce pupal size and adult emergence. The reproductive potential of treated second instar larvae might also have been affected because the fecundity is positively correlated with pupal size (Carriere 1992).

Table 4. Effect of Dipel 2X on laboratory strain fecundity and hatchability of *S. littoralis* treated as 2nd inatar larvae.

Concentration (ppm)	Mean no. of eggs/female	Mean no. of hatched eggs	% Hatchability	% Sterility
2.0	620	352	56.77	39.06
4.0	409	196	47.92	48.56
8.0	460	138	30.0	67.80
Control	966	900	93.16	6.84

The results of the study described here have important implications for management of the Egyptian cotton leafworm with Dipel 2X (B.t). Although differences between instars were greater for second instar than any other instars. Thus, the fourth instar can't be the target for control of *S. littoralis* with foliar applications.

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تأثير العمر اليرقى على حساسية دودة ورق القطن لفعل بكتيريا باسليس ثيرنجنسس كيرستاكى

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تمت دراسة حساسية الأعمار المختلفة ليرقات دودة ورق القطن لفعل بكتيريا باسليس ثيرنجنسس كيرستاكى معمليا. أجرى التقييم الحيوى باستخدام طريقة غمر الأوراق، كما أستخدمت طريقة انحدار التركيز مقابل نسبة الموت وذلك لتقدير الحساسية النسبية.

أظهرت الدراسة أن العمر اليرقى الثانى أكثر الأعمار حساسية وأن العمر اليرقى الرابع أقلها حساسية فى كلا من اليوم الثانى والخامس من وقت المعاملة، كما لوحظ عدم وجود نمط ثابت للتأثير على الأعمار المتتالية. كما تمت دراسة التركيزات التحت مميتة لفعل بكتيريا باسليس، أظهرت النتائج تأثير سلبى على اليرقات الحية، فعند معاملة العمر اليرقى الثانى حدث انخفاض فى وزن اليرقات المعاملة بالإضافة الى انخفاض فى وزن العذارى وخروج الفراشات والتأثير على الكفاءة التناسلية والنسبة الجنسية للفراشات الناتجة بعد المعاملة. من خلال النتائج يعتبر العمر اليرقى الثانى هو العمر المستهدف لفعل بكتيريا باسليس ثيرنجنسس.