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BIOACTIVITY OF DIPEL 2X, A COMMERCIAL PREPARATION OF *BACILLUS THURINGIENSES* BERLINER AGAINST THE COTTON LEAFWORM *SPODOPTERA LITTORALIS* (BOISD.)

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Abstract

The insecticidal activity as well as the latent effect of Dipel 2x , a commercial preparation of *Bacillus thuringienses* var. *Kurstaki* (Berliner) were evaluated in the laboratory . The 2nd instar larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.) were fed on a diet surface treated with various doses of Dipel 2x, i.e. 64, 192, 320, 5120 and 6400 IU/ml at 27±2°C and 55 - 60% R.H. Data of initial and latent mortality revealed that Dipel 2x had slight insecticidal activity against the cotton leafworm. The ability of larvae to recover decreased with the increase in concentration or / and feeding time . Both larval and pupal duration were remarkabley prolonged with dose increase, while pupation percentage was reduced . Remarkable latent adverse effects were detected on adult emergence, fecundity and egg viability, particularly with doses above 320 IU/ml.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is one of the most destructive lepidopterous pests in Egypt, where it causes serious damage to most of vegetable and field crops. The chemical control by organic insecticides had been extensively used during the last three decades. As a result of recent restrictions on the usage of chemical pesticides , microbial control agents are receiving now a great deal of consideration, as a possible safer substitues and as a mean of reducing chemical insecticides.

The insecticidal action of *B. thuringiensis* var. *Kurstaki* Berliner had been tested against a wide variety of lepidopterous insects and showed considerable mortality particularly against those infesting cotton, i.e.*S. exigua*, *S. littoralis* and *Heliothis armigera* (Salama *et al.*, 1981) and *Spodoptera littoralis* (Abul - Nasr and Abdallah 1970; sneh *et al*. 1981; Sneh and Gross, 1983; Beroza *et al.*, 1984).

The accurate measurement of the potency of *B. thuringiensis* is a matter of particutlar concern when the preparations are to be used for biological control . Several workers have reportd differences in insecticidal activity in accordance to the tested preparations of *B. thuringiensis* (Heimple and Angus 1960; Angus 1965; Angus and Norris 1968). In this respect , Burgerjon (1964) indicated that more information was needed about the spectrum of the insecticidal activity of various *B. thuringiensis* preparations and suggested that the response of different genera or species of Lepidoptera might vary according to the serotype tested or even between successive lots produced from a single serotype. Accordingly the advent of commerical preparation of *B. thuringiensis* has created an increasing need to evalute critically the insecticidal activity of such preparation as well as its effect on different biological aspects of the traget pest.

MATERIALS AND METHODS

The insecticidal activity of the commerical preparation of *B. thuringiensis* var. kurstaki formulated as Dipel 2x wettable powder (32000 IU/mg) was investigted against the larval stage of the cotton leafworm. Larvae used in the tests were obtained from a colony raised on a semi - artificial diet . Laboratory experiments were carried out at the Plant Protection Research Institute, Dokki, Egypt.

Second instar larvae were transferred from the mass rearing on the untreated diets in plastic cups and individually tested on diet surface - treated with *B. thuringienses* preparation, following the procedure described by Ignoffo (1966) where Hamilton repeating syringe (finger control release of 100 ul volume) was used to dispense the tested dose of bacterium suspensions. Treatment doses, calculated on the basis of diet surface were prepared to represent 64, 192, 320 , 5120 and 6400 IU/ml . A hundred larvae, distributed in five replicates were used for each of the tested doses or/and control (20 larvae/ replicate or cup), except for the higher doses (320, 5120 and 6400 IU/ml) where additional five replicates were used to

provide adequate number of moths.

Daily inspections were carried out and the number of individuals that managed to develop (3rd- 6th larval instars, pupae and adult) were recorded. Moreover, pupal weights, larval and pupal duration were also determined.

Adult fecundiy was determined by placing female and male together in a glass jar (1 lb) provided with a piece of cotton wool soaked with 5% sucrose solution for moth feeding and the jar was internally surrounded by an ovipostion site .

Each jar was replicated 5 times. The glass jars were inspected daily to count survived moths and number of eggs/egg - mass. All testing cups and glass jars were held at 27 $\pm$ 2° C and 55 - 60% R.H. To derermine the latent effects of *B. thuringiensis* on fertility of eggs deposited by moths that developed from treated larvae , three bathches, each of 100 eggs were collected during the first 3 days of oviposition. Eggs were incubated at 27$\pm$2° C until eclosion and percentage of hatchabilty was recorded.

RESULTS AND DISCUSSION

Table 1. presents the results of feeding the cotton leafworm larvae on various doses of Bacillus thuringinensis kurstaki. The progressive doses that ranged between 64 and 6400 IU/ml resulted in 1-5 % larval mortality within 24h and 2 - 15 % within 48 h of initial exposure indicating the slight intial bioactivity of *B. thuringiensis*. However, increasing the dosage by 100 times resulted in only 9 - fold increase in toxicity within the first 48 h of exposure. Furthermore, continuous exposure for longer time (10 days) resulted in 5 and 57% kill for the doses 64 and 6400 IU/ml, respectively thus exhibiting 11.4 - fold increase in mortality. The decrease in susceptibility of 2 nd instar larvae indicated that a very high dose (6400IU/ml) was needed to achieve aceptable larval mortality within 10 days of feeding. It is of interest to denote that larvae which survived higher doses were generally smaller than those exposed to lower doses . In this repect, Hamed (1980) and Rizk *et al.*, (1981) recorded that *B. thuringiensis* had a moderate effect on *A. ipsilon* larvae. Hamed (1985) showed that the concentration prolonged larval duration of *S. littoralis*.

Table 1. Average percent mortality of *Spodoptera littoralis* 2 nd instar larvae fed continuously on a semi- synthetic diet treated with various doses of *B. thuringiensis* (Dipel 2x).

Dose (IU/ml)	Larval mortality % days				Average mortality %
	1	2	5	10	
64	1	2	2	5	78
192	2	6	7	13	80
320	3	8	12	20	85
5120	5	11	29	38	86
6400	5	18	36	57	94
Untreated	--	4	6	10	44

Feeding the 2nd instar larvae on untreated diet resulted in 56% pupation, each pupae averaged 324 mg body weight. On the other hand feeding on diet treated with doses ranging from 64 to 5120 IU/ml had no influence on either pupation percent or pupal body weight except at the dose 6400 IU/ml where a slight pupation (6%) was recorded while higher pupation rates were observed at the doses ranging from 64 - 5120 IU/ ml (Table 2). Inhibition of feeding was remarkably obvious at the dose 6400 IU/ml resulting in the least pupal body weight. Cessation of feeding with subsequent effects on larvae or pupal deelopment after treatment *with B. thuringiensis* was reported for *S. littoralis* by Hamed (1985) and Bekhit (1985).

Table 2 presents the developmental time of larvae that survived various doses of *B. thuringiensis* . The time was measured from the start of feeding the 2nd larval instar until pupation. A remarkable and progressive elongation in larval duration was indicated . Such elongation was associated with dose increase.

As for pupal duration the increase in *B. thuringiensis* dose resulted in an increase in pupal duration. Bekhit (1985) and Hamed (1985) reported that the concentrations of Bt prolonged the larval duration of *S. littoralis* , reduced the percentages of pupation, pupal weight and adult emergence.

Table 2. Larval duration, pupal duration and pupal weight of *S. littoralis* fed as 2nd instar larvae on *B. thuringiensis.*

Dose (IU/ml)	Duration (days)		Pupation %	Pupal weight (mg)±SE
	Larval period (2nd-6th instar) ± SE	Pupal stage ± SE		
64	17.79±0.6198	7.6±0.9393	22	381±0.0397
192	17.86±0.5157	8.9±0.5090	20	340±0.0300
320	17.92±0.3900	9.3±1.5330	15	330±0.0614
5120	18.00±0.0000	10.1±0.4570	14	314±0.0470
6400	18.20±0.8090	10.0±0.0000	6	259±0.0400
Untreated	16.46±9340	7.3±0.4714	56	324±0.0530

Table 3 shows that the various doses affected remarkably adult emergence. However, the reduction in adult emergence increased with the increase of the applied doses, it ranged from 85% at 192 IU ml to 0% with the highest dose.

Regarding the effect on fecundity, doses as low as 64 - 320 IU/ ml did not affect the number of eggs laid (1343 - 1171 eggs / female) compred with the control (1040 eggs/ female) . On the other hand 5120 IU/ml drastically affected fecundity and the deposited eggs were reduced by 58.94%. Following the effect to egg fertility, no hatchability occurred for eggs treated with doses above 320 IU/ml .

Table 3. Latent effect of *Bacillus thuringiensis* on cotton leafworm fecundity and egg viability.

Dose (IU/ml)	Adult emergence %	No.of eggs/ female ±SE	Egg hatchabilty %
64	81.8	1343±237	50.3
192	85.0	1206±225	49.2
320	53.3	1171±163	0.0
5120	50.0	427±105	0.0
6400	0.0	---	---
Untreated	100.0	1040±190	100.0

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التأثير الحيوي للمبيد البكتيري Diple 2x علي دودة ورق القطن

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أجري إختبار لدراسة النشاط الإبادي والآثار المتأخرة للمبيد البكتيري Dipel 2x علي العمر الثاني لدودة ورق القطن في المعمل ، حيث استخدمت ٥ تركيزات مختلفة كمعاملة سطحيه علي بيئة صناعية وغذيت عليها اليرقات لمدة ٢٤ ساعة ثم نقلت بعد ذلك إلي بيئة غير معاملة حتي التعذير.

أوضحت النتائج أن نسبه موت اليرقات تزداد بزيادة التركيز ، كذلك لوحظ أن مدة عمر الطور اليرقي وطور العذراء أطول في التركيز الأعلي ، كما اختبرت أيضا نسبه التعذير وخروج الفراشات ووضع البيض ونسبة الفقس . وقد لوحظ أنه بالنسبة للتعذير ونسبة خروج الفراشات وخصوبتها فإنها تقل بزيادة التركيز، وكذلك كان التأثير علي الفقس واضحا حيث كانت نسبة الفقس ١٠٠ ٪ في الحشرات الغير معاملة بينما تأثرت نسبه فقس البيض وانخفضت بزيادة التركيز.