Minufiya J. Agric. Res. Vol.36 No. 5:1313-1322 (2011) "http://www.mujar.net"

EFFECT OF THE BIOINSECTICIDE *Bacillus thuringiensis kurstaki* ON THE BIOLOGICAL ASPECTS OF COTTON LEAFWORM, *Spodoptera littoralis* (Boisd.)

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(Received: Jul. 6, 2011)

ABSTRACT: *The experiments were conducted to evaluate the effect of different concentrations of Bacillus thuringiensis kurstaki (Dipel 2X®) on the 4th instar larvae of Spodoptera littoralis (Boisd.). The highest percent of mortality of the 4th instar larvae was achieved at the concentrations of 4000 and 2000 ppm after 96 hrs of treatment. While, the least mortality percentages were recorded with the other concentrations (250, 500, 1000). The pupation percentage was gradually reduced to the minimum rate with the highest concentrations (2000, 4000ppm). Results also cleared that there was a gradual significant decrease in adult emergence process, as the concentration increased (P=0.0002). Slight significant difference (P= 0.034) in the larval period was observed after treating 4th instar larvae at the highest concentration (2000, 4000) compared to the control. Significant differences (P= 0.0001) were noticed in pupal duration between the control and all tested treatments except the lowest concentration (250ppm). The pupal weight significantly differed among the all treatments including the untreated control (P=0.0001). There was a wide and significant variation in the fecundity of S. littoralis among different treatments (P=0.0001). Number of laid eggs /female reduced as the concentration increased. On the other hand, the egg hatchability was reduced significantly (P=0.0004) in all tested treatments compared to the control. The experimental results proved that this biopesticide, (Dipel 2x) could be used as an alternate control method in combating the pest and implicated in the Integrated Pest Management (IPM).*

Key words: *Spodoptera littoralis, Bacillus thuringiensis, bioinsecticide, biological aspects*

INTRODUCTION

In Egypt, many field crops as well as various vegetables are attacked by numerous insect pests. Lepidopterous insects in general and the cotton leafworm, *Spodoptera littoralis* (Boisd.), in particular, are the most dangerous pests in this respect. In fact, the cotton leaf-worm is a major limiting factor affecting crop and vegetable production, not only in Egypt, but also in many other countries. *S. littoralis* is similarly one of the most destructive agricultural lepidopterous pests within its subtropical and tropical range (Hosny *et al.* 1986). It can attack a lot of economically important crops all year round. Of these crops are cotton, leguminous crops, and various vegetables. Chemical control of *S. littoralis* has been extensively used especially on cotton and resistance to this compounds developed. Since then, numerous other organophosphorus, synthetic pyrethroids, insect growth regulators and other nonconventional insecticides have been used, with many reports of resistance and cross resistance development in many cases (Issa *et al.*, 1984 and Abo-El-Ghar *et al.*, 1986). More attention should be paid to the use of bioinsecticides such as compounds based on bacteria, fungi, and viruses (Rao *et al.*, 1990). These groups have unique modes of action (Ascher, 1993 and Thompson *et al.*, 1999) and their properties may differ considerably from the conventional agents which growers are familiar. The most widely used microbial pesticides are those based on preparations of the bacterium *Bacillus thuringiensis* (*Bt*) (Lambert and Peferoen, 1992). The toxicity of *Bt* is due to the production of crystalline protein protoxins, known as δ-endotoxins (Broderick *et al.*, 2006). Solubilized protoxins are activated by midgut proteases and bounded with the receptors of the epithelial cells (Pigott and Ellar, 2007). The toxins insert themselves into the cell, where they form pores that lead to cell lysis, subsequently causing insect death (De Maagd *et al.*, 2003). Commercial *Bt* products generally consist of a mixture of spores and crystals, produced in large fermenters and applied as foliar sprays, much like synthetic insecticides. (Sanchis *et al.*, 1999). Application of bioagents sharply increased the mortality of 4th instars larvae. Also, there was a delay in the killing effect due to latent period of the tested bioagents and decrease of pupation and adult emergence. Treatment of 4^{th} instar larvae with *B. thuringiensis*, prolonged larval duration, while led to reduction in the number of laid eggs and hatchability (Abd El-Kareem *et al.* 2010).

The aim of this study was to evaluate the effects of *B. thuringiensis (Bt)* biopesticide (Dipel 2X®) on the biological aspects of different stages of *S. littoralis* insect pest.

MATERIALS AND METHODS

This study was carried out in the Entomology Research Laboratory in Department of Plant Protection, Faculty of Agriculture, Al-Azhar University (Cairo, Egypt).

Insect rearing

Adults of *Spodoptera littoralis* (Boisduval) were obtained from Entomology Research Laboratory, Plant Protection Institute, Agricultural Research Center (ARC), Dokki, Giza, Egypt. Insects were reared under an environmental controlled condition at temperature of 25±1°C and 60±5% relative humidity (RH) at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. The larvae were reared on castor leaves in individual containers to prevent contamination (Santharam, 1985). Fresh castor leaves changed every 48 hrs for larval feedings till the end of larval stage. Fourth instar larvae were used for the bioassay experiments. The emerged adults were supplemented by 10% sucrose solution in cotton pad suspended in the jar for adult feeding. Small muslin cloth were exposed to adults for egg laying.

Biopesticide (Bt.):

The commercial bioinsecticide (Dipel 2X®) *Bacillus thuringeinsis kurstaki* (Btk) 32000 IU/mg, was applied in concentrations of 0, 250, 500, 1000, 2000 and 4000 ppm.

Bioassay:

For bioassay, castor leaves were submerged in the *B. thuringeinsis* (Btk) solution of each concentration. *S. littoralis* larvae were exposed to treated castor leaves for feeding and checked daily. Treated castor leaves were changed by fresh leaves after 48 hrs, then fresh leaves offered to larvae till the end of the larval stage. The treatments were replicated three times in each tested concentration and also the control. In order to compare the LC50 values, toxic curves were made using probit analysis for tested bioinsecticide (Finney, 1971). The effect of *B. thuringeinsis* on biological aspects of *S. littoralis* was focusing on the following parameters namely; larval mortality rate, larval duration (days), pupation (%), pupal weight (g), pupal duration (day), adult emergence (%), number of laid eggs and hatchability (%).

Statistical analysis

The effect *B. thuringeinsis* on *S. litoralis* biological parameters were subjected to statistical analysis by Analysis of variance (ANOVA) test using a computer software SAS (SAS Institute, 2000). Means were determined and compared by Duncan multiple range test at 0.05% probability level (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of *B. thuringiensis* on percentage of mortality:

Data presented in figure (1) showed the effect of different concentrations of *B. thuringiensis kurstaki* (Dipel 2X®) on the 4^{th} instar larvae of *S. litoralis*. it noticed that the highest effectiveness of mortality was achieved with the highest concentrations (4000 and 2000 ppm) after 96 hrs of treatment, while the least concentrations (250 and 500 ppm) induced lower mortality rates on the tested larvae. The tested concentrations did not result in instant mortality rates due to the latent effect of this bioagent especially in the older instars (LC50 value= 1930.22 ppm) and (Slope = 1.0566). The obtained data are in agreement with those obtained by Hernandez (1988) who reported that three subspecies of *B. thuringiensis* achieved larval mortality of *Spodoptera frugiperda* averaged 80, 100 and 70%, respectively. Similar results obtained by David *et al.* (2000) reported that a significant mortality of *B. thuringiensis*

Berliner formulations resulted with all tested concentrations to all immature stages tested compared to the control on the cotton wood leaf beetles in both laboratory and field experiments. Results also greatly correspond with findings of Mabrouk, (2001); Mabrouk and El-Abbas, (2002), Hanafy *et al.*, (2005); Abdel-Aziz, (2007); and Abd El-Kareem, (2007) who reported similar observations.

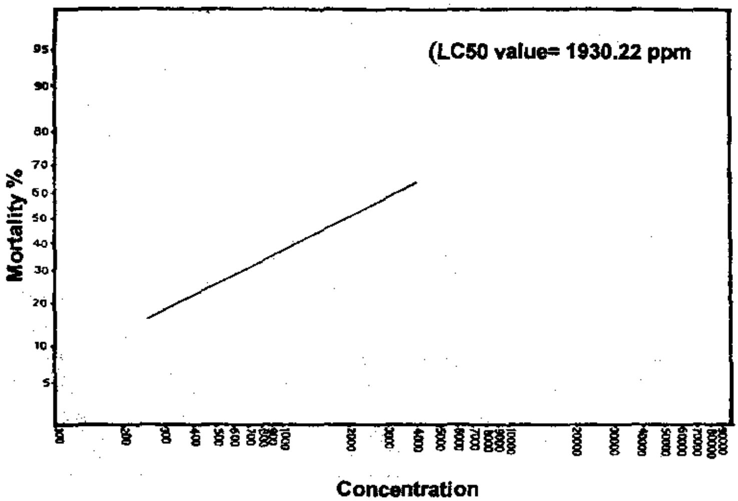

Figure (1): Toxic effect of the *B. thuringiensis* (Dipel 2X®) against the 4th instar larvae of *S. littoralis* after 96 hrs of exposure.

Effect of *B. thuringiensis* on the pupation:

The pupation was reduced in the highest concentrations (4000 and 2000 ppm) averaged 69.99 and 70.0%, respectively. Pupation percentage of control was 86.29%, whereas, pupation percentage at the lowest concentration (250 ppm) was79.49%. Statistical analysis indicated that there were no significant differences in pupation percentage among all tested concentrations including the control (Table 1). Results are in agreement with those obtained by Abo-El-Ghar *et al.* (1995) who reported that use of *Bacillus thuringiensis* and Abamectin against cotton leafworm *S. littoralis*, achieved considerable decrease of pupation (36%) after Abamectin treatment, and a high reduction of moth fecundity (87.4%).

Table (1): Pupation and the adult emergence percentages of *S. littoralis* 4th instar larvae treated with *B. thuringiensis, kurstaki* (Btk).

Concentration ppm	Pupation %	Adult emergence %
control	86.29a	98.33a
250	79.49a	86.66ab
500	73.61a	80.66bc
1000	72.22a	69.33cd
2000	70.0a	65.00d
4000	69.99a	63.66d
P=0.05	0.8069	0.0002
F value	0.44	12.67
LSD 0.05	29.62	11.88

Means in a column with the same letter are not significantly different

Effect of *B. thuringiensis kurstaki* (Btk) on adult emergence of cotton leaf worm:

Obtained results cleared that there was a significant gradual decrease in adult emergence as the concentration increased (P=0.0002). The highest percentage (98.33%) of moth emergence was recorded in untreated larvae. While, the lowest percentage of adult emergence (63.66 and 65%) were recorded with the highest concentrations of 4000 and 2000 ppm, respectively (Table 1). Similar trend was obtained by Osman and Mahmoud (2008) who stated that the emergence of moths was highly affected by *B. thuringiensis* treatments. Abd El-Latif (2001); Gamil (2004) and Mohamed (2006), they found that adult emergence was affected after treatment with bacterial or viral agents.

Effect of *B. thuringiensis* on the larval , pupal duration and pupal weight:

The effect of *B. thuringiensis kurstaki* (Btk) on larval and pupal duration of *S. littoralis* treated as 4th instar larvae were evaluated and recorded as shown in table (2). Slight significant difference (P=0.034) in the larval period were observed after treating 4th instar larvae at highest concentration (11.66 days) compared to the control (10.36days). Significant differences (P= 0.0001) were noticed in pupal duration between the control (14.03days) and all treatments except the lowest concentration (250 ppm) averaged 13.8 days. Pupal duration was shortened gradually as the concentration increased reached to the shortest pupal period in the highest concentration of *B. thuringiensis* averaged 9.93 days (Table 2). Similar findings was obtained by Gamil (2004) and Mohamed (2006) who found that the development time of larvae and pupae were extended as well as adult emergence after treatment with bacterial or viral agents. These data also agree with youssef *et al.* (1991) who stated that the larval duration of *Agrotis ipsilon* was prolonged when treated by 1m of juvenile hormone analogue.

On the other hand, pupal weights were significantly (P=0.0001) reduced as the concentration increased. The highest pupal weight observed in the untreated larvae (281.1mg) concequantly pupal weight reduced till reached to the lowest pupal weight (231.7mg) in the highest concentration. Results are in agreement with those of Osman and Mahmoud (2008) who cleared that pupal weight was decreased by the treatment of larvae with *B. thuringiensis* (Diple2X®).

Table (2): Effect of biological agent of *B. thuringiensis kurstaki* (Btk) on larval, pupal durations and pupal weights of *S. littoralis*.

Concentration ppm	Larval duration (days)	Pupal duration (days)	Pupal weight (mg)
control	10.36a	14.03a	281.1a
250	10.53a	13.8a	274.8b
500	10.83ab	12.73b	255.43c
1000	11.03ab	10.76c	243.73d
2000	11.46b	10.16cd	241.6d
4000	11.66b	9.933d	231.76e
P=0.05	0.034	0.0001	0.0001
F value	3.51	52.04	199.98
LSD 0.05	0.84	0.78	4.26

Means in a column with the same letter are not significantly different

Fecundity and hatchability:

There was a great significant variation in the fecundity of *S. littoralis* among different treatments (P=0.0001). Number of laid eggs/female clearly reduced as the concentration increased. Results in Table (3) illustrated that the highest number of laid eggs/female was recorded in the control (1690.66 eggs), while, the lowest was recorded with the highest concentration (4000ppm) (710.66 eggs).

On the other hand, the egg hatchability was reduced significantly (P=0.0004) in all tested treatments compared to the control. The highest hatchability percentage (96.07%) was recorded in the control, whereas, low hatchability percentage (62.14%) was recorded in the highest concentration (4000 ppm) (Table 3). Reduction of hatchability 24 and 48 hrs after treatment of eggs compared to the control were recorded with Dipel were −78.4 and−73.3% (Osman and Mahmoud 2008). Temerak and Sayed (2001) cleared that unhatched eggs of *Deudorix livia* treated with Spinosad at 10 to 40 ml/100 liters ranged from 20 to 29% in 1999 and from 17 to 23% in 2000. Also, Fang *et al.*, (2002) who stated that spinosad killed all exposed *Rhyzopertha domicinca* adults and significantly suppressed progeny production (84-100).

Table (3): Effect of larval treatment with *B. thuringiensis kurstaki* (Btk) on fecundity and fertility of *S. littoralis*

Concentration ppm	No. of laid eggs/female	Hatchability %
control	1690.66a	96.07a
250	1714.0a	87.61ab
500	1203.0b	81.71bc
1000	1034.33bc	74.58cd
2000	939.33cd	69.69de
4000	710.66d	62.14e
P=0.05	0.0001	0.0004
F value	23.749	11.144
LSD 0.05	259.94	11.39

Means in a column with the same letter are not significantly different

Finally, it could be concluded that, the information based on these results may be help to prove that the biopesticides, particularly microbial pesticides can be used as an alternate control method in combating the pest. Its wide application as a biological pesticide could be taken up after exploring its toxicity and field trials.

The results assured that the 4th instar larvae were susceptible to *Bacillus thuringiensis kurstaki*. Furthermore, larval and pupal duration were harmfully affected only at higher concentrations. Laying eggs and hatchability% were significantly greatly affected by (Btk) treatment.

Acknowledgement

The author wish to express his deep thanks to Dr. Mohamed Fawzy for his kind help in this manuscript.

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تأثير المبيد الحيوي *Bacillus thuringiensis kurstaki* على النواحي الحيوية *Spodoptera littoralis* (Boisd.) لدودة ورق القطن

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الملخص العربي

تم إجراء التجارب لتقييم تأثير تركيزات مختلفة للمبيد الحيوي (Dipel 2x®) *Bacillus thuringiensis kurstaki* على العمر اليرقي الرابع ليرقات دودة ورق القطن، وقد وجد أن أعلى معدل للموت كان في أعلي تركيزين ٤٠٠٠ ، ٢٠٠٠ جزء في المليون بعد ٩٦ ساعة من المعاملة بينما كان أقل معدل للموت في التركيزات الأقل. قلت نسبة التعذير تدريجياً مع تزايد التركيزات كما قل معنوياً نسبة خروج الفراشات بزيادة التركيز، بينما لوحظ وجود فرق معنوي في مدة العمر اليرقي بين التركيز الأعلى والكنترول كما اختلفت مدة التعذير معنوياً بين كل المعاملات والكنترول ماعدا أقل تركيز، وكذلك كان هناك فروقاً معنوية في أوزان العذارى بين التركيزات المختلفة.

وجدت فروق معنوية بين التركيزات المختلفة بالنسبة لعدد البيض الموضوع وكذلك نسبة الفقس في البيض.

تدل النتائج المتحصل عليها على إمكانية استخدام المركبات الحيوية خصوصا الميكروبية منها كطرق مكافحة بديلة يمكن إدراجها في برامج المكافحة المتكاملة .