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Efficiency of the bioagent *Bacillus thuringensis Kurstaki* on the lesser cotton leafworm, *Spodoptera exigua* (Hb)

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ABSTRACT

The present experiments were carried out at the plant protection research station, Kaha, Qalubia Governorate, Egypt to study the efficiency of the bioagent *Bacillus thuringensis* var. *Kurstaki* against the 2nd instar larvae of the lesser cotton leafworm, *Spodoptera exigua* (Hb). Results revealed that the 2nd instar larvae were more susceptible to *B. thuringiensis* towards 4th instar larvae. Accumulative mortality percentage of the 2nd instar larvae of *S. exigua* treated with the different concentrations of *B. thuringeinsis kurstaki,* increased gradually with increasing the time elapsed after treatment. There was a stronger larvicidal effect on the 2nd instar larvae giving $2.889x10^6$ and $6.267x10^{13}$spore/ml for LC_{50} and LC_{90} respectively. The mean larval duration for both LC_{50} and LC_{90} of *B. thuringeinsis* var. *kurstaki* was elongated compared to the control treatment. Pupal duration was insignificantly affected when 2nd instar larvae of *S. exigua* were pretreated with LC_{50} and LC_{90} of *B. thuringeinsis kurstaki*. The percentage of pupation was significantly decreased than in case of the control for both treatments, respectively. On the other hand, the percentage of pupae that succeeded in surviving and reaching the adult stage decreased in case of treated larvae than in case of the control for both treatments.

INTRODUCTION

In Egypt, many pests of economic importance infest cotton throughout the season. Lepidopterous insects are the most destructive pests of the cotton plant. *Spodoptera exigua* attacks cotton plant and its danger increases from year to year where it causes considerable damage to the crop. It attacks cotton during March and April. *Bacillus thuringiensis* is the most widely used biopesticide among many other available methods to control these pests, Arunsiri *et al.* (2003).

MATERIALS AND METHODS

I-Rearing technique:

Different instars of larvae of *Spodoptera exigua* were collected from Kaha region Qualubia Governorate, Egypt. Larvae were kept in glass jars (9.5cm diameter, 15cm height) with castor bean oil leaves, *Ricinus communis* as a source of feed. Third or fourth instar larvae were reared individually in separate units as plopotes (5x5cm heights x weights) to avoid cannibalism and virus infection. Each plopote contains at the bottom a thin layer of saw dust. Fresh castor oil leaves were offered daily till pupation. The pupae were collected from the plopotes then placed in glass jars until adult emergence. Couples of female and male moths were kept in glass jars covered with muslin. In each jar, 2-3 strips of paper were placed for ovipostion. Food was provided daily as a cotton pad soaked with 10% honey solution. The eggs were collected and kept in clean glass jars till hatching.

2-Isolation and cultivation of bacteria

2-1-Isolation from soil:

Bacillus thuringiensis kurstaki was isolated from soil collected from different localities in Egypt. Isolation was according to Thiery and Francon (1997).

2-2-Isolation from dead and abnormal larvae:

Bacillus thuringiensis kurstaki isolated from dead and abnormal larvae of Family: Noctuidae related in feature to *S. exigua.* The technique used is according to Campell and Roberts (1971) for isolation of *B. t.* var. *kurstaki* from watery larvae (dead and abnormal).

3- Identification and purification of bacterial strain:

Identification of the strain is by means of examining the dishes with the naked eye. Different shaped colonies appear which may be raised or flat with different colors (white or creamy). To purify the strain of *Bacillus thuringiensis* var. *kurstaki,* single colonies were selected according to their morphological characteristics; being grayish white, opaque with entire edge and having a granular surface, as described by Heimpel (1967). A loop of pure bacterial colony is placed on a slide. A bacterial film is made, then stained with gram stain and examined under the light microscope. The bacterial strain was identified at the Plant Pathology Research Center, Giza, Egypt.

4 - Bacterial count:

The concentration of *B. thuringiensis* var. *kurstaki* was $2.7x10^{10}$ spores/ml, detected by serial dilutions test for the suspension of the selected isolates, in the laboratory of the Plant Protection Institute, Agriculture Research Center, Doki, Giza.

5 - Preparation of the suspension:

Five concentrations of *B. thuringiensis* var. *kurstaki* were used ($2.7x\ 10^4$- $2.7x\ 10^6$- $2.7x\ 10^8$- $2.7x\ 10^{10}$- $2.7x\ 10^{12}$ spores/ml) in distilled water.

6 - Pathogenecity tests:

Pathogenecity tests with *Bacillus thuringiensis* var. *kurstaki* were carried out at five concentrations ($2.7x\ 10^4$- $2.7x\ 10^6$- $2.7x\ 10^8$- $2.7x\ 10^{10}$- $2.7x\ 10^{12}$ spores/ml) against the second instar larvae of *Spodoptera exigua* by leaf dipping technique according to Makkar and El Mandarawy (1996). The larval mortality percentages were recorded after 24h to 9days. The mortality percentages of treated larvae were corrected against those of the control by Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected mortality} = \frac{\text{observed mortality\% - Control mortality\%}}{\text{100 - Control mortality\%}} \times 100$$

7 -Statistical analysis:

All data obtained from the above experiments were analyzed statistically using complete randomized blocks design. Student t-test as statistical analysis of the obtained data was used by COSTAT program, for Windows.

RESULTS AND DISCUSSION

The toxicity of the entomopathogenic bacteria *B. thuringiensis* var. *kurstaki* against larvae of *S. exigua* revealed that 2nd instar larvae were more susceptible to *B. thuringiensis* than 4th instar larvae. This shows that younger larvae are generally more susceptible than older larvae. These results coincide with the findings of Atwa *et al*, (1984), SungChae and YongGyun (2006).

1- Bioassay:-

Accumulative mortality percentage of the 2nd instar larvae of *S. exigua* treated with the different concentrations of *B. thuringeinsis Kurstaki*.

Data in Table (1) shows that the mortality percentages after 9 days were 30.81%, 47.49%, 54.49%, 61.38% and 67.82% for the concentrations $2.7x10^4$, $2.7x10^6$, $2.7x10^8$, $2.7x10^{10}$ and $2.7x10^{12}$ spore/ml, respectively.

Table1: Effect of *B. thuringiensis kurstaki* on the accumulated mortalities of the 2nd instar larvae:

Conc. Spore/ml.	Mortality % days after treatment				
	1st	3rd	5th	7th	9th
$2.7x\ 10^4$	17.24	17.24	24.14	30.81	30.81
$2.7x\ 10^6$	20.69	27.36	30.82	40.82	47.49
$2.7x\ 10^8$	27.59	37.59	44.49	47.82	54.49
$2.7x10^{10}$	31.03	34.36	44.71	61.38	61.38
$2.7x10^{12}$	31.03	37.7	41.16	54.49	67.82

The LC_{50} and LC_{90} as well as regression lines were calculated. *Bacillus thuringeinsis kurstaki* exhibited a stronger larvicidal effect on 2nd instars of *S. exigua* giving an LC_{50} and LC_{90} of $2.889x10^6$ and $6.267x10^{13}$ spore/ml, respectively, [Table2]. The slope values were 0.92 for 2nd instar larvae, proving the homogenecity of the treated insects [Fig.1].

Table 2: LC_{50} and LC_{90} values of *B. thuringiensis kurstaki* against 2nd instar larvae of *S. exigua*

Microbial control:	LC_{50}:	LC_{90}:	Slope
B. thuringiensis kurstaki:	2.889x10	$6.27x10^{1}$	0.92

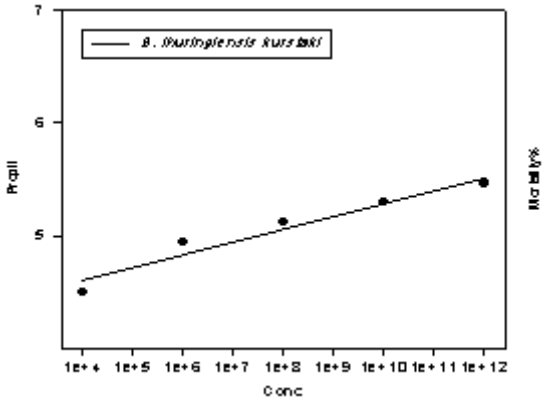

Fig.1: Toxicity regression line of *B. thuringiensis kurstaki* on 2nd instar larvae of *S. exigua.*

Our results clearly indicate that treatment of 2nd instar larvae of *S. exigua* by *B. thuringiensis* var. *kurstaki* induced bacterial disease and increased the larval mortality (Youngjin *et al*., 2002) .The toxicity of *B. thuringiensis* var. *kurstaki* against 2nd instars of *S. exigua* clearly indicates that there is a positive relationship between the concentrations of *B. thuringeinsis kurstaki* and toxicity. The LC_{50} and LC_{90} of *B. thuringeinsis kurstaki* recorded agree with GuiLan *et al*. (2002) and Namvar *et al.* (2003).

2- Biological studies:

Data in Table (3) show that the mean larval duration of *S. exigua* was 8.33 and 8.00 days for LC_{50} ($2.889x10^6$) and LC_{90} ($6.267x10^{13}$), respectively, compared to the control (7.67 days)**.** The mean pupal durations were 12.67 and 12.33 days for LC_{50} and LC_{90} of *B. thuringeinsis kurstaki*, respectively, compared to the control (12.00 days)**.** The percentage of pupation was 46.67 and 26.67% for LC_{50} ($2.889x10^6$ spore/ml) and LC_{90} ($6.267x10^{13}$spore/ml) of *B. thuringeinsis kurstaki*, respectively, compared to the control treatment which recorded 93.33%. The percentage of pupae that succeeded to survive and give adults was decreased when compared to the control group. The percentage of adult emergence treated by LC_{50} and LC_{90} of *B. thuringeinsis kurstaki* was 78.57 and 50% respectively, while the percentage of adult emergence in the control was 100%. The deformation percentage was 22.73%, while the percentage of adult emergence in the control was 100%**,** without any deformations.

The results show an increase in the larval duration when using the LC_{50} of *B. thuringeinsis kurstaki* which agrees with Salama *et al*. (1981), Hou and Chou (1993); Abd El-latif (2001) and Hatem (2006) who reported that the biological insecticide *Bacillus thuringiensis* gives high toxicity against *S. littoralis* treated as 4th instar larvae, and reported that the duration of the larval instars was increased, while the pupal duration was reduced. Generally, increasing of the concentrations of *B. thuringeinsis kurstaki* reduced adult emergence compared to the control.

Table 3: Effect of LC_{50} and LC_{90} of *B. thuringiensis kurstaki* on larval duration, Pupal duration, Pupation%, Adult emergence% and Deformation% of *S. exigua* treated as 2nd instar larvae.

LCvalues	Larval duration (Days±S.E)	Pupal duration (Days±S.E)	Pupation %	Adult emergence%	Deformation %
LC_{50}	$8.33±0.67^{ns}$	$12.67±0.89^{ns}$	46.67	78.57	-
LC_{90}	$8.00±0.01^{ns}$	$12.33±0.33^{ns}$	26.67	50	22.73
Control:	7.67±0.33	12.00±0.58	93.33	100	-

° Pupation% based on the number of treated larvae.

• Pupal-adult deformation.

ns**:** non significant, (student-t test).

REFERENCES

Abd El-Latif, E. M. A. (2001): Integrated pest Management for cotton in Dakahlia governorate. Ph. D Thesis, Fac. Agric, Mansoura univ. Cairo, Egypt.

Abbott, W. S. (1925): A method for computing the effectiveness of an insecticide. J. Econ. Entoml., 18: 265-267.

Arunsiri, T. A.; M. Suphantharika and U. Ketunuti (2003): Preparation of spray-dried wettable powder formulations of *Bacillus thuringiensis* based biopesticides. Journal of Economic Entomology, 96(2): 292-299.

Atwa, W. A.; M. A. Abdel-Rahman and A. Z. El-Sharkawy (1984): Effect of *Bacillus thuringiensis* Berliner on the biology of silkworm *Philosamia ricini* (boids.) (Lepidoptera: Saturiniidae). 5th Ann.Conf. Of the African association of Insect scientists. pp.13-21.

Campell R. I. and D. W. Roberts (1971): In vivo temperature-dependent development of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) mycosis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: pyralidae). J. inver. Patholo. 46, 305-311.

GuiLan, N.; Y. JianPing; Z. DaSheng and Y. ZhiMing (2002): Characterization of *Bacillus thuringiensis* WY-190 showing high performance in killing *Spodoptera exigua.* Chinese Journal of Biological Control. 2002; 18(4): 166-170.

Hatem, A. E. (2006): Comparacion de los efectos de insecticidas selectivos sobre el desarralloy repoduccion de *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Thesis dextoral vniversided de Cardoba.240.

Heimpel, A. M. (1967): A taxonomic key proposed for the species of crystalliferous bacteria .J. Invert. Pathol., 9: 364 – 375.

Hou, F. R. and Chou Tao-Mei (1993): Applications of *Bacillus thuringiensis* preparations against the diamondback moth, *Plutella xylostella* (L.), in Taiwan. In "The biopesticide *Bacillus thuringiensis* and applications in developing countries." Salama, S.H.; Morris, N. O. and Rached, E. (Eds.). pp.167–178.National Research Center, Cairo, Egypt and international Development Research Center (IDRC), Ottawa, Canada.

Makkar, A. W. and El-Mandarawy B. R. Monna (1996): Laboratory studies for increasing the efficacy of a bioinsecticide against *Spodoptera littoralis* larvae. Annals of Agric. Sci., Moshtohor, 34 (4), 1925-1934.

Namvar, P.; M. H. Safaralizadeh and A. A. Pourmirza (2003): Studies on the susceptibility of *Spodoptera exigua* (Hubner) larvae to *Bacillus thuringiensis* under greenhouse conditions. Journal of Science and Technology of Agriculture and Natural Resources. 2003; 7(1): 215-221.

Salama, H. S.; S. M. Foda; A. El-Shabrawy; M. Matter and M. Khalafallah (1981): Development of some lepidopterous cotton pests as affected by exposure to sublethal levels of endotoxins of *Bacillus thuringiensis* for different periods J. Invert. Pathol, 38: 220 – 229.

SungChae, J. and K. YongGyun (2006): Synergistic effect of *Xenorhabdus nematophila* K1 and *Bacillus thuringiensis* subsp. *aizawai* against *Spodoptera exigua* (Lepidoptera: Noctuidae). Biolo. Control; 39(2): 201-209.

Thiery, I. and Francon E. (1997): Manual of techniques in insect pathology. 1st Ed. Ch III pp 55-77, edited by Lacey A. L. , Academic press, Toronto.

Youngjin, P.; K. Kilho and K. Yonggyun (2002): A pathogenic bacterium, *Enterococcus faecalis*, to the beet armyworm, *Spodoptera exigua*. Journal of Asia Pacific Entomology. 5(2): 221-225.

ARABIC SUMMARY

كفاءة باسيلس ثورنجينسيس كمبيد حيوى على دودة ورق القطن الصغرى.

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2- معهد بحوث وقاية النبات – مركز البحوث الزراعية – الدقى

أجريت التجارب المعملية بمعهد بحوث وقاية النباتات والتجارب الحقلية بمحطة بحوث وقاية النباتات قها – قليوبية موسمي 2008 / 2009 بهدف دراسة كفاءة استخدام مسببات الامراض (البكتريا) على دودة ورق القطن الصغرى.

أظهرت النتائج ان تأثير البكتريا *باسيلس ثرنجينسيس*التى تم عزلها من التربة واليرقات المصابة بها كان أكثر تاثيراً على العمر الثاني ليرقات دودة ورق القطن الصغرى عن العمر الرابع.

كما أظهرت النتائج أن النسبة المئوية للموت للعمر الثاني قد زادت تدريجيّا بإستخدام التركيزات المختلفة من البكتريا، وقد اوضحت النتائج أن LC_{50} هو10^{6}x2.889 وLC_{90} هو10^{13}x6.27 جرثومة/ملليتر، وأدت المعاملة ب LC_{50} وLC_{90} إلى زيادة العمر اليرقي الثاني مقارنة بالضابط، ولم يتأثر عمر طور العذراء بإستخدامهما.

كما أظهرت النتائج ان النسبة المئوية للدخول فى طور العذراء قد تناقصت بمقدار 46.67% و26.67% عند المعاملة ب LC_{50} و LC_{90}على التوالى.