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ACUTE AND CUMULATIVE INSECTICIDAL ACTIVITY OF *Bacillus thuringiensis* var. aegyptiaca. ON SOME GROWTH CRITERIA OF *Spodoptera littoralis* (Boisd)

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ABSTRACT

A field trial was carried out at Sakha Agricultural Research Station, at Kafr El-Sheikh Governorate during 2002 season in order to evaluate the potency of *Bacillus thuringensis* on both the 2nd and the 4th larval instar of *S. littoralis*. Both of the acute and cumulative toxicity was evaluated. Moreover the effect of the tested compound on the pupation and adult emergence was evaluated. In all cases the 2nd larval instar was more sensitive to the tested compound. Moreover, the toxic effect of the tested biocide was instar dependent, 100 ppm had the superior in its toxic effect on the 2nd larval instar, the effect was vise versa with 4th larval instar. Regarding the effect of time after feeding, there are an obvious accumulative effects with all treatments either on the 2nd or with the 4th. As for larval instar period permeated after treatment to collect the treated leaves, the effect also was larval dependent the most toxic effect was recorded on the 2nd larval instar when larvae fed on leaves with treated 100 ppm and collected after 5 days from treatment. On the other hand, also mortality increased and reached 100% with some case required along period, and so this compound at any tested concentration can't used alone for controlling *S. littoralis* specially in severe infestation but help in achieving good and satisfactory results when combined with other practices of control within IPM programme.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) causes serious damage to most of vegetables and field crops.

Chemical control in the field faced serious difficulties because this insect had developed resistance to most of the commercial pesticides beside the harmful effects on the environment. The environmental hazards of insecticides used are still controversial (Evarts, 1990). So, increasing emphasis aimed to establish alternative means for controlling the cotton leafworm *S. littoralis* was stated by many investigators. Entomologists try to develop new approaches in this respect in order to replace wide spectrum toxicant with other less or non poisonous control agents. It is well known that one of the promising approaches is the use of microbial control agents. Those agents are receiving a great deal of consideration application, as possible safer substitutes and as a mean of reducing chemical insecticides (Hamid, 1991 and Zaki, 1991). The development of the microbial control agent *Bacillus thuringiensis* for possible use against *S. littoralis* has occurred during the last two decades (Abdeen *et al.*, 1986, Lee Susan *et al.*, 1990, Abou Bakr *et al.*, 1993, Abdel-Hafez *et al.*, 1994, Abdel-Halim and Sawsan, 1997, Romeilah and Abdel-Meguid, 2000, Sondos *et al.*, 2000 and Abdel-Kerim and

Tarek, 2002). Efficacy of *B. thuringiensis* had been tested against a wide variety of Lepidopterus insect pests particularly *S. littoralis* within integrated pest management programme, either alone or with conventional insecticides in order to minimize pesticide applications (Ali El-Moursy *et al.*, 2000).

The aim of the current study is to evaluate the efficiency of *Bacillus thuringiensis* as a biocide. On the second and fourth instar larvae of *S. littoralis* (either the acute or the latent effects), as well as the effect of this compound on the pupation, adult, emergence, and insect numbers in the second generation was also evaluated.

MATERIALS AND METHODS

Tested insect:

Laboratory strain of the *S. littoralis* larvae was used in this study, which obtained as a second generation of the field strain. This strain was reared in the laboratory as described by El-Defrawy *et al.* (1964) at Sakha Agricultural Research Station.

Bioinsecticide (Agreen):

A selective bacterial insecticide containing 3200 IU of *B. thuringensis* var. aegyptica/mg of productive.

Bioassay :

Field experiments were carried out in Sakha Agricultural Research Station at Kafr El-Sheikh Governorate. In this study cotton Giza 86 was cultivated on March 2002 season. The treatments were arranged in a completely randomized block design with four replicates of half feddan each. Cotton plants were subjected to normal agricultural practices. Spray was performed on October after noon) using a knapsack sprayer with one nozzle (mod CPS) in which concentrations of 100, 200 and 400 ppm were prepared using 200 L water/feddan. According to the protocol of the Ministry of Agriculture for bioassaing biocides. Samples (cotton leaves) from treated plants were collected at 0, 1, 2, 3, 4 and 5 days after treatment feeding with larvae treated leaves was performed for only 24 hours after starvation period of about 24 hours, then normal feeding on untreated leaves was conducted. Individuals in the check groups were fed on untreated leaves along the experimental period. For every concentrations 50 larvae in 5 replicates of ten larvae each were used under laboratory conditions of 27 ± 1°C and 70 ± 2 %R.H. Died individuals were recorded and removed daily and percent of moralities after 3, 5.7 days and during the whole period was calculated according to Abott's formula (1925). Moreover, the newly formed pupae and adults emergence were recorded daily, as well as the percentages of pupation and adults emergences were also calculated .

RESULTS AND DISCUSSION

Acute and cumulative effect of *B. thuringiensis* on *S. littoralis* larvae:

Data in Tables (1) & (2) summarized the efficiency of *B. thuringiensis* as a biocide on the 2nd and 4th larval instar of *S. littoralis*. Data revealed that in general the 2nd larval instar was more sensitive to the tested compound that T than 4th one. In other words, comparing the percentage of mortality obtained with the two instars at any intervals and concentrations always reflected the aforementioned trend of results. In term of figures comparing the percent of mortalities during the larval period when the 2nd larval instar fed on treated leaves for 24 hrs. of intervals of 0, 1, 2, 3, 4 and 5 days they found to be 96, 80, 80, 100 and 96 while corresponding values with the same concentration were 56, 46, 30, 52, 40 and 54 at the same periods respectively. These results may be due to the immune systems which may developed with growth and can faced the effect of the used biocide partially.

It is of interest that the relation between the effect and the concentration was instar dependent. In other words while 100 ppm of the used biocide was superior in its toxic effect on the second larval instar wit most cases or at least its effect was nearly the same recorded with other tested concentrations, the case was vice versa with the 4th larval instar.

Regardly the effect of time after feeding and the resulted effect. In general it can concluded that with all treatments there are an obvious accumulative effects with time in all treatments either on the 2nd or 4th larval instar but this cumulative effect was more noticeable with the 2nd than the 4th instar.

As for the relation between the effect of the period permitted after treatment to collect the treated leaves, the effect also was larval instar dependent. While the effect increased with days after treatment with the 2nd instar, on the other hand, the recorded effect was slightly changed with the same criteria.

However all the exhibited differences between the effect recorded with the two tested larval instars may be attributed as referred before to differences in the immune system of the two instars or other biochemical or physiological differences which effect the response to the tested compound.

In general the resulted data reflected that the most toxic effect was recorded on the 2nd instar when larvae fed on leaves treated with 100 ppm and collected after 5 days from treatment. It can concluded that in all cases the current biocide could be effective in certain cases but the results are not satisfactory to be recommended alone for controlling *S. littoralis* larvae since the resulted effect on the 4th instar ranged between slight to moderate. On the other hand, also the observed effect on the 2nd larval instar increased to reach 100% with some cases required a long period and so it is not suitable or effective completely with sever infestation. The obtained results was in harmony with (Abou Bakr *et al.,* 1993, Ali El-Moursy *et al.,* 2000 and Sondos *et al.,* 2000), they emphasized that there was a difference in the sensitivity of a given pest as well as the susceptibility of the treated instars.

Table (1): Bioinsecticidal activity (acute and accumulative) of *B. thuringiensis* on the 2nd larval instar of *S. littoralis* feeding on cotton leaves collected at different intervals from treatment.

Concentration (ppm)	Days of collecting treated leaves after spraying with different concentration																			
	Zero				1st				2nd				3rd				4th				5th			
	% mortality after :																			
	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T
100	84	84	86	96	36	56	64	80	52	64	68	80	70	94	100	100	42	52	62	98	58	90	96	96
200	48	50	54	80	48	58	72	86	12	16	24	56	46	72	100	100	22	36	64	92	54	92	98	98
400	56	56	56	68	36	58	62	80	22	26	40	74	56	78	100	100	24	29	56	92	52	90	90	100

T = % mortality during larval period from feeding

Table (2): Bioinsecticidal activity (acute and cumulative) of *B. thuringiensis* on the 4th larval instar of *S. littoralis* feeding on cotton leaves collected at different intervals from treatment.

Concen-tration (ppm)	Days of collecting treated leaves after spraying with different concentration																			
	Zero				1st				2nd				3rd				4th				5th			
	% mortality after :																			
	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T	3 days	5 days	7 days	T
100	30	40	44	56	26	28	28	46	4	10	12	30	8	14	36	52	8	20	40	40	16	44	54	54
200	24	34	36	50	0	0	0	8	8	24	36	62	4	12	26	36	10	16	38	38	14	40	46	46
400	28	42	52	78	10	10	20	34	10	24	28	50	6	18	26	46	14	24	54	54	18	40	54	54

T = % mortality during larval period from feeding.

William and Whalon, 1992 reported that *B. t.* produces a number of insect toxins, the most distinctive of which are protein crystals formed during spor/ulation, following activation, these toxins bind with high affinity to receptors and on the mid gut epithelium. Mackay (1988) emphasized that resistance may be relatively specific to the toxin used in selection, and it can be managed with insect age.

Effect of *B. thuringiensis* on pupation and adult emergence of *S. littoralis*:

Percentages of reduction of both pupation and adult emergence comparing with those obtained when, larvae were fed on untreated cotton leaves were calculated and the obtained data are presented in Tables (3) and (4).

In general all treatments led to a reduction in the percentage of pupation and adult emergence in ratios differed with time, concentration and larval instar but the reduction percentage of both pupation and adult emergence were recorded when the 2nd larval instar fed on leaves collected after 3 days from spraying with any concentration.

It is obvious that as previously observed the effect was more pronounced when the experiments conducted on the 2nd larval instar than that of the 4th one. This result reemphasized the aforementioned interpretation that the 2nd larval instar is more sensitive to the tested compound due to biochemical, physiological and/or immune factors. In term of figures for example percentages of reduction of pupation obtained with the 2nd instar (at 100 ppm level) ranged from 75.75 to 100 comparing with 15.15 to 68.48 in the case of the 4th instar at the same level of concentration but the reduction percentage of both pupation and adult emergence was higher when the 2nd larval instar fed on leaves collected after 3 days from spraying with any concentration. The same trend of results was found with the percentage of adult emergence. At the same level of concentration (100 ppm), the calculated reduction of adult emergence percentage of reduction ranged from 79.39 to 100 when treatment performed on the 2nd larval instar comparing with 28.28 to 73.21 with the 4th larval instar.

Table (3): Percentages of reduction of pupation and adult emergence of *S. littoralis* after 2nd instar larvae feeding on cotton leaves collected at different intervals from treatment with *B. thuringiensis*.

Concentration ppm	Days of collecting treated leaves after spraying											
	Zero		1st		2nd		3rd		4th		5th	
	%											
	1	2	1	2	1	2	1	2	1	2	1	2
100	80.61	83.45	75.75	90.30	75.75	80.69	100	100	88.85	84.83	90.30	93.10
200	75.76	91.72	83.03	90.90	51.52	75.17	100	100	46.67	48.97	61.21	61.38
400	61.21	69.66	73.33	83.45	68.48	90.30	100	100	90.30	94.48	100	100

(1) = % of reduction of pupation than control
(2) = % of reduction of adult emergence than control.

Table (4): Percentages of reduction of pupation and adult emergence of *S. littoralis* after 4th instar larvae feeding on cotton leaves collected at different intervals from treatment with *B. thuringiensis*.

Concentration ppm	Days of collecting treated leaves after spraying											
	Zero		1st		2nd		3rd		4th		5th	
	%											
	1	2	1	2	1	2	1	2	1	2	1	2
100	46.67	66.88	68.48	55.85	15.15	31.03	41.82	44.83	27.27	28.28	44.04	73.20
200	39.39	70.41	9.09	17.24	53.93	64.14	22.42	31.03	36.97	64.14	36.97	61.38
400	73.22	83.45	20.0	11.72	39.39	47.59	34.55	44.83	51.52	75.17	44.25	58.62

(1) = % of reduction of pupation than control
(2) = % of reduction of adult emergence than control.

Regarding to the relation between periods permitted after treatment to collect leave and the resulted effect on pupation and adult emergence, it is clear that these is no steady relation either with the 2nd or the 4th larval instar. In other words there is an obvious fluctuation in the resulted reduction percentage of pupation at 100 ppm level were 80.61, 75.75, 75.75, 100, 88.85 and 93.10. The obtained results with the 4th larval instar were 46.82, 68.48, 15.15, 41.82, 22.77 and 44.24 for pupation. The corresponding values for adult emergence were 66.89, 55.86, 31.03, 44.83, 28.28 and 73.22, respectively.

As for the effect of concentration, also there is no constant relation between the effect and correlation either with the 2nd or the 4th larval instar.

As a net result all treatments resulted in a reduction of pupation and adult emergence but these results revealed that as previously mentioned this effect required time a long the life period to be detected. And so, this compound at any tested concentration can't used alone for controlling *S. littoralis* larvae, but help in achieving good and satisfactory results when combined with other practices of control or insecticides within IPM programme.

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التأثير الفورى والتراكمى لبكتريا باسيلس ثورنجنسيز على بعض خـواص نمـو دودة ورق القطن

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أجريت التجربة الحقلية فى مزرعة محطة البحوث الزراعية بسخا خلال موسـم ٢٠٠٢ بغرض تقييم كفاءة المستحضر البكتيرى باسيلس ثورنجنسيز على كل من العمرين الثانى والرابع لدودة ورق القطن (فورى وتراكمى) بالاضافة الى التأثير على نسـبة التعـذير ونسـبة خـروج الفراشات. وقد أوضحت النتائج أن العمر الثانى كان أكثر حساسية من العمر الرابع ، كما وجد أن سمية التركيز ١٠٠ جزء فى المليون أعطت اعلى نسبة أبادة فى العمر الثانى بينما كـان التـأثير معتدل أو طفيف على العمر الرابع. وقد وجد تأثير تراكمى واضح فى سمية المستحضر على كلا العمرين مع طول الوقت. كما أظهرت النتائج أن تغذية اليرقات بورق معامل لأيام مختلفة اعطـت نسبة موت ١٠٠% فى يرقات العمر الثانى المغذاة على ورق معامل بعد خمس أيام مـن الـرش. وبصفة عامة وجد أن المستحضر يعطى نسبة موت تصل الى ١٠٠% ولكن بعد وقـت طويـل. ولهذا لا يصلح هذا المستحضر كوسيلة لمقاومة دودة ورق القطن باستخدام أى تركيز وخاصة فى حالة الاصابة الوبائية. ولكن ينصح باستخدامه كوسيلة فعالة وآمنة ضمن برنامج مكافحة متكامل.