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EVALUATION OF MICROBIAL CONTROL OF THE COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.) ON TOMATO PLANTS UNDER LABORATORY CONDITIONS

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ABSTRACT

Laboratory experiments were carried out to evaluate the effect of some microbial agents against the 2nd and 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) on tomato plants. Mortality percent of the 2nd and 4th instar larvae of *S. littoralis* increased with increasing duration after treatment with *Bacillus thuringiensis kurstaki*, *S. littoralis* NPV and *Beauveria bassiana*, as well as, increasing the concentration used. Positive correlation in mortality percentage of *S. littoralis* 2nd and 4th instar larvae with tested concentrations of *B. thuringiensis kurstaki*, whereas the higher concentration revealed higher mortality. Also, data indicated that the 2nd instar larvae of *S. littoralis* was more susceptible to *B. thuringiensis kurstaki*, *S. littoralis* NPV and *B. bassiana* than the 4th instar larvae.

INTRODUCTION

Vegetables are important components of the human diet since they provide essential nutrients required for most of the reactions occurring in the body. A high intake of vegetables (five or more servings per day) has been encouraged not only to prevent consequences due to vitamin deficiency but also to reduce the incidence of major diseases such as cancer, cardiovascular diseases and obesity. Like other crops, vegetables are attacked by insect pests during production and storage leading to damages that reduce the quality and the yield Hamam (2003). In order to reduce the loss and maintain the quality of vegetables, pesticides are used together with other pest management techniques during cropping to control pests. The use of pesticides have increased because they have rapid action, decrease toxins produced by food infecting organisms and are less labour intensive than other pest control methods. However, the use of insecticide, during production often leads to the presence of pesticide residues in vegetables after harvest Eman *et al* (1988). The presence of pesticide residues is a concern for consumers because pesticides are known to have potential harmful effects to other non-targeted organisms than pests. The major concerns are their toxic effects such as interfering with the reproductive systems and foetal development as well as their capacity to cause cancer and asthma (Gilden *et al.,* 2010).

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is one of the most serious and destructive pests, to cotton and many other field and vegetable crops. It is known that, *S. littoralis* the most important insect in agricultural systems in Egypt, especially vegetables.

The effect of natural compounds on *S. littoralis* have attracted the attention of many workers of the world including Egypt (Moawad , *et al.* 1996, Emam 1998, Reyad 2001 and Hamam 2003)

The present work aims to investigate the effect of some microbial agents against the cotton leafworm, *S. littoralis* on tomato plants.

MATERIALS AND METHODS

Insect rearing:

The cotton leafworm, *Spodoptera littoralis* (Boisd.) larvae were obtained from the laboratory culture of Plant Protection Research Institute Dokki, Giza. Newly hatched larvae from a single egg batch were introduced to pots containing the synthetic diet by using a soft hairbrush then transferred to plastic "poly pots" with dimensions of 5cm deep and 10cm internal diameter, containing the diet with 0.5 cm thick, which on cooling gave a very smooth surface. Ten larvae were reared in each plastic cup until reaching pupae. Freshly emerged moths were coupled in small glass jars provided with a filter paper as a site for egg-laying, and supplied with 10% sugar or honey solution in a piece of soaked cotton wool. All experiments were carried out under laboratory constant conditions of temperature 26±2ºC and 60±5% RH.

The following microbial agents were tested:

a. Biovar, an entomopathogenic fungi (3200 viable spore/mg), containing the fungus *Beauveria bassiana*. It was applied at a rate of 200 mg/100 liter of water.
b. Protecto, W- P based on *Bacillus thuringiensis subsp. Kurstaki* (32x 10^3 I. U/mg). Active ingredient 9.4% inert ingredient (Carrier) 90.6%.
c. Viroset, active Ingredient: *Spodoptera littoralis* Nuclear Polyhedrosis Virus .

Virulence of entomopathogens on *S. littoralis*:

To study the virulence of entomopathogens on the cotton leafworm, *S. littoralis*, newly hatched larvae from a single egg batch was introduced to pots containing treated synthetic diet. Then the 2^{nd} and 4^{th} instar larvae were selected carefully to be used in this study. The tested larvae were almost similar in the weight and size.

Leaf dipping technique:

To evaluate the entomopathogen virulence against the 2^{nd} and 4^{th} instar larvae of *S. littoralis*, leaf dipping technique was used. Tested entomopathogen concentrations were prepared as follows:-

Beauveria bassiana was prepared at 32 x 10^3 ,16 x 10^3 , 8 x 10^3 , 4 x 10^3 , and 2 x 10^3 conidia /ml. *B. thuringeinsis kurstaki* was prepared at concentrations of 64 x 10^3 , 32 x 10^3 , 16 x 10^3 , 8 x 10^3 and 4x 10^3 CFU /ml. while *S. littoralis* nuclear polyhedrosis virus was prepared at concentrations of 28 x 10^3 , 14 x 10^3 , 7 x 10^3 , 3.5 x 10^3 and 1.75x 10^3 PIB /ml . Second and fourth instar larvae were exposed for 48h to treated tomato leaves, *Lycopersicom esculentum* by using dipping technique with conidial suspension. Four replicates were used for tested concentration of assayed entomopathogens. Each replicate in different treated concentrations and check contain twenty and ten larvae of both the 2nd and 4th instar, respectively. After preparation of tested concentrations of the three pathogens, tomato leaves were dipped in each concentration separately and left for air dryness. Then the treated leaves were offered to *S. littoralis* larvae for feeding for 48 hours. After feeding period, the mortality percentage was recorded and treated tomato leaves were replaced with another clean leaves for feeding whereas mortality counts were recorded every two days for ten days.

Statistical analysis

Cumulative mortality percentages were corrected using Abbott's formula (Abbott, 1925). The IC_{50} and IC_{90} values were calculated according to method of (Finney, 1971). Probit analysis was used to analyze data from bioassay experiments, such as the proportions of insects killed by several concentrations of an insecticide or at several time intervals at one or more concentrations of an insecticide. Results of Probit analysis are reported typically as a concentration or time required to kill a certain proportion of the test insects (for example, LC_{50} , LT_{50}); the slope and intercept of the regression line of the Probit-transformed data were also reported.

In the equation of a straight line (when the equation is written in the useful form):

$$Y= a + bX$$

The slope is the number "b" multiplied on X, "a" is the Y intercept, where the line crosses the Y-axis

Y = Mortality percentage

X = Concentration or time

RESULTS AND DISCUSSION

Effect of *Bacillus thuringiensis kurstaki* on 2nd and 4th larval instars:

Data in Table (1) showed that mortality percent of the 2nd instar larvae of *Spodoptera littoralis* increased with increasing durations after treatment of *B. thuringiensis kurstaki*. After the 2nd day of application, mortality percent was 40.25% at a concentration of $64x10^3$ CFU/ml, but it was 28.51, 12.75, 10.23 and 9.51% at concentrations 32,16,8 and $4x10^3$ CFU/ml, respectively.

There was positive correlation in mortality percentage of *S. littoralis* 2nd instar larvae with tested concentrations of *B. thuringiensis kurstaki*, whereas the higher concentration revealed higher mortality. For example, at $64x10^3$ CFU/ml, mortality percentage ranged between 40.45 and 95.79%, but it ranged 28.51- 84.42%, 12.75-61.65%, 10.23-33.76% and 9.51-12.83% at concentrations of 32,16,8 and 4x103 CFU/ml, respectively (Table, 1).

Data in Table (2) indicated that the 4th instar larvae of *S. littoralis* was less susceptible to *B. thuringiensis* than the 2nd instar larvae. Mortality percentage of the 4th instar larvae increased with increasing the duration after treatment as well as increasing the concentration used of *B. thuringiensis*.

After the 4th day of treatment, mortality percent was only 18.58 % at the highest concentration, and 4.11 % at the lowest one. Six days post treatment, mortality percentage at a concentration of $64x10^3$ CFU/ml wad 22.95%, but it was 19.35%, 12.04%, 9.75% and 7.25% at concentrations of 32, 16, 8 and $4x10^3$ CFU/ml, respectively. Mortality percentage ranged between 8.66 and 39.67% after the 8th day of treatment, while it ranged between 10.59% and 48.15% after 10 days (Table 2).

These results agree with those recorded by Naveen *et. al*. (2006) who found that the mortality of *Heliothis armigera* was higher in the 2nd instar than in the 4th instar larvae in all treatments. Similar results were obtained in case of *Spodoptera exigua* under both laboratory and greenhouse conditions.

Table (1): Corrected accumulative mortality percentage of S. *littoralis* 2nd instar larvae fed on tomato leaves immersed in different concentrations of *B. thuringiensis kurstaki* .

Conc. (CFU/ml) X 10^3 ml	Accumulative mortality% indicated days after treatment				
	2	**4**	**6**	**8**	**10**
4	9.51	8.25	12.68	9.38	12.83
8	10.23	12.00	16.78	29.02	33.76
16	12.75	20.23	29.85	58.40	61.65
32	28.51	39.75	70.65	83.57	84.42
64	40.45	65.54	82.72	65.92	95.79

Table (2): Corrected accumulative mortality percentage of S. *littoralis* 4th instar larvae fed on tomato leaves immersed in different concentrations of *B. thuringiensis kurstaki* .

Conc. (CFU/ml) X 10^3 ml	Accumulative mortality% indicated days after treatment				
	2	**4**	**6**	**8**	**10**
4	0.00	4.11	7.25	8.66	10.59
8	0.00	6.53	9.75	14.91	15.85
16	0.00	10.85	12.04	15.62	19.22
32	0.00	15.71	19.35	22.92	31.15
64	0.00	18.58	22.95	39.67	48.15

Effect of *Spodoptera littoralis* nuclear polyhedrosis virus (SlNPV) on 2nd and 4th instar larvae:

Data in Table (3) showed that the mortality percent of the 2nd instar larvae of *S. littoralis* increased with increasing duration after treatment of *S. littoralis* larvae with NPV. After the 2nd day of application, *S*. *littoralis* NPV resulted in 20.54%, 18.50%, 8.75%, 6.23% and 3.51% mortality at concentrations of 28, 14, 7, 3.5 and $1.75x10^3$ PIB/ml, respectively. Mortality percentage was lower than 50% even at the highest concentration till the 6th day after treatment. It ranged 8.45-32.34% and 9.60-42.62% after the 4th and 6th days of treatment, respectively.

There was positive correlation in mortality percentage of *S. littoralis* 2nd instar larvae with tested concentrations of *S. littoralis* NPV, whereas the higher concentration revealed higher mortality.

Eight days post treatment the 2nd instar larvae of *S. littoralis* fed on treated tomato leaves suffered, mortality of 57.21% at a concentration of

28x10^3 PIB/ml, but it was 44.28%, 31.93%, 21.32% and 13.12% for the at concentrations of 14, 7, 3.5 and 1.75x10^3 PIB/ml, respectively. Mortality percentages were 81.30%, 66.72%, 49.02%, 31.52% and 17.41% for *S. littoralis* at the concentrations of 28, 14, 7, 3.5 and 1.75x10^3 PIB/ml, respectively (Table 3).

Data in Table (4) indicated that mortality percentage of the 4^{th} instar larvae increased with increasing the duration after treatment, as well as, increasing the concentration used of *S. littoralis* NPV. Mortality percentages after the 4^{th} day of treatment were 7.40%, 6.11%, 4.28%, 2.15% and 0.0% at concentrations of 28, 14, 7, 3.5 and 1.75x10^3 PIB/ml, respectively. After the 6^{th} day of treatment, mortality percentage ranged between 3.50% and 18.82%, while it ranged 6.30- 27.08% and 5.95-32.57% after the 8^{th} and 10^{th} days of feeding the 4^{th} instar larvae of *S. littoralis* on treated tomato leaves, respectively (Table 4).

Thus, it could be concluded that the 2^{nd} instar larvae of *S. littoralis* are more susceptible than the 4^{th} instar. Cumulative mortality percentage increased with increasing durations post treatment for both the 2^{nd} and 4^{th} instars. Higher concentration usage of *S. littoralis* NPV resulted in a higher larval mortality.

These results agree with those obtained by Mabrouk *et al.* (1996) who investigated the efficacy of different isolates of *S. littoralis* nuclear polyhedrosis virus (SLNPV). They recorded a positive correlation between the concentrations of the pathogen and the percentage of larval mortality. Second instars were more susceptible to NPV than 4^{th} instars , in general, mortality was low during the first three days following the NPV treatments, but increased gradually thereafter.

Table (3): Corrected accumulative mortality percentage of *S. littoralis* 2^{nd} instar larvae after feeding on tomato leaves immersed in a preparation of nuclear polyhedrosis virus.

Conc. (PIB/ml) X 10^3 ml	Accumulative mortality% indicated days after treatment				
	2	**4**	**6**	**8**	**10**
1.75	3.51	8.45	9.60	13.12	17.41
3.5	6.23	10.23	14.80	21.32	31.52
7	8.75	13.55	17.05	31.93	49.02
14	18.50	23.00	37.45	44.28	66.72
28	20.54	32.34	42.62	57.21	81.30

Table (4): Corrected accumulative mortality percentage of *S. littoralis* 4^{th} instar larvae after feeding on tomato leaves immersed in a preparation of nuclear polyhedrosis virus.

Conc. (PIB ml) X 10^3 ml	Accumulative mortality% indicated days after treatment				
	2	**4**	**6**	**8**	**10**
1.75	0.00	0.00	3.50	6.30	5.95
3.5	0.00	2.15	6.12	9.79	9.99
7	0.00	4.28	9.54	14.28	15.74
14	0.00	6.11	13.35	20.07	23.32
28	0.00	7.40	18.82	27.08	32.57

Effect of entomogenous fungus, *Beauveria bassiana* on 2nd and 4th instar larvae:

Data in Table (5) show that mortality percent of the 2nd instar larvae of *S. littoralis* increased with increasing the duration after treatment of *B. bassiana*. Mortality percentage was 10.11% at a concentration of $32x10^3$ CFU/ml after the 2nd day of application, but it was 22.24%, 30.25%, 44.09% and 64.47% after 4, 6, 8 and 10 days of treatment at the same concentrations, respectively. Mortality percentage ranged between 9.12% and 53.31% at concentration of $16x10^3$ conidia.ml, while it ranged from 6.60 - 41.88%, 5.20 - 31.10%, and 3.21 - 21.74% at concentrations of 8, 4 and $2x10^3$ conidia/ml, respectively.

There was positive trend in mortality percentage of *S. littoralis* 4th instar larvae with tested concentrations of *B. bassiana*, as the higher concentrations resulted in higher mortalities. For example, at $32x10^3$ condia/ml, mortality percentage ranged between 8.50% and 38.12%, but it ranged from 6.15-30.18%, 5.50-22.15%, 4.25-14.00% and 0.00%-10.65% at concentrations of 16,8,4 and $2x10^3$ conidia/ml, respectively (Table 6). After the 4th and 6 days of treatment, mortality percent was lower than 20% even at highest concentration. It ranged between 0.00% and 8.50% in the 4th day post treatment, and from 3.65 to 18.62% in the 6th day after feeding of *S. littoralis* larvae on treated tomato, respectively. Generally, the 4th instar larvae of *S. littoralis* reflected lower susceptibility (or more tolerance) towards *B. bassiana* as compared to the 2nd instar larvae.

Table (5): Corrected accumulative mortality percentage of S. *littoralis* 2nd instar larvae fed on tomato leaves immersed in different concentrations of *B. bassiana*.

Conc. (conidia/ml) X 10^3 ml	Accumulative mortality% indicated days after treatment				
	2	4	6	8	10
2	3.21	6.20	10.11	19.04	21.74
4	5.20	8.00	14.28	24.37	31.10
8	6.60	12.25	19.60	30.41	41.88
16	9.12	19.50	25.44	37.08	53.31
32	10.11	22.24	30.25	44.09	64.47

Table (6): Corrected Accumulative mortality percentage of S. *littoralis* 4th instar larvae fed on tomato leaves immersed in different concentration of *B. bassiana* .

Conc. (condia/ ml) X 10^3 ml	Accumulative mortality% indicated days after treatment				
	2	**4**	**6**	**8**	**10**
2	0.00	0.00	3.65	5.25	10.65
4	0.00	4.25	7.50	10.50	14.00
8	0.00	5.50	9.10	12.80	22.45
16	0.00	6.15	12.16	18.50	30.18
32	0.00	8.50	18.62	25.60	38.12

Reviewing above mentioned results, it could be concluded that the 2nd instar larvae of *S. littoralis* are more susceptible to *B. bassiana* than the 4th instar larvae. This difference of virulence against larval instars depends on instar response difference due to it's integument composition and fungus ability to penetrate the cuticle layer. Furthermore, it is known that early instars are the most susceptible, with percent mortality ranging up to 100 depending on the dosage and the isolate (Feng, *et. al.* 1985).

Potency of entomopathogens against the 2nd and 4th instar larvae of *S. littoralis*

Data in Table (7) showed Potency of entomopathogens against the 2nd and 4th instar larvae of *S. lttoralis* after 10 days post treatment with treated tomato leaves using leaf dipping technique expressed as LC_{50}, and LC_{90}. It clear from data the 2nd instar larvae was more susceptible to different entomopathogens than the 4th instar larvae of *S. lttoralis.*

For the 2nd instar larvae *S. lttoralis* NPV was the most effective followed discendingly by *B. thuringiensis* and *B. bassiana*, respectively. The LC_{50} value was 7.26×10^3 PIB/ml for *S. littoralis* NPV, while it was 12.01×10^3 CFU/ml and 26.20×10^3 conidia/ml for *B. thuringiensis* and *B. bassiana*, respectively

Regarding the 4th instar larvae *B. thuringiensis* was the most effective followed discendingly by *S. lttoralis* NPV and *B. bassiana*, respectively. The LC_{50} value was 83.47×10^3 conidia /ml for *B. thuringiensis*, while it was 86.79×10^3 PIB /ml and 125.41×10^3 conidia /ml for *S. lttoralis* NPV and *B. bassiana*, respectively

Reviewing the above mentioned results, it appear that the potency of pathogen differ according to the larval instar of *S. littoralis*. While the 2nd instar larvae were more susceptible to the three tested pathogens than the 4th instar larvae. Furthermore, efficacy the same pathogen on larval instars of insect differ from instar to the other. This difference depending on different factors. For example, in the case of *B. thuringiensis* because of their peptidic nature and insecticidal activities, effect of midgut pH on insecticidal protein soulubility, binding success of Cry1Ab toxin to larval brush border membrane vesicles (BBMV) (Hofmann, *et. al*. 1988 Aronson, *et. al*. 1991; Tabashnik , *et. al.* 1994; Grochulski, *et. al*. 1995; Tang, *et. al.* 1996; Soberon, et. al. 2000; Oppert, *et. al*. 1997).

Tacking into account the potency of S. littoralis NPV, it is clear that the pathogenicity decreased with increasing larval instars. This agree with El-Saadany *et al*. (1992b). They indicated that high larval density method was superior to the Oxford method as a means of infecting larvae of *S. littoralis*. The percentage mortality of heavy larvae was less than that of lighter larvae and the net yield of PIBs was considerably higher in older larvae. Treating larvae individually resulted in higher net yields of PIB compared with the Oxford method.

As for *B. bassiana*, The difference of virulence against larval instars depends on instar response difference due to it's integument composition and fungus ability to penetrate the cuticle layer. Furthermore, it is known that first instars are the most susceptible, with percent mortality ranging up to 100% depending on the dosage and the isolate (Feng, *et. al.* 1985).

Table (7): LC_{50}, LC_{90}, Confidence Limite and Slope Values of *B. thuringiensis kurstaki*, *S. littoralis NPV* and *B. bassiana* after feeding the 2nd and 4th instar larvae of *S. littoralis* on treated castor bean leaves for 48 hours at indicated days after treatment

Days after treatments	LC_{50} x 10^3	LC_{90} x 10^3	Confidence Limit		Slope ± SE	Intercept (a) ± SE
			Lower x 10^3	Upper x 10^3		
2nd instar larvae						
B. thuringiensis	12.01	41.56	3.19	30.18	2.54±0.54	2.43±0.70
B. bassiana	26.20	572.65	16.99	50.60	3.64±0.29	3.64±0.28
S. littoralis NPV	7.26	50.80	5.41	9.81	3.69±0.22	1.52±0.24
4th instar larvae						
B. thuringiensis	83.47	1466.99	46.31	335.88	1.02±0.25	3.02±0.34
B. bassiana	125.41	2973.44	59.16	1083.48	0.93±0.25	3.04±0.34
S. littoralis NPV	86.79	2150.34	34.64	1838.3	0.92±0.27	3.21±0.28

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تقييم المكافحة الميكروبية لدودة ورق القطن على نباتات الطماطم تحت الظروف المعملية

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تم دراسة تأثير المعاملة بثلاثة مستحضرات من الممرضات الحشرية وهي بكتيريا الباسيلس،وفيروس البوليهيدروليسيس، وفطر البيوفاريا علي العمر اليرقي الثاني والرابع لدودة ورق القطن علي نباتات الطماطم. أوضحت النتائج إرتفاع نسبة موت العمر اليرقي الثاني والرابع لدودة ورق القطن بزيادة الفترة الزمنية بعد المعاملة وبزيادة تركيز الممرضات الحشرية الثلاث. كما أوضحت النتائج أيضا أن العمر اليرقي الثاني أكثر حساسية من العمر اليرقي الرابع للمركبات الثلاث المختبرة .

قام بتحكيم البحث

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