EFFICACY OF SOME BIOLOGICAL AND CHEMICAL INSECTICIDES ON THE COTTON LEAF WORM, *SPODOPTERA LITTORALIS* (BOISD.)

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Abstract

The present study aimed to evaluate the effect of Emamectin benzoate and *Bacillus thuringiensis* as bioinsecticides as well as the organophosphorus compound, chlorpyrifos on the 2nd and 4th instar larvae of *S. littoralis* under laboratory conditions. The results showed a decrease in both larval and pupal duration after larvae treatment. Results also showed a decrease in the pupation and the adult emergence percentage compared to the control. Moreover, adult longevity of male and female moths was affected. In addition, mean number of eggs laid and hatched /female as a result of treatment was reduced. Results showed also that the treatment with tested compounds presented and biochemical components impact on the activity of certain enzymes, e.g. total carbohydrates, total proteins, digestive enzymes, Acetylcholinesterase (AChE) and Glutathione S-transferase (GST).

Keywords: Cotton leaf worm, *Spodoptera littoralis*, *Bacillus thuringiensis* var. *kurstaki*, Emamectin benzoate, organophosphorus, chlorpyrifos and activity of certain enzymes.

INTRODUCTION

Cotton plants are liable to be attacked by a great number of insect species, several of which cause serious damage to cotton plant. The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is considered one of the most destructive insect pests that attack cotton plants and many other crops in Egypt (Hosny *et al*., 1986). *S. littoralis* larvae feed on leaves, stems and flowers of plants. The control of this pest is based mainly on foliage treatments with chemical synthetic insecticides (Dent, 2000).

Emamectin benzoate is the salt of emamectin isolated from the soil microorganism, *Streptomyces avermitilis*. It,s effects on the nervous system of arthropods is by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis. Also, it effects GABA and glutamate-gated chloride channel agonist (Dunbar *et al.,* 1998).

The entomopathogenic bacteria *Bacillus thuringiensis* var. *kurstaki* represents a good example for biological control. This bacterium, proved to be a highly successful for controlling some agricultural insect pests (Dent, 2000).

Chlorpyrifos is a broad spectrum OP insecticide that has been used in the home and on the farm. Therefore, one can be exposed to these compounds as residues in agricultural products (Ncibi *et al.* 2008).

In the present study the effectiveness of some bio and chemical insecticides against the cotton leaf worm, *S. littoralis* was examined in order to find the best compounds for controlling this economic pest in an integrated pest management program under laboratory conditions.

MATERIALS AND METHODS

1. Rearing of *S. littoralis* (Boisd):

The laboratory strain was obtained from the Research Division of the Cotton Leaf worm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin cloth held in position with rubber bands and incubated under laboratory conditions at 27º±2ºC, 60 ± 5% RH, and 8:16 LD photoperiod. They were fed on castor oil leaves and examined daily. After pupation, the pupae were collected; sexed and emerged moths were placed in pairs in glass globes supplied with leaves of tafla, *Nerium oleander* (L.) as an oviposition site.

2. Tested compounds:

The potency of three new bio pesticides and one insectside was evaluated for their effect on *S. littoralis* larvae: -

A- Emamectin benzoate is the salt of emamectin isolated from the soil microorganism, *Streptomyces avermitilis*. a naturally occurring soil actinomycete used as a biological pesticide the trade names:

1. Biolrve® 5% EC, was obtained from Suez Canal Company for commercial and agricultural development (e.c.c). Co., LTd.
2. Trimph®1.9% EC, was obtained from Bridge Trade Company. Co., LTd.

While the chemical structure of emamectin benzoate: $C_{49}H_{75}NO_{13}$. C7H6O2

B- *Bacillus thuringiensis* var. *kurstaki* with the trade name W-Bus® 8% WP, was obtained from Afcoo Egypt Fertilizers and Chemicals Company. Co., LTd.

C- Organophosphorus insecticide Chlorpyrifos with the trade name Linker® 48% EC. The chemical was obtained from Sam trade Company. Co., LTd. The chemical structure: $C_9H_{11}Cl_3NO_3PS$ (O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate)

3. Bioassay:

The insecticidal activity of the four chemicals was assessed on newly ecdysed 2nd and 4th instar *S. littoralis* larvae.

1. A series of aqueous concentrations of Biolrve and Trimph were prepared which were (0.00097, 0.00048, 0.00024, 0.00012 and 0.00006 ppm.).
2. W-Bus: A series of dilution were prepared from 1 gm. of the product obtained as a wettable powder, (40, 20, 10, 5 and 2.5 ppm.).
3. Linker: A series of aqueous concentrations were prepared which were (2.5, 1.25, 0.625, 0.312and 0.156 ppm.).

Clean dry castor oil leaves were dipped for 15 s. in the different concentrations of the tested compounds, then left for air dryness for 1 h under room temperature and then offered to 2nd and 4th instar larvae in clean jars, twenty larvae of *S. littoralis* were placed on the treated leaf surface, while in control treatment leaves dipped in sterile distilled water. Five replicates were used for each tested concentration. Jars containing larvae were kept in the incubator. Mortality was recorded after 24 hours for Linker insecticides and 48 hours for bioinsecticides.

Mortality percentage was corrected according to Abbott's formula (Abbott, 1925). LC_{50} were estimated according to Finney (1971) using "LdP Line®" software. [http://embakr.tripod.com/ldpline/ldpline.htm].

4. Biological studies:

Newly ecdysed 2nd and 4th instars larvae were offered castor oil leaves treated with the determined LC_{50} of each of the tested compounds and the following parameters were recorded: larval and pupal duration of each instar, and percentage of pupation. Pupae were sexed and then placed in pairs in the glass globes. Subsequently, percentage of adult emergence, longevity of moths and the fecundity and fertility of eggs/female were determined. A control was set comprising a similar number of untreated moths.

5. Biochemical studies:

Biochemical studies were done in order to examine the host immune response in terms of monitoring activity of different enzymes, which include detoxification enzymes, upon treatment with both bio and chemical insecticides, separately.

Sample preparation

One gram of 4th instar larvae of *S. littoralis,* treated with LC_{50} of compounds under investigation, was homogenized in 5ml. distilled water using chilled glass Teflon tissue homogenizer (ST – 2 Mechanic-Preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15min. at 5°C and supernatants were kept at -20°C till use.

Enzymes' protein and carbohydrate Assay

1. Total carbohydrates content was determined according to the method of Crompton and Birt (1967)

2. Total proteins content was determined according to the method of Bradford (1976).

3. Digestive enzymes (amylase, invertase and trehalase) were determined according to the method described by Ishaaya and Swirski (1976)

4. Acetylcholinesterase (AChE) activity: was measured according to the method described by Simpson *et al*. (1964).

5. Glutathione S-transferase (GST) activity: was measured according to the method of Habig *et al.* (1974).

RESULTS AND DISCUSSION

Data in table (1&2) showed larval mortality rates due to treatment of the 2nd and 4th instar larvae with different concentrations of the used Biolrve, Trimph, W-Bus and Linker. LC_{90}, LC_{75}, LC_{50} and LC_{25} values were determined for both 2nd and 4thinstar larvae Fig. (1&2). Our results were agreed with those reported by Abou-Taleb, *et al.,* (2009) who reported that the toxicity of Emamectin benzoate against the different instar larvae of laboratory and field strains of *S. littoralis* was increased with increasing the concentration and exposure time and decreased by increasing the insect instar.

Table 1. Toxicity of the tested compounds against the 2nd instar larvae of cotton leafworm, *S. littoralis*.

Compound	LC_{90} (ppm.)	LC_{75} (ppm.)	LC_{50} (ppm.)	LC_{25} (ppm.)	Slope± S.E
Biolrve	0.0009	0.0004	0.0001	0.00005	1.523± 0.165
Trimph	0.0007	0.0002	0.00007	0.00002	1.288±0.159
W-Bus	38.525	12.997	3.886	1.162	1.286± 0.153
Linker	0.866	0.41573	0.183	0.081	1.9± 0.176

Table 2. Toxicity of the tested compounds against the 4th instar larvae of cotton leafworm, *S. littoralis*.

Compound	LC_{90} (ppm.)	LC_{75} (ppm.)	LC_{50} (ppm.)	LC_{25} (ppm.)	Slope± S.E
Biolrve	0.0009	0.0004	0.0002	0.00006	1.715± 0.159
Trimph	0.0014	0.0005	0.0002	0.00006	1.441±0.149
W-Bus	294.027	61.849	10.942	1.936	1.685± 0.138
Linker	1.383	0.603	0.24	0.095	0.897±0.171

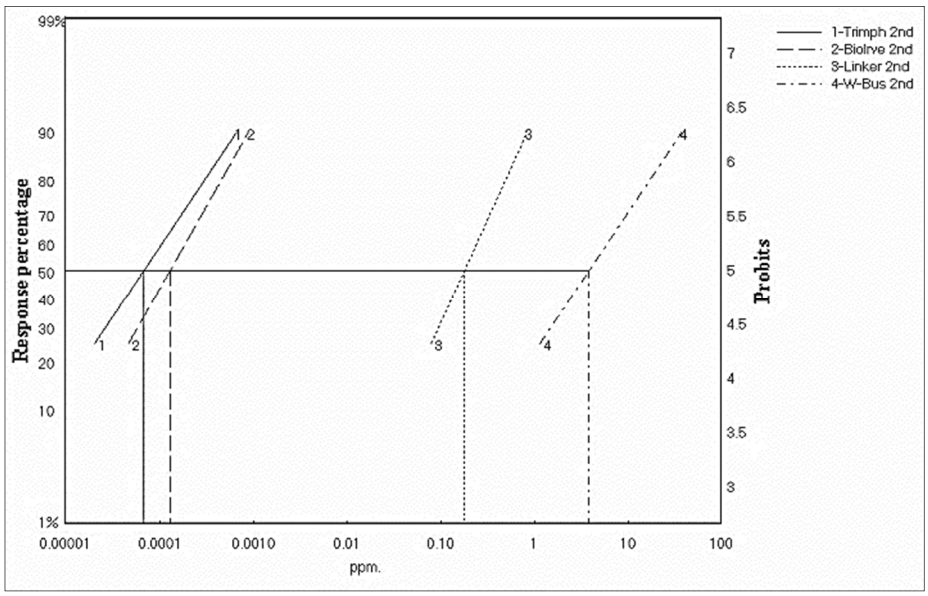

Fig. 1. Toxicity of Biolrve, Trimph, W-Bus and Linker on 2nd instar larvae of *S. littoralis*

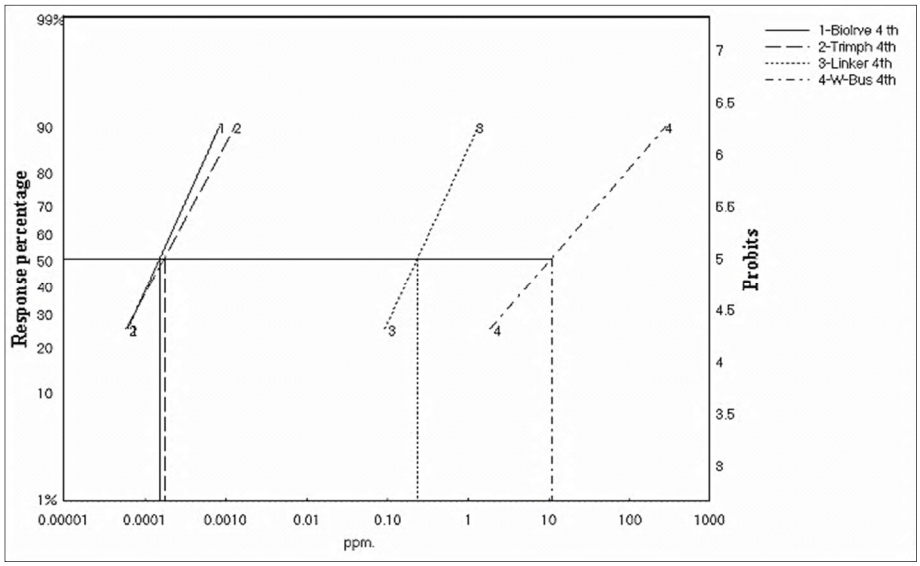

Fig. 2. Toxicity of Biolrve, Trimph, W-Bus and Linker on 4th instar larvae of *S. littoralis*

Results presented in table (3) showed the effect of compounds at LC_{50} on the mean larval duration, pupation percentage, pupal duration and percentage of adult emergence.

Treatment of the 2nd and 4th instar larvae with tested compounds led to variable effect on the mean larval duration. As shown in table (3), treatment with Biolrve, Trimph and W-Bus increased the mean larval duration. On the other hand, treatment with Linker decreased the mean larval duration compared to the untreated check.

Treatment of 2nd and 4th instar larvae with all tested compounds at LC_{50} level caused an obvious reduction in pupation percentage. Treatment of both 2nd and 4th instar larvae with all tested compounds had reduced the percentage of pupation to nearly the half compared to the control, pupation percentage of 40% was the lowest recorded with Linker. A significant decrease in pupal duration has also been observed after treatment of the 2nd and 4th instar larvae with all tested compounds compared to the control Table (3). Meanwhile, percentage of adult emergence was slightly decreased than the control, adult emergence of 76% and 80% was the lowest recorded when Biolarve was tested for both treatment2nd and 4th instar larvae respectively, less than the control. These are shown in previous studies with El-Zahi (2013), he mentioned that the latent effects of emamectin benzoate against 4th larval instar of *S. littoralis* were significantly decreased in pupal duration, pupal weight, pupation and adult emergence percentages.

Table 3. Effect of Biolrve, Trimph, W-Bus and Linker on larval duration, pupation rate and duration of 2nd and 4th instar larvae of *S. littoralis.*

Treatments	Larval duration days ± S.E		pupal duration days± S.E.		Pupation%		Adult emergence %	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Biolrve	18±0.3**	16±0.5**	11.3±0.3**	10±1.3***	46	47	76	80
Trimph	18±0.1**	16.6±0.1**	11±1.5**	10±1.7***	46	45	84.4	87.6
W-Bus	17±0.3*	15.3±0.5*	12.6±0.6**	11.0±1.6**	42	44	90	88
Linker	15.3±0.3*	12±0.3**	12.2±0.1*	13.3±0.1*	40	40	82.6	82.2
Control	16.0±0.2	14±1.1	13.6±0.5	14.0±1.7	100	100	100	100

*: Significant at P> 0.05 **: highly significant at P> 0.01***: Very highly significant at P> 0.001.

Table (4) showed the latent effect of treatment of 2nd and 4th instar larvae with the LC_{50} level of used compounds on the adult longevity, the mean number of laid and hatched eggs/female. All tested compounds have significantly shortened the mean adult longevity for both males and females.

All used compounds caused highly significantly decrease in the mean number of eggs/female. Linker was the most effective compound, followed by W-Bus, Trimph and finally Biolrve. There was a significant reduction in the mean number of hatched eggs/female. The lowest number of hatched eggs resulted from females treated as 2nd instar larvae with Linker (Table 4). Furthermore, our results are in the same trend with those obtained by (Abo-El-Ghar *et al.*, 1995), with *B. thuringiensis* and emamectin against cotton leaf worm *S. littoralis*, with a pronounced decrease of pupation (36%) after emamectin treatment, and a high reduction of moth fecundity (87.4%). Fetoh *et al.* (2015), mentioned that the latent effects of emamectin benzoate against 2nd and 4th larval instar of *S. littoralis* significantly decreased egg masses (24, 48 and 72 h old) of the Egyptian cotton leaf worm *S. littoralis* infesting tomato crop under field and semi field conditions.

Table 4. Effect of Biolrve, Trimph, W-Bus and Linker on adult longevity, fecundity and fertility of 2nd and 4thinstar larvae of *S. littoralis.*

Treatments	Mean adult longevity (days) ± S. E.				Mean no. of eggs/female ± S.E.		Mean no. hatched eggs/female ± S.E.	
	2nd		4th		2nd	4th	2nd	4th
	♂	♀	♂	♀				
Biolrve	12.3±0.4**	11±0.28**	13.3±1.2**	12.0±1.7**	882±12.5***	793±14.2***	682±4.7***	646±15.3***
Trimph	11±1.0***	10.3±0.57***	12±1.1***	11±1.5**	737±16.8***	703±18.4***	528±8.5***	622±4.04***
W-Bus	12.6±0.4**	11.7±0.4***	15±1.0*	12±1.7**	639±10.2***	682±15.7***	599±4.9***	668±6.11***
Linker	10±1.1***	9.3±0.57***	11.7±0.3***	10.3±0.1***	576±9.1***	512±13.38***	415±3.6***	462±21.3***
Control	15.6±1.15	14.6±0.58	16.0±1.0	14.3±0.57	2921±60.6	2154±15.1	2103±4.04	1857±12.11

*: Significant at P> 0.05 **: highly significant at P> 0.01***: Very highly significant at P> 0.001.

Results given in table (5) indicated that all tested insecticides led to increase in total carbohydrates which more obvious with Biolrve and Linker compared with control. Total carbohydrates content were 20.2,20.65,28.3 and 40.9 (mg/g.b.wt) for Biolrve, Linker, Trimph and W-Bus respectively, while it was 47.6 (mg/g.b.wt) with control.

The total protein content of *S. littoralis* 6th instar larvae treated as 4th instar larvae were decreased with all tested insecticides. The total protein were 41.7, 62.2, 70.9 and 78.1 (mg/g.b.wt) with W-Bus, Linker, Trimph and Biolrve, respectively, compared with control 80 (mg/g.b.wt). The obtained results are in agreement with the results of Kamel *et al.* (2010) who studied the effect of commercial formulations of *Bt* (Agerin, Dipel 2X and Dipel DF) on larvae of *S. littoralis.*

Data also similar to data obtained by Assar, *et al.* (2016) who found that the total protein content of 4th instars of *S. littoralis* was decreased with all tested insecticides. The total protein content was 30.3, 27.9 and 26.9 (mg/g.b.wt) with emamectin benzoate, hexaflumuron and teflubenzuron, respectively.

Three digestive enzymes; amylase, trehalase and invertase were determined in 6th instar larvae of *S. littoralis* treated as 4th instar larvae treated with LC_{50} of the bio insecticide W-Bus. Data in table (6) showed that amylase activity was 219.3 in untreated larvae, this level was relatively unaffected in treated larvae with LC_{50} of W-Bus i.e. 213.6 µg glucose/min /gm. also trehalase activity was unaffected in treated larvae compared to the control.

Meanwhile, invertase activity was significantly decreased from 653 in untreated larvae to 571 µg glucose/min /gm. in larvae treated with W-Bus. The results in the present study are agree with those obtained by El- Sheikh (2012). *B. thuringiensis* significantly decreased the invertase activity in *S. littoralis* compared to the untreated larvae as 27.7%, 27.5% and 32.9 %, respectively, compared to untreated one.

Acelylcolinesterase (AchE) activity in table (7) was significantly activated with Linker and Trimph 314.6 and 288 (ug Ach Br /min/g.b.wt), respectively, while it was inhibited with Biolrve 247, as compared with control 246.1 (ug Ach Br/min/g.b.wt). Our results are in agreement with Abd El-Kareem, (2012) who recorded an increase in acetylcholinesterase activity 12 hours post treatment with carbamate insecticide. [Increased AChE activity has been reported in the lesser grain borer *Rhyzoperth adominica* (Guedes *et al.,* 1997)].

Glutathione S-transferase (GST) activity table (7) showed that Trimph caused significant reduction in the GST enzyme activity post treatment. Meanwhile, Biolrve activity was significantly increased from 46.5(µmole/min/ml) in untreated larvae to

55.2 (µmole/min/ml) in the GST enzyme activity post treatment. Glutathione S-transferase (GST) is a group of soluble detoxification enzymes which catalyze the conjugation of reduced glutathione with various compounds containing an electrophilic center, including insecticides. Structure of GST consists of three amino acids, glutamic acid, cysteine and glycine. GST also play an important role in stress physiology, and have been implicated in intracellular transport and various biosynthetic pathways (Wilce and Parker, 1994). The present study showed that neonicotinoid insecticides (imidacloprid and acetamiprid) caused significant reduction in the GST enzyme activity 24 h post treatment. Wang *et al.* (2009) found that GST might be unimportant or less important in conferring spinosad resistance in the *S. exigua* field population. They suggested that the bio-chemical mechanisms of insect resistance to spinosad might be related with the species of insect pests.

Table 5. Effect of the bioinsecticides on total proteins and total carbohydrates activity in 6thinstar larvae of *S. littoralis.*

Tested Compounds	**Total carbohydrates (gm./gb.w.) (Mean ±S.D)**	**Total proteins (gm./gb.w.) (Mean ±S.D)**
W-Bus	40.9 ± 1.30^{b}	41.7 ± 1.59^{d}
Biolrve	20.2±1.46^{d}	78.1 ± 2.66^{a}
Trimph	28.3± 1.28^{c}	70.9± 2.02^{b}
Linker	20.65±1.4^{d}	62.2± 1.76^{c}
Control	47.6 ±2.27^{a}	80 ±3.3^{a}

-Means of the same column followed by different letters are significantly different, P≤0.05, b.w. = body weight.

Table 6. Effect of the bioinsecticides on digestive enzymes activity (amylase, invertase and trehalase) in 6th instar larvae of *S. littoralis*.

Tested Compounds	**Mean(µg glucose / min / g.b.wt) ±S.D**		
	Amylase	**Invertase**	**Trehalase**
W-Bus	213.6±4.1^{a}	571±11.5^{b}	416.3±5.85^{a}
Control	219.3±8.1^{a}	653±13.45^{a}	410.6±9^{a}

-Means of the same column followed by different letters are significantly different, P≤0.05, b.w. = body weight.

Table 7. AChE and GST activity in 6thinstar larvae post treatment.

Tested Compounds	AChE activity (µg ACh Br/min/ml) (Mean ±SD)	GST activity (µmole/min/ml) (Mean ±S.D)
BioIrve	247±3.6^{c}	55.2±2.8^{a}
Trimph	288±7.2^{b}	25.2±1.6^{c}
Linker	314.6± 13.79^{a}	42.1±1.9^{b}
Control	246.1±8.5^{c}	46.5±2.87^{b}

-Means of the same column followed by different letters are significantly different, P≤0.05.

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فاعلية بعض المبيدات الحيوية والكيميائية علي دودة ورق القطن

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قسم بحوث دودة ورق القطن– معهد بحوث وقاية النباتات – مركز البحوث الزراعية

تهدف هذه الدراسة الي دراسة تآثير مبيدات إيمامكتين بنزوات وهي مبيدات معدلة من البكتريا المعزوله من التربة *Streptomyces avermitilis* ومركب اخر من بكتيريا الباسيلس ثيرونجنسيس بالاضافه لمركب كلوربيريفوس علي العمر الثاني والرابع لدودة ورق القطن تحت الظروف المعملية. أدت المعاملة بالتركيز نصف المميت بالمركبات محل الدراسة إلى خفض في متوسط العمر اليرقي والعذري وانخفاض في نسبه التعذير ونسبه خروج الفراشات مقارنه باليرقات غير المعاملة، كما أدت أيضا إلى خفض متوسط عمر الطور البالغ مقارنه بالكنترول. أدت المعاملة أيضا إلى خفض الكفاءة التناسلية للفراشات الناتجة من اليرقات المعاملة بالمبيدات محل الدراسة حيث ظهر ذلك في انخفاض متوسط عدد البيض ومتوسط الفقس الناتج مقارنة بالكنترول بالإضافة إلى ذلك، أظهرت النتائج أن المعاملة بالمركبات المختبرة أدت إلى تأثيرعلى نشاط بعض الإنزيمات.

الكلمات المفتاحية:

دودة ورق القطن،بكتيريا الباسيلس ثيرونجنسيس، كلوربيريفوس، بكتريا *Streptomyces avermitilis* ،إيمامكتين بنزوات، نشاط بعض الانزيمات.