

Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jpmp.journals.ekb.eg

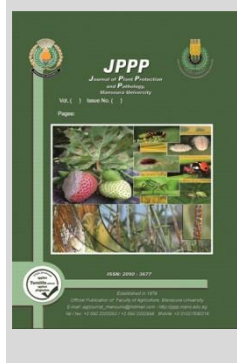
Toxicological and Physiological Impacts of Some Insecticides on the Cotton Leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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ABSTRACT

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is a serious pest of cotton and other crops in Egypt. Bioassay was carried out to evaluate the toxicity of some biochemical insecticides (emamectin benzoate, spinosad, and *Bacillus thuringiensis*) against 2nd larval instar of *S. littoralis* (Boisd.). The LC₅₀ values determined for emamectin benzoate, spinosad, and *Bacillus thuringiensis* against *S. littoralis* larvae were 1.38, 114.09, and 54.19 ppm respectively. Based on the LC₅₀ values, emamectin benzoate was more toxic to *S. littoralis* than the other compounds. Biochemical studies were done to detect the effect of tested compounds on total carbohydrates, proteins, lipids, amylase, invertase, trehalase, and alpha and beta-esterase enzymes. The total results indicated that carbohydrates, total proteins, and lipids were decreased significantly after treatment with *Bacillus thuringiensis* than the other compounds. The tested insecticides significantly reduced the amylase, invertase, and trehalase activity. A significant increase in alpha and beta esterase was induced by all tested compounds.

Keywords: Cotton leafworm, *Bacillus thuringiensis*, pesticides, chemical constituents of larvae, enzymes.

INTRODUCTION

Throughout the year, several economically significant crops are attacked by cotton leaf worm *Spodoptera littoralis* (Boisd.), as one of the most significant lepidopteron pests that results in financial losses. Because cotton satisfies both local and export demands and makes a major contribution to industry, employment, agriculture, and export revenue, it is essential to the nation's economy FAO (2022). Cotton leaf worm is reported as a pest on a variety of other commercially significant crops, fruits, and vegetables. Its broad host range, about 40 plant families and high reproductive potential, make it a detrimental pest of Egyptian crops Ahmed *et al.*, (2019) and Zaka *et al.* 2014).

Resistance to several pesticides has been developed in the last decades due to the extensive use of pesticides against *S. littoralis*, making control of it more challenging Ismail *et al.*, (2020).

The three most widely used biochemical insecticides in the world are *Bacillus thuringiensis*, spinosad, and emamectin benzoate. According to Aziz and Mohamed (2019), emamectin benzoate is a semisynthetic side of the bioinsecticide abamectin. This compound has a wide range of applications for controlling lepidopteran insects, such as *Spodoptera littoralis*. Emamectin affects muscles and stops the feeding of insects resulting in the insect's death Fritz *et al.*, (1979).

Nowadays, all researchers do their best to develop new agents with novel biochemical targets to overcome resistance problems in the insect Ismail, (2020). Spinosad is a neurotoxic insecticide that affects insects' nervous system when they touch it or swallow it. Their muscles twitch wildly, paralysis and eventual death result. It is also not harmful to mammals Barazani, (2001).

Entomopathogenic bacteria *B.t var. kurstaki* represents an effective example of biological control. According to Dent (2000), this bacterium has a remarkable effect in managing certain insect pests in agriculture. The target organ of insecticides is the midgut, at which digestion and absorption occur. Insecticides can penetrate the perimicrovillar membrane, causing harm and epithelial cell destruction. Castro *et al.*, (2021 and Santos-Junior *et al.*, (2020)

The current work aimed to evaluate the efficiency of the sub-lethal rates of these insecticides against the cotton leafworm larvae and the enzymatic changes resulting from the treatment.

MATERIALS AND METHODS

Tested compounds

1. Protecto 9.4% WP (*Bacillus thuringiensis var. kurstaki*, (32000 I.U. /mg) bacterial insecticides. It was provided by Bioinsecticide Production Unit, Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt
2. Master Top 25% SC. Active ingredients (spinosad), spinosyns group. It was provided by Syngenta Company.
3. Core 10% EC. Active ingredients (emamectin benzoate), avermectin group. It was provided by United Phosphorus Ltd.

Insect culture

Larvae in the current study were reared in the laboratory of the cotton leafworm research department, Sakha station. Insects were reared on leaves of castor according to Abdel-Salam and Hassan (1962). Insects were kept under laboratory conditions. Metayi *et al.*, (2015).

Bioassay

The toxicity of the tested compounds was detected by the leaf-dipping technique Abdel-Halim *et al.*, (2019). leaves were immersed in the insecticidal preparations for 10 sec then let to dry. Treated leaves were transferred to cups, that have 10

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DOI: 10.21608/jppp.2024.314081.1253

starved larvae. Control was treated with distilled water. Each treatment was represented by five replicates. Mortality percentages were recorded after two, four and six days after treatment Shaver *et al.*, (2018a). Mortality rates were calculated by using the Abbott formula Abbott, (1925) and probit analysis was performed according to Finney (1971) by using LdPLine[®] software.

Biochemical Studies

Insect analysis: -

Insect samples were performed according to Amin (1998). They were grinded in distilled water (50 mg /1 ml), then, centrifuged at 8000 r.p.m. for 15 min at 2 °C. The pellet was left and the supernatant was stored at 5 °c without the loss of activity. Samples were used for:

- a.determination of total carbohydrates as described byDubois *et al.*, (1956)
- b. determination of total protein as described by Brad Ford.(1976)
- c.determination of lipids according to Knight *et al.*, (1972)
- d. determination of α - and β -esterases according to Van Asperen, (1962)
- e.The activities of invertase, amylase, and trehalase were evaluated according to Ishaaya (1971) and Ishaaya and Swirski (1970).

Statistical data analysis

The determined toxicity and biochemical parameters were analyzed. based on four replicates, and the values are expressed as mean \pm standard error. The data were statistically analyzed separately. for each experiment and were subjected to analysis of variance (ANOVA) using costat software. Means were compared according to Snedecor and Cochran (1980). and were considered significant at $P \leq 0.05$. Differences between the treatments were determined by Duncan's Multiple Range Test ($P \leq 0.05$) Duncan (1955).

RESULTS AND DISCUSSION

Table (1) shows the LC₅₀ values of the compounds against 2nd instar larvae recording 1.38, 114.09, and 54.19 ppm, for emamectin, benzoate spinosad, and *Bacillus thuringiensis*, respectively. Based on the LC₅₀ values emamectin benzoate is more toxic to *S. littoralis* than other compounds. Spinosad and *B. thuringiensis* were lower than mamectin benzoate. Our results agree with those of E I-Moursy *et al.* (2000) as the latent effect of the Delfin (*B. thuringiensis*) compound was lower than pyrethroid. El-Naggar (2013) showed that the spinetoram was more toxic than spinosad.

Table 1.Susceptibility of *Spodoptera littoralis* larvae to *Bacillus thuringiensis*, emamectin benzoate and spinosad.

Insecticide	LC ₅₀ (ppm.)	95% Conf. limits (ppm.)		Slope \pm SE
		Lower limit	Upper limit	
Emamectin benzoate	1.38	0.84	2.09	1.87 \pm 0.34
Spinosad	114.09	47.32	2.77	1.56 \pm 0.43
<i>Bacillus thuringiensis</i>	54.19	46.27	63.56	0.73 \pm 0.26

Results in Table (2) showed that the tested compounds led to a decrease in total carbohydrates which is obvious in *B.t var. kurstaki* compared with control. Total carbohydrate contents were 45.3, 52.2, and 60.3 (mg/g.b.wt) for *B.t var. kurstaki*. Emamectin benzoate, Spinosad, respectively, while it was 73.1 with control. According to Chapman (1998), carbohydrates are essential for the insect's development, flight

muscles, metabolism, metamorphosis, and embryonic development. Our results coincide with Abd El-Kareem *et al.*, (2022) who explained, that under toxicant stress, the carbohydrate content shortage could increase the metabolism. Also, low carbohydrates in stress conditions could activate the glycogenolysis and glycolytic to provide excess energy. Li, *et al.*, (2018) studied the metabolism of carbohydrates in *Spodoptera exigua* larvae infected with the *Heliothis virescens* ascovirus. They found that carbohydrate content was significantly lower in the infected larva than in the control.

Aly and Ali (2024), disagree with our results. They showed that thiocyclam increased the carbohydrate content in 2nd instar, while novaluron and thiocyclam increased the carbohydrate content in 5th instar compared with control and other tested insecticides.

The total protein content of 2nd instar larva decreased with all compounds. The total proteins were 35.8, 41.0, and 45.2 mg/g.b.wt with *B.t var. kurstaki*, emamectin benzoate, and spinosad, respectively, by comparing with control (47.3 mg/g. b. wt). El-Barky *et al.* (2008) reported similar findings, they used spinetoram on *S. littoralis* and found that total proteins significantly decreased. A decreased total protein value was also seen in *S. littoralis* treated with spinetoram, which is associated with the inhibitory impact on the neurosecretory receptors that control protein secretion Hamouda and Dahi (2008). According to De França *et al.* (2017), insecticides, particularly those of the methomyl class, target ion channels and muscle ryanodine receptors. This reduces nutritional indicators including proteins, carbs, and lipids that impair *Agrotis ipsilon* larvae development Xu *et al.*, (2016). During the pupal stage, the protein hemolymph acts as a supply of protein required for the adult stage's development. Wilkinson (1976) showed that proteins aid in microsomal enzyme synthesis, which aids in the removal of toxins that are ingested by insects. Proteins are the primary biological constituents of insects that bind foreign substances.

All compounds caused a reduction in lipids that was more obvious with *Bacillus thuringiensis* than with the other compounds. They were 37.3, 37.6, and 41.5 (mg/g.b.wt) for *B.t var. kurstaki*, emamectin benzoate, and spinosad, respectively as compared with control (48.0 mg/g.b.wt). Lipid accumulation is directly related to a shortage of juvenile hormones, according to Hill and Izatt's (1974) findings. Administering the tested insecticide does not affect the corpora allata, which is the place where the juvenile hormone is secreted.

Table 2. Effect of LC₅₀ of tested compounds on total proteins, carbohydrates, and lipids in the *Spodoptera littoralis* larvae treated as 2nd instar larvae

Tested compound	Total proteins (µg/g b.w.)	Total carbohydrate (µg/g b.w.)	Total lipids (µg/g b.w.)
	(Mean \pm S.E.)	(Mean \pm S.E.)	(Mean \pm S.E.)
<i>B.t var. kurstaki</i>	35.8 \pm 0.3 ^c	45.3 \pm 1.4 ^d	37.3 \pm 1.2 ^c
Emamectin benzoate	41.0 \pm 1.3 ^b	52.2 \pm 0.7 ^c	37.6 \pm 0.7 ^c
Spinosad	45.2 \pm 0.6 ^b	60.3 \pm 0.7 ^b	41.5 \pm 0.7 ^b
Control	47.3 \pm 1.1 ^a	73.1 \pm 0.9 ^a	48.0 \pm 1.1 ^a
Df	3	3	3
F-value	73.0	430.79	72.3
P-value	0.0000***	0.0000***	0.0000***

Means in the same column with the same letter(s) are not significantly different. (P < 0.05)

* Very Highly significant effect.

Our results in Table (3) showed a decrease in the activity of amylase, invertase, and trehalase enzymes. These results match with those of Salem *et al.*, (2023a) who observed a remarkable decrement in amylase activity after treating 4th instar larvae of *S. frugiperda* with lethal doses of spinerotam. Also, amylase, invertase, and trehalase enzyme activity were decreased when *Helicoverpa armigera*, larvae were exposed to a spectrum of pesticides Al-shannaf *et al.*, (2012). The same finding in treated *S. littoralis* has also been detected by earlier investigators (Rashwan, 2013 and Salem *et al.*, 2023b).

Table 3. Effect of LC₅₀ of tested compounds on amylase, invertase, and trehalase in the *Spodoptera littoralis* larva treatment as 2nd instar larvae

Tested compound	Amylase	Invertase	Trehalase
	(µg glucose/min./gm b.w.)	(µg glucose/min./gm b.w.)	(µg glucose/min./gm b.w.)
	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.
<i>B.t var. kurstaki</i>	200.6 ± 0.7 ^c	563.0 ± 2.1 ^d	383.6 ± 2.7 ^b
Emamectin benzoate	206.0 ± 0.6 ^b	583.0 ± 3.0 ^b	405.0 ± 2.9 ^a
Spinosad	211.2 ± 0.6 ^a	560.3 ± 0.7 ^d	341.5 ± 0.7 ^b
Control	213.6 ± 0.9 ^a	652.6 ± 1.4 ^a	409.6 ± 2.6 ^a
Df	3	3	3
F-value	155.0	4831.0	463.0
P-value	0.0000***	0.0000***	0.0000***

Means in the same column with the same letter(s) are not significantly different. (P < 0.05)

* Very Highly significant effect.

Table (4) shows values of Alpha and beta esterase. Alpha esterase was activated by emamectin benzoate (217.6) as compared with control (196.3). Also, activation occurred with emamectin benzoate 221.6 in beta esterases, while it was 208.0 with control. Alpha esterases were highly activated with emamectin benzoate (217.6) followed by *B.t var. kurstaki* (206.0) and Spinosad (200.5). In beta esterases, there was a significant increase in emamectin benzoate (221.6), and spinosad (215.3) compared with control (208.0), while there was no significant change between *B.t var. kurstaki* (211.6) and control (208.0).

Table 4. Effect of LC₅₀ of tested compounds on alpha and beta esterases in the *Spodoptera littoralis* larvae treated as 2nd instar larvae

Tested compound	α-esterase (µg α-naphthol/min./g.b.wt)	β-esterase (µg α-naphthol/min./g.b.wt)
	(Mean ±S.E.)	(Mean ±S.E.)
<i>B.t var. kurstaki</i>	206.0 ± 0.6 ^b	211.6 ± 1.3 ^a
Emamectin benzoate	217.6 ± 6.3 ^c	221.6 ± 14.6 ^c
Spinosad	200.5 ± 1.3 ^b	215.3 ± 0.6 ^b
Control	196.3 ± 2.6 ^a	208.0 ± 10 ^a
Df	3	3
F-value	176.33	426.29
P-value	0.0000	0.0000

Means in the same column with the same letter(s) are not significantly different. (P < 0.05)

* Very Highly significant effect.

According to Vanhaelen *et al.* (2001), esterases are essential for the detoxification of both naturally occurring and chemically manufactured pesticides. Mahmoud, *et al.*(2024) found that esterase activities significantly increased in larvae treated with spinosad, chlorpyrifos, and methomyl.

Enzymes involved in metabolism, like esterase and mixed-function oxidase (MFO), can have their functions changed by insecticides. This matches the findings documented by Abd El-Mageed and Elgohary (2006), who

verified that the exposure of the 4th instar larva of *S.littoralis* to spinosad varies the esterase activity. Similar to the study of Abd El-Samei *et al.* (2019), spinosad's LC25 was found to significantly activate α- and β-esterases in larvae in their 3rd and 5th instars after 48 hours. Bakr *et al.*, (2013), explained that IGRs may result in varying degrees of alterations in the α and βesterases in *S. littoralis*.

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التأثيرات السُمِّية والفسيولوجية لبعض المبيدات الحشرية على دودة ورق القطن

سوزان محمد سعد بدر¹ ونيفين محمد فايز¹

قسم بحوث دودة ورق القطن - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الجيزة - مصر

المخلص

دودة ورق القطن هي آفة تصيب القطن والعديد من المحاصيل في مصر. تم إجراء اختبار حيوي لتقييم سمية بعض المبيدات الحشرية الكيمائية الحيوية (إيمامكتين بنزوات، سبينوساد وباسيلاس ثيورينجنسس) ضد طور اليرقي الثاني لدودة ورق القطن. كانت قيم LC50 ضد دودة ورق القطن للمركبات (إيمامكتين بنزوات، سبينوساد وباسيلاس ثيورينجنسس) هي 1.38، 0.9، 1.14، 1.9، 0.4 جزء في المليون على التوالي، وكان إيمامكتين بنزوات هو الأكثر سمية لدودة ورق القطن. أجريت دراسات بيوكيميائية للكشف عن تأثير المركبات المختبرة على إجمالي الكربوهيدرات والبروتينات والدهون وأنزيمات الأميليز والإنفرتاز والتريهاليز وألفا وبيتا استريز. أشارت النتائج إلى أن إجمالي البروتينات ومحتوى الدهون انخفض بشكل ملحوظ مع جميع المركبات المختبرة، بينما حدث انخفاض ملحوظ في الكربوهيدرات في الباسيلاس ثيورينجنسس أكثر من المركبات الأخرى. أدت المبيدات الحشرية المختبرة إلى تقليل نشاط الأميليز والإنفرتاز والتريهاليز بشكل ملحوظ. حدثت زيادة كبيرة في نشاط ألفا وبيتا استريز بواسطة جميع المركبات.