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The Toxicity and Biochemical Activity of Spinosad, Emamectin Benzoate and Dinotefuran on *Spodoptera littoralis* (Boisd.)

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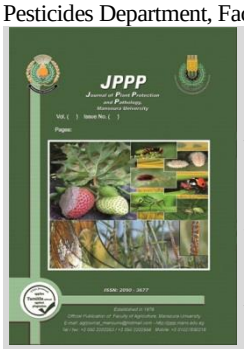

ABSTRACT

The failure of the traditional insecticides to control cotton leafworm *Spodoptera littoralis* led to severe losses in different crops as a result of resistance development. This study aimed to evaluate the toxicity and biochemical activity of newly emerged insecticides, spinosad, emamectin benzoate and dinotefuran on 4th instar larvae of *Spodoptera littoralis.* The results clearly illustrated that emamectin benzoate was more toxic than spinosad and dinotefuran according to LC_{50} values 0.8, 201.7 and 3979.6 ppm, respectively. Additionally, the tested compounds resulted in a significant reduction in Ache activity by 50, 42 and 63.2%, respectively. On the contrary, there was significant increase of α – E by 5.3, 90.9 and 75.2%, β- E by 33.3, 29.7 and 44%, AST by 163.6, 181.8 and 63.6%, ALT by 263.2, 210.5 and 157.89%, ACP by 100, 200 and 200% and ALP activities by 250, 320 and 158%, respectively for the mentioned insecticides in comparing with untreated larvae.

Keywords: Spinosad, emamectin benzoate, dinotefuran, biochemical activity, *Spodoptera littoralis.*

INTRODUCTION

Cotton leafworm, *Spodoptera littorals* (Boisd.) is considered as one of the most damaging pests for many different crops worldwide (Horowitz *et al*., 1994, Smagghe and Degheele 1997). *S. littoralis* is a polyphagous pest, active all the year, attacking cotton and more than 150 hosts crops and vegetables, causing severe damage to different parts of plant. (Temerak, 2006). Earlier studies revealed the difference in susceptibility to different groups of insecticides between the local strains of *S. littoralis* (Gutierrez-Moreno *et al.,* 2019). Spinosad is a bioinsecticide extracted from the metabolites of *Saccharopolyspora spinosa* naturally (Mertz and Yao 1990). Spinosad act based on affecting the nicotinic acetylcholine receptors and H- Glutamate receptor sites of the nervous system, leading to persistent stimulation of motor neurons causing stop feeding, muscle tremors, paralysis and death (Semiz *et al.,* 2006). Emamectin benzoate (EB) belongs to avermectin group of chemicals which produced through the process of soil microorganisms' fermentation, *Streptomyces avermilitis* (Crouch *et al.,* 1997). The mode of action based on the blocking of y-amino butyric acid-stimulated (GABA) chloride channels and open non-neurotransmitter- gated chloride channels (Martin, *et al.,* 2002) leading to paralysis and finally to death. Dinotefuran is a synthetic organic insecticide belonged to neonicotinoids group. The insecticidal action of neonicotinoids is focused on nicotinic acetylcholine receptors (nAChRs) (Karlin, 2002; Tomizawa and Casida, 2005).

The appearance of resistance to any insecticide could be due to enzymes action, which is resistant to the insecticide or able to degrade it to nontoxic metabolites (Al-Elimi 1994). For these reasons, the current study has been carried out to evaluate the effect of the sub lethal concentrations (LC_{25}) of the tested insecticides (spinosad, emamectin benzoate and dinotefuran) through the activity of esterase's (α&β-E), phosphatases (acid & alkaline phosphatase), transaminases (AST & ALT) as well as acetylcholine esterase (Ache) on 4th instar larvae of *S. littoralis*.

MATERIALS AND METHODS

Insecticides used:

Common names	Trade names	Empirical Formulas	The tested concentrations
Spinosad	tracer 24 % SC	$C_{41}H_{65}NO_{10}$	100, 200, 300 and 400 ppm.
Emamectin benzoate	pasha 1.9% EC	$C_{56}H_{81}NO_{15}$	0.01, 1, 10 and 50 ppm
Dinotefuran	Oshin 20% SG	$C_7H_{14}N_4O_3$	2000, 3000, 4000 and 5000 ppm.

Insect Rearing:

A sensitive strain of the cotton leafworm, *Spodoptera littorals* (Boisd.) was reared in the laboratory of pesticides department, Faculty of Agriculture, Mansoura University, Egypt. Fresh castor leaves were offered to larvae as a diet.. Rearing insects was performed according to the technique described by (EL -Defrawi et al, 1964). The culture of the cotton leafworm was initiated from freshly collected egg masses supplied from the department of cotton leafworm, Plant Protection Research Institute, Dokki, Egypt.

Toxicity bioassays:

Toxicity bioassays of the tested insecticides against fourth-instar larvae of S. littorals were conducted under the laboratory mentioned above conditions (EL -Defrawi *et al*, 1964). To determine LC_{25} and LC_{50} values, different concentrations of each insecticide as mentioned above were tested against the 4th instar larvae using the leaf dipping technique. Fresh castor bean leaves were dipped in each concentration for 30 seconds and then left to dry for one hour. The treated leaves were left with 4th instar larvae in glass jars

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covered with muslin for 24 hours. Then, the larvae got into clean glass jars containing fresh untreated leaves for 3 days. Each concentration had 4 replications and each replicate contained 10 larvae (40 larvae /treatment). Untreated larvae (as a check) were fed on castor bean leaves dipped in distilled water. Mortality was recorded after 4 days of treatment.

By using Abbott's formula (1925) the average of mortality percentage was corrected.

Biochemical Assay

Samples preparation for enzyme analysis:

Castor leaves were dipped for 30 seconds in insecticide solution for each of the tested compounds at the LC_{25} levels (0.06, 27.9 and 3042.6 ppm), respectively for emamectin benzoate, spinosad and dinotefuran. Before leaves were offered to larvae, they were left to dry for one hour.

Larvae were fed for 24 hours on the treated leaves, then transferred to fresh untreated leaves for three days. Removing the prolegs by forceps then applying gentle pressure with fingers hemolymph was collected and kept in cold tubes until the biochemical assay was determined (Sookar *et al*, 1999 and Abd El-Mageed 2002).

Determination of esterase enzymes:

Carboxylesterase assay:

Following the method described by Van Asperen (1962) the activity of α- and β- esterases (carboxylesterase) were determined.

Acid and alkaline phosphatases (ACP-ALP) assays: According to the method described by Powell & Smith (1954) each of acid and alkaline phosphatases were determined

Aspartate aminotransferase (AST/GOT) method:

According to the international federation of clinical chemistry (IFCC), 1986, aspartate aminotransferase was determined.

Determination of Acetylcholine esterase (Ache) enzyme activities:

Acetylcholine esterase (Ache**)** was measured according to the method described by Sympthon *et al.*, (1964).

Statistical analysis:

- Lc_{25} and Lc_{50} were determined according to Finney *et al*, (1971) using "LD-P-Line "software.
- According to Abbott's formula (Abbott,1925) mortality percentages were corrected.
- Means were tested for significance by the one-way analysis of variance (ANOVA) using COSTAT statistics software version 6.4.0.0, the differences between means were tested using the least significant difference (LSD) test at $p < 0.05$ (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Efficiency of spinosad, emamectin benzoate and dinotefuran on toxicological parameters of *S. littoralis:*

The illustrated results in Table (1) summarized the efficiency of spinosad, emamectin benzoate and, dinotefuran at different concentrations against 4th instar larvae of *S. littoralis*. The study results emphasized the different effects of the selected three insecticides. The toxicity of these insecticides is based on the concentration and mode of action.

In the current study it was found that LC_{50} of spinosad on 4th instar larvae of *S. littoralis* was 201.7 ppm. Many authors determined the LC_{50} of spinosad such as Abdel-Aal *et al*, (2007), Ragaei and Sabry (2011) found that LC_{50} values were 7.83 ppm and 70.7 ppm, respectively. The previous results may be explained by the increased ability of the insect to become tolerant for high concentrations of the spinosad. The LC_{50} value for emamectin benzoate was 0.8 ppm. The results obtained in the current study were comparable with those found by Hafez (2021) reported that LC_{50} value for 4th instar larvae treated with emamectin benzoate was 1.8 ppm. Similarly, Amin *et al*, 2022 found that LC_{50} value for larvae treated with emamectin benzoate was 0.05 ppm. In addition, LC_{50} value for dinotefuran was 3979.6 ppm. These results disagree with the results reported by Ismail *et al*, (2012) explained that LC_{50} value of dinotefuran was 0.40 ppm against 2nd instar larvae of *S. littoralis*. Also, Hamama *et al*, (2015) tested the efficiency of imidacloprid on 2nd and 4th instar larvae of cotton leafworm. They reported that LC_{50} value was 0.263 ppm.

Table 1. Toxicity of spinosad, emamectin benzoate and dinotefuran on 4th instar larvae of *S. littoralis*.

Treatments	Lc_{25} (ppm)	Lc_{50} (ppm)	Slope ± SE
Spinosad	27.9	201.7	0.5±0.05
Emamectin benzoate	0.06	0.8	0.7± 0.23
Dinotefuran	3042.6	3979.6	0.01± 0.86

Biochemical effects of spinosad, emamectin benzoate and dinotefuran on 4th instar larvae of *S. littoralis* treated with LC_{25}

Results listed in Table 2 summarized the impact of spinosad, emamectin benzoate, and, dinotefuran on serum enzyme activities treated with LC_{25} compared with control. Treatment of 4th instar larvae with sub-lethal concentration of spinosad resulted in a significant decrease in Ache levels at 50% compared with untreated larvae.

Some researchers stated that hemolymph is a good organ for studying enzyme activities and gave information about the mechanism of resistance (Abdel-Samie *et al*, 1979; Sookar *et al*., 1999). The change in the response for the insecticides could be related to the increase in α-e, β-e, ALT, AST, ACP, ALKP, and a decrease in Ache (Abd El-Mageed *et al*, 2005). Spinosad targets nicotinic acetylcholine receptors with Ache. Abdel-Mageed and El-gohary (2006) reported that the change in spinosad response could be related to the reduction in Ache activity. Ache has a primary function in neurotransmission and it is the target site for several neurotoxic pesticides (Salgado *et al*., 1998). Similar results were found by Ismail *et al* (2012) found that larvae treated with spinosad showed less effectiveness in Ache levels at 24.4% as an inhibitor of Ache activity.

Obtained results showed that larvae treated with spinosad recorded a significant increase in beta and alpha esterases (β, α-E) at 5.3 and 33.3 % respectively, compared with the control.

Esterases have a major function in the detoxification of chemically and naturally synthetic insecticides (Vanhaelen *et al*, 2001). Insecticides may alter the functions of metabolic enzymes such as esterase. This agrees with the results reported by (Abd El- Mageed and Elgohary 2006) confirmed that the activity of β esterase of *S. littoralis* 4th instars larvae was significantly varied post-exposure to spinosad for 4 days. This suggested that α-β esterases were enclosed in the hydrolysis of spinosad using esters and

amides hydrolysis. In the same trend (Assar *et al*, 2016) found a significant rise in α-β esterase activity.

The results obtained during this study illustrated that larvae treated with spinosad recorded a significant increase in ACP & ALP levels at 250 and 100%, respectively, compared with non-treated larvae.

Phosphatases are extracellular enzymes that catalyze phosphate monoesters under acid or alkaline conditions (Trowsdale *et al*, 1990). Acid phosphatases play a role in carbohydrate metabolism. This enzyme is inside the membrane of lysosomes. So, damaging the membrane of lysosomes can lead to leakage into muscle and increase its levels (Trdan 2013). Chemical and biosynthetic insecticides enhance the activities of acid and alkaline phosphatases. ACP's main function is to provide phosphate ions from mononucleotide and ribonucleic proteins for a diversity of metabolic processes (Etebari *et al.*, 2005). ALP is mainly active in tissues with active membrane transport, such as intestinal epithelial cells (Ferreira and Terra, 1980). El-Sheikh (2012) found that there were different levels of significant changes in phosphatase activities (acid-alkaline) after exposure of 4th instar larvae of S. littoralis to spinosad. In the current study, there was a significant increase in ALT & AST enzyme levels at 263 and 163.6% in larvae treated with spinosad in comparison with control.

Transaminases (AST and ALT) help in the process of energy production according to (Azmi *et al*., 1998). The increase in AST and ALT activities in larvae treated with insecticides could be associated with reversible binding between the site of action on the enzymatic surface and insecticide. (Salem *et al*, 2023). Results, too revealed that transaminases may play an important part in insecticidal toxicity (Al-Elimi 1994 and Abd El- Mageed 2002).

The obtained results agree with the results reported by Mery *et al*, (2019) who observed a significant increase in ALT- AST and α-β esterase enzymes in larvae treated with spinosad at LC_{25} levels. On the other hand, (Assar *et al*, 2016) found that there was a significant reduction in Ache, ACP, AST, and ALT levels in larvae treated with spinosad. Emamectin benzoate is considered an inhibitory neurotransmitter, which stops feeding within hours of ingestion and causes paralysis of the Lepidoptera. (Anonymous, 2003). In addition, larvae treated with emamectin benzoate recorded a significant reduction in Ache levels at 42 % compared with untreated larvae. These obtained results agreed with the findings of (Abd-El-Aziz and El-gohary 2013) observed that there was a significant reduction in the Ache enzyme after the treatment with emamectin benzoate. Furthermore, after the treatment with emamectin benzoate, there were significant increases in beta & alpha esterase, AST, ALT, ACP & ALP at 29.7, 89.4, 181.8, 210.5, 200 and 320 %, respectively in treated larvae. A similar effect was observed by (Assar *et al*, 2016) showed that beta esterase levels increased after the treatment with emamectin benzoate. On the contrary, the obtained data are not in agreement with (Assar *et al*, 2016) observed that AST, ALT, ACP, and α-E were decreased after the treatment with emamectin benzoate. Neonicotinoids act as stimulants of the nicotinic acetylcholine receptors leading to an overstimulation of the cholinergic synapse and finally, insect death (Le Questel *et al*, 2011). The obtained results showed that the group treated with dinotefuran recorded a significant reduction of – 63.2%. Similar effects were observed by (Ismail *et al*, 2012) reported a high reduction in Ache at -60.2% in 2nd instar larvae of *S. littoralis*. On the contrary, (Hamama *et al*, 2015) observed that there was a significant increase in Ache levels in larvae treated with imidacloprid and acetamiprid. In addition, Marwa *et al* (2017) found that larvae treated with thiacloprid showed a significant increase in Ache activity, while larvae treated with imidacloprid showed a significant decrease in ALT, AST, and Ache activities. Moreover, both insecticides as well as acetamiprid recorded significant increases in esterase (β, α- E) which are comparable with the obtained results. On the other hand, Yasmeen *et al* (2023) reported that 3rd instar larvae of *Chrysoma megacephaly* treated with imidacloprid showed a significant reduction in ACP and ALKP activities.

Table 2. Effect of spinosad, emamectin benzoate and dinotefuran on some biochemical parameters of 4th instar larvae of *S. littoralis* (Values are presented as mean ± SD).

Treatments	Ache (U/L)	Alpha esterase (α-E)	Beta esterase (β-E)	Alkaline Phosphatase (µg/ml)	Acid phosphatase (µg/ml)	ALT /GPT (IU/l)	AST/ GOT (IU/l)
Spinosad	19±1^{c}	11.9±1^{c}	11.2±1^{a}	175±1^{c}	0.6±0.1^{bc}	69±1^{b}	29±1^{c}
Emamectin benzoate	22±1^{b}	21.4±1^{a}	10.9±1^{ab}	210±1^{a}	0.9±0.1^{a}	59±1^{c}	31±1^{b}
Dinotefuran	14±1^{e}	19.8±1^{ab}	12.1±1^{a}	129±1^{d}	0.9±0.1^{a}	49±1^{d}	18±1^{d}
Control	38±1^{a}	11.3±1^{c}	8.4±1^{c}	50±1^{f}	0.3±0.1^{d}	19±1^{f}	11±1^{f}
LSD ($p< 0.05$)	1.7	2.1	2.1	1.7	0.1	1.7	1.7

µg alpha- beta naphthol hydrolyzed / instar/ min.

CONCLUSION

In the present study, spinosad, emamectin benzoate and dinotefuran were evaluated against 4th instar larvae of cotton leafworm according to their LC_{50} values. In addition to the biochemical evaluation in larvae treated with sub lethal concentrations LC_{25}. The results indicated that emamectin benzoate was more efficient than spinosad and dinotefuran, respectively.

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السميه والنشاط البيوكيميائي للاسبينوساد والايمامكتين بنزوات والداينو تيفوران علي دوده ورق القطن.

ريم رفعت سالم ، عادل عبد المنعم صالح ، ليلي رجب الجوهري و محمد صبحي حماده*

قسم المبيدات- كلية الزراعة – جامعة المنصورة

الملخص

أجري هذا البحث بهدف تقييم السميه والنشاط البيوكيميائى لثلاث مركبات هما الاسبينوساد والايمامكتين بنزوات ودايتو تيفوران على العمر اليرقى الرابع لدوده ورق القطن – بالاضافة الي قياس تأثير التركيزات الغير مميته للمركبات المختبرة علي نشاط انزيمات الاستيريزات (ألفا وبيتا استيريز) وانزيمات الفوسفاتيزات (الفوسفات الحامضي والقاعدي) والانزيات الناقلة لمجاميع الأمين(الاسبرتات-الألانين). وقد أشارت النتائج المتحصل عليها بوضوح الي أن الايمامكتين بنزوات كان أكثر سمية من الاسبينوساد والداينو تيفوران ضد العمر اليرقي الرابع طبقا لقيم التركيز النصفى المميت والتي كانت 8, و 21.7 و3979.6 جزء من المليون علي التوالي. علاوة علي ذلك تسببت المركبات المختبرة في انخفاض معنوي في نشاط انزيم الاستيل كولين استيريز بمعدل 50 و 42 و 63.2 %علي التوالي. علي النقيض كان هناك زيادة معنوية في نشاط جميع الانزيمات المختبرة مثل الفا استيريز بمعدل 5.3 و 90.9 و 75.2% وفي بيتا استيريز بمعدل 33.3 و 29.7 و 44% وفي الاسبرتات بمعدل -163.6و 181.8 و 63.6% وفي الالانين بمعدل 263.2 و 210.5 و 157.89% وفي الفوسفات الحامضي بمعدل 100و 200 و 200% وفي الفوسفات القاعدي بمعدل 250 و 320 و 158% علي التوالي للمركبات المذكورة مقارنة باليرقات الغير معاملة.

الكلمات الدالة : الاسبينوساد – ايمامكتين بنزوات -داينو تيفوران- النشاط البيوكيمائي – دوده ورق القطن.