Biochemical and Biological Influences of Sub-Lethal Concentrations of Emamectin Benzoate and Certain IGR Insecticides against *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ABSTRACT

Toxicity and sub-lethal effects of Emamectin benzoate and IGR 's (lufenuron - flufenoxuron) were evaluated against the 2nd instar larvae of cotton leafworm, *Spodopter alittoralis*. The biochemical influences of these agrochemicals with the polyphenol oxidase (PPO) of the treated larvae were investigated (invivo). Emamectin benzoate (LC_{50} = 0.007 mg L^{-1}) was approximately 34 and 83 times more toxic than lufenuron (LC_{50} = 0.24 mg L^{-1}) and flufenoxuron (LC_{50} = 0.58 mg L^{-1}), respectively. Lufenuron was approximately 2 times more toxic than flufenoxuron. The 2nd instar larvae treated with the sub-lethal concentrations (LC10 and LC_{25}) of emamectin benzoate, lufenuron or flufenoxuron showed significant ($P < 0.05$) reduce of larval body weights, pupation%, pupal mean weight and adult emergence%. While the average time of the pupation for the larvae that given concentrations equivalent to the LC_{10} and LC_{25} of lufenuron and flufenoxuron, were significantly longer than the untreated larvae. On the other hand larval duration for the larvae that given concentrations equivalent to the LC_{10} and LC_{25} of emamectin benzoate did not differ significantly compared with the untreated larvae. The fecundity and fertility were strongly reduced in all insecticide treatments compared with check untreated. The PPO activity was partially decreased in the treated larvae (with all insecticide treatments) in a concentration dependent manner. These results suggest that, sublethal concentrations of emamectin benzoate, lufenuron and flufenoxuron may reduce the population growth of S. littoralis by affecting its development and reproduction.

Key words: *Spodoptera littoralis*; IGRs; Emamectin benzoate; Sub-lethal effects and PPO.

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisduval), is one of the most destructive agricultural lepidopterous pests of cotton and vegetable plants in Egypt (Hatem *et al*., 2009). The insect occurs during the whole cycle of cotton, causing considerable damage by feeding on leaves, fruiting points, flower buds and occasionally, bolls. So, it requires several insecticide applications to control.

Commonly, control of this pest has largely been depending on the use of neurotoxic insecticides including organophosphates, carbamates and pyrethroids (Ahmad *et al*., 2009).The intensive application of these insecticides has give rise to *S. littoralis* populations resistant to all of these groups (Abou-Taleb, 2010). Selective insecticides with modes of action differed from those insecticide groups are highly desirable in integrated pest management (IPM) programs. Among these insecticides are insect growth regulators (IGRs) and avermectin insecticide group.

IGRs were developed to mimic, block or otherwise interact with the hormonal system of insects (Oetken *et al*., 2004). These include the juvenile hormone analogues, the ecdysone agonists and the chitin synthesis inhibitors (CSIs) (Graf, 1993; Tunaz and Uygun, 2004). Among the chitin synthesis inhibitors, acylureas (BPUs) are gaining importance in insect pest control because they are relatively harmless to natural enemies (Furlong *et al*., 1994).

Chemical modifications on the abamectin (avermectins) structure resulted in the discovery of emamectin benzoate (Mrozik, 1994). Emamectin benzoate is very effective against a broad spectrum of lepidopteran pests including the Egyptian cotton leafworm (Abou-Taleb *et al*., 2009). It modulates specific glutamate-gated anion channels in synapses and muscle cells (Roberts and Hutson, 1999), thereby increasing the influx of chloride ions. This hyperpolarizes the cell and prevents depolarization of the neuromuscular endplate beyond the threshold level (Davies and Rodger, 2000).

The use of insecticides may result in multiple sub-lethal effects on insect pests (Singh and Marwaha, 2000 and Sabri *et al* 2017), such as physiological effects may be manifested as reductions in life span (Stark and Rangus, 1994), development rate (Cripe *et al*., 2003), fertility (Liu and Trumble, 2005), fecundity (Zalizniak and Nugegoda, 2006), changes in sex ratio (Couty*et al*., 2001). These sub-lethal effects as well as mortality must be considered when examining the total effects of

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insecticides (Yin *et al*., 2008). Moreover, these sub-lethal doses may interfere with some biochemical process such as the related enzymes activity.One of the related enzymes to IGR's mode of action are phenoloxidases that related to critical steps of melanization reactions, which are crucial for the sclerotization of a new cuticle after ecdysis (Andersen, 2005) and for the encapsulation of pathogens in the hemolymph (Soderhall and Cerenius, 1998).

So, the objectives of the present study were to investigate the sub-lethal effects of lufenuron, flufenoxuron and emamectin benzoate on some biological aspects of *S. littoralis,* including development time, fecundity, larval and pupal weights and adult emergence. As well we investigate the effect of the sub-lethal concentrations of these insecticides on the polyphenol oxidase (PPO) activity.

MATERIALS AND METHODS

Experimental insect:

A susceptible strain of the *S. littoralis* has been reared for many years in the Plant Protection Research Station, Agricultural Research Center, Alexandria, Egypt. Larvae were fed castor oil leaves under controlled laboratory conditions (25 ± 2 °C, RH 65 %) for several years avoiding exposure to any pesticides according to the method of Eldefrawi *et al*., (1964).

Test insecticides and other chemicals:

Formulated emamectin benzoate (Proclaim® 5% SG), lufenuron (Match® 5% EC) and flufenoxuron (Cascade® 10% EC) were obtained from Syngenta Co. Bovine serum albumin (BSA), Folin–Ciocalteu phenol reagent and pyrocatechol were purchased from Sigma–Aldrich Chemical Co., USA. All chemicals were used without further purification.

Bioassay and determination of sub-lethal concentrations:

A leaf dip bioassay method (Eldefrawi *et al*., 1964) was used. Castor oil leaf disks (35 mm in diameter) were dipped in the desirable concentration of insecticide prepared in distilled water contained 0.05% (V/V) triton X-100 for 10 sec., and dried at room temperature. For each tested insecticide a series of 6-8 concentrations were tested. The control containing pure water was also used with Triton X-100 as an emulsifier (0.05% V/V)). The treated castor oil leaf disks were introduced to ten 2nd instar larvae (2.3 ± 0.1 mg/larva), which had been starved for two hrs. The cups were covered with lids and maintained at 25 ± 2 °C. Each concentration was replicated five times. After 24 hrs, fresh untreated castor oil leaf pieces were added to each cup. Mortality was recorded after 24 96 hrs and corrected according to Abbott equation (Abbott, 1925). The sub-lethal concentrations (LC_{25} and LC_{10}) were calculated from the Ldp-line equation obtained after 96 hrs and used for the subsequent experiments.

Latent effects of sublethal concentrations of emamectin benzoate, lufenuron and flufenoxuron against *S. littoralis*:

Castor oil leaves were soaked in the previously determined LC25 and LC_{10} equivalent concentrations for tested insecticides. Four hundred 2nd instar larvae (2.3 ± 0.1 mg / larva) in 4 replicates were used for each treatment and provided with treated leaves. After 24 hrs, surviving larvae were transferred to jars containing fresh untreated leaves and observed daily for pupation and eclosion. Pupae were sexed and weighed 24 hrs after formation. Larval, pupal and adult durations and survivorship were determined, as well as, larval & pupal weights and the percentages of adult emergence. Resulted adults were placed in plastic cups provided with a folded sheet paper as oviposition site. Two adult males were kept with one adult female to maximize the probability of successful mating. The sub-lethal effects of various insecticides on fecundity (total number of eggs / female) and fertility (hatchability percentages of eggs) were determined. Mating of untreated adults of both sexes were used as control. Initially, 12 mating were planned for each insecticide treatment as well as control. The mating cups were check daily and egg masses were removed until female death. The total number of eggs /female for each mating and hatched eggs percentages were evaluated.

Polyphenol oxidase (PPO) activity assay:

Crude enzyme preparation: Surviving larvae treated with LC_{25} and LC_{10} values of each insecticide, after 96 hrs of treatment and the untreated (control) were starved for two hrs. Then three grams of total larvae from each treatment or control were homogenized in 10 ml of 0.1M potassium phosphate buffer (pH 7.0) using Polytron Kinemetica on ice. The homogenate was filtered through two layers of cheesecloth and centrifuged at 10,000 rpm for 10 min at 4 ºC using Cryofuge 20-3, Heraeus Christ centrifuge. The supernatant was used as the crude enzyme extract. Lowry et al. (1951) method was used to determine protein content in the supernatant comparing to the standard curve of BSA.

Enzyme activity determination: The activity of PPO was determined according to Zhi-qing*et al*. (2008) by mixing of 1.5 ml 0.2 M pyrocatechol, 1.4 ml of 0.05 M phosphate buffer, pH 6.8 and 0.1 ml enzyme extract, respectively. The mixture was incubated at 25 ºC for 30 min and the absorbance was measured at 420 nm using a Sequoia-Turner Model 340 spectrophotometer. The

specific activity of PPO was calculated as OD_{420} / min/mg protein.

Statistical analysis:

The LC_{50}'s and their 95% fiducial limits of the tested insecticides were obtained using the POLO program (Russell *et al*., 1977) based on Finney (1971). All other quantitative estimations were replicated four times and the values are expressed as mean ± standard error. The SAS 8.0 software was used for analysis of the data obtained from each experiment and the means were tested for significant differences by Duncan's multiple range tests at P = 0.05.

RERSULTS

Toxicity of emamectin benzoate, lufenuron and flufenoxuron against 2nd instar *S. littoralis* larvae:

Susceptibility of 2nd instar larvae of *S. littoralis* to emamectin benzoate, lufenuron and flufenoxuron after 96 hrs of exposure is presented in Table (1). Emamectin benzoate (LC_{50} = 0.007 mg L^{-1}) was approximately 34 and 83 times more toxic than lufenuron (LC_{50} = 0.24 mg L^{-1}) and flufenoxuron (LC_{50}= 0.58 mg L^{-1}), respectively. Lufenuron was approximately 2 times more toxic than flufenoxuron. The LC_{25} and LC_{10} values after 96 hrs of exposure are 0.002 and 0.001 mg L^{-1} for emamectin benzoate, 0.064 and 0.028 mg L^{-1} for lufenuron, and 0.16 and 0.06 mg L^{-1} for flufenoxuron.

Sub-lethal effects of emamectin benzoate, lufenuron and flufenoxuron on some biological aspects of *S. littoralis*:

Sub-lethal effects of tested insecticides against *S. littoralis* larvae are presented in Tables (2, 3 and 4). The average weight of treated larvae was decreased significantly compared to control during the observation period. It is clear that, the higher concentration of all tested insecticides (LC_{25}) were significantly the highest in the effect in reducing the larval weight. When larvae were treated with emamectin benzoate at LC_{25} (0.002 mgL^{-1}), the larval weight averages were 32.6, 126.7 and 422.3 mg / larva compared to 44.7, 186.2 and 602.5 mg / larva in control after 5, 10 and 15 days of treatment, respectively. The larval weight averages were 34.1, 137.2 and 426.5 mg / larva, and 37.4, 142.5 and 431.0 mg / larva when larvae were treated with LC_{25} of lufenuron (0.064 mgL^{-1}) and flufenoxuron (0.16 mgL^{-1}) after 5, 10 and 15 days of treatment, respectively (Table 2).

The average time to the pupation for larvae given concentrations equivalent to the LC_{10} and LC_{25} of lufenuron and flufenoxuron treatments were significantly longer than those given the control treatment. These times were 22.2 and 23.7 days for lufenuron and 22.4 and 23.1 days for flufenoxuron at LC_{10} and LC_{25}, respectively, where it was 18.4 days for control. Larval duration for larvae given concentrations equivalent to the LC_{10} and LC_{25} of emamectin benzoate treatments did not differ significantly compared to larvae given control treatment (Table 2).

Table 1. Toxicity of emamectin benzoate, lufenuron and flufenoxuron against the 2nd instar larvae of *S. littoralis* after 96 hrs of exposure

Insecticide	LC_{50} (mg L^{-1})	Confidence limits	Slope ± SE	LC_{25} (mg L^{-1})	LC_{10} (mg L^{-1})
Emamectin benzoate	0.007	(0.006 – 0.009)	1.70 ± 0.08	0.002	0.001
Lufenuron	0.240	(0.18 – 0.34)	1.66 ± 0.11	0.064	0.028
Flufenoxuron	0.580	(0.42 – 80)	1.54 ± 0.12	0.16	0.06

Table 2. Effect of sub-lethal concentrations of emamectin benzoate, lufenuron and flufenoxuron on the larval weight, larval duration and pupation percentage of the 2nd instar larvae of *S. littoralis*

insecticide	Conc. (mg L^{-1})	Days after treatment			Larval duration (days) ± SE	Pupation% ± SE
		5	10	15		
		Mean weight (mg\Larva) ± SE				
Control	-	44.7^{a} ± 1.5	186.2^{a} ± 3.2	602.6^{a} ± 5.9	18.4^{b} ± 1.3	98.1^{a} ± 1.0
Emamectin benzoate	0.001	38.2^{b} ± 2.4	160.9^{c} ± 5.2	510.2^{c} ± 4.5	17.8^{b} ± 0.6	82.0^{b} ± 1.6
	0.002	32.6^{c} ± 2.1	126.7^{e} ± 1.9	422.3^{d} ± 6.7	19.3^{b} ± 1.5	77.6^{c} ± 2.6
Lufenuron	0.028	38.2^{b} ± 1.4	166.3^{c} ± 3.0	501.3^{c} ± 3.8	22.2^{a} ± 1.8	72.6^{de} ± 1.2
	0.064	34.1^{c} ± 0.7	137.2^{d} ± 4.3	426.5^{d} ± 3.6	23.7^{a} ± 1.7	61.7^{f} ± 0.40
Flufenoxuron	0.06	40.0^{b} ± 1.2	174.6^{b} ± 2.9	532.6^{b} ± 6.2	22.4^{a} ± 1.4	76.1^{cd} ± 1.6
	0.16	37.4^{b} ± 1.4	142.5^{d} ± 5.3	431.0^{d} ± 4.8	23.1^{a} ± 0.8	69.3^{e} ± 1.7

Within a column, means possessing the same letter do not differ significantly at *P* = 0.05.

Sub-lethal concentrations of tested insecticides had a considerable effect on pupation (Table 2). The LC_{25} treatment of lufenuron gave the lowest pupation percentage 61.7% compared to 98.1% in control treatment. Also, significant decrease in pupation percentage was achieved with the LC_{10} of lufenuron (72.6%). The LC_{10} and LC_{25} of emamectin benzoate and flufenoxuron gave (82.0 and 77.6%) and (76.1 and 69.3%) of pupation, respectively compared to control treatment (98.1%).

As shown in Table (3), all treatments significantly reduced the mean of pupal weight compared to control treatment. The averages of pupae weight were 275.2 and 251.1 mg / pupa in the LC_{10} and LC_{25} of emamectin benzoate treatments, respectively, compared to 293.5 mg / pupa in the control treatment. In case of LC_{10} and LC_{25} of lufenuron treatments, pupae weight averages were 265.6 and 240.4 mg / pupa, respectively. Finally, pupae weight averages were 267.6 and 248.5 mg / pupa in the LC_{10} and LC_{25} of flufenoxuron treatments, respectively. However, pupal duration did not changed significantly in all treatments compared to control (Table 3). Reduction percentage in the adult emergence rates were significantly occurred in all treatments. Adult emergence percentages were 88.4 and 76.2% in the LC_{10} and LC_{25} of emamectin benzoate treatments, respectively, compared to 97% in the control treatments. The LC_{25} of both lufenuron and flufenoxuron caused the highest reduction in the adult emergence, where the adult emergence percentages were 65.6 and 66.4, respectively.

Table(4) represents the effect of insecticide treatments on the adult fecundity, fertility and longevity. Flufenoxuron and lufenuron at LC_{25} caused the highest effect on the adult fecundity where the average number of eggs laid / female were 328.5 and 340.8 / female, respectively, compared to 544.3 / female in control treatment. Also, lufenuron and flufenoxuron at the LC_{10} significantly decreased the adult fecundity where the average number of eggs laid / female were 385.3 and 347.6 / female, respectively. Emamectin benzoate at LC_{10} and LC_{25} was significantly decreased the average of eggs laid / female to 507.5 and 495.5 / female, respectively, compared to 544.3 / female in control treatment. The fertility (percentages of hatched eggs) were significantly decreased as a result of all insecticides treatments. Whereas the percentages of hatched eggs were (88.5 and 85.8%), (76.3 and 64.2%) and (74.6 and 67.7%) for the LC_{10} and LC_{25} of emamectin benzoate, lufenuron, and flufenoxuron, respectively, compared to 97.4% in the control treatment. On the other hand, adult longevity did not differ significantly in all insecticides treatments compared with the control treatment.

Table 3. Effect of sub-lethal concentrations of emamectin benzoate, lufenuron and flufenoxuron on the pupal weight, pupal duration and adult emergence of the 2nd instar larvae of *S. littoralis*

Insecticide	Conc. (mg L^{-1})	Pupal mean weight (mg/pupa) ± SE	Pupal duration (days) ± SE	Adult emergence % ± SE
Control	-	293.5^{a}± 3.4	8.4^{a}± 0.6	97.0^{a} ± 0.2
Emamectin benzoate	0.001	275.2^{b}± 2.7	8.2^{a}± 0.4	88.4^{b}± 2.3
	0.002	251.1^{c} ± 3.1	8.3^{a}± 0.4	76.2^{d}± 1.5
Lufenuron	0.028	265.6^{b} ± 2.7	8.3^{a}± 0.5	82.4^{c} ± 2.4
	0.064	240.4^{e}± 3.2	8.4^{a}± 0.5	65.6^{e}± 1.8
Flufenoxuron	0.06	267.6^{b}± 2.4	8.5^{a}± 0.4	79.3^{cd}± 2.1
	0.16	248.5^{c}± 1.9	8.4^{a}± 0.5	66.4^{e}± 1.7

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$

Table 4. Effect of sub-lethal concentrations of emamectin benzoate, lufenuron and flufenoxuron on adult fecundity, fertility and longevity of the 2nd instar larvae of *S. littoralis*

Insecticide	Conc. (mg L^{-1})	Fecundity (No. eggs laid / female) ± SE	fertility (percentage of egg hatch) ± SE	Adult longevity (days) ± SE
Control	-	544.3^{a}± 22.5	97.4^{a}± 2.1	6.5^{a}± 0.4
Emamectin benzoate	0.001	507.5^{b}± 24.5	88.5^{b} ± 3.5	6.1^{a}± 0.3
	0.002	495.5^{b}± 14.7	85.8^{b}± 4.2	6.2^{a}± 0.5
Lufenuron	0.028	385.3^{c}± 26.7	76.3^{c}± 2.9	6.7^{a}± 0.3
	0.064	340.8^{d} ± 23.2	64.2^{d} ± 3.5	6.3^{a}± 0.6
Flufenoxuron	0.06	347.6^{d}± 12.4	74.6^{c}± 1.8	6.8^{a}± 0.5
	0.16	328.5^{d}± 21.9	67.7^{d} ± 2.6	6.4^{a}± 0.4

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

Effect of emamectin benzoate, lufenuron and flufenoxuron on the PPO activity in the *S. littoralis* larvae:

Data of the *in vivo* effects of emamectin benzoate, lufenuron and flufenoxuron when applied to the 2nd instar larvae of *S. littoralis* on the PPO activity after 4 days of treatment are presented in Table (5). Data showed that all treatments significantly decreased the PPO activity compared to the control. The highest inhibition was recorded with LC_{25} of lufenuron followed by flufenoxuron and emamectin benzoate with inhibition percentages of 60.3, 58.7 and 44.6, respectively.

DISCUSSION

Insect management strategies must be directed towards the use of insecticides that are none or less toxic to all environmental components including the beneficial arthropods. Emamectin benzoate is less toxic to most beneficial arthropods, especially when exposure occurs beyond one day after application (Lasota and Dybas, 1991). Also, IGRs which act as chitin synthesis inhibitors or juvenile hormone analogs have been regarded as excellent integrated control insecticides because of their specificity to target pests and their general safety to vertebrates (Deakle and Bradly 1982). In field application of insecticides, some insects may expose to sub-lethal concentrations of the applied insecticide. Many sub-lethal effects on insect pests can result from that exposure.

In the present study, emamectin benzoate was approximately 34 and 83 times more toxic than lufenuron and flufenoxuron, respectively. Lufenuron was approximately 2 times more toxic than flufenoxuron against the 2nd instar larvae of *S.littoralis* after 96 hrs of exposure. Argentine *et al*. (2002) reported high toxicity of emamectin benzoate against six species of Lepidoptera (LC_{90} ranged from 0.0050 to 0.0218 µg/ml). Previous studies also showed that emamectin benzoate was more toxic than lufenuron and flufenoxuron against the 2nd, 3rd and 4th instar larvae of *S. littoralis* (Saad *et al*., 2011).

Emamectin benzoate at the two tested concentrations (LC_{10} and LC_{25}) significantly decreased the average weight of treated larvae, reduced pupation percentage, suppressed the pupal mean weight and reduced adult emergence rate compared to control during the observation period. Abro*et al*. (1993) found that abamectin significantly reduced weight gain (food consumption) by fourth instars of *P. xylostella* at LC_5. In another study, when *Lymantria dispar* L. larvae treated with either 5.0 or 1.0 ppm of abamectin had led to significantly reduced frass output and weight gain, indicating feeding cessation, also, delayed molting by at least 1 d. Avermectin at all sublethal concentrations appeared to act as depressants (Deecher *et al*., 1990). In some dipteran larvae, sub-lethal doses of ivermectin have been reported to reduce pupation and emergence of adults (Strong, 1989). Emamectin benzoate at LC_{10} and LC_{25} when used against the 2nd instar larvae, was also significantly decreased the average of eggs laid / female (fecundity) and percentage of egg hatch (fertility). Strong (1993) mentioned that ivermectin residues in sheep dung affected the reproduction of treated insects. So, Kruger and Scholtz (1995) reported that ivermectin diminished the fertility in adults of *Musca nevilli* which developed in dung from treated cattle.

Benzoylphenylureas (BPUs), are considered safe insecticides for humans and other mammals, since chitin is absent in these species (Apperson *et al*., 1978). In this study, lufenuron and flufenoxuron negatively affect most of the growth and development parameters when the 2nd instar larvae of *S. littoralis* were treated by two sub-lethal concentrations. Lufenuron and flufenoxuron caused significant decrease of the larval body weight, increase of the average time to pupation for treated larvae, decrease of pupation percentage, suppress the mean pupal weight and reduce the adult emergence, in a concentration dependent manner, compared to control treatment.

Table 5. *In vivo* effect of sub-lethal concentrations of emamectin benzoate, lufenuron and flufenoxuron on the PPO activity extracted from the treated 2nd instar larvae of *S. littoralis* after 4 days of exposure

Insecticide	Conc. (mg L^{-1})	Specific activity (OD_{420}/mg protein/hr) ± SE	Inhibition % ± SE
Control	-	18.4^{a} ± 1.6	
Emamectin benzoate	0.001	14.0^{b} ± 0.8	23.78 ± 0.76
	0.002	10.2^{d} ± 0.8	44.54 ± 0.16
Lufenuron	0.028	11.5^{cd} ± 0.5	37.34 ± 0.91
	0.064	7.3^{e} ± 0.5	60.28 ± 0.25
Flufenoxuron	0.06	12.7^{bc} ± 0.7	30.85 ± 0.74
	0.16	7.6^{e} ± 0.4	58.61 ± 0.48

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

1: No of eggs laid/femal 2:% of egg hatch

Abdel Rahman *et al*. (2007) reported that, when the 3rd instar larvae of *S. littoralis* were treated with lufenuron the affected larvae ceased feeding within 48 hrs. This may be the main reason for larval and pupal weight reduction. Our results are in agreement with the data of Adel (2012), who found a profoundly inhibition in the weight gain of *S. littoralis* treated larvae by lufenuron. Similar results also were recorded by Bakr*et al*. (2010) with flufenoxuron. They mentioned that, flufenoxuron significantly increased the larval and pupal durations, on the other hand decreased the percentages of pupation and adult emergence of *S. littoralis*. Same results were recorded with chlorfluazuron and lufenuron against *S. littoralis* larvae, where all treatments succeeded to decrease the percent pupation, pupal weight and adult emergence (Sammour *et al*., 2008).

Chlorfluazuron and lufenuron had been reported to significantly reduce the *S. littoralis* fecundity and fertility (Sammour *et al*., 2008). In the present study, *S. littoralis* fecundity and fertility were significantly suppressed as a result of lufenuron and flufenoxuron treatments compared to control. Previous results confirmed what we have acquired from the results. Yamada *et al*. (1993) reported that, chlorfluazuron attenuated the fecundity and emergence rates on the development stage of the *Plutella xylostella*. Adel (2012) also reported that, lufenuron decrease the fecundity and fertility of the eggs produced by the adult progeny.

In our study, the two concentrations of emamectin benzoate, lufenuron and flufenoxuron significantly inhibit PPO of treated *S. littoralis* larvae in a concentration dependent manner. Nasr *et al*. (2010) recorded same results with pyriproxyfen and buprofezin against *S. littoralis* larvae. They mentioned that, the inhibition of PPO of *S. littoralis* by pyriproxyfen and buprofezin was concentration dependent. In general, further investigations were needed to clarify the interaction of IGR compounds with insect PPO activity and its impact on the insect growth. However, other studies are needed to investigate the effect of these sub-lethal doses on the development of resistance.

Finally, our results verified the lethal and sublethal effects of emamectin benzoate, lufenuron and flufenoxuron on the larval stage of *S. littoralis*. The sublethal concentrations of these insecticides negatively affect fertility and fecundity of *S. littoralis*. These effects are very important from a practical point of view, because offspring can then be reduced and the insect population can be negatively affected. Fortunately, the mode of action of emamectin benzoate, lufenuron and flufenoxuron is different from pyrethroid, carbamate and organophosphate insecticides. So, emamectin benzoate, lufenuron and flufenoxuron can be used for *S. littoralis* control and resistance management programs.

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الملخص العربي

التأثيرات البيوكيميائية والبيولوجية للجرعات التحت مميتة لمبيدات الإيماميكتين بنزوات وبعض منظمات النمو الحشرية ضد دودة ورق القطن

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تم تقييم السمية وتأثيرات الجرعات التحت المميتة لمبيدات الإيماميكتين بنزوات واللو فينورون وفلوفينوكسيرون على يرقات دودة ورق القطن (العمر الثانى). كما اختبر تأثيرهذه المبيدات الحشرية على انزيم البولى فينول أوكسيديز فى اليرقات المعاملة . كان تركيز مبيد الإيماميكتين بنزوات اللازم لقتل ٥٠% من الأفراد المعاملة (٠,٠٠٧ مليجرام/لتر) مما يدل على انه أكثر سمية حوالى ٣٤ و٨٣ مرة مقارنة بمبيد اللوفينورون ٠,٢٤ مليجرام/لتر) وفلوفينوكسيرون ٠,٥٨مليجرام/لتر) على التوالى. بينما كانت سمية اللوفينورون ضعف سمية الفلوفينو كسبرون. وقد أظهرت النتائج انخفاض معنوى فى وزن اليرقات ونسبة تحول اليرقات لعذارى ومتوسط وزن العذارى ومعدل خروج الحشرات الكاملة عند معاملة يرقات العمر الثانى لدودة ورق القطن بتركيزات LC_{25} وLC_{10} من الإيماميكتين بنزوات واللو فينورون وفلوفينوكسيرون . كما انخفضت فترة تحول اليرقات لعذارى عند تركيزات LC_{10} و LC_{25} ا من اللوفينورون او فلوفينوكسيرون بينما كان وقت التحول اطول منه لليرقات الغير معاملة. وعلى الجانب الاخرلم يختلف طول فترة العمر اليرقى اختلاف معنوى فى كل من اليرقات المعاملة بكلا التركيزين LC_{10}و LC_{25} او برقات الكنترول. بينما انخفضت نسبة الخصوبة ونسبة فقس البيض انخفاضا معنويا فى كل المعاملات مقارنة بالكنترول.كذاك اظهرت النتائج اتخفاض نشاط اتزيم البولى فينول أوكسيديز فى كل المعاملات وهذا الانخفاض يتناسب طرديا مع التركيز.وتشير النتائج إلى أن التركيزات التحت مميتة من الإيماميكتين بنزوات واللو فينورون وفلوفينوكسيرون قد تؤدى الى انخفاض معدلات الزيادة فى تعداد يرقات دودة ورق القطن وذلك عن طريق التأثير على تطورها وتكاثرها.