Toxicological, Biochemical and Histological Studies on the Fall Armyworm, Spodoptera frugiperda Treated with Five Insecticides

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Abstract

Saleh, H.A., S.F. Abd El-Rahman, M.A. Batt and H.R.K. Ali. 2024. Toxicological, Biochemical and Histological Studies on the Fall Armyworm, *Spodoptera frugiperda* Treated with Five Insecticides. Arab Journal of Plant Protection, 42(4): 489-496. <u>https://doi.org/10.22268/AJPP-001280</u>

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) is one of the most important, invasive, and destructive pests. It was reported for the first time on maize plants in 2019 in upper Egypt. A bioassay was conducted to evaluate the toxicity of 5 insecticides (Protecto 9.4% WP, Pesover 90% SP, Punkron 20% EC, Uphold 36% SC and Full 48% EC) against 2^{nd} and 4^{th} instar larvae of this pest under laboratory conditions. All tested insecticides were more efficient against the 2^{nd} instar larvae than the 4^{th} instars. Punkron was the most efficient pesticide against the 2^{nd} instar larvae, with LC₅₀=0.0039 g/L, whereas Uphold showed higher toxicity than the other insecticides against the 4^{th} instar larvae (LC₅₀=0.0524 g/L) 5 days after treatment. The effect of the tested insecticides on the activities of acetylcholine esterase (AChE), carboxylesterase (CE), glutathione S-transferase (GST) and Alpha & beta esterase enzymes was determined. All tested insecticides induced the activity of AChE and CE in the 4^{th} instar larvae, except for the insecticide Full (Chlorpyrifos). All insecticides induced histological alterations (e.g., structure of the muscle layers, disorganization in the epithelial cells, and cell fragmentation) in the midgut, damaging the digestive cells and peritrophic matrix, affecting digestion and nutrient absorption. The tested insecticides in this study may play a prominent role in the integrated management program of the fall armyworm.

Keywords: Spodoptera frugiperda, insecticides, bioassay, biochemical activity, histology.

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a major lepidopteran pest of maize and it is native to the tropical and subtropical regions of the western hemisphere from the United States to Argentina (Sparks, 1979). In Africa, the first report of the pest on maize plants was recorded in 2016 (Goergen *et al.*, 2016; Montezano *et al.*, 2018). In Egypt, it appeared for the first time in 2019 on maize fields at Aswan Governorate, Upper Egypt (Mohamed *et al.*, 2022).

Larvae of this insect attacks about 186 species of cultivated plants but the greatest damage was observed in maize and sorghum plants as main hosts, along with other crops such as cotton, soybean, sugarcane, rice, wheat, cowpea, groundnut, potato, and other vegetable crops (Casmuz *et al.*, 2010; Hardke *et al.*, 2015). *S. frugiperda* caused losses in maize yield from 8.3 to 20.6 million tons per year, in just 12 of African maize countries. (Montezano *et al.*, 2018). In Brazil, the same pest caused up to 34% reduction in maize grain which equaled 400 million US\$ (Lima *et al.*, 2010). Therefore, it is important to use insecticides and there is a need to develop sustainable IPM programs against *S. frugiperda* for safe, ecofriendly management (Abrahams *et al.*, 2017; Ramanujam *et al.*, 2020).

Insects respond to insecticides toxic action either through modulating detoxification enzyme activity or increasing target site insensitivity, as in nerve conduction enzyme acetylcholine esterase (AChE). Different susceptibilities of insect pests to insecticides have been related to the levels of metabolizing enzymes (Fergani *et al.*, 2020; Liu *et al.*, 2017). Also, the insecticide resistance was observed in different insect species associated with high activities of hydrolases such as non-specific and specific esterases and many detoxifying enzymes (including GST, AChE and CaE) (Mohamed *et al.*, 2016).

The current study was conducted to evaluate toxicity of some insecticides against *S. frugiperda* and their effect on it's the enzymes activity and histology of under laboratory conditions.

Materials and Methods

Toxicity of five insecticides on fall armyworm, *S. frugiperda* larvae

Insect Culture - A stock culture of *S. frugiperda* larvae was collected from infested maize fields at Abjij, Faiyum District, Faiyum Governorate (29°17′09.5N, 30°48′54″E). Insects were reared under controlled conditions in an incubator at $25\pm2^{\circ}$ C, $65\pm5\%$ R.H. and 8 hr light:16h dark at Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. Larvae were supplied daily with fresh and cleaned castor bean leaves, *Ricinus communis* L. as a source of food until pupation. Larvae were reared separately from the 3rd instar until the pupation. This was done in small glass vials (7 cm height × 2 cm diameter each) covered with fine muslin. The pupae were kept in clean jars (500 ml) tell adult emergence. Emerged adults were kept in chimney glass cages. Each cage was provided piece of cotton

https://doi.org/10.22268/AJPP-001280

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in vials filled with 20% honey solution as food. The cage was placed in Petri dish and covered with muslin cloth and rubber band (El-Defrawi *et al.*, 1964). The cages were provided with a maize seedling, 18 cm length, as placed for egg laying. The seedlings were replaced daily and collect the deposited eggs. The eggs were maintained at previous conditions until hatching. The newly hatched larvae were reared for three successive generations on the castor leaves to obtain a sensitive strain.

Insecticides used - Five insecticides (Table 1) were tested against fall armyworm *S. frugiperda* larvae, as well as their effect on the insect enzymes activity were investigated.

Bioassay of tested insecticides - The concentrations of tested insecticides were 0.50, 0.25, 0.125 and 0.0625 (ml/L or g/L). These concentrations were evaluated against 2nd and 4th instar S. frugiperda larvae by leaf dipping technique. Castor leaf discs $(8 \times 6 \text{ cm})$ were dipped in these concentrations for 2 minutes and offered to larvae after they completely dried under room temperatures. The control was treated by only distilled water. After larvae were starved for 8h, they were fed for 24 hr on the treated discs except for Protecto (B. thuringiensis) treatment, they were fed for 48 hours, then all larvae were transferred to fresh untreated leaves. The five tests were replicated three times/dose and 10 individuals were used per replicate. The experiment was conducted in an incubator at 25±2°C and 65±5% R.H. Numbers of dead larvae were recorded at 1, 3, 5, 7 and 10 days after treatment to calculate mortality percentages and determine LC₅₀ and LC₉₀ values.

Effects of the insecticides on detoxification enzymes in larvae

Sample preparation -The biochemical assay was carried out on the 4th instar larvae treated with LC_{50} concentration of all tested insecticides. 24 h after treatments, 0.5 g of live treated larvae was frozen until processed. The frozen larvae were homogenized in distilled water using a Teflon homogenizer and then centrifuged at 8000 rpm for 15 minutes at 2°C in a refrigerated centrifuge. The supernatant of each sample was kept in a deep-freezer at -20°C until used for biochemical analysis using three replicates for each sample.

AchE activity - Acetylcholine esterase activity was measured according to the method described by Simpson *et al.* (1964), using acetylcholine bromide (Ach Br) as substrate. The reaction mixture contained 200 μ l enzyme solution, 0.5 ml 0.067 M phosphate buffer (pH 7.0) and 0.5 ml AchBr (3 mM). The decrease in Ach Br resulting from hydrolysis by AchE was recorded at 515 nm, and Carboxyl esterase activity was measured according to Simpson *et al.* (1964), using methyl-n-butyrate (MeB) as substrate. The decrease in MeB resulting from hydrolysis by carboxyl esterase was recorded at 515 nm.

GST activity - Glutathione S-transferase (GST) catalyses the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-Lglutathione was detected as described by the method of Habig *et al.* (1974). Determination of α -esterase and β -esterase activity -Esterases activity was done according to Van Asperen (I962) using α -naphthyl acetate or β -naphthyl acetate as substrates, respectively.

Effect of the tested insecticides on histological structure of mid gut

Preparation of mid gut for light microscopy - The histological effects of the five insecticides against 4th instar larvae were carried out by feeding them for 24 hr on each treated leaves with (LC_{50}) insecticides concentration in addition to the control (distilled water). Mid guts of 4th instar larvae were immersed in a saline solution (0.9% NaCl solution), then dissected and the obtained tissues were immediately fixed in a Bouin's solution for 24 hours. The midguts were dehydrated in a graded ethanol series (70, 80, 90 and 95%) and cleared in Xylene for few seconds. The tissues were then left in paraffin wax serials, and then sectioned to 3 µm slices. The sections were deparafnized, rehydrated and stained in hematoxylin for 12 min and in alcoholic eosin for 5 min. They were then dehydrated, cleared in xylene and each was photographed by a lightmicroscope (Vinha et al., 2021). The sections were photographed at 200x magnification.

Statistical analysis

The corrected mortality was calculated using Abbott's formula (1925) and Probit analyses for larval mortality were performed according to Finney (1971) to determine the lethal concentrations (LC₅₀ and LC₉₀) and slope values of the mentioned compounds were also determined. All data were statistically analyzed using analysis of variance (ANOVA), and treatments mean significance was measured by Duncan's test (P<0.01). F and LSD values were determined using SPSS version 14 software (Snedecor & Cochran,1967).

Results and Discussion

Toxicity of tested insecticides

The LC_{50} and LC_{90} values for 2nd instar *S. frugiperda* larvae treated with the insecticides are shown in Table 2. The LC_{50} values ranged from 0.0039 (ml/L) for Punkron to 0.1493 (ml/L) for Full insecticide. Depending on the LC_{50} and LC_{90} values, Punkron was the most effective insecticide on the larvae (0.0039 and 0.1806 ml/L, respectively) followed by Uphold. Whereas Protecto was the least efficient of the tested insecticides.

The efficacy of treatments on the fourth instar larvae depending on the LC_{50} values showed that Uphold insecticide was the most effective (0.0524 ml/L) followed by Punkron (0.0979 ml/L), Pesover (0.4282 g/L), Full (3.4944 ml/L) then Protecto (14.5597 g/L). IN addition, the results obtained showed that *B. thuringiensis* had confidence limits with LC_{50} from 4.0924 to 52.0618 g/L (Table 2). Generally, Uphold and Punkron insecticides were more effective than the other three products treatments 5 days after treatment, which was in agreement with previous findings (Ahissou *et al.*, 2021; Idrees *et al.*, 2022; Kumar & Mohan, 2020; Shareef *et al.*, 2022; Sileshi *et al.*, 2022; Vinha *et al.*, 2021).

Table 1. Trade name and active ingredients of the five tested insecticides investigated in this study.

Trade name	Active ingredient	Group	Rate/feddan
Protecto 9.4% WP	Bacillus thuringiensis var. kurstaki	Bacterial agent	400 g
Pesover 90% SP	Methomyl	Carbamate	300 g
Punkron20% EC	Lufenuron	Benzoylurea	40 ml
Uphold 36% SC	Methoxyfenozide30% + Spinetoram 6%	Diacylhydrazine + Spinosyns	125 ml
Full 48% EC	Chlorpyrifos	Organophosphate.	1000 ml

Table 2. Toxicity of the tested insecticides against the 2^{nd} and 4^{th} instars larvae of *S. frugiperda* five days after treatment.

	Instar		Confide	nce limits	Confidence limits					
Treatment	stage	LC50	Lower	Upper	LC ₉₀	Lower	Upper	Slope		
Protecto 9.4% WP	2 nd	0.0893 (g/L)	0.0593	0.1151	45.1007 (g/L)	5.3942	116.3900	0.4608 ± 0.1940		
	4^{th}	14.5590 (g/L)	4.0920	52.0610	145.2110 (g/L)	89.1600	795.1700	0.6407 ± 0.1675		
Pesover 90% SP	2^{nd}	0.0746 (g/L)	0.0019	0.1940	0.3362 (g/L)	0.2786	0.4371	2.2271±0.3221		
	4^{th}	0.4280 (g/L)	0.2230	1.6300	3.6196 (g/L)	1.1580	5.1900	1.3827±0.2936		
Uphold 36% SC	2^{nd}	0.0229 (ml/L)	0.0008	0.0540	0.3524 (ml/L)	0.0553	0.8990	1.0802 ± 0.5413		
	4^{th}	0.0524 (ml/L)	0.0130	0.0970	4.9879 (ml/L)	1.9970	45.5400	0.6478 ± 0.1413		
Punkron 20% EC	2^{nd}	0.0039 (ml/L)	0.0003	0.0092	0.1806 (ml/L)	0.0951	1.1744	0.7697 ± 0.2091		
	4^{th}	0.0970 (ml/L)	0.0650	0.1960	4.2587 (ml/L)	1.1920	45.7000	0.7823 ± 0.1372		
Full 48% EC	2^{nd}	0.1493 (ml/L)	0.0514	0.3471	0.9161 (ml/L)	0.7098	1.2569	1.6270 ± 0.3180		
	4 th	3.494(ml/L)	2.4220	6.2810	57.246 (ml/L)	23.2600	65.0900	1.0427±0.1520		

Effects of the insecticides on detoxification enzymes in larvae

It was inevitable to assess the effect insecticides on $\alpha \& \beta$ esterase and detoxification enzymes (AChE, CE and GST), that the insects use to defend themselves against the insecticides in 4th instar *S. frugiperda* larvae.

Acetvl cholinesterase - Acetvl cholinesterase (AChE) is a very important element in the insect's nervous system. Results summarized in Table 3 revealed a highly significant stimulation of ACh E activity by the tested insecticides. The Pesover insecticide significantly increased the enzyme reactions by 14.09-fold compared to the control. Furthermore, Pesover induced the highest activity of AChE (418.7 ug Ach Br/min/g.b.wt.) followed by Uphold (165.3 ug Ach Br/min/g.b.wt.). However, Full (30.7 ug Ach Br /min/ g.b.wt), Punkron (37.7 ug Ach Br/min/g.b.wt.) and protecto (47 ug Ach Br /min/ g.b.wt.) treatments were not significantly different compared with 29.7 ug Ach Br/min/g.b.wt. in the control. These results agreed with the findings of other workers (Abd-El-Aziz et al., 2020; Abd El-Rahman et. al., 2019a); On other hand, Rahman et al. (2022) found that, AchE activity in 3rd instar S. frugiperda larvae was significantly decreased (60.25%) in treated larvae with the higher concentrations (500 ppm) of copper nanoparticles, 24 h after treatment.

Carboxylesterases (CEs) - Results obtained (Table 3) showed significant changes in the activity of the Carboxylesterase enzyme resulting from the treatment of the 4th instar larvae with the tested insecticides Uphold, Punkron and Full with significant increase of 355.7, 281.0 and 238.3 ug per g.b.wt., respectively as compared with the control. However, there were no significant differences between the

enzyme activity in the Protecto and Pesover treatments and the control which were 150.3, 181.0 and 167.3 ug per g.b.wt., respectively. Carboxylesterases (CEs) in insects play an important role in the biotransformation and detoxification of exogenous structures such as insecticides that are hydrolyzed and/or sequestered. These enzymes might contribute to insecticide resistance (Montella *et al.*, 2012).

Glutathione S-transferase (GST) - Tested insecticides decreased the activity of the GST enzyme (Table 3). Protecto produced the lowest activity $(4\pm0.400 \text{ mmol})$ subconjugated/min/g.b.wt.). There was no significant difference between the Uphold and Punkron treatments or between Pesover or Full treatments. Uphold and Punkron treatments inhibited GST reaction in S. frugiperda, suggesting a weak resistance against these insecticides, and thus they are effective against S. frugiperda and can be used in control programs. Insect GSTs are known to be specifically or preferentially expressed into major detoxification organs such as fat body, midgut, epidermis and malpighian tubules (Huang et al., 2011). Glutathione Stransferases (GSTs) metabolize and detoxify a wide variety of toxic chemical compounds, environmental pollutants and oxidative stress products. GSTs play a major role in the detoxification of reactive metabolites of methylcholanthrene by mediating catalytic binding with GSH to form a detoxified complex and converting them into less toxic water-soluble products which are then eliminated (Dasari et al., 2018; Singh et al., 2001). Pengsook et al. (2022) found that, when S. exigua larvae were treated with Piper ribesioides, the extract with ethyl acetate inhibited the glutathione-s-transferase (GST) enzyme 1.30-fold 24 h posttreatment.

Table 3. Effect of five insecticides on enzymes Activity of 4th instar larvae of *S. frugiperda*.

	Acety	lcholines	sterase												
	AchE (ugAchBr/min/g			Carboxylesterases			GST (mmol			Alpha Esterases (ug a-			Beta Esterases (ug β-		
	(be	(body weight) (ug Meb/min/g.b.wt)				subcojugated/min/g.b.wt)			naphthol/min/g.b.wt.)			naphthol/min/g.b.wt.)			
		Change Activity		Change Activity		Change Activity		Change Activity			Change Activity				
Treatment	Mean	%	ratio	Mean	%	ratio	Mean	%	ratio	Mean	%	ratio	Mean	%	ratio
Protecto	47.0 c	58.24	1.58	150.3 c	-10.16	0.89	4.0 d	-82.83	0.17	1061.0 a	95.28	1.95	295.0 a	29.38	1.29
9.4% WP															
Pesover	418.7 a	1309.76	14.09	181.0 c	8.18	1.08	15.5 b	-33.47	0.66	1069.3 a	96.81	1.97	248.0 b	8.77	1.08
90% SP															
Uphold	165.3 b	456.56	5.56	355.7 a	112.61	2.12	10.6 c	-54.50	0.45	670.0 b	23.32	1.23	261.0 a	14.47	1.14
36% SC															
Punkron	37.7 c	26.93	1.26	281.0 b	67.96	1.67	10.5 c	-54.93	0.45	717.0 b	31.97	1.32	267.7 b	17.41	1.17
20% EC															
Full	30.7 c	3.36	1.03	238.3 bc	42.43	1.42	17.5 b	-24.89	0.75	413.3 c	-23.93	0.76	158.7 c	-30.35	0.69
48% EC															
Control	29.7 c	-	-	167.3 c	-	-	23.3 a	-	-	543.3 d	-	-	228.0 b	-	-

Mean values followed by same letter in the same column are not significantly different at P=0.01.

% Change compared to the control =(Test –Control /Control) x 100

Activity ratio= enzyme activity of the compound /enzyme activity of control

Alpha and **β-esterase in** S. frugiperda larvae - Results obtained (Table 3) showed that all treatments led to an increase in Alpha esterase $(\alpha - E)$ activity except the Full treatment which decreased the activity by 23.93% lower than the control. The highest activity was determined in larvae treated with Pesover and Protecto and reached 1.95 and 1.97fold compared to the control. On the other hand, the highest activity of B-esterase was found with Protecto treatment $(295\pm18.735 \ \mu g \ \beta$ -naphthol/min/g.b.wt.) compared to Punkron and Uphold which reached 267.7±12.42 and $261\pm11.53 \ \mu g \beta$ -naphthol/min/g.b.wt, respectively (Table 3). Whereas, Full insecticide produced the lowest activity (158.7±10.26 ug β-naphthol/min/g.b.wt.), and these results were in agreement with studies reported earlier (Abd EL-Naby, 2019; Abd El-Rahman et al., 2019b; Fergani et al., 2020; Kady et al., 2007; Pengsook et al., 2022).

Effect of the tested insecticides on histological structure of mid gut

The normal mid-gut ultrastructure in control - The histological structures of normal mid-gut of 4^{th} larval instar of *S. frugiperda* are shown in Figure 1. The midgut consists of a single epithelial layer cell (ec) and goblet cells (gc). The epithelium layer rest on basement membrane (bm) and the membrane is externally surrounded by circular and longitudinal muscle layers. In addition, epithelium layers are protected from food particles by a peritrophic membrane (pm) surrounding the lumen (lu) (Sakr & El-Nabi, 2007; Roel *et al.*, 2010).

Protecto 9.4% WP insecticide - The histopathological effects of the Protecto treatment on the midgut of 4^{th} instar larvae of *S. frugiperda* is shown in Figure 2. Epithelium cells were the most affected compared with the untreated mid gut, where the epithelial cells showed histolysis with vacolated cytoplasm. The regenerative-cells (rc) were separated from each other, and the goblet cells secretions were increased.

Pesover 90% SP insecticide- The transverse section in the midgut of 4th instar larvae of *S. frugiperda* fed on castor-bean

leaves treated with LC_{50} of Pesover, is shown in Figure 3. The regenerative cells (rc) lost their integrity between the columnar cells. The peritrophic membrane (pc) was unattached and disintegrated, with disorganization of regenerative cells (rc) leaving a cleft. The basement membrane (bm) was separated from the base of the columnar cells (cc). In addition, the goblet cells became disorganized.



Figure 1. Light micrographs of the normal mid-gut of 4^{th} larval instars of *S. frugiperda* (200X). ec: epithelial cell, bm: basement membrane, gc: goblet cell, ln: Lumen, pr= peritrophic membrane.

Uphold 36% SC insecticide - Examination of mid-gut section of 4^{th} larval instar of *S. frugiperda* treated with LC₅₀ of Uphold is shown in Figure 4. The Uphold treatment resulted in numerous histological alterations compared to those of the control (Figure 1). In addition, this treatment proved to be the most effective against the midgut epithelial cells which caused the appearance of a large cavity in cells,

and slight increase in size. The base epithelial cells were separated from the basement membrane.

Punkron 20% EC insecticide- After treatment with LC_{50} of Punkron insecticide, examination of *S. frugiperda* mid-gut section showed that the most effect was observed on the apical surface of the midgut epithelium cells with less epithelial cells vacuolization. The cytoplasm was highly vacuolated in treated larva (Figure 5). In addition, peritrophic membrane separation, and the walls between columnar cells were completely disappeared, and the columnar cells started to disintegrate.



Figure 2. Light micrographs of the mid-gut of 4^{th} larval instars of *S. frugiperda* after treatment with Protecto 9.4% WP, (200X). ec: epithelium cells, gc: globlet cells, rc: regenerative cells.



Figure 3. Light micrographs of the mid-gut gut of 4th larval instars of *S. frugiperda* after treatment with Pesover 90% SP (200X). rc: regenerative cells, cc: columnar cells, pm: peritrophic membrane (pm), bm: basement membrane.



Figure 4. Light micrographs of the mid-gut of 4^{th} larval instars of *S. frugiperda* after treatment with Uphold 36% SC (200X). A large cavity is observed in the epithelial cells (ec).bm: basement membrane.



Figure 5. Light micrographs of the mid-gut of 4th larval instars of *S. frugiperda* after treatment with Punkron 20% EC (200 X). ec: epithelial cells, pm: peritrophic membrane, cc: columnar cells.

Full 48% EC insecticide- The histological effects of Full 48% EC on the midgut are shown in Figure 6 and characterized by the following: (i) e apical surface of the midgut epithelium was irregular, (ii) disruption of the peritrophic membrane, (iii) goblet-cells secretion increased with ruptures of basement, and (iv) separation of the peritoneal membrane.

Likewise, Shu *et al.* (2021) studied the effect of Camptothecin (CPT) on histology of 3^{rd} larval instar *S. frugiperda*. The severity of histological damage to the gut was dose dependent. In larvae fed on a diet containing 5.0

 μ g/g CPT, only the basement membrane was left in the intestinal wall of the midgut, and nearly all functional cells disappeared. Vinha *et al.* (2021) found that deltamethrin caused disorganization in the striated border, cytoplasm vacuolization, and cell fragmentation in the midgut of *S. frugiperda* larvae, damaging the digestive cells and peritrophic matrix. Additionally, Amin *et al.* (2022) reported that the columnar cells began to disintegrate; the regenerative cells lost their integrity between the columnar cells separated from the basement membrane in *S. littoralis* larvae when treated with Emamectin benzoate and its nanoform and the silver nanoparticles.

It can be concluded from this study that the five insecticides applied against the cotton leaf worm, *S. frugiperda* showed that Punkron and Uphold treatments were the most efficient compared to the other insecticides against 4th instar larvae. Accordingly, Punkron and Uphold insecticides could be included as a part of integrated pest management of *S. frugiperda*.



Figure 6. Light micrographs of the mid-gut of 4^{th} larval instars of *S. frugiperda* after treatment with Full 48% EC (200 X). ec: epithelial cells, pm: peritrophic membrane, bm: basement membrane.

الملخص

صالح، حسام أ.، سهير عبد الرحمن، محمد بط وهدى علي. 2024. دراسات سميّة وكيميائية-حيوية ونسيجية على دودة الحشد الخريفية (Spodoptera frugiperda). مجلة وقاية النبات العربية، 42(4): 489-489. <u>https://doi.org/10.22268/AJPP-001280</u>.

تعد دودة الحشد الخريفية (Spodoptera frugiperda) واحدة من أهم الآفات الغازية المدمرة. سجلت هذه الآفة لأول مرّة في مصر على نباتات الذرة في عام 2019 في صعيد مصر. تمّ إجراء اختبار حيوي لتقييم سمية 5 مبيدات حشرية (Pw WP ، Protecto 9.4% WP) على يرقات العمر الثاني والرابع لهذه الآفة تحت ظروف المختبر . أوضحت النتائج أن جميع المبيدات المختبرة كانت عالية الكفاءة ضدّ يرقات العمر الثاني مقارنة بيرقات العمر الثاني والرابع لهذه الآفة تحت ظروف المختبر . أوضحت النتائج أن جميع المبيدات المختبرة كانت عالية الكفاءة ضدّ يرقات العمر الثاني مقارنة بيرقات العمر الرابع. كان المبيد Punkron أكثر المبيدات فعاليةً ضدّ يرقات العمر الثاني، وكانت قيمة 2000 ع/ليتر ، بينما أظهر يرقات العمر الثاني مقارنة بيرقات العمر الرابع. كان المبيد Punkron أكثر المبيدات فعاليةً ضدّ يرقات العمر الثاني، وكانت قيمة 2000 ع/ليتر ، بينما أظهر المبيد Uphold سميّة أعلى من المبيدات الحشرية الأخرى ضدّ يرقات العمر الرابع، حيث بلغت قيمة 2000 للاثني، وكانت قيمة 2000 ع/ليتر ، بينما أظهر دراسة تأثير المبيدات الحشرية الأخرى ضدّ يرقات العمر الرابع، حيث بلغت قيمة 2000 ع/ليتر وذلك بعد 5 أيام من المعاملة. كذلك تعت المبيد عائير المبيدات الحشرية الأخرى ضدّ يرقات العمر الرابع، حيث بلغت قيمة 2000 ع/ليتر (CSP) وأنزيمات وراسة تأثير المبيدات الحشرية المختبرة إلى تحفيز نشاط أنزيمات ACA و عال على الرابع، ومن ناحيةٍ أخرى خفضت المبيدات تعتر فى طبقات العمر الرابع، وقد أنت حميع المبيدات الحشرية المختبرة إلى تحفيز نشاط أنزيمات ACA و عال في يرقات العمر الرابع، ومن ناحيةٍ أخرى خفضت المبيدات المشرية نشاط GST مع زيادة نشاط أنزيمات ألفا وبيتا استيراز في العمر اليرقي نفسه. كما تسببت جميع المبيدات الحشرية بحدوث تغييرات نسيجية على نحو حدوث تعتر فى طبقات العضلات، وتشوّه في الخلايا الظهارية، وتقتيت الخلايا في الأمعاء وأدى ذلك إلى إزلاف خلايا الجهاز الهضمي ما أثر على عملية الهضم وامتصاص العناصلات، وتشوّه في الخلايا الظهارية، وتقتيت الخلايا في الأمعاء الوسطى، وأدى ذلك إلى إتلاف خلايا الجهاز الهضمي ما أثر على عملية الهضم وامتصاص العناصر الغذائية. يمكن للمبيدات الحشرية المختبرة في هذه الدراسة أن تلعب دوراً بارزاً في برنامج الإدارة المتكاملة الخريفية. كلمات صاص العناصر الغذائية. ميدات الخشرية المنيماط ك

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Received: February 7, 2023; Accepted: December 7, 2023

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تاريخ الاستلام: 2023/2/7؛ تاريخ الموافقة على النشر: 2023/12/7