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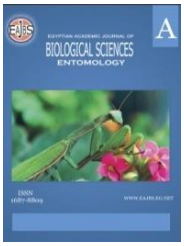

Biochemical and Histopathological Impacts Induced by the Lethal Toxicity of Chlorpyriphos, Methomyl, and Spinosad against the Fall Armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Egypt

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* is one of the most highly invasive and damaging agricultural pests. In Egypt, maize grains have enormous economic importance. Infestations of *S. frugiperda* in maize cause a significant reduction in maize yield. This research aimed to assess the potential impact of LC_{50} values for chlorpyrifos, methomyl, and spinosad, set at 470, 105.5, and 2.5 ppm, respectively, on the biochemical and histopathological responses of *S. frugiperda*. The results indicated that the exposure of fall armyworm larvae to lethal concentrations of such insecticides resulted in a significant decrease in the total protein, carbohydrate, and lipids associated with a significant increase in α, β-esterase, and acetylcholinesterase. Moreover, a noted elevation in acid and alkaline phosphatases, Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), phenoloxidase, and chitinase activities occurred. For the digestive enzymes, a significant decrease in amylase activity has occurred while the activities of invertase and trehalase have changed only with significant differences among spinosad and methomyl treatments. However, chlorpyrifos exhibited non-significant variations in the activities of invertase and trehalase. By the cross-section of the midgut larvae, distinct histological damage of the midgut was distinguished by cytoplasmic vacuolation, and necrosis with sloughing of epithelial lining from the basement membrane toward its lumen. In conclusion, all the treatments of insecticides, chlorpyrifos, methomyl, or spinosad, significantly affected the biochemical aspects and histopathology of *S. frugiperda* larvae**.**

INTRODUCTION

Spodoptera frugiperda is a crucial pest of several cultivations, maize in particulate. *S. frugiperda* recorded its first onset in Western Africa in 2016 (Goergen *et al*., 2016), and it speedily expanded all over the continent (Prasanna *et al.*, 2018) and resumed extending to Asia (Maino *et al.,* 2021). Because of its widespread, armyworm is currently the most destructive agricultural pest affecting maize in Egypt and other nations Bakry *et al*. (2023).

In agriculture, it has become a general trend for the application of variable classes of pesticides to control varieties of insects (Quazi *et al*., 2011). Noteworthy, pesticides are intentionally entered into agriculture with variable formulations to keep crops safe against insects and others (Yang *et al*., 2007). Earlier studies investigated the diversity of susceptibility to different insecticides among local strains of fall armyworm (Gutiérrez-Moreno *et al*., 2019). Among a variety of chemical insecticides, the most commonly utilized insecticides worldwide are methomyl and chlorpyrifos. The two insecticides could cause overstimulation of the nervous system because of irreversible inhibition of acetylcholinesterase (AChE) through profound cholinergic poisoning in insects, ultimately leading to the death of insects. Bioinsecticides are well studied because they are more efficient in plant protection as an alternative method to conventional pesticides. Spinosad is a bioinsecticide extracted naturally from the metabolites of *Saccharopolyspora spinosa* (Mertz and Yao 1990). Spinosad dominates a unique action on the insects, affecting the nicotinic acetylcholine receptors and H-Glutamate receptor sites of the nervous system, leading to persistent stimulation of motor neurons inducing stop feeding, muscle tremors, and paralysis and later on, death (Semiz *et al*., 2006).

Many defensive responses and biochemical mechanisms are implicated in the metabolic detoxification of insecticides (Hemingway *et al*., 1996). These mechanisms principally comprised insecticide detoxification, behavioral resistance, and target site insensitivity therefore it is no longer vulnerable to insecticide inhibition (Benelli *et al*., 2019). Among the most critical metabolic mechanisms for lacking insect resistance; is the inhibition of detoxification enzymes. In insects, esterases (α-β-EST) are considered the most common metabolic mechanism and mixed function oxidase (Dong *et al*., 2016), and hydrolysis of esterases is a critical process for the progression of resistant insects that recurrently occurs to diverse groups of chemical compounds (Gong *et al*., 2022). Further, AChE is a specified molecular goal for organophosphorus and carbamate.

Insecticides primarily affect the midgut, the target organ where digestion and absorption occur (Castro *et al*., 2021). Insecticides can cross the perimicrovillar membrane inciting damage and destruction of the epithelial cells (Santos-Junior *et al*., 2020).

Accordingly, the ongoing research was designed to evaluate the potential effect of lethal concentrations of short-term exposure of *S. frugiperda* larvae exposed to certain pesticides on the biochemical and histopathological profile.

MATERIALS AND METHODS

Insecticides:

Three pesticides were utilized in this study. Two insecticides, methomyl (Lannate® 90) and chlorpyrifos (Dofos 48%), have chemical bases. Insecticide produced from biological sources, Spinosad (Tracer 24%), was also utilized. The three pesticides' LC_{50s} were applied in accordance with (Salem *et al*., 2023)**.** The LC_{50s} values for chlorpyrifos, methomyl, and spinosad were 470, 105.5, and 2.5 ppm, respectively.

Preparation of Homogenate Samples for Physiological Analysis:

The 4^{th} instar larvae of *S. frugiperda* were treated with the LC_{50} values of the

tested insecticides for 24 hours. Three replicates were assigned for each treatment. Thereafter, the survived larvae of each treatment were prepared as described by Amin (1998). They were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, which are referred to as enzyme extract, can be stored for at least one week without appreciable loss of activity when stored at less than 0 ^{0}C. The same sample of untreated larvae was used as a control check.

Main Metabolic Contents and Enzymes Assay:

The produced supernatants underwent biochemical assessment, and the total level of carbohydrates, protein, and lipids was quantitatively determined using methods outlined by Singh and Sinha (1977), Lowry *et al*. (1951), and Barnes and Blackstock (1973), respectively. Biomarkers of digestive enzymes for assay of invertase and amylase were expressed by the procedure of Ishaaya and Swirski (1970) and Ishaaya (1971), respectively using 3,5-dinitrosalicylic acid reagent. Moreover, the trehalase enzyme was assessed by a procedure similar to that for invertase and amylase as mentioned by Noelting and Bernfeld (1948). While, activities of α&β-esterases and acetyl cholinesterase (AChE) in *S. frugiperda* were done spectrophotometrically by the technique described by Van Asperen (1962) and Simpson *et al*., (1964), respectively. The average concentrations of both acid and alkaline phosphatase were determined according to Laufer and Schin (1971). Moreover, biomarkers of the activities of glutamate oxaloacetate transferase (GOT/AST) and glutamate pyruvate transferase (GPT/ALT) were quantified according to Reitman and Frankel **(**1957). Eventually, colorimetric determination for phenoloxidase and chitinase enzymes was proceeded according to Ishaaya **(**1971).

Histopathological Studies:

Untreated and treated 4th instar larvae of *S. frugiperda* were dissected and underwent fixation in a 10% neutral-buffered formalin solution. After processing, they were embedded in paraffin wax. To facilitate histopathological analysis, the paraffin-embedded tissues were sliced into 4-5 µm sections using a microtome. These sections were subsequently stained using the hematoxylin and eosin (HE) staining method; and then prepared for observation and photomicroscopy (Suvarna et al., 2018).

Statistical Analysis*:*

The statistics were carried out via one-way ANOVA of IBM SPSS Statistics (Version 27). Data were described in the mean ± SE. Using the Duncan test to compare differences between untreated and other treated groups. However, the significance point was set at $p < 0.05$.

RESULTS

Effect of LC_{50} Values of Chlorpyrifos, Methomyl, and Spinosad on Biochemical Parameters of The Fourth Instar Larvae of *S. frugiperda*:

Effect on Total Carbohydrate, Protein, and Lipid Levels:

Based on the obtained data in Table (1), evaluation of the total carbohydrate content, indicated that spinosad had the lowest carbohydrate concentration (185.31±2.57 mg/100mg b.wt.) followed by methomyl (194.78±3.03 mg/100mg b.wt.). In comparison with the untreated control group (211.93± 4.44 mg/100 mg b.wt.), in chlorpyrifos treated group; carbohydrate did not significantly change (202.31±3.79 mg/100mg b.wt.). In total protein, both spinosad and chlorpyrifos compounds recorded a notable significant depletion (360.48± 3.24, 353.45±2.07 mg/100 mg b.wt.), respectively followed by methomyl (382.14±5.42 mg/100 mg b.wt.) as compared with control (391.45±5.98 mg/100 mg b.wt.). Regarding total lipids, it decreased with a more significant value among methomyl and

chlorpyrifos (302.12± 4.71, 314.11±4.36 mg/100 mg b.wt.), respectively, followed by spinosad (320.25±5.39 mg/100 mg b.wt.) in comparison with control (351.56±4.20 mg/100 mg b.wt.).

Table 1: Total carbohydrate, proteins, and lipids content (mg/g body weight) in 4th instar *S. frugiperda* larvae 24 hours post treatment with LC_{50s} of tested insecticides.

Component	Control	Chlorpyrifos	% Increase or decrease than control*	Methomyl	% Increase or decrease than control*	Spinosad	% Increase or decrease than control*
Carbohydrates	211.93±4.44^{a}	202.31±3.79^{ab}	-4.54	194.78±3.03^{bc}	-8.09	185.31±2.57^{c}	-12.56
Proteins	391.45±5.98^{a}	353.45±2.07^{b}	-9.71	382.14±5.42^{a}	-2.38	360.48±3.24^{b}	-7.91
Lipid	351.56±4.20^{a}	314.11±4.36^{bc}	-10.65	302.12± 4.71^{c}	-14.06	320.25±5.39^{b}	-8.91

Means have the different letters in the same row are significant (P<0.05).

*%Increase or decrease than control $= \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$

The Alterations in The Activities of Carbohydrate Hydrolyzing Enzymes:

Data in Table (2), exhibited that in all treatments by insecticides; the mean level of amylase activity in the supernatant of the homogenated *S. frugiperda* larvae was lesser than that obtained in the control untreated larvae. The activity of amylase was decreased significantly with spinosad and chlorpyrifos followed by methomyl. It was 48.32 ±0.2, 49.19 ±1.19 and 52.37 ±1.08, respectively µg /ml compared with the control 62.83±1.04 µg /ml.

Data expressed in Table 2, indicated a significant reduction in the activity of amylase in *S. frugiperda* resulted from treatment by spinosad and methomyl as compared to control.The activity of the invertase enzyme tended to record the highest reduction after treatment by spinosad followed by methomyl (33.46±0.10 and 35.26±0.04 µg/ml) with the exception of chlorpyrifos (38.18±0.14 µg/ml**)** that recorded the non-significant reduction in invertase enzyme when compared with control group (37.32± 0.19 µg/ml).

The activity of the trehalase enzyme was obviously decreased in spinosad compared to control larvae. It was 17.06± 0.39 µg/ml compared to control (22.94±1.11 µg/ml). The activity of trehalase was significantly increased in methomyl treatment. It was 27.15±1.11 µg/ml compared to the control. While trehalase enzyme was non-significantly changed among all chlopyrifos after 24 hours post-treatment. It was 20.38±0.56 µg/ml as shown in Table (2).

Table 2: Effect on the activities of the digestive Enzymes in 4th instar *S. frugiperda* larvae 24 hours post treatment with LC_{50} of tested insecticides.

Component	Control	Chlorpyrifos	% Increase or decrease than control*	Methomyl	% Increase or decrease than control*	Spinosad	% Increase or decrease than control*
Amylase (µg glucose/ml/min)	62.83±1.04^{a}	49.19±1.19^{c}	-21.71	52.37±1.08^{b}	-16.85	48.32±0. 2^{c}	-23.09
Invertase (µg glucose/ml/min)	37.32±0.19^{a}	38.18±0.14^{a}	2.30	35.26±0.04^{b}	-5.52	33.46±0.10^{c}	-10.34
Trehalase (µg glucose/ml/min)	22.94±1.11^{b}	20.38±0.56^{b}	-11.16	27.15±1.11^{a}	18.35	17.06±0.39^{c}	-25.63

Means have the different letters in the same row are significant (P<0.05).

*%Increase or decrease than control $= \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$

Effect on Esterases and Acetylcholinesterase Activities:

The current findings in Table (3), show that the toxicity of chlorpyrifos, spinosad, and methomyl resulted in significant elevation ($p \leq 0.05$) in α-Esterase (217.40±3.94, 176.95± 3.88 and 120.63±4.73 nmole/min/mg Pt.), respectively, as compared with control

group (73.20±2.40 nmole/min/mg Pt.). However, the activity of β- Esterase displayed a significant increase ($p \leq 0.05$) after treatment by spinosad, chlorpyrifos, and methomyl (268.38±6.33, 116.91±3.86 and 93.95±2.14 nmole/min/mg Pt.), respectively, in comparison with control (43.15±1.55 nmole/min/mg Pt.). Significant elevation ($p \leq 0.05$) in Ach enzyme was detected for spinosad-exposed *S. frugiperda* larvae when compared with control (210.93±3.44 nmole/min/mg Pt.) later; on the other hand, chlorpyrifos and methomyl significantly elevated ($p \leq 0.05$) Ach activity (526.36±6.44b and 436.53±5.18 nmole/min/mg Pt.), respectively.

Table 3: Enzymes activities in 4th instar *S. frugiperda* larvae 24 hours post treatment with LC_{50s} of tested insecticides.

Component	Control	Chlorpyrifos	% Increase or decrease than control*	Methomyl	% Increase or decrease than control*	Spinosad	% Increase or decrease than control*
α –Esterase	73.20±2.40^{d}	217.40±3.94^{a}	196.99	120.63±4.73^{c}	64.8	176.95± 3.88^{b}	141.73
β –Esterase	43.15±1.55^{d}	116.91±3.86^{b}	170.94	93.95±2.14^{c}	117.73	268.38±6.33^{a}	521.97
Acetyl Choline-esterase	210.93±3.44^{d}	526.36±6.44^{b}	149.54	436.53±5.18^{c}	106.95	642.15±6.87^{a}	204.44

Means have the different letters in the same row are significant (P<0.05).

*%Increase or decrease than control $= \frac{\text{Treated - Control}}{\text{Control}} \times 100$

Effect on Phosphatase Activities:

The results in Table (4), indicated a positive effect of chemically and biologically synthesized insecticides on the activities of acid and alkaline phosphatase (µg/ml). In which, exposure to chlorpyrifos, afterward spinosad and methomyl (30.13±0.97, 22.45±0.91 and 19.83±0.93 µg/ml), (11.23±0.33, 9.18±0.15 and 8.65±0.33 µg/ml), respectively, caused a significant increase ($p \leq 0.05$) in such enzymes when compared with non-exposed larvae (12.53± 0.63 and 5.58±0.23 µg/ml), respectively.

Effect on Activities of GOT/AST and GPT/ALT:

The results found in Table (4) revealed that spinosad and chlorpyrifos provided a significant increment ($p \leq 0.05$) in GOT/AST activity (39.45±1.27 and 36.89±0.66 IU/l), respectively, in comparison with the un-exposed group (27.53±0.53IU/l). However, the activity of GOT/AST was insignificantly changed in methomyl treated group (28.98±1.10 IU/l).

As for the activity of GPT/ALT, a more significant rise (63.71±0.09 IU/l) at $p \leq 0.05$ was noticed in spinosad compared to chlorpyrifos and methomyl, which also, displayed a significant increase in GPT/ALT (62.13±0.07 and 44.81±0.18 IU/l), respectively.

Effect on Enzyme Activities of Phenoloxidase and Chitinase:

As compared with control and other treated larvae, a significant difference was detected in the phenoloxidase enzyme in spinosad (13.47±0.35 O.D. units/min/g.b.wt.) at ($p \leq 0.05$). Following, a significant increase was discovered in the phenoloxidase enzyme of larvae treated with chlorpyrifos and methomyl (17.23±0.43 and 12.44±0.47 O.D. units/ min/g.b.wt.), respectively in comparison with an untreated group (9.52±0.65 O.D. units/min/g.b.wt.).

Chitinase level (ug NAGA/min/g.b.wt.) was insignificantly differed in methomyl and chlorpyrifos (13.78±0.7 and14.69±0.58 ug NAGA/ min/g.b.wt.), respectively. Nevertheless, a significant increase ($p \leq 0.05$) was found at the lethal concentration of spinosad (17.53±0.39 ug NAGA/ min/g.b.wt.) when compared with control (12.88±0.49 ug NAGA/ min/g.b.wt.) as exhibited in Table (4).

Table 4: Enzymes activities in 4th instar *S. frugiperda* larvae 24 hours post treatment with LC_{50s} of tested insecticides.

Component	Control	Chlorpyrifos	% Increase or decrease than control*	Methomyl	% Increase or decrease than control*	Spinosad	% Increase or decrease than control*
Acid phosphatase	12.53±0.63^{c}	30.13±0.97^{a}	140.46	19.83±0.93^{b}	58.26	22.45±0.91^{b}	79.17
Alkaline Phosphatase	5.58±0.23^{c}	11.23±0.33^{a}	101.25	8.65±0.33^{b}	55.02	9.18±0.15^{b}	64.52
(AST/GOT)	27.53±0.53^{b}	36.89±0.66^{a}	34.0	28.98±1.10^{b}	5.27	39.45±1.27^{a}	43.3
(ALT/GPT)	33.56±0.29^{d}	62.13±0.07^{b}	85.13	44.81±0.18^{c}	33.52	63.71±0.09^{a}	89.84
Phenoloxidase	9.52±0.65^{c}	17.23±0.43^{a}	80.99	12.44±0.47^{b}	30.67	13.47±0.35^{b}	41.49
Chitinase	12.88±0.49^{b}	14.69±0.58^{b}	14.05	13.78±0.7^{b}	6.99	17.53±0.39^{a}	36.10

Means have the different letters in the same row are significant (P<0.05).

*%Increase or decrease than control $= \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$

Histopathological Findings:

By using H& E satin, histological observations of the mid-gut of the control *S. frugiperda* showed normal organization of the midgut structure with the basement membrane, and normal epithelial cell (Table 5 and Fig. 1 A, B). Histological lesions of the midgut of the 4th instar larva of *S. frugiperda* treated with chlorpyrifos exhibited intense necrosis with sloughing of all epithelial cell layers from the basement membrane toward the lumen, also, cytoplasmic vacuolization was detected (Table 5 and Fig. 2 A, B, C, D). However, histological damage appeared in the mid-gut of the 4th instar larva of *S. frugiperda* treated with methomyl distinguished by destruction and necrosis of the epithelial layer, elongation of epithelial cell from one side, degenerative changes with epithelial vacuolation, and thickened basement membrane (Table 5 and Fig. 3 A, B, C, D). Moreover, the mid-gut treated with spinosad detected severe histopathological changes like cytoplasmic vacuolization of the epithelial cells, completely destroyed peritrophic membrane and muscle layer, destroyed tissues with detachment of epithelial cells and sloughed inside fill the lumen (Table 5 and Fig. 4 A, B, C, D).

Table 5: The histological lesions scores of the midgut of 4th instar larva of *Spodoptera frugiperda* classified into absent, (-), mild (+), moderate (++), and severe (+++) according to severity of lesions.

Groups / Lesions	Control	Methomyl	Chlorpyrifos	Spinosad
Necrosis of epithelial cells	-	++	++	+++
Cytoplasmic vacuolization	+	+++	++	++
Detachment and sloughed Epithelial layer	-	+++	++	++
Cell and nucleus elongation	-	++	+++	++
Destruction of peritrophic membrane	-	++	++	+++
Thinning of basement membrane	-	++	+++	++
Destruction of muscle layer	-	++	++	+++
Lumen full of necrosed debris	-	+++	++	+++

Absent, (-), mild (+), moderate (++), and severe (+++)

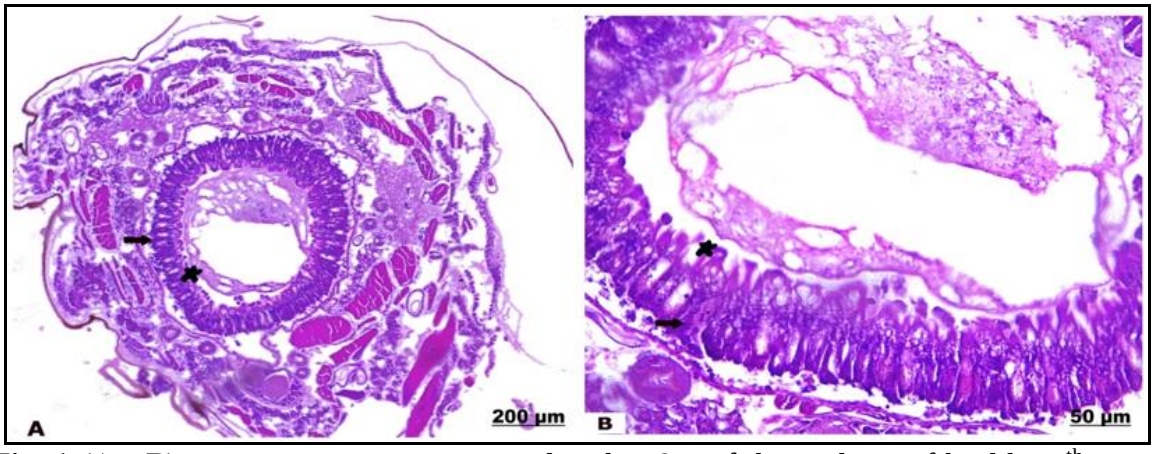

Fig. 1 (A→B): Transverse sections stained with H&E of the mid-gut of healthy 4th instar larva of *Spodoptera frugiperda* shows normally organized epithelial cell layer (arrow), and intact epithelial brush border (star). Scale bar = 50 & 200 µm

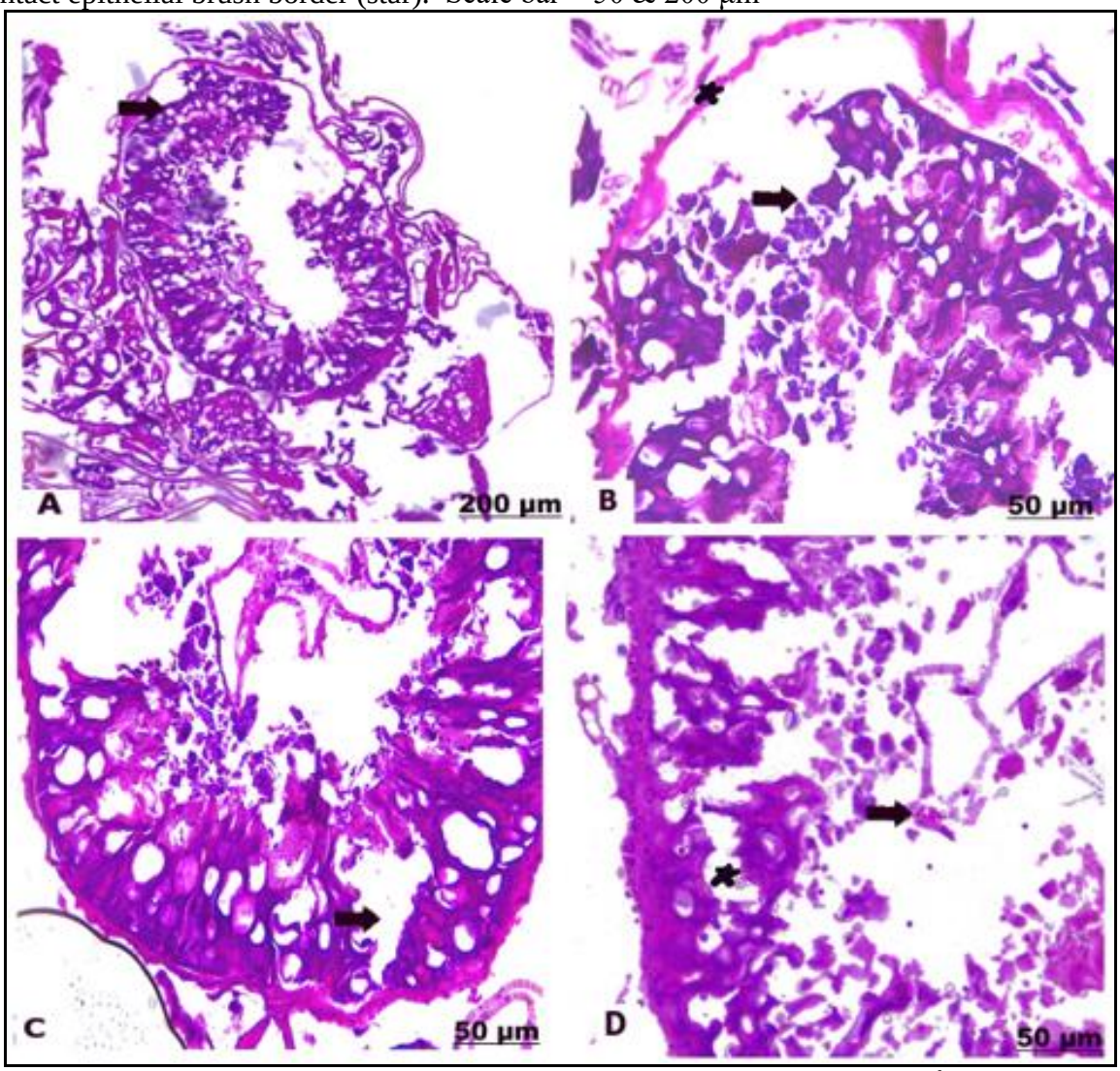

Fig. 2 (2 A→D): Transverse sections stained with H&E of the mid-gut of 4th instar larva of *Spodoptera frugiperda* treated with Chlorpyrifos. **A, B)** show detachment with sloughing of epithelial layer (arrow) from the basement membrane (star) into the lumen (head arrow). **C)** shows severe epithelial vacuolation. **D)** shows vacuolated epithelial tissues (arrow) with necrosed tissues filled lumen (star). Scale bar = 50 & 200 µm

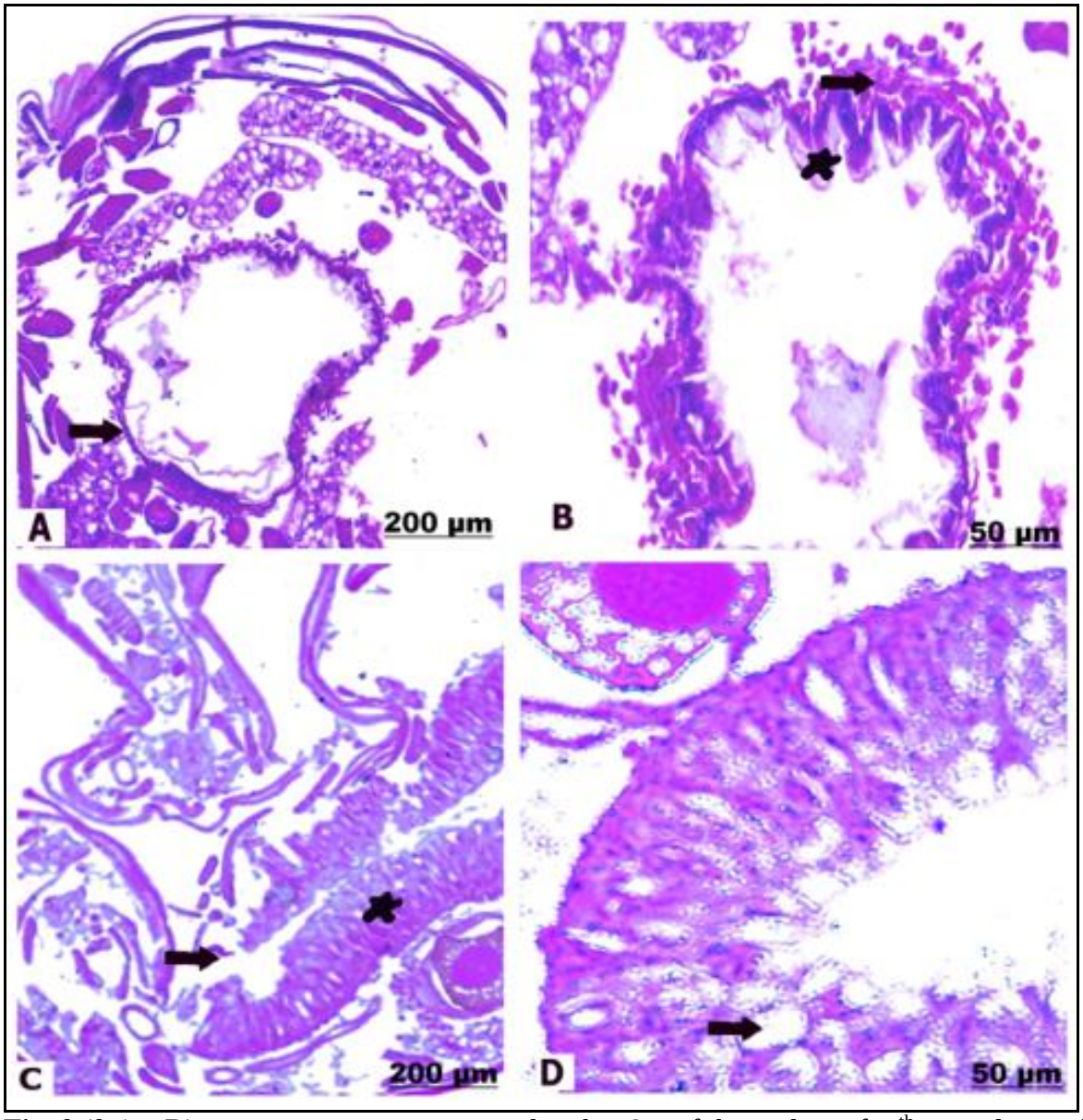

Fig. 3 (3 A→D): Transverse sections stained with H&E of the midgut of 4th instar larva of *spodoptera frugiperda* treated with Methomyl. **A)** shows destructive damage with thining of the epithelial cell layer. **B)** shows thickening of the basement membrane (arrow) with proliferation of epithelial cells (star). **C)** shows epithelial necrosis (arrow) with elongated cell layer (star) in other side. D) shows prominent cytoplasmic vacuolization. Scale bar = 50 & 200 µm

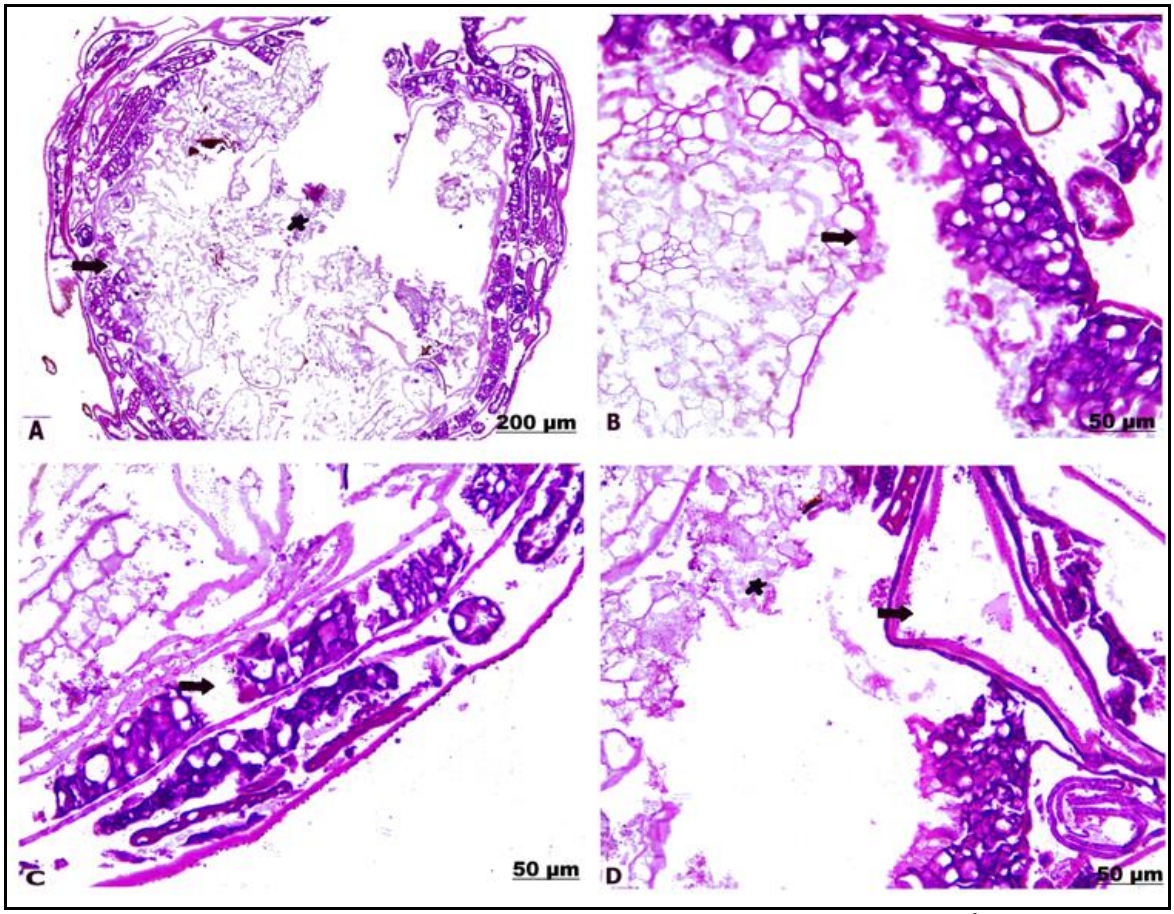

Fig. 4 (A→D): Transverse sections stained with H&E of the midgut of 4th instar larva of *Spodoptera frugiperda* treated with Spinosad. **A)** shows necrosis with vacuolization of the epithelial cell (star) and filled lumen with cell debris (star). **B)** shows detachment of epithelial tips to fill lumen. **C)** shows cytoplasmic vacuolization. **D)** shows destructive muscle layer (arrow), besides sloughing of epithelial lining (star).
Scale bar = 50 & 200 µm

DISCUSSION

The present investigation commenced with an examination of biochemical and histopathological consequences of chlorpyrifos (organophosphorus insecticide), methomyl (carbamate insecticides), and spinosad (bioinsecticide) against the 4th instar larvae of the *S. frugiperda*. The study's results emphasize the different effects of the selected insecticides. It is evident that the toxicity of these insecticides is based on the concentration and mode of action. Insecticides are successful tools used for the management of fall armyworms worldwide notably in Africa (Sisay *et al*., 2019). Proteins are the main components of all living cells for the maintenance of many activities. The body of insects has several kinds of proteins, each with an accurate and distinguished task (Tunaz and Uygun 2004). All tested insecticides showed a decrease in carbohydrates, protein, and lipid levels. Chlorpyrifos recorded the greatest decrease in protein content, owing to the organophosphate chlorpyrifos being more toxic than other pesticides (Rico *et al*., 2011). Also, *Spodoptera littoralis* treated with spinetoram detected a lowered value of total protein related to the inhibitory effect on the neurosecretory receptors responsible for protein secretion (Hamouda and Dahi 2008). Insecticides, notably methomyl class aim at the ryanodine receptors of musculatures and the ion channels (De França *et al*., 2017), reducing

nutritional markers such as proteins, carbohydrates, and lipids affecting larvae development of *Agrotis ipsilon* (Xu *et al*., 2016)**.**

Carbohydrate metabolism by hydrolyzing enzymes plays a principal part in the digestion and exploitation of carbohydrate by insects (Wyatt, 1967); it is principally controlled by enzymes of amylase, invertase and trehalase. Our results showed a significant reduction in carbohydrate hydrolyzing enzymes among classes of insecticides. Organophosphates insecticides could impair the average amylase activity (Vijay Gundi *et al*., 2007). An exposure of American bollworm larvae, *Helicoverpa armigera* to various ranges of insecticides accompanied by a significant decrease in the activity of amylase, invertase and trehalase enzymes (Al-shannaf *et al*., 2012). Similar results are consistent with Salem *et al.*, (2023) who observed a remarkable decrement in amylase activity after treating 4th instar larvae of *S. frugiperda* with lethal doses of spinerotam. Moreover, the activities of amylase, trehalase, and invertase enzymes in cotton leafworm larvae exposed to spinosad and triflumuron were mostly decreased than control larvae (Mead *et al*., 2008).

Treatment of 2nd instar larvae of *S. frugiperda* with *Bacillus thuringiensis* subspecies *kurstaki* (Btk), emamectin benzoate and lufenuron caused a considerable decrease as compared to control in amylase and invertase enzyme activity (Aly *et al.*, 2023). This was attributed to a disturbance in carbohydrate metabolism followed by a disturbance in the activity of carbohydrate enzymes in the treated larvae. The same observation in treated *S. littoralis* has also been detected by earlier investigators (El-Barky *et al*., 2008; Dahi *et al*., 2009; Rashwan, 2013 and Salem *et al*., 2023).

Esterases have a major role in the detoxification of chemically and naturally synthetic insecticides (Vanhaelen *et al*., 2001). Esterase activities significantly increased in spinosad, chlorpyrifos, and methomyl. Insecticides may alter the functions of metabolic enzymes such as esterase and mixed-function oxidase (MFO). This is in agreement with the results recorded by (Abd El-Mageed and Elgohary 2006) who confirmed that the activity of β-esterase of 4th instars *Spodoptera littoralis* larvae was significantly varied post LC_{50} of spinosad exposure for four days. This similarity to the same investigation by Abd El-Samei *et al*., 2019 found that LC_{25} of spinosad caused a significant activation in α-and β-esterases after 48 hours in the 3rd and the 5th instar larvae. This suggested that α- and β-esterase were enclosed in the hydrolysis of spinosad utilizing esters and amides hydrolysis. The hydrolysis rate of an ester bond of spinosad insecticide is the main factor affecting the toxicity of insecticides (Siegwart *et al*., 2015). Unlike *Culex pipiens* L. and *Anopheles multicolor* Cambouliu when subjected to the spinetoram at LC_{50} for 24 hours a significant decrease has been accelerated in the α-and β-esterases activities (El-Kady *et al*., 2008). An exposure of 4th instar *Tuta absoluta* larvae to chlorpyrifos induced an increase in the esterase activities after 24 to 48 hours (Zibaee 2016).

AChE is the principal objective site in insects for carbamates and organophosphorus groups **(**Nathan *et al*., 2008**)**. Spinosad targets nicotinic acetylcholine receptors with AChE concurrently, in addition to working on a novel site that varies from the main AChE site (Salgado *et al*., 1998). During the current investigation, the activity of AChE of FAW larvae increased throughout the experiment following the treatment with spinosad, chlorpyrifos, and methomyl. The increase in AChE activities can relate to the mode of action of most pesticides. Likewise, spinetoram significantly elevated the AChE activity *of Spodoptera littoralis* (Fahmy and Dahi 2009). Beside this, the potential biochemical resistance of insects to chlorpyrifos can be reflected by the increase in the activity of the acetylcholinesterase enzyme (Yuliani *et al*., 2020). The elevated AChE concentration assists in insect resistance following insecticide application (Charpentier and Fournier 2001). Contrariwise to Palanikumar *et al*., (2014) who recorded that chlorpyrifos remarkably inhibited acetylcholinesterase. Phosphatases are extracellular enzymes that

catalyze phosphate monoesterase (Trowsdale *et al*., 1990). Acid phosphatase plays a role in carbohydrate metabolism. This enzyme is present inside the membrane of lysosomes. Therefore, damaging the membrane of lysosomes can lead to leakage into muscle and increase its levels (Trdan 2013). Presently, chemical and biosynthetic insecticides enhance the activities of acid and alkaline phosphates. Similar to Abou-Taleb (2010) who observed an increase in alkaline phosphatase of Cotton Leafworm larvae exposed to methomyl, spinosad, spinetoram, and chlorpyrifos.

The increased GOT/AST and GPT/ALT activities in larvae treated with insecticides might be associated with reversible binding between the site of action on the enzymatic surface and insecticide. Salem et al., (2023) followed up on the biochemical effects of spinetoram on the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of *S. frugiperda* and documented parallel findings.

Chitinases share in the hydrolysis process of chitin for the production of N-acetyl-d-glucosamine and chitobiose which are categorized into exochitinases and endochitinases (Hirose *et al*., 2010). Phenoloxidase plays an important role in the immune system of insects exhibiting monophenol monooxygenase and diphenoloxidase activity. Also, has a contributory role in the melanization process in animals and plants (Lu and Jiang 2007). Spinosad and chlorpyrifos recorded the highest significant increases in the chitinase enzyme of FAW larvae. This is confirmed by the crucial increase in the activity of chitinase enzyme after exposure of American bollworm larvae, *H. armigera* to Dipel DF bioinsecticide than other chemical classes, fundamentally, chlorfluazuron, and pyriproxyfen (Al-Shannaf *et al*., 2012). Previous researchers documented the alteration in the chitinase in homogenized larvae. Phenoloxidase activity was more significantly activated in chlorpyrifos, followed by spinosad and methomyl; the activity of phenoloxidase is often affected by pesticides as external stressors (James and Xu 2012).

In the present investigation, histopathological changes were noticed in the larvae midgut of *S. frugiperda* after exposure to chlorpyrifos, methomyl, and spinosad characterized by intense necrosis with sloughing of all epithelial cell layers from the basement membrane toward the lumen**,** also, cytoplasmic vacuolization was detected. The results were in agreement with Ahmed and El-Sobki (2021) who recorded histological damage of the midgut of *Rhynchophorus ferrugineus* treated with LC_{50} of methomyl, chlorpyrifos and spinosad. Where methomyl showed vacuolar degeneration and necrosis with desquamation of the basement membrane from the epithelial lining. Also, there was cell degeneration, besides thinning, necrosis and desquamation of the epithelial lining and the basement membrane were found in the midgut of larvae treated with chlorpyrifos. On the other hand, the histopathological changes were cruel and graver in spinosad-treated larvae. The changes were intense vacuolar degeneration, and necrosis, as well as proliferation of epithelial lining with separation of the basement membrane. Cellular alterations in the organelles of the midgut of red palm weevil (RPW) larvae *Rhynchophorus ferrugineus* were reported for spinosad through histopathological changes (Abdelsalam *et al*., 2016), with varied intensity in the cell organelles changes (Abdelsalam *et al*., 2020)**.** The increased vacuolization of cytoplasm in the digestive cells of *P. nigrispinus* subjected to the pesticides based on the time exposure may be due to the detoxification process occurring against substances (Plata-Rueda *et al*., 2020). Our result also agreed with Salem *et al.,* (2023) stated that considerable histological damage was seen in the midgut of the fourth instar larvae of *Spodoptera frugiperda* treated with spinetoram. Degenerative alterations in the epithelial cell, such as cytoplasmic vacuolization, and the necrotic sloughing and desquamation of the epithelial cell from the basement membrane were the signs of this damage.

Conclusion:

Based on the discussed biochemical findings, the exposure of *S. frugiperda* to lethal concentrations of the three studied insecticides exhibited disturbance in the biochemical activities and the histological structure of the midgut. Correspondingly, remarkable effectiveness was detected with a well-defined reduction in concentrations of carbohydrate, protein, and lipids, contrary to the prominent increase observed in the activities of esterases, AChE, phosphatases, and both transaminases AST and ALT. Exposure to FAW is accompanied by increased phenoloxidase and chitinase enzyme activities. Substantially, spinosad was more effective on the biochemical manifestation of *S. frugiperda* than chlorpyrifos and methomyl insecticides.

Declarations:

Ethical Approval: The experimental procedure concerning this work was conducted and approved by the Institutional Review Board for Animal Experiments of South Valley University according to the Ethical Guidelines for the Animals Handling in laboratory experiments of the Faculty of Science, South Valley University, Qena, Egypt (Approval No. 008/03/2023).

Competing interests: The authors have no competing interests to declare that are relevant to the content of this article

Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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