Efficacy and biochemical analysis of sub-Lethal concentrations of some insecticides against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT

The study assessed the toxicity and biochemical effects of emamectin benzoate, lufenuron, chlorpyrifos, and spinosad on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) 2nd and 4th instar larvae. Results revealed that, 2nd instar larvae were more sensitive than 4th instar larvae to the four insecticides. Emamectin benzoate exhibited the highest toxicity (LC_{50}: 0.166 ppm and 0.23 ppm), followed by lufenuron, chlorpyrifos, and spinosad. Biochemical analysis revealed significant metabolic disruptions. Total soluble protein levels increased after emamectin and spinosad treatments but decreased with lufenuron. Carbohydrate levels rise with emamectin and lufenuron but dropped with chlorpyrifos and spinosad, while lipid content decreased across all treatments, indicating high energy demands. Glutathione S-transferase (GST) activity increased substantially, reflecting enhanced detoxification processes, particularly with emamectin. Alpha and beta esterase activities also increased, further supporting detoxification mechanisms. Alkaline phosphatase activity showed pronounced increases, especially with emamectin, while acid phosphatase levels declined, suggesting disrupted lysosomal activity. Acetylcholinesterase (AChE) activity decreased in all treatments, with chlorpyrifos causing the greatest inhibition. Significant increases in GOT/AST and GPT/ALT activities indicated cellular damage and metabolic stress, with chlorpyrifos showing the strongest effect, followed by emamectin. These findings highlight the varying toxicity levels and metabolic disruptions caused by the tested insecticides, providing valuable insights for supporting management strategy of *S. frugiperda*.

Key words: *Spodoptera frugiperda*, emamectin benzoate, lufenuron, chlorpyrifos, and spinosad, ALP, GST, AChE, ACP, Biochemical studies.

INTRODUCTION

Spodoptera frugiperda, commonly known as the fall armyworm, is a significant agricultural pest native to the Americas. It has a broad host range, feeding on numerous crops such as maize, rice, and wheat, and is known to cause severe yield losses (Goergen *et al*., 2016 and Montezano *et al*., 2018). According to the FAO (2019), this pest was recently detected in Egypt in 2019, where it caused substantial damage to maize and other crops. The rapid spread of the fall armyworm and its ability to develop resistance to insecticides has made it a major challenge for pest management worldwide (Yu, 1991, 1992 and Yu *et al*., 2003). In general, pesticides are essential for managing insect pests and diseases, thereby safeguarding and enhancing agricultural production (Prodhan *et al*., 2015 and Adamson *et al*., 2020). Emamectin benzoate, a semi-synthetic derivative of avermectins, is widely used as an insecticide due to its high efficacy against lepidopteran pests. It works by binding to glutamate-gated chloride channels in the insect nervous system, causing an influx of chloride ions that lead to paralysis and eventual death (Fisher & Mrozik, 1992 and Bai & Ogbourne, 2016). Its targeted mode of action and minimal impact on non-target organisms make it a popular choice in integrated pest management programs (Lasota and Dybas, 1991). Lufenuron, an insect growth regulator belonging to the benzoylurea class, is primarily used to control lepidopteran and coleopteran pests. It inhibits chitin synthesis in insects, disrupting exoskeleton formation during molting, which leads to mortality (Oberlander and Silhacek, 1998). This action of such insecticides preventing developmental insect stages, making lufenuron an effective component of pest management strategies with minimal impact on beneficial organisms (Smagghe *et al*., 2004). Chlorpyrifos, an organophosphate insecticide, is widely used to control a variety of agricultural pests. It inhibits acetylcholinesterase (AChE), an essential enzyme in the nervous system, leading to the accumulation of acetylcholine at synapses, causing overstimulation, paralysis, and insect death (Sultatos, 1994). However, concerns over its environmental persistence and potential non-target effects have prompted stricter regulations (Eaton *et al*., 2008). Spinosad is a biologically derived insecticide composed of spinosyn A and D, produced by *Saccharopolyspora spinosa* through fermentation. It primarily targets nicotinic acetylcholine receptors and, to a lesser extent, GABA receptors, affecting pests through ingestion or topical exposure (Thompson *et al*., 1995 and Salgado, 1997). Spinosad is used on over 200 crops to control pests such as caterpillars in cotton, loopers in cabbage, leafminers, leafrollers, and thrips in citrus (Bret *et al*., 1997 and Thompson *et al*., 2000). Biochemical studies in insects are crucial for understanding their physiological and

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metabolic responses to environmental stressors, including pesticides. These studies reveal the mechanisms of insecticide action and resistance, aiding in the development of effective pest management strategies. Additionally, they provide insights into the detoxification pathways and enzymatic adaptations of insects, which are essential for designing targeted and sustainable control methods (Wei *et al*., 2020 and Xu *et al*., 2015). Considering the above information, the current study aims to evaluate the toxicity of emamectin benzoate, lufenuron, chlorpyrifos, and spinosad by determining their LC_{50} values and assessing their sub-lethal effects through biochemical analysis in second and fourth instar larvae of laboratory strains of *S. frugiperda*.

MATERIAL AND METHODS

Tested insecticides

Emamectin benzoate (Proclaim® 5% SG) and lufenuron (Match® 5% EC) were obtained from Syngenta Co. Chlorpyrifos (Linker® 48% EC) was obtained from Sam trade Co., LTd. while spinosad (Tracer® 24% SC) was obtained from Dow Agro Sciences Co.

Insect rearing

The larvae of fall armyworm were collected from maize fields in Beheira Governorate and reared under controlled conditions at the Faculty of Agriculture, Damanhour University. Rearing followed standardized protocols as outlined by Dahi et al. (2020). To establish a laboratory strain, the larvae were maintained for multiple generations in the laboratory. Newly molted second and fourth instar larvae were selected for use in the current study.

Bioassays

Bioassays were performed on 2nd and 4th instar larvae of the fall armyworm (S. frugiperda) to assess the effectiveness of the four insecticides. A range of concentrations for each insecticide was prepared using their commercial formulations as follows: emamectin benzoate (1, 0.75, 0.5, 0.25, 0.1 and 0.05 µg/ml); lufenuron (5, 4, 3, 2, 1, and 0.5 µg/ml); chlorpyrifos (15, 13, 11, 9, 7, and 5 µg/ml); and spinosad (50, 45, 40, 35, 30, and 25 µg/ml). The LC25, LC50 and LC90, slope values and q2 were determined using the leaf-dipping method. Fresh castor leaves were cut into 2 cm^2 discs, dipped for 20 seconds in the respective concentrations, and allowed to dry under laboratory conditions before being fed to the larvae. For each concentration, 20 larvae from each instar were used, with four replicates per concentration. Larvae in the control group were fed on leaves dipped in water only. Newly molted 2nd and 4th instar larvae were exposed to treated leaves in glass jars covered with muslin for 24 h for chlorpyrifos and 72 h. for emamectin benzoate, lufenuron and spinosad. Mortality rates were corrected using Abbott's (1925) formula. The data were analyzed using Probit analysis as described by Finney (1971). LdP-line, Ehab Softwaren (http://www.ehabsoft.com/ldpline/).

Biochemical studies

After 48 hours of feeding 2nd and 4th instar larvae of S. frugiperda on castor bean leaves treated with chlorpyrifos, emamectin benzoate, lufenuron, and spinosad at their respective LC25 concentrations, surviving larvae showing toxic symptoms were selected for next step of biochemical analysis. These larvae were anesthetized and rinsed with 5 mL of acetone to remove surface residues, weighed, and homogenized in phosphate buffer (pH 7) using a Teflon tissue homogenizer surrounded by crushed ice. The homogenates were then centrifuged at 8000 rpm for 20 minutes at 4°C, and the supernatant was used to measure the larval biochemical parameters.

Larval biochemical parameters

Enzyme assays were conducted to measure various biochemical parameters. Total soluble protein was quantified following the Bradford (1976) method, total lipids were measured according to Knight et al. (1972), and total carbohydrates were determined as per Singh and Sinha (1977). Acetylcholine esterase (AChE, EC 3.1.1.7) activity was assessed using acetylcholine bromide as a substrate based on the method of Simpson et al. (1964). Glutathione S-transferase (GST, EC 2.5.1.18) activity was determined spectrophotometrically at 340 nm according to Habig et al. (1974). Alkaline phosphatase (ALP, EC 3.1.3.1) and acid phosphatase (ACP, EC 3.1.3.2) activities were measured from larval hemolymph as described by Laufer and Schin (1971). Finally, non-specific α and β esterase activities were determined using α-naphthyl acetate and β-naphthyl acetate as substrates, following the method of Van Asperen (1962). The activities of glutamate oxaloacetate transferase (GOT/AST) and glutamate pyruvate transferase (GPT/ALT) were quantified according to Reitman and Frankel (1957).

Statistical analysis

All quantitative estimations of biochemical parameters were based on four replications, and the results were expressed as mean ± SD. The data were statistically analyzed using one-way ANOVA (SAS, 2001), followed by the least significant difference (LSD) test to determine significant differences between the different insecticides.

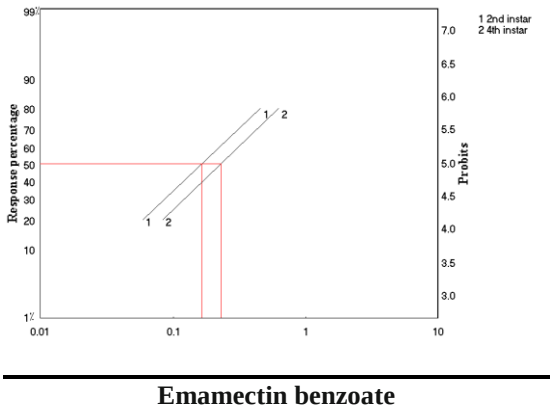
RESULTS AND DISCUSSION

Toxicity study

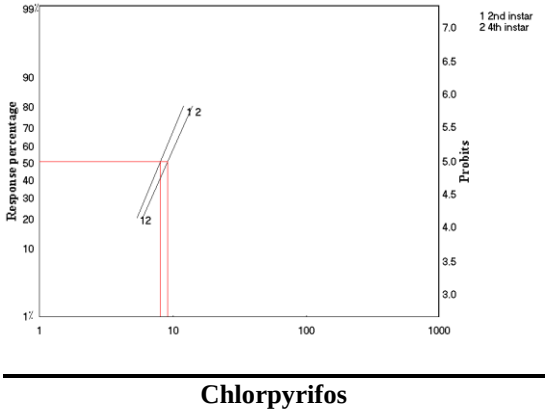
As presented in Table (1) and Figure (1), the bioassay results for the 2nd instar larvae of *S. frugiperda* revealed that 2nd instar larvae were more sensitive than 4th instar larvae to the four insecticides. The tested insecticides can be ranked by their LC_{50} values as follows: emamectin benzoate (0.166 ppm), lufenuron (1.571 ppm), chlorpyrifos (8.109 ppm), and spinosad (21.065 ppm). For the 4th instar larvae, the LC_{50} values were 0.23, 1.898, 9.177, and 37.771 ppm for the same insecticides, respectively. These findings align with Amein and Abdelal (2023), who reported LC_{50} values of 0.18 ppm for teflubenzuron, 0.019 µg/ml for emamectin benzoate, and 0.6046 µg/ml for α-cypermethrin against *S. frugiperda* larvae. Similarly, Aly *et al*. (2024) observed that emamectin benzoate exhibited the highest toxicity (LC_{50}: 0.0079 ppm), followed by Btk (LC_{50}: 1.6857 ppm) and lufenuron (LC_{50}: 3.2155 ppm). Fiaboe *et al*. (2023) also reported an LC_{50} of 0.019 ppm for emamectin benzoate. Attia *et al*. (2023) investigated the efficacy of bio-insecticides on *S. frugiperda* and determined an LC_{50} of 6.982 ppm for spinosad in 3rd instar larvae after 24 hours of exposure. Furthermore, Gichere *et al*. (2022) assessed the susceptibility of *S. frugiperda* populations across thirteen counties in Kenya to various insecticides. Their findings highlighted high toxicity of spinetoram, spinosad, lufenuron, and pyridaben, while indoxacarb, deltamethrin, lambda-cyhalothrin, imidacloprid, and abamectin showed relatively lower toxicity.

Table 1. Insecticides toxicity on 2nd and 4th larval instar of susceptibility *S. frugiperda*

Insecticides	Instar	LC_{25} (Confidence limits)	LC_{50} (Confidence limits)	LC_{90} (Confidence limits)	Slope	X^2
Emamectin benzoate	2nd	0.073 (0.055-0.091)	0.166 (0.138-0.195)	0.784 (0.626-1.045)	1.901 ±0.154	4.341
	4th	0.103 (0.055-0.14)	0.23 (0.151-0.331)	1.067 (0.782-2.215)	1.924 ±0.154	5.972
Lufenuron	2nd	0.852 (0.692-1)	1.571 (1.377-1.781)	5.018 (4.1-6.612)	2.541 ±0.236	6.581
	4th	0.941 (0.463-1.193)	1.898 (1.239-2.73)	7.19 (5.876-17.98)	2.215 ±0.2	9.332
Chlorpyrifos	2nd	5.866 (4.205-6.548)	8.109 (6.591-9.405)	15.003 (13.48-21. 5)	4.796 ±0.429	8.12
	4th	6.503 (5.823-7.069)	9.177 (8.594-9.772)	17.655 (15.79-20.62)	4.51 ±0.424	3.095
Spinosad	2nd	21.065 (18.27-23.15)	26.78 (24.66-28.44)	42.271 (39.68-46.19)	6.467 ±0.739	4.408
	4th	32.065 (36.58-33.29)	37.771 (36.58-39.01)	51.559 (48.94-55.21)	9.483 ±0.771	2.438



Emamectin benzoate



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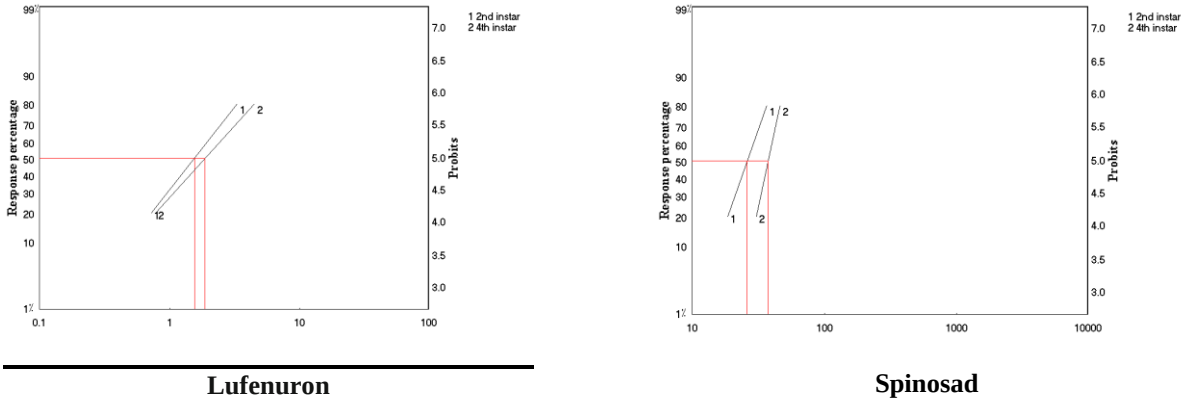

Lufenuron **Spinosad**

Fig. 1. Toxicity lines of chlorpyrifos, lufenuron, emamectin benzoate, and spinosad against 2nd and 4th S. frugiperda larvae

Effect of the tested insecticides on the main cell contents

Table (2) shows that treating 2nd instar *S. frugiperda* larvae with LC_{25} of lufenuron resulted in a decrease in total soluble protein levels from 6.37 to 4.03 mg/ml, representing a 36.74% reduction compared to the control. Conversely, treatments with chlorpyrifos, emamectin benzoate, and spinosad increased total soluble protein levels to 6.45, 7.52, and 7.58 mg/ml, corresponding to increases of 1.2%, 18.05%, and 18.99%, respectively, relative to the control. For 4th instar larvae (Table 3), emamectin benzoate and spinosad treatments caused increases in total soluble protein by 16.03% and 9.98%, respectively, while chlorpyrifos and lufenuron treatments led to reductions of 1.54% and 12.38%, respectively. Regarding carbohydrates, lufenuron and emamectin benzoate treatments increased levels by 46.83% and 7.8%, respectively, in 2nd instar larvae and by 44.46% and 13.6%, respectively, in 4th instar larvae. In contrast, chlorpyrifos and spinosad treatments decreased carbohydrate levels by 23.9% and 16.1%, respectively, in 2nd instar larvae and by 17.57% and 15.9%, respectively, in 4th instar larvae. Lipid content decreased across all treatments for both instars. In the 2nd instar, lipid reductions were 20.89%, 41.51%, 30.03%, and 48.83% for chlorpyrifos, lufenuron, emamectin benzoate, and spinosad, respectively. In the 4th instar, these reductions were 20.02%, 42.34%, 53.08%, and 57.98%, respectively.

The observed effects of insecticide treatments on the biochemical parameters of *S. frugiperda* larvae indicate significant metabolic disruptions, which likely contribute to the mortality and physiological impairment of the larvae. The reduction in soluble protein levels following lufenuron treatment in 2nd instar larvae suggests that lufenuron impairs protein synthesis or promotes protein degradation. This may be linked to its mode of action as a chitin synthesis inhibitor, which could indirectly affect metabolic processes essential for protein maintenance (Mondal and Parween, 2000). In contrast, the increase in soluble protein levels after treatments with chlorpyrifos, emamectin benzoate, and spinosad suggests enhanced protein synthesis or retention. This may be a compensatory response to stress, where the larvae attempt to repair tissue damage or produce detoxifying enzymes. The particularly significant increases with emamectin benzoate and spinosad in both instars may reflect a more pronounced stress response compared to chlorpyrifos (Buss and Callaghan, 2008). The rise in carbohydrate levels following lufenuron and emamectin benzoate treatments in both instars indicates a disruption in carbohydrate metabolism. This could be due to an increase in glycogen mobilization as a stress response, possibly linked to energy demands for detoxification or repair mechanisms. The higher increase with lufenuron suggests a stronger metabolic disruption. In contrast, chlorpyrifos and spinosad caused reductions in carbohydrate levels in both instars. This indicates a depletion of carbohydrate reserves, likely due to excessive energy expenditure or impaired carbohydrate synthesis. The decrease could also signify inhibited glycolysis or disruptions in pathways regulating carbohydrate metabolism, ultimately weakening the larvae (Sparks *et al*., 2001 and Desneux *et al*., 2007).

The reduction in lipid levels across all treatments highlights a common metabolic effect of these insecticides. Lipids are critical energy reserves, and their depletion suggests increased energy demands to counteract the toxic effects of the insecticides (Singh *et al*., 2018). The most pronounced decreases, observed with spinosad and emamectin benzoate, suggest these treatments impose a severe energetic burden, likely accelerating lipid catabolism.

Table 2. Effect of emamectin benzoate, lufenuron, chlorpyrifos and spinosad on some biochemical aspects of *Spodoptera frugiperda* 2nd instar larvae

Insecticides	Total soluble protein	Carbohydrates	Lipid
Control	6.37±0.5[b]	4.1±0.3[b]	7.66±0.59[a]
Chlorpyrifos	6.45±0.28[b]	3.12±0.29[c]	6.06±0.28[b]
Lufenuron	4.03±0.31[c]	6.02±0.25[a]	4.48±0.39[c]
Emamectin benzoate	7.52±0.52[a]	4.42±0.18[b]	5.36±0.83[b]
Spinosad	7.58±0.33[a]	3.44±0.28[c]	3.92±0.13[c]
F value	51.847	73.352	33.215
LSD	0.60138	0.39794	0.76335

Means within the same column that are followed by different letters indicate a significant difference (P<0.05).

Table 3. Effect of emamectin benzoate, lufenuron, chlorpyrifos and spinosad on some biochemical aspects of *Spodoptera frugiperda* 4th instar larvae

Insecticides	Total soluble protein	Carbohydrates	Lipid
Control	22.14±2.09[ab]	9.56±0.61[c]	18.68±0.73[a]
Chlorpyrifos	21.8±1.83a[b]	7.88±0.43[d]	14.94±0.71[b]
Lufenuron	19.4±1.36[b]	13.83±1.1[a]	10.77±0.71[c]
Emamectin benzoate	25.69±5.46[a]	10.86±0.51[b]	8.63±0.62[d]
Spinosad	24.35±1.66[a]	8.04±0.54[d]	7.85±0.25[d]
F value	2.810	53.555	209.382
LSD	4.37438	1.00812	0.950385

Means within the same column that are followed by different letters indicate a significant difference (P<0.05).

The differential impact on lipid reduction between insecticides suggests variations in their modes of action. Spinosad, for instance, is known to act on the nervous system, which could lead to heightened metabolic activity and increased lipid utilization (Sparks *et al*., 2001).

Effect on glutathione S-transferase enzymes activity.

In untreated 2nd instar larvae, the glutathione S-transferase (GST) enzyme activity was measured at 22.3 µmol/min/mg, approximately one-quarter of the activity observed in untreated 4th instar larvae, which was 91.1 µmol/min/mg. Treatment of the 2nd and 4th instar larvae with their respective LC_{50} doses of Indoxacarb resulted in a significant increase in GST activity. In the 2nd instar, GST activity increased to 29.61, 23.69, 55.46, and 29.9 µmol/min/mg following treatment with chlorpyrifos, lufenuron, emamectin benzoate, and spinosad, respectively. These changes correspond to percentage increases of 32.78%, 6.23%, 148.7%, and 34.98%. For the 4th instar, GST activity rose to 119.1, 98.67, 215.06, and 120.04 µmol/min/mg after treatment with the same insecticides, showing percentage increases of 30.74%, 8.38%, 136.07%, and 31.77%, respectively.

The observed increase in glutathione S-transferase (GST) enzyme activity following treatment with the four tested insecticides can be attributed to its role in the detoxification mechanisms of insects. GST enzymes are critical for metabolizing and neutralizing toxic compounds, including insecticides, by catalyzing their conjugation with glutathione. The elevated GST activity in treated larvae suggests an overproduction of the enzyme as a physiological response to counteract the toxic effects of the insecticides. Similar findings have been reported by Sarita *et al.* (2010) and Wang *et al.* (2010), who observed significantly higher GST activity in insecticide-treated larvae compared to untreated controls. These studies support the hypothesis that increased GST activity is a common adaptive mechanism employed by insects to mitigate chemical stress.

Effect on acetyl choline esterase, alpha and beta esterase activity.

Treatment with LC_{25} concentrations of chlorpyrifos, lufenuron, emamectin benzoate, and spinosad resulted in a reduction in acetylcholine esterase (AChE) activity in *S. frugiperda* larvae. In the 2nd instar, AChE activity decreased by 39.44%, 9.06%, 9.9%, and 28.66%, respectively, compared to the control. Similarly, in the 4th instar, the reductions were 33.48%, 11.67%, 14.69%, and 25.79%, respectively. Regarding alpha and beta esterase activity, larvae treated with the same insecticides showed significant increases in both enzyme types. For alpha esterase, activity increased by

7%, 18.32%, 28.42%, and 11.88% in the 2nd instar, and by 8.5%, 14.09%, 22.85%, and 17.79% in the 4th instar for chlorpyrifos, lufenuron, emamectin benzoate, and spinosad, respectively. Similarly, beta esterase activity increased by 10.25%, 8.25%, 20%, and 6.75% in the 2nd instar, and by 10.9%, 10%, 20%, and 6.83% in the 4th instar following treatments with the respective insecticides. Esterases play a critical role in the detoxification of both synthetic and natural insecticides, as highlighted by Vanhaelen *et al*. (2001). These findings indicate that insecticide treatments can significantly modulate metabolic enzymes like esterases, contributing to their detoxification pathways. The observed results align with previous studies. Abd El-Mageed and Elgohary (2006) reported significant variations in beta esterase activity in 4th instar *S. littoralis* larvae after exposure to spinosad for four days. Similarly, Assar *et al*. (2016) documented increased alpha and beta esterase activity in *S. littoralis* treated with emamectin, spinetoram, hexaflumuron, and teflubenzuron. Recent findings by Salem *et al*. (2024) also reported similar increases in esterase activity in *S. littoralis* treated with spinosad, emamectin benzoate, and dinotefuran. These consistent results highlight the insecticides' ability to modulate esterase activity, underscoring their significant impact on metabolic enzyme function in pest management strategies.

Effect on alkaline phosphatase and acid phosphatase:

In untreated larvae, alkaline phosphatase activity was significantly higher in 4th instars (92.05 µg phenol/ml/min) compared to 2nd instars (16.95 µg phenol/ml/min). Similarly, acid phosphatase activity was much greater in 4th instar larvae (5.96 µg phenol/ml/min) compared to 2nd instars (1.68 µg phenol/ml/min). As shown in Tables (4 and 5), alkaline phosphatase activity in 4th instar larvae increased from 92.05 to 93.19, 126.99, 221.6, and 176.48 µg phenol/ml/min following treatment with LC_{25} of chlorpyrifos, lufenuron, emamectin benzoate, and spinosad, representing increases of 1.23, 37.96, 140.74, and 91.72%, respectively. In 2nd instar larvae, the enzyme activity also significantly increased from 16.95 to 17.95, 57.28, 58.01, and 33.32 µg phenol/ml/min, corresponding to increases of 5.9, 237.94, 242.24, and 96.58%, respectively.

Conversely, acid phosphatase activity decreased in treated 2nd instar larvae by 13.69, 19.64, 6.55, and 10.12% and in 4th instar larvae by 22.32, 31.88, 9.23, and 10.07% after treatment with LC_{25} of chlorpyrifos, lufenuron, emamectin benzoate, and spinosad, respectively.

The increase in ALP activity in both 2nd and 4th instar larvae treated with LC_{25} doses of chlorpyrifos, lufenuron, emamectin benzoate, and spinosad indicates that these insecticides may stimulate processes requiring enhanced phosphate metabolism. The substantial increase in ALP activity, particularly with emamectin benzoate (140.74% in 4th instars and 242.24% in 2nd instars), suggests that this compound has a pronounced effect on the larvae's metabolic systems. Such an increase could result from a compensatory mechanism triggered by stress or damage to cellular structures, as ALP is often linked to tissue remodeling and detoxification. The relatively milder increases observed with chlorpyrifos and spinosad might reflect differences in their modes of action or toxicity levels. The reduction in ACP activity in both instars across all treatments suggests a potential suppression of lysosomal activity or altered hydrolysis processes. Acid phosphatases are commonly associated with lysosomes, playing roles in the degradation of cellular components. The observed decrease could indicate that insecticides interfere with normal lysosomal function, potentially leading to reduced protein or membrane turnover. This effect could hinder normal metabolic and detoxification processes, contributing to larval mortality. These findings align with earlier studies, such as El-Sheakh *et al*. (1990), who observed increased alkaline phosphatase activity in 4th instar *S. littoralis* larvae treated with ofunac and sumithion. Similarly, El-Kordy *et al*. (1995) reported increased alkaline phosphatase activity in 4th and 6th instar larvae of *S. littoralis* after treatment with pyriproxyfen, flufenoxuron, and teflubenzuron. Also, Aly *et al*. (2024) observed a notable disparity in the activity of digestive enzymes, such as amylase and invertase, as well as detoxifying enzymes, including glutathione S-transferase (GST) and acetylcholinesterase (AChE), in *Spodoptera frugiperda* larvae treated with lufenuron, emamectin benzoate, and Bacillus thuringiensis.

Effect on GOT/AST and GPT/ALT:

The data presented in Tables (4 and 5) indicate that treatment with chlorpyrifos, lufenuron, emamectin benzoate, and spinosad resulted in a significant increase ($p \leq 0.05$) in GOT/AST activity compared to untreated control larvae. Chlorpyrifos induced the highest increase, followed by spinosad and emamectin benzoate, while lufenuron exhibited the least effect. Similarly, regarding GPT/ALT activity, a more pronounced increase ($p \leq 0.05$) was observed in larvae treated with chlorpyrifos and emamectin benzoate, underscoring their stronger impact on this enzyme. Spinosad caused a moderate increase, whereas lufenuron again showed the least effect, consistent with its impact on GOT/AST.

The observed elevation in AST and ALT activities in *S. frugiperda* larvae reflects metabolic stress and

cellular damage induced by these insecticides. Transaminases (AST and ALT) play a crucial role in energy production, as described by Azmi *et al*. (1998). These findings corroborate the results of Magdy *et al.* (2019), who reported a significant increase in ALT, AST, and α-β esterase enzyme activity in on *S. littoralis* larvae exposed to spinosad at LC_{25} levels. However, they contrast with Assar *et al.* (2016), who noted a significant reduction in AChE, ACP, AST, and ALT levels in larvae treated with spinosad in the same insect.

Chlorpyrifos demonstrated the highest toxicity, significantly elevating both enzyme activities, followed by emamectin benzoate and spinosad, which caused moderate increases. Lufenuron, an insect growth regulator, exhibited the least impact, suggesting lower toxicity. These results emphasize the differential toxicity of insecticides, with chlorpyrifos emerging as the most disruptive to biochemical processes. Monitoring enzyme activity provides critical insights into insecticide-induced stress and can inform safer pest management strategies.

Table 4. Effect of emamectin benzoate, lufenuron, chlorpyrifos and spinosad on some biochemical aspects of *Spodoptera frugiperda* 2nd instar larvae

Enzymes \ Insecticide	Control	Chlorpyrifos	Lufenuron	Emamectin benzoate	Spinosad	F value	LSD
AChE	105.88±4.37^{a}	64.12±5.311^{d}	96.29±5.04^{b}	95.4±5.11^{b}	75.53±3.89^{c}	51.221	7.1957
GST	22.3±2.71^{d}	29.61±2.9^{bc}	23.69±2.85^{cd}	55.46±6.56^{a}	29.9±4.05^{b}	43.409	6.1526
ALP	16.95±0.95^{c}	17.95±1.04^{c}	57.28±4.24^{a}	58.01±3.62^{a}	33.32±2.95^{b}	195.478	4.3529
ACP	1.68±0.12^{a}	1.45±0.06b^{c}	1.35±0.26^{c}	1.57±0.05^{ab}	1.51±0.08ab^{c}	3.284	0.2074
α-Esterase	286.57±10.06^{e}	306.62±4.89^{d}	339.07±5.04^{b}	368±9.72^{a}	320.61±4.06^{c}	74.132	10.8994
β-Esterase	570.19±14.23^{c}	628.61±12.12^{b}	617.21±5.97^{b}	684.22±17.07^{a}	608.7±14.94^{b}	37.801	20.2136
AST/GOT	11.91±0.94^{c}	16.61±1.11^{a}	14.4±1.01^{b}	15.02±0.82^{b}	16.55±0.69^{a}	17.32149	1.396176
ALT/GPT	18.49±1.4^{c}	23.42±1.62^{a}	19.16±1.49^{c}	24.08±1.21^{a}	21.33±1.02^{b}	13.2895	2.05427

ALP: Alkaline phosphatase (µg phenol/ml/min); GST: Glutathione ST (µmol/min/mg); AChE: Acetyl choline-esterase (µg AchBr/ml/min); ACP: Acid phosphatase (µg phenol/ml/min). Means within the same row that are followed by different letters indicate a significant difference (P<0.05).

Table 5. Effect of emamectin benzoate, lufenuron, chlorpyrifos and spinosad on some biochemical aspects of *Spodoptera frugiperda* 4th instar larvae

Enzymes \ Insecticide	Control	Chlorpyrifos	Lufenuron	Emamectin benzoate	Spinosad	F value	LSD
AChE	504.7±12^{a}	335.74±9.314^{d}	445.79±23.14^{b}	430.54±15.64^{b}	374.54±14.99^{c}	69.211	23.6907
GST	91.1±2.98^{d}	119.1±2.86^{b}	98.67±4.92^{c}	215.06±3.29^{a}	120.04±6.21^{b}	548.607	6.4157
ALP	92.05±2.86^{d}	93.19±2.79^{d}	126.99±11.46^{c}	221.6±30.04^{a}	176.48±3.44^{b}	59.478	21.9604
ACP	5.96±0.34^{a}	4.63±0.59^{b}	4.06±0.71^{b}	5.41±0.28^{a}	5.36±0.24^{a}	172.088	0.7093
α-Esterase	468.36±5.55^{d}	508.16±6.57^{c}	534.34±15.82^{b}	575.4±10.95^{a}	537.63±7.78^{b}	62.206	15.1414
β -Esterase	681.62±12.22^{c}	755.91±21.04^{b}	749.78±13.44^{b}	817.94±14.66^{a}	728.19±28.3^{b}	27.249	28.50549
AST/GOT	23.54±1.78^{d}	35.47±1.86^{a}	26.3±1.23^{c}	29.51±0.74^{b}	31.46±0.93^{b}	44.30036	2.0861
ALT/GPT	32.6±2.62^{d}	49.13±2.74^{a}	33.66±1.81^{d}	42.37±1.09^{b}	39.24±1.38^{c}	44.11094	3.0655

ALP: Alkaline phosphatase (µg phenol/ml/min); GST: Glutathione ST (µmol/min/mg); AChE: Acetyl choline-esterase (µg AchBr/ml/min); ACP: Acid phosphatase (µg phenol/ml/min). Means within the same row that are followed by different letters indicate a significant difference (P<0.05).

CONCLUSION

The study evaluated the efficacy of emamectin benzoate, lufenuron, chlorpyrifos, and spinosad against 2nd and 4th instar *S. frugiperda* larvae. Emamectin benzoate exhibited the highest toxicity, followed by lufenuron, chlorpyrifos, and spinosad. Insecticide treatments significantly disrupted metabolic processes, including protein, carbohydrate, and lipid levels, as well as enzyme activities like GST, AChE, ALP, and ACP. These disruptions contributed to larval mortality and physiological impairments. The findings highlight the potential of these insecticides, particularly emamectin benzoate, in managing *S. frugiperda*.

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الملخص العربي

الفعالية والتحليلات البيوكيميائية للتركيزات تحت القاتلة لبعض المبيدات الحشرية *ضد* حشرة دودة الحشد الخريفية ***Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)**

بلال سليمان محمد سليمان

هدفت الدراسة إلى تقييم سمية والتأثيرات البيوكيميائية لكل من الإيمامكتين بنزوات، واللوفينورون، والكلوربيريفوس، والاسبينوساد على يرقات العمرين الثاني والرابع من حشرة دودة الحشد الخريفية*Spodoptera frugiperda* . أظهرت النتائج أن يرقات العمر الثاني كانت أكثر حساسية لجميع المبيدات مقارنةً بيرقات العمر الرابع. أظهر الإيمامكتين بنزوات أعلى سمية ليرقات الحشرة، تلاه اللوفينورون، والكلوربيريفوس، والاسبينوساد. أظهرت التحليلات البيوكيميائية اضطرابات كبيرة في الأنشطة الأيضية. ارتفعت مستويات البروتينات الكلية بعد التعرض للإيمامكتين والسبينوساد، بينما انخفضت مع اللوفينورون. ازدادت مستويات الكربوهيدرات مع الإيمامكتين واللوفينورون، في حين انخفضت مع الكلوربيريفوس والاسبينوساد. كما لوحظ انخفاض في محتوى الدهون في جميع المعاملات، مما يشير إلى زيادة استهلاك الطاقة.

ارتفعت نشاطات إنزيم الجلوتاثيون–إس–ترانسفيراز (GST)بشكل ملحوظ، خصوصًا مع الإيمامكتين، مما يدل على تعزيز عمليات إزالة السموم. كما ازدادت أنشطة إنزيمات الأستريز (ألفا وبيتا)، مما يدعم دورها في إزالة السموم. سجلت انزيمات الفوسفاتاز القاعدي زيادة ملحوظة، خاصة مع الإيمامكتين، بينما انخفضت مستويات الفوسفاتاز الحمضي، مما يشير إلى اضطرابات في النشاط الليسوسومي. انخفض نشاط إنزيم الأستيل كولين إستريز (AChE)في جميع المعاملات، وكان الكلوربيريفوس الأكثر تأثيرًا في هذا الجانب. بالإضافة إلى ذلك، أظهرت أنشطة إنزيمي GOT/AST و GPT/ALT زيادات كبيرة، مما يدل على وجود تلف خلوي وضغط أيضي، مع تسجيل الكلوربيريفوس أقوى تأثير، يليه إيمامكتين. تشير هذه النتائج الي رؤى مهمة حول الاستجابات البيوكيميائية المختلفة ومستويات السمية للمبيدات المدروسة، مما يساعد في تطوير استراتيجيات أكثر فعالية لمكافحة*S. frugiperda*..