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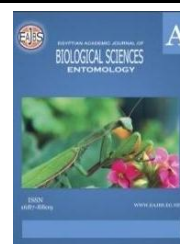
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Lethal, Biological, and Biochemical Impacts of Two Insecticides on the 2nd Instar Larvae of The Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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ABSTRACT

The fall armyworm *Spodoptera frugiperda*, a destructive insect pest, poses a significant threat to agricultural crop production, particularly maize. Traditional chemical insecticides have drawbacks, such as resistance building and environmental pollution. Proper selection and understanding of the potential consequences of insecticides are crucial for efficient control. The current study assesses the efficiency of spinetoram as a bioinsecticide compared with chlorpyrifos as an organophosphorus insecticide on the 2nd larval instar of *S. frugiperda* under laboratory conditions. The LC₅₀ values of chlorpyrifos and spinetoram were 181.0 and 0.23 ppm, respectively, depending on commercial products. The results revealed that the toxicity of chlorpyrifos and spinetoram significantly increased the larval period and decreased the pupation percentage, leading to elevated larval mortality. Both tested insecticides increased pupal durations and decreased pupal weights. Moreover, both spinetoram and chlorpyrifos significantly decreased the mean number of eggs laid. Fundamentally, the two applied insecticides significantly altered carbohydrates and lipids levels; however, spinetoram significantly lowered the total protein level. Furthermore, there is a significant difference in the activity of the digestive enzymes invertase and amylase. The activity of phenoloxidase, glutathione S-transferases, and chitinase were significantly varied. Based on the results, these insecticides offer a broad range of effectiveness in the control of *S. frugiperda*, but spinetoram superior chlorpyrifos in some biological and biochemical changes. Spinetoram is a safer, sustainable approach, and more environmentally friendly biocide that can be used instead of chlorpyrifos in integrated pest management strategies for controlling the fall armyworm.

INTRODUCTION

The fall armyworm *Spodoptera frugiperda*, (J. E. Smith, 1797) is a destructive insect pest; it was first introduced to Africa in 2016 (Goergen *et al.*, 2016). Currently, more than 30 countries have detected the fall armyworm within their borders, suggesting that it will set as an endemic, multi-generational pest in the African Continent (Prasanna *et al.*, 2018). Subsequent investigations have indicated that FAW is one of the major problems for agricultural crop production, especially maize due to its ability to rapidly breed, migrate, and feed on a wide range of host plants. These factors make it very difficult to control (Assefa

and Ayalew, 2019; Early *et al.*, 2018).

Conventional insecticides are commonly utilized to control insect pests in agricultural fields since they are more potent effective, easy to apply, and provide satisfactory results. Despite some disadvantages, chemical involvement is vital for maintaining high outcomes in modern agriculture (Environmental Protection Agency, 2021). These insecticides probably develop several negative consequences, such as selection of resistant lineages, environmental pollution, higher costs, and substantially, death of natural enemies. The reduction in the number of beneficial arthropods due to the usage of non-selective insecticides may cause serious problems for crops globally. One of the problems is the resurgence of new pests and the eruption of secondary pests, especially due to the decrease of the natural enemies that maintain pest populations under the level of economic threshold (Fernandes *et al.*, 2008). Insect pests have developed resistance to conventional insecticides; therefore, using different types of insecticides resulted in appropriate pest control and impairs the progress of insecticide resistance (Torres-Vila *et al.*, 2002). The use of bioinsecticides has proven to be one of the primary means to protect crops, their products, and the environment from pesticide pollution.

Chlorpyrifos is a wide spectrum chemical insecticide that can damage insects by causing neurotoxicity and ultimately neuronal death (Smegal, 2000). It provokes its toxic action by blocking certain vital enzymes of the nervous system, such as cholinesterase (ChE) (Ware and Whitacre, 2004). Application of neurotoxic insecticides to control *S. frugiperda* larvae subsequently can widen control efficacy. However, these insecticides are also used for eggs (Tavares *et al.*, 2011) and adult stages (Pratissoli *et al.*, 2004). Nirmal and Manjit (2008) stated that chlorpyrifos and spinetoram confer higher efficacy against the third instar of cotton bollworm larvae when compared with other insecticides, such as endosulfan, cypermethrin and acephate.

Spinetoram is bioinsecticide, and naturally created by the soil bacteria, actinomycetes *Saccharopolyspora spinosa* (Shimokawatoko *et al.*, 2012). It is a safe insecticide; that excites high toxicity to *Helicoverpa armigera* (Rafiee *et al.*, 2008). Spinetoram is a spinosyn derived from spinosad, and it targets the acetylcholine receptors (nAChR) in the insect nervous systems (Galm and Sparks, 2016). Recently, spinetoram has been suggested for using in integrated FAW management programs due to its broad insecticidal range against a variety of pests, toxicological effectiveness, eco-friendly effects, and effective method of action on synaptic transmission (Gao *et al.*, 2022; Mohanan *et al.*, 2022; Wakil *et al.*, 2023; Salem *et al.*, 2024).

Insects use a variety of defense mechanisms against insecticides; one of them is the detoxifying enzymes which play a vital role against insecticides (Li *et al.*, 2013; Wang *et al.*, 2014). They can change harmful substances into innocuous or quickly eliminated substances (Ahmad *et al.*, 2007; Panini *et al.*, 2016). Thus, in order to comprehend both the biological adaptive ability and the mechanism of resistance that may arise, it is required to examine the enzyme activities relevant to the insecticide (Ercan *et al.*, 2022).

The present study was conducted to compare the lethal effects of two selected insecticides: chlorpyrifos (a conventional insecticide) and spinetoram (a bioinsecticide) against the 2nd larval instar of *S. frugiperda*, under laboratory conditions with regard to biological and biochemical investigations.

MATERIALS AND METHODS

Insect Rearing:

Spodoptera frugiperda larvae were collected from an infested maize field at South Valley University farm in Qena governorate, Egypt. In the insectary of the Department of

Zoology at Faculty of Science, South Valley University, the protocol for insect rearing followed that described by Dahi *et al.* (2020). The larvae were reared in the laboratory under controlled conditions (25±2°C, 65±5% RH) for up to four generations to obtain a laboratory strain. Newly second instar larvae of *S. frugiperda* were utilized in the experiment.

Insecticide Treatments:

Two insecticides were applied on the 2nd instar larvae in this experiment: chlorpyrifos (Dofos 48 %, Chema_industries - Elisra_Industries) and spinetoram (Radiant 12 % SC, Dow Agro Sciences). The concentrations of the insecticides used were: 450, 350, 250, 150 and 50 ppm for chlorpyrifos, and 1.5, 1, 0.5, 0.25, 0.125 and 0.0625 ppm for spinetoram.

Bioassay of the Tested Insecticides on *S. frugiperda* 2nd instar larvae:

Newly molted 2nd instar larvae of *S. frugiperda* were utilized to assess the larvicidal efficacy of chlorpyrifos and spinetoram. Fresh castor oil leaves were immersed in each of the prepared concentrations of the tested compounds and subsequently allowed to dry at room temperature. In the control, distilled water was used to immerse the leaves. For each treatment, forty healthy 2nd instar larvae were divided by four replications (one replicate = 10 larvae per cube pack). Larvae were supplied with contaminant leaves with the tested insecticides. The number of larvae in the control group was comparable. The mortality rate of the larvae was assessed after 24 hours.

Biological Investigation:

Some biological aspects were determined in the 2nd instar larvae of *S. frugiperda* treated with the calculated LC₅₀ of the two tested insecticides. The tested biological parameters included the larval development period post-treatment, larval mortality and pupation percentages, the pupal stage duration, the pupal weight of male and female, sex ratio, percentage of adult emergence, male and female moth longevity, fecundity, and fertility.

Preparation for Biochemical Analysis:

Following the application of the tested insecticides, samples of the treated larvae were efficiently prepared for biochemical analysis. The samples were prepared according to the methodology outlined by Amin (1998). The samples were homogenized in distilled water (50 mg /1 ml). Homogenates underwent centrifugation at 8000 r.p.m. for 15 minutes at a temperature of 2 °C using a refrigerated centrifuge. The pellets were discarded, and the supernatants, referred to as enzyme extract, can be stored for at least one week without significant loss of activity when maintained at temperatures below -20.

Biochemical Estimations:

Total carbohydrates were determined in the acid extract of the sample using the phenol-sulphuric acid method as described by Dubois *et al.* (1956). The extraction and preparation of total carbohydrates for assay were conducted following the methodology established by Crompton and Birt (1967). Total lipids were estimated using the method described by Knight *et al.* (1972), which involved the preparation of a phosphovanillin reagent. This was achieved by dissolving 0.6 g of pure vanillin in 10 ml of ethanol and then diluting it to a final volume of 100 ml with distilled water. Subsequently, 400 ml of concentrated phosphoric acid was added. Total proteins were quantified using the method of Bradford (1976). The determination of digestive enzymes was conducted following the methodology outlined by Ishaaya and Swirski (1976). Phenoloxidase activity was assessed according to Ishaaya (1971). The activity of acetylcholinesterase (AChE) was assessed following the method by Simpson *et al.* (1964), utilizing acetylcholine bromide (AChBr) as the substrate. Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) through the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione, was detected as described

in Habig *et al.* (1974). Chitinase activity was measured using the 3, 5-dinitrosalicylic acid reagent, as demonstrated by Ishaaya and Casida (1974).

Statistical Analysis:

One-way analysis of variance (ANOVA) combined with Tukey's post hoc test was run to conduct statistical analysis using the statistics package for social sciences (SPSS) version 27. Values of LC₉₀, LC₇₅, LC₅₀, and LC₂₅ were obtained by probit analysis using LdP Line^R software (<http://www.ehabsoft.com/ldpline>). The analytical data were presented as mean $\pm$ standard error (SE). Statistical significance variation was set at $p < 0.05$.

RESULTS

Toxicity of Insecticides:

The recorded results exhibited variations in the efficiency of the two applied insecticides, chlorpyrifos and spinetoram, against the 2nd larval instar of *S. frugiperda*, as shown in Table 1. The LC₅₀ values of chlorpyrifos and spinetoram were 181.0 ppm and 0.23 ppm, respectively. Accordingly, spinetoram recorded high toxicity followed by chlorpyrifos. Additionally, the values of LC₂₅, LC₇₅ and LC₉₀ were represented in Table, 2 and Figures 1 and 2.

Table 1 Toxicity of chlorpyrifos and spinetoram against the 2nd larval instar of *Spodoptera frugiperda*

Insecticides	Concentrations (ppm)	Mortality %	LC ₅₀ (ppm)	Slope
Chlorpyrifos	50	10	181.0	2.17
	150	47		
	250	60		
	350	73		
	450	80		
	Control	0.0		
Spinetoram	0.0625	13	0.23	1.63
	0.125	41		
	0.25	54		
	0.5	69		
	1.0	85		
	Control	0.0		

Table 2: LC₂₅, LC₅₀, LC₇₅, and LC₉₀ values (ppm) of the two insecticides against the 2nd larval instar of *Spodoptera frugiperda*

Insecticides	LC ₂₅	LC ₅₀	LC ₇₅	LC ₉₀	R	Homogeneity LC ₉₀ /LC ₅₀ ratio
Chlorpyrifos	89	181.0	370.0	705	0.99	388.0
Spinetoram	0.09	0.23	0.59	1.39	0.99	6.09

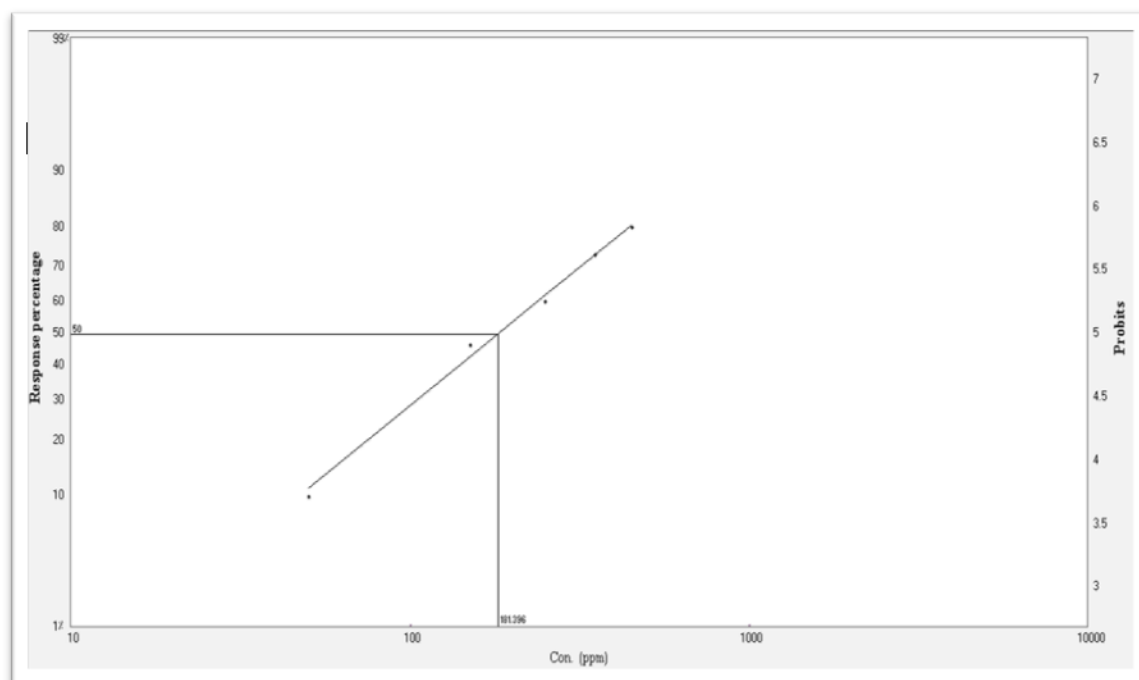


Fig. 1 Concentration-mortality regression line for the 2nd larval instar of *Spodoptera frugiperda* treated with chlorpyrifos.

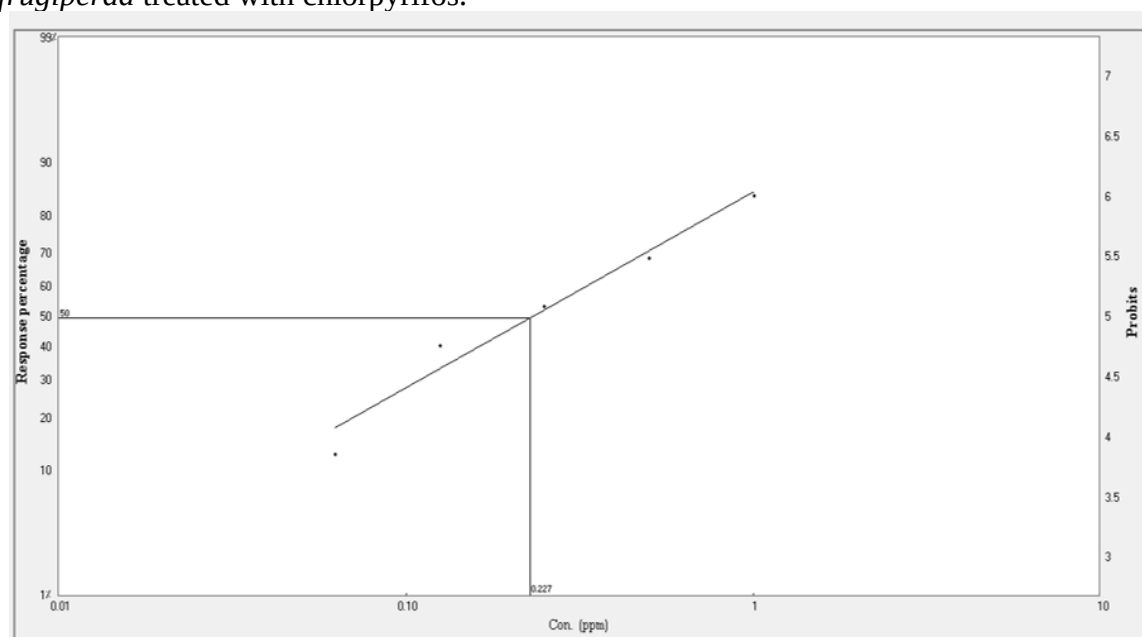


Fig. 2: Concentration-mortality regression line for the 2nd larval instar of *Spodoptera frugiperda* treated with spinetoram.

Effects on the Biological Aspects of the 2nd Larval Instar of *S. frugiperda*:

Treatment of the 2nd larval instar of *S. frugiperda* with LC₅₀ of spinetoram induced a highly significance in the larval period followed by chlorpyrifos (Table 3). The larval duration for spinetoram was 22.47 ± 0.21 days, while it was 18.97 ± 0.35 days for chlorpyrifos, as opposed to the control (12.75 ± 0.14 days). Notably, application with the two insecticides decreased the pupation to (52% and 53% for chlorpyrifos and spinetoram respectively), compared with the control percentage (97%). Mortality percent was higher in the treated larvae; it was 48% and 47% for chlorpyrifos and spinetoram respectively, compared with 3.0% in the control group.

The effects of the LC₅₀ of chlorpyrifos and spinetoram on some pupal indices were summarized in Table 4. Both pupal duration and pupal weights exhibited significant variations after treatment. The tested compounds induced a highly significant rise in pupal duration, chlorpyrifos and spinetoram recorded 13.07 ± 0.27 and 10.50 ± 0.11 days/pupa, respectively when compared with 8.81 ± 0.15 days for control. In addition, the average pupal weight revealed the highest significant decrease in spinetoram (0.148 ± 0.003 g) followed by 0.179 ± 0.01 g for chlorpyrifos while it was 0.231 ± 0.01 g in the control group. Spinetoram treatment induced larval malformation (6.0%), while no malformation was noticed in chlorpyrifos application. No pupal mortality was recorded in the treated groups or in the control group. Furthermore, an emergence of 100% was reported for both untreated and treated larvae.

Effects of LC₅₀ of chlorpyrifos and spinetoram on adult longevity and fecundity were shown in Table 5. The application increased the longevity of females to 11.75 ± 0.48 and 12.0 ± 0.41 days for chlorpyrifos and spinetoram respectively, compared to 10.0 ± 0.0 days in the control group. Otherwise, males' longevity didn't exhibit significant differences. Also, nonsignificant changes were recorded for pre-oviposition, oviposition and post-oviposition periods. The mean number of eggs decreased significantly by chlorpyrifos and spinetoram (1039.5 ± 43.26 eggs/female and 1070.75 ± 62.83 eggs/female, respectively), compared to the control (1277.25 ± 59.98 eggs/female). Hatchability showed nonsignificant differences in chlorpyrifos and spinetoram treatments (96.0% and 95.0%, respectively) compared to 96.0% in the control. At the same level, the incubation period was 2.5 days in the two insecticide treatments, similar to the control group.

Table 3 Biological aspects of the 2nd instar of *S. frugiperda* larvae after treated with LC₅₀ of chlorpyrifos and spinetoram

Biological aspects	Control	Chlorpyrifos	Spinetoram
Larval duration (days)	12.75 ± 0.14^c	18.97 ± 0.35^b	22.47 ± 0.21^a
Pupation (%)	97.0	52.0	53.0
Larval mortality (%)	3.0	48.0	47.0

Values are mean $\pm$ standard error

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

Table 4 Effect of LC₅₀ of chlorpyrifos and spinetoram on *S. frugiperda* pupae following their treatment as the 2nd instars larvae

Biological aspects	Control	Chlorpyrifos	Spinetoram
Pupal duration (days)	8.81 ± 0.15^c	13.07 ± 0.27^a	10.50 ± 0.11^b
Female Pupal duration (days)	8.67 ± 0.06^c	13.06 ± 0.41^a	10.81 ± 0.19^b
Male Pupal duration (days)	8.92 ± 0.29^c	13.06 ± 0.30^a	10.19 ± 0.06^b
Normal pupae %	100	100	94.0
Malformed pupae %	0.0	0.0	6.0
Pupal weight (gm.)	0.231 ± 0.01^a	0.179 ± 0.01^b	0.148 ± 0.003^c
Female Pupal weight (gm.)	0.249 ± 0.01^a	0.192 ± 0.01^b	0.162 ± 0.00^c
Male Pupal weight (gm.)	0.204 ± 0.01^a	0.165 ± 0.03^b	0.133 ± 0.00^c
Pupal mortality (%)	0.0	0.0	0.0
Emergence (%)	100	100	100

Values are mean $\pm$ standard error

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

Table 5 Percentage of adult emergence, life span and reproductive potential of moths emerging from the 2nd instars *S. frugiperda* larvae treated with LC₅₀ chlorpyrifos and spinetoram

Biological aspects	Control	Chlorpyrifos	Spinetoram
Sex ratio % (♂ : ♀)	0.95:1	1.06:1	0.89:1
Adult longevity (days)	10.25 ± 0.14 ^b	10.63 ± 0.47 ^{ab}	11.25 ± 0.14 ^a
Female longevity (days)	10.0 ± 0.0 ^b	11.75 ± 0.48 ^a	12.0 ± 0.41 ^a
Male longevity (days)	10.50 ± 0.29 ^a	9.50 ± 0.5 ^a	10.50 ± 0.29 ^a
Pre- oviposition period (days)	2.25 ± 0.25 ^a	2.50 ± 0.29 ^a	2.50 ± 0.29 ^a
Oviposition period (days)	7.0 ± 0.41 ^a	8.0 ± 0.41 ^a	8.25 ± 0.63 ^a
Post-oviposition period (days)	0.75 ± 0.25 ^a	1.50 ± 0.63 ^a	1.50 ± 0.65 ^a
Fecundity (No. eggs/female)	1277.25 ± 59.98 ^a	1039.5 ± 43.26 ^b	1070.75 ± 62.83 ^b
Hatchability (%)	96	96	95
Incubation period (days)	2.5 ^a	2.5 ^a	2.5 ^a

Values are mean ± standard error

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

Effects on Metabolic Nutrients of the 2nd Larval Instar of *S. frugiperda*:

The latent effects of chlorpyrifos and spinetoram on some metabolic reserves of the 2nd instar *S. frugiperda* larvae after LC₅₀ exposure was elucidated in Figure 3. Treatment with chlorpyrifos showed significant declines in the levels of carbohydrates and lipids (5.97 ± 0.09 and 3.73 ± 0.16 mg/g body weight "b.wt", respectively) compared with the control values (12.4 ± 0.21 and 6.20 ± 0.25 mg/g b.wt, respectively). Spinetoram resulted in a further significant decrease in carbohydrate levels (8.17 ± 0.12 mg/g b.wt) but no significant changes in lipid levels (5.83 ± 0.18 mg/ml). The total protein level was significantly lowered after spinetoram application, recording 10.20 ± 0.12 mg/g b.wt compared to the control level (15.4 ± 0.84 mg/g b.wt). Meanwhile, chlorpyrifos displayed no significant decline in total protein level (14.0 ± 0.26 mg/g b.wt).

Effects on Some Biochemical Parameters of the 2nd Larval Instar of *S. frugiperda*:

The effects of LC₅₀ of the two insecticides on some enzymes in the 2nd instar *S. frugiperda* larvae were illustrated in Figures 4 and 5. The results showed a highly significant decrease in the levels of amylase (16.0 ± 1.73 µg glucose/minute/g b.wt) after the application of chlorpyrifos, followed by 33.67 ± 3.53 µg glucose/minute/g b.wt for spinetoram, in comparison with the control group (75.67 ± 3.84 µg glucose/minute/g b.wt). Also, chlorpyrifos and spinetoram induced a significant drop in the levels of invertase enzyme (73.0 ± 3.51 , 113.33 ± 4.41 µg glucose/minute/g b.wt respectively), compared to 233.33 ± 8.82 µg glucose/minute/g b.wt in the control. Whereas, the levels of phenoloxidase enzyme were non-significantly varied in chlorpyrifos and spinetoram treatments (6.68 ± 0.08 and 6.87 ± 0.15 optical density "O.D." units/minute/g b.wt, respectively) when compared to the control levels (5.97 ± 0.19 O.D. units/minute/g b.wt). In Figure 4, 2nd instar *S. frugiperda* larvae treated with chlorpyrifos resulted in a significant increase in the activities of AchE (320.0 ± 11.54 µg AchBr/minute/g b.wt) when compared to the control (195.0 ± 7.64 µg AchBr/minute/g b.wt), contrary to 200.67 ± 6.36 , the mean value of spinetoram. The LC₅₀ of spinetoram significantly decreased the GST level (42.67 ± 1.2 mmol sub. conjugated/minute/g b.wt) and chlorpyrifos (44.0 ± 1.15 mmol sub. conjugated/minute/g b.wt) as compared to the control (58.33 ± 2.03 mmol sub. conjugated/minute/g b.wt). Chlorpyrifos induced high significant decrease in the chitinase levels (42.0 ± 2.85 µg N-acetylglucoseamine "NAGA"/minute/g b.wt), followed by spinetoram (118.67 ± 4.1 µg NAGA/minute/g b.wt) when compared to their control values (181.0 ± 7.37 µg NAGA/minute/g b.wt).

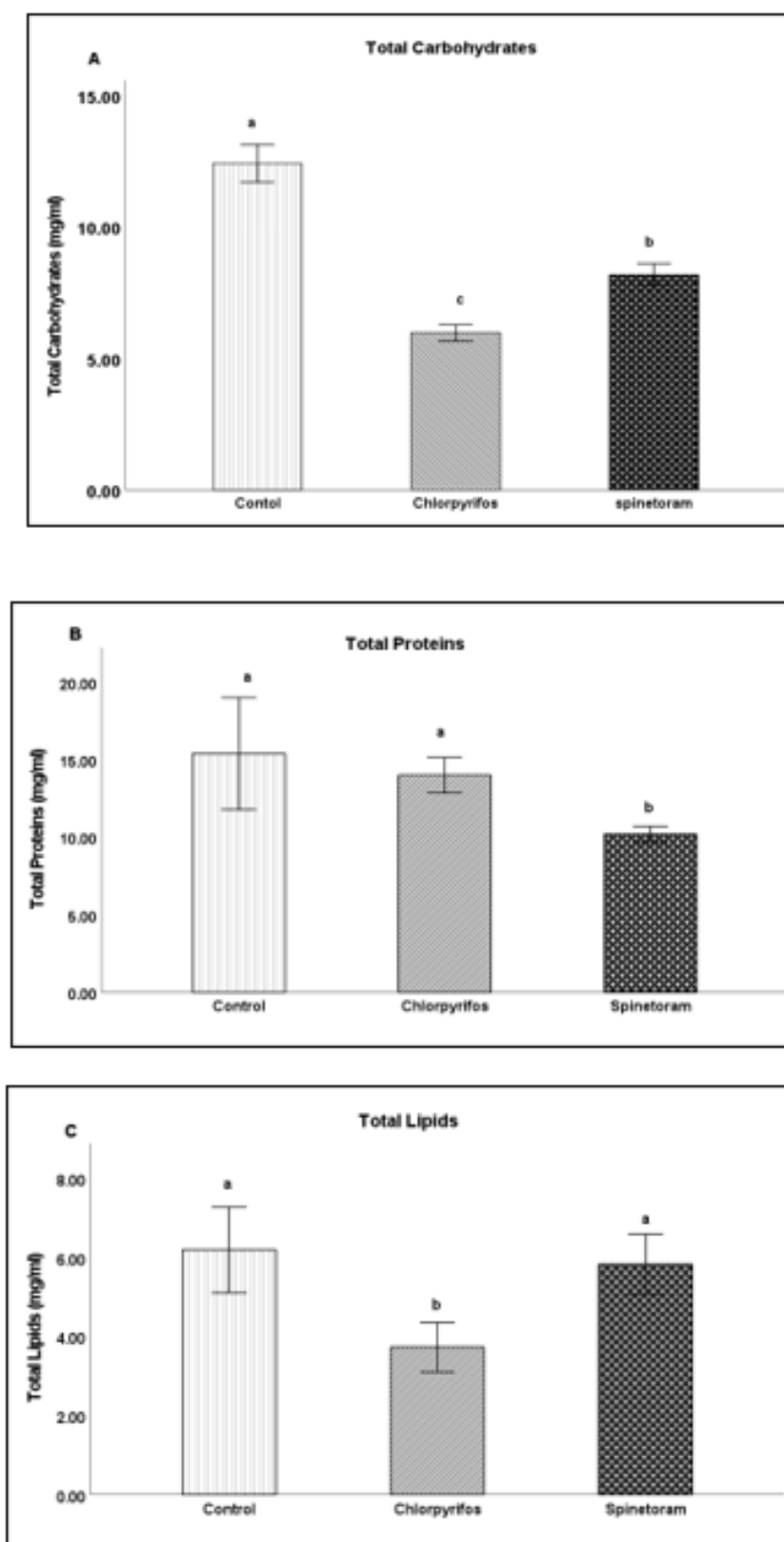


Fig. 3 (A) Total carbohydrates, (B) proteins and (C) lipids contents in the 2nd instar *Spodoptera frugiperda* larvae 24 hours post-treatment with LC₅₀ of chlorpyrifos and spinetoram. Means with different letters are significantly different (Tukey's test: P < 0.05).

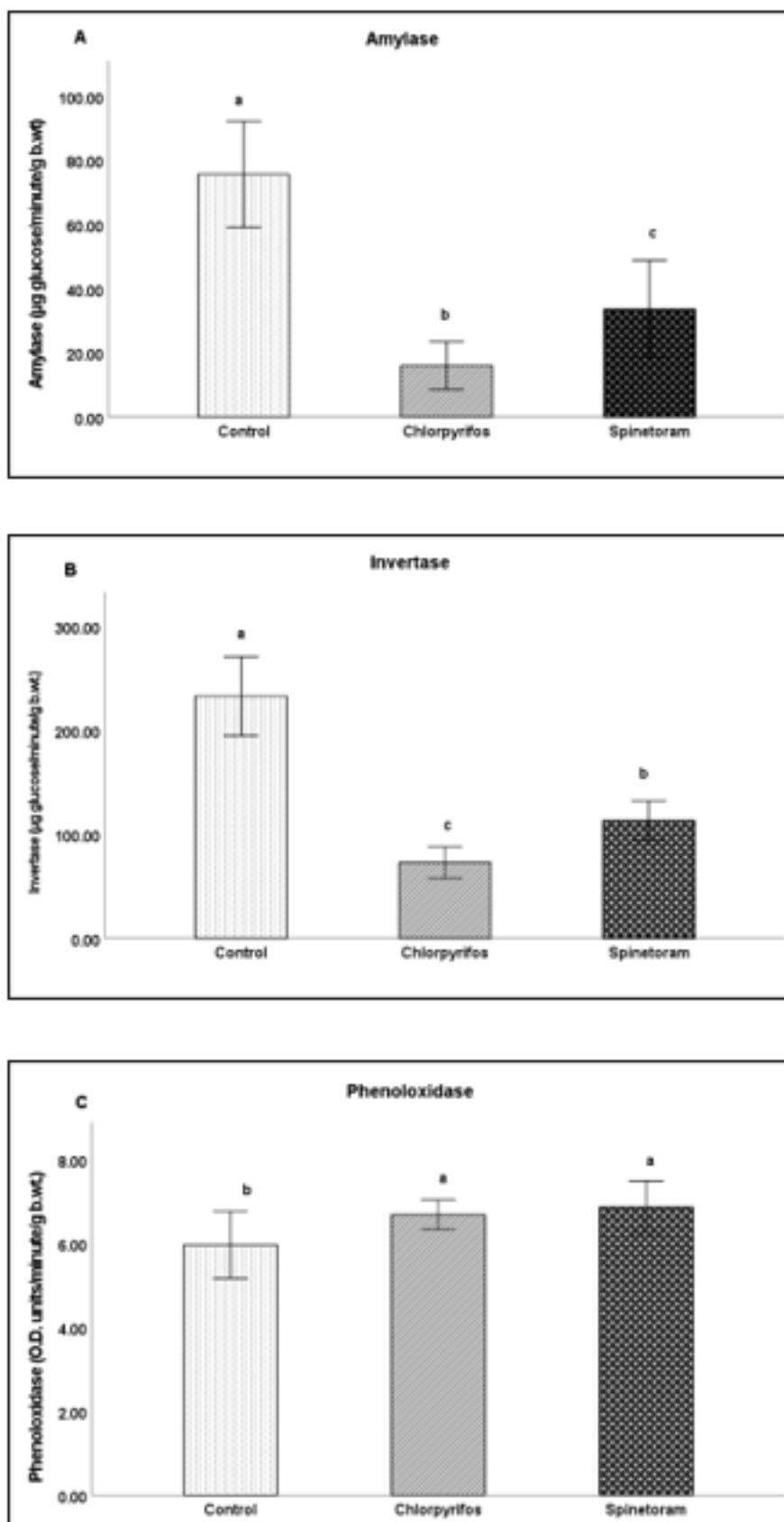


Fig. 4 (A) Amylase, (B) Invertase, and (C) Phenoloxidase activities in the 2nd instar *Spodoptera frugiperda* larvae- 24 hours post-treatment with LC₅₀ of chlorpyrifos and spinetoram. Means with different letters are significantly different (Tukey's test: P < 0.05).

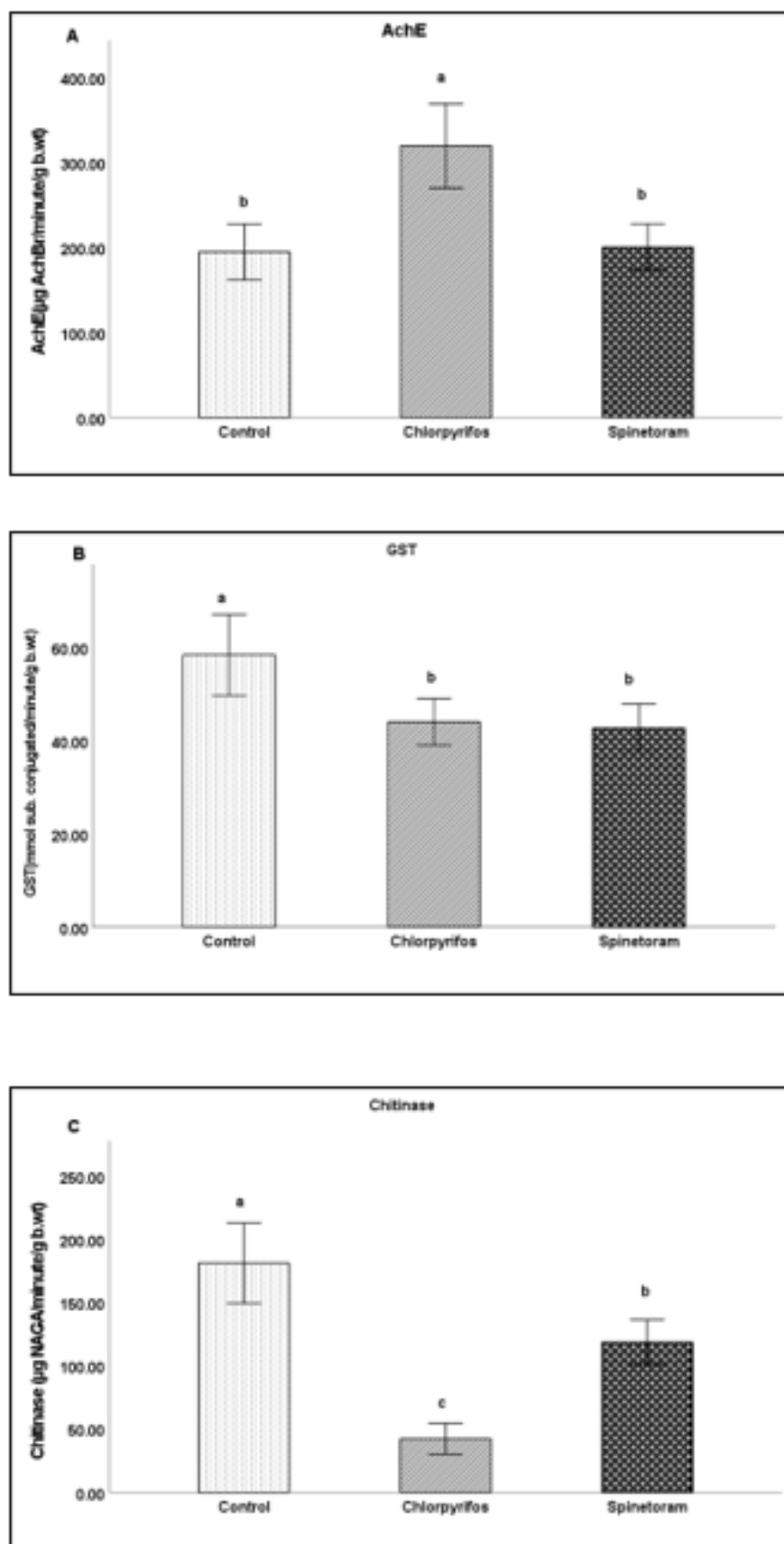


Fig. 5 (A) AChE, (B) GST, and (C) Chitinase activities in the 2nd instar *Spodoptera frugiperda* larvae- 24 hours post-treatment with LC₅₀ of chlorpyrifos and spinetoram. Means with different letters are significantly different (Tukey's test: P < 0.05).

DISCUSSION

A variety of insecticides, such as organophosphates, insect growth regulators (IGRs), and synthetic pyrethroids, has demonstrated effectiveness in pest control. Nonetheless, the excessive application of these insecticides across multiple generations of insects annually, the continuous availability of host crops, and the emergence of pest resistance to various classes of insecticides have been documented (Mokbel *et al.*, 2019). Due to the consequences of resistance, there has been a significant demand for more selective and effective insecticides for pest control across various crops. As a result, agrochemical products have introduced new categories of insecticides (Ghosal *et al.*, 2016). The biorational spinetoram insecticide provides substantial protection while maintaining reduced toxicity to the surrounding environment and mammals. Spinetoram has been studied as a means to decrease the reliance on conventional synthetic insecticides in pest management (Küçüksari and Tunaz, 2021).

The current findings indicate that spinetoram exhibited greater toxicity than chlorpyrifos, because its LC₅₀ value is lower. The lower the LC₅₀ value, the higher the toxicity and vice versa.. This could be supported by El-Naggar (2013) who reported that spinetoram exhibited more toxicity than spinosad against 4th instar *Spodoptera littoralis* larvae. Aslam *et al.* (2004) indicated that chlorpyrifos was the most effective insecticide for controlling cotton bollworms among the insecticides tested. Also, El-Khayat *et al.* (2012) observed that chlorpyrifos treatment against 2nd and 4th instar cotton leafworm larvae led to significant mortality.

The biological observations caused by the application of chlorpyrifos and spinetoram, indicated a significant increase in the pupal and larval periods and an elevation in the mortality percentage. Furthermore, a marked decrease in the pupation percentage and weight, and an increase in duration of the pupal stage were detected. Additionally, chlorpyrifos and spinetoram treatment diminished the lifespan of female individuals. Spinetoram and chlorpyrifos caused reduction of the eggs number and a notable malformation rate appeared only with spinetoram treatment. These findings are in accordance with Salem *et al.* (2024) who determined that spinetoram toxicity resulted in significant biological alterations in 4th instar larvae of *S. frugiperda*. The growth of larvae and pupae exhibited notable increases, accompanied by heightened mortality rates, adult malformations, decreased fecundity, and diminished hatchability.

Hannig *et al.* (2009) showed that chlorpyrifos had a negative impact on the adult fecundity and longevity of individuals that emerged from the treated 3rd instar cotton bollworm larvae, *Helicoverpa armigera*. Vojoudi *et al.* (2011) stated that the application of chlorpyrifos on 3rd instar cotton bollworm larvae resulted in a notable reduction in the lifespan of the adult insects. Furthermore, chlorpyrifos notably decreased the pupal weight and the main number of eggs laid by female cotton bollworm in comparison to control larvae. The reduction in fecundity was observed in a prior study by Santos *et al.* (2017), in which chlorpyrifos was found to extend the developmental periods of both pupal and larval stages in *H. convergens*.

The latent effects of pesticides resulted in a notable decrease in metabolic reserves (Hussain *et al.*, 2018). The current findings indicated that both chlorpyrifos and spinetoram have significantly reduced the contents of carbohydrates, spinetoram reduced total proteins and while clorpyrofos reduced total lipids. In other study by Megahed *et al.* (2013), spinosad led to a decrease in total protein as a result of the inhibition of RNA and DNA synthesis. The use of spinetoram on 4th instar larvae of *S. frugiperda* resulted in notable alterations in the profiles of total proteins, carbohydrates, and lipids (Salem *et al.*, 2024). The decrease in carbohydrates may result from an elevated metabolism to produce more energy in toxicant

stress conditions (Franeta *et al.*, 2018). A compensatory mechanism for decreased energy during pesticide stress was linked to a protein lack (Nath *et al.*, 1997). One possible explanation for the decrease in total lipids is that the detoxifying process causes the larvae to expend a significant amount of energy Xu *et al.* (2016).

Our results indicated a notable decrease in the activities of amylase and invertase, moreover, a noticeable reduction in GST and chitinase levels following the exposure to chlorpyrifos and spinetoram. In insects, glutathione-S-transferase (GSTs) serves as a detoxification enzyme (Claudianos *et al.*, 2006). Fetoh and Asiry (2013) identified a decrease in the chitinase level of the fourth larval instar of *S. littoralis* following treatment with chlorpyrifos. Consistent with our findings, Ali *et al.* (2017) observed a decrease in GST activities after treatment with chlorpyrifos and spinosad targeting *Bemisia tabaci*. The decrease during the experimental exposure may be linked to the sequence of actions of chemicals and pathogens against the treated insects. Shenouda *et al.* (2019) indicated that spinosad significantly decreased GST and phenoloxidase activities, whereas chlorpyrifos only reduced the level of phenoloxidase. Conversely, spinosad resulted in a notable increase in chitinase, albeit not to a significant extent.

Acetylcholinesterase serves as a significant target for carbamates and organophosphorus insecticides within the central nervous system of insects (Nathan *et al.*, 2008). Also, it is responsible for catalysing the decomposition of the neurotransmitter acetylcholine inside the insect nervous system (Bourne *et al.*, 2016). In the present study, chlorpyrifos induced an increase in AchE levels, consistent with the findings of Fetoh and Asiry (2013) who reported elevated AchE activity in the treated 4th larval instar of *S. littoralis*, whereas spinosad did not demonstrate significant differences. Gao *et al.* (2020) reported that spinetoram was highly effective against the 4th instar larvae of *S. frugiperda*, as compared with chlorantraniliprole, and the activities of CarE, MFO and AchE increased after exposure to different concentrations of spinetoram. Also, Salem *et al.* (2024) stated highly significant elevations in the activities of AchE in the larvae of *S. frugiperda*.

Given everything mentioned above, as well as long-term effects on the biology of successive insect stages and pertinent biochemical disruptions motivate us to embrace and suggest spinetoram as a powerful agent for *S. frugiperda* biocontrol strategies instead of chlorpyrifos, paying closer attention to the potential long-term effects of spinetoram's use in future research.

Conclusion

The current investigation highlights the efficacy of chlorpyrifos and spinetoram in control the invasive *S. frugiperda* in Egypt. The two selected insecticides were significantly effective against the 2nd instar larvae. Chlorpyrifos and spinetoram exhibited a similar effect on some biological and physiological aspects in the treated larvae. These insecticides offer a broad range of effectiveness in destroying pests, but spinetoram superior chlorpyrifos in some biological and biochemical changes. Spinetoram is a safer, sustainable approach, and more environmentally friendly biocide that can be used instead of chlorpyrifos in integrated pest management strategies for controlling fall armyworm instars.

Abbreviations

FAW	Fall Armyworm
LC ₅₀	Median Lethal Concentration
RH	Relative Humidity
AchE	Acetylcholinesterase
AchBr	Acetylcholinbromide
GSH	Glutathione
GST	Glutathione S-transferase
CarE	Carboxyl Esterase

MFO Mixed Function Oxidase
 NAGA N-acetylglucosamine
 IGRs Insect Growth Regulators

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 animal handling in laboratory experiments of the Faculty of Science, South Valley University, Qena, Egypt (Approval number: 021/11/22).

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REFERENCES

- Ahmad, I., Astari, S. and Tan, M. (2007). Resistance of *Aedes aegypti* (Diptera: Culicidae) in 2006 to pyrethroid insecticides. *Pakistan Journal of Biological Science*, 10: 3688-3692. DOI: <https://doi.org/10.3923/pjbs.2007.3688.3692>
- Ali, S., Zhang, C., Wang, Z., Wang, X. M., Wu, J. H., Cuthbertson, A. G., Shao, Z. and Qiu, B. L. (2017). Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). *Scientific Reports*, 7: 1–14. DOI: <http://dx.doi.org/10.1038/srep46558>.
- Amin, T. R. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd.). Ph.D. thesis, Faculty of science, Cairo University. 164 pp.
- Aslam, M., Razaq, M., Rana, S. and Faheem, M. (2004). Efficacy of different insecticides against bollworms on cotton. *Journal of Research Science, Bahauddin Zakariya, Multan, Pakistan*, 15: 17-22. DOI: <https://api.semanticscholar.org/CorpusID:88307086>
- Assefa, F. and Ayalew, D. (2019). Status and Control Measures of Fall Armyworm (*Spodoptera frugiperda*) Infestations in Maize Fields in Ethiopia: A Review. *Cogent Food & Agriculture*, 5, Article ID: 1641902. DOI: <https://doi.org/10.1080/23311932.2019.1641902>
- Bourne, Y., Sharpless, K. B., Taylor, P. and Marchot, P. (2016). Steric and dynamic parameters influencing in situ cycloadditions to form triazole inhibitors with crystalline acetylcholinesterase. *Journal of the American Chemical Society*, 138: 1611–1621. DOI: <https://doi.org/10.1021/JM051132B>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72:248-254.

- Claudianos, C., Ranson, H., Johnson, R. M., Biswas, S., Schuler, M. A., Berenbaum, M. R., Feyereisen, R. and Oakeshott, J. G. (2006). A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. *Insect Molecular Biology*, 15: 615–636. DOI: <https://doi.org/10.1111/j.1365-2583.2006.00672.x>
- Crompton, M. and Birt, L. M. (1967). Changes in the amounts of carbohydrates, phosphagen, and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*. *Journal of Insect physiology*, 13: 1575-1595. DOI: [https://doi.org/10.1016/0022-1910\(67\)90180-1](https://doi.org/10.1016/0022-1910(67)90180-1)
- Dahi, H. F., Salem, S. A. R., Gamil, W. E. and Mohamed, H. O. (2020). Heat requirements for the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) as a new invasive pest in Egypt. *Egyptian Academic Journal of Biological Science: A. Entomology*, 13(4): 73-85. DOI: <https://doi.org/10.21608/eajbsa.2020.120603>
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356. DOI: <https://doi.org/10.1021/ac60111a017>
- Early, R., Moreno, P. G., Murphy, S. T. and Day, R. (2018). Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. *Neo Biota*, 40: 25-50. DOI: <http://dx.doi.org/10.3897/neobiota.40.28165>
- Environmental Protection Agency (2021). <https://www.epa.gov/caddis-vol2/insecticides>.
- El-Khayat, E. F., Desuky, W. M. H., Azab, M. M. and Khedr, M. M. A. (2012). Toxic impact of some insect growth regulators and biocides in relation to chlorpyrifos to cotton leafworm, *Spodoptera littoralis* (Boisd.). *Egyptian Journal of Agriculture Research*, 90(1): 55-65. DOI: <https://dx.doi.org/10.21608/ejar.2012.159548>
- El-Naggar, J. B. A. (2013). Sublethal effect of certain insecticides on biological and physiological aspects of *Spodoptera littoralis* (Boisd.). *Nature and Science*, 11(7): 19-25. DOI: <https://doi.org/10.21608/eajbsa.2020.115948>
- Ercan, F. S., Baş, H. and Azarkan, S. Y. (2022). In silico detection of cucurbitacin-E on antioxidant enzymes of model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae) and variation of antioxidant enzyme activities and lipid peroxidation in treated larvae. *Beni-Suef University Journal of Basic and Applied Sciences*, 11: 130. <https://doi.org/10.1186/s43088-022-00310-3>.
- Fernandes, M. E. S., Fernandes, F. L., Picanço, M. C., Queiroz, R. B., Silva, R. S. and Huertas, A. A. G. (2008). Physiological selectivity of insecticides to *Apis mellifera* (Hymenoptera: Apidae) and *Protonectarina sylveirae* (Hymenoptera: Vespidae) in citrus. *Sociobiology*, 51: 765-774. DOI: <https://www.researchgate.net/publication/273665664>
- Fetoh, B. E. A. and Asiry, K. A. (2013). Biochemical effects of chlorpyrifos organophosphorous insecticide, camphor plant oil and their mixture on *Spodoptera littoralis* (Boisd.). *Phytopathology and Plant Protection*, 46(15): 1848-1856. DOI: <http://dx.doi.org/10.1080/03235408.2013.779073>
- Franeta, F., Mirčić, D., Todorović, D., Milovac, Ž., Granica, N., Obradović, S. and Perić-Mataruga, V. (2018). Effects of different insecticides on the antioxidative defense system of the European corn borer (*Ostrinia nubilalis* Hübner) (Lepidoptera: Crambidae) larvae. *Archives of Biological Science*, 70(4): 765-773.
- Galm, U. and Sparks, T. C. (2016). Natural product derived insecticides: discovery and development of spinetoram. *Journal of Industrial Microbiology and Biotechnology*, 43: 185-193. DOI: <https://doi.org/10.1007/s10295-015-1710-x>
- Gao, Z., Batool, R., Xie, W., Huang, X. and Wang, Z. (2022). Transcriptome and Metabolome Analysis Reveals the Importance of Amino-Acid Metabolism in *Spodoptera*

- frugiperda* Exposed to Spinetoram. *Insects* 13(9): 852. <https://doi.org/10.3390/insects13090852>.
- Gao, Z. P., Guo, J. F., He, K. L. and Wang, Z. Y. (2020). Toxicity of spinetoram and its effects on the detoxifying enzyme and acetyl cholinesterase activities in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *Acta Entomologica Sinica*, 63(5): 558-564. DOI: <https://doi.org/10.16380/j.kcxb.2020.05.004>
- Ghosal, A., Dolai, A. K. and Chatterjee, M. (2016). Plethora (Novaluron+Indoxacarb) insecticide for the management of tomato fruit borer complex. *Journal of Applied and Natural Science*, 8(2): 919–922. DOI: <https://doi.org/10.31018/jans.v8i2.897>
- Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A. and Tamo, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. PLoS ONE, DOI: <http://dx.doi.org/10.1371/journal.pone.0165632>.
- Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249: 7130–7139. DOI: [https://doi.org/10.1016/S0021-9258\(19\)42083-8](https://doi.org/10.1016/S0021-9258(19)42083-8)
- Hannig, G. T., Ziegler, M. and Marcon, P. G. (2009). Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Management Science*, 65(0): 969-74. DOI: <https://doi.org/10.1002/ps.1781>
- Hussain, A., Khana, M. F., Mukhtar, O., Faheema, M. and Alib, U. (2018). Comparative Toxicity of Spinetoram and Nitenpyram against Earthworm and their Effects on Protein Contents and Cholinesterase Activity. *Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences*. 61(2): 103-111. DOI: 10.52763/PJSIR.BIOL.SCI.61.2.2018.103.111
- Ishaaya, I. (1971). Observation on the phenoloxidase system in the armored scale *Aonidiella aurantii* and *Chrysomphalus aonidum*. *Comparative Biochemistry and Physiology*, 39 B, 935-943. DOI: [https://doi.org/10.1016/0305-0491\(71\)90117-9](https://doi.org/10.1016/0305-0491(71)90117-9)
- Ishaaya, I. and Casida, J. E. (1974). Dietary TH-6040 alters composition and enzyme activity of housefly larval cuticle. *Pesticide Biochemistry and Physiology*, 4: 484–490. DOI: [https://doi.org/10.1016/0048-3575\(74\)90073-X](https://doi.org/10.1016/0048-3575(74)90073-X)
- Ishaaya, I. and Swirski, E. (1976). Trehalase, invertase and amylase activities in the black scale, *Saissetia oleae*, and their relation to host adaptability. *Journal of Insect Physiology*, 16: 1025-1029. DOI: [https://doi.org/10.1016/0022-1910\(76\)90087-1](https://doi.org/10.1016/0022-1910(76)90087-1)
- Knight, J. A., Anderson, S. and Rawle, J. M. (1972). Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clinical Chemistry*, 18: 199-202. DOI: <https://doi.org/10.1093/clinchem/18.3.199>
- Küçüksarı, F. and Tunaz, H. (2021). Residual Contact Toxic Effects of Spinosyn Insecticide, Spinetoram Against German Cockroach (*Blattella germanica*) Adults. *KSU Journal of Agriculture and Nature*, 24 (4): 795-804. DOI: 10.18016/ksutarimdoga.vi.000000
- Li, S. G., Li, M. Y., Huang, Y. Z., Hua, R. M., Lin, H. F., He, Y. J. and Liu, Z. Q. (2013). Fumigant activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S-transferase activities in adult *Sitophilus zeamais*. *Journal of Pest Science*, 86: 677-683. DOI: 10.1007/s10340-013-0520-z
- Megahed, M. M. M., El-Tawil, M. F., El-Bamby, M. M. M. and Abouamer, W. L. (2013). Biochemical effects of certain bioinsecticides on cotton leaf Worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Journal of Agriculture and Biological Science*, 9(6): 308-317. DOI: <https://www.researchgate.net/publication/310505419>
- Mohanan, A., Chandrakar, G., Gupta, K. and Nagdev, P. (2022). Bio-efficacy of spinetoram insecticide against diamond back moth (*Plutella xylostella* Linn.) in cabbage. *The*

- Pharma Innovation Journal* SP-11(9): 1110-1114.
- Mokbel, E. M. S., Fouad, E. A. and El-Sherif, S. A. N. (2019). Resistance monitoring of cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) against certain alternative insecticide. *Journal of Biological Chemistry and Environmental Science*, 14(1):319–333.
- Nath, B. S., Suresh, A., Varma, B. M., Surendra Kumar, R. P. (1997). Changes in protein metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. *Ecotoxicology and Environmental Safety*, 36(2): 169-173. DOI: <https://doi.org/10.1006/eesa.1996.1504>
- Nathan, S. S., Choi, M. Y., Seo, H. Y., Paik, C. H., Kalaivani, K. and Kim, J. D. (2008). Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown plant hopper *Nilaparvata lugens* (Stål). *Ecotoxicology and Environmental Safety*, 70: 244–250. DOI: <https://doi.org/10.1016/j.ecoenv.2007.07.005>
- Nirmal, S. H., Manjit, S. M. (2008). Sub-lethal influences of different insecticides on oviposition preference of *Helicoverpa armigera* (Hübner). (Lepidoptera: Noctuidae) on cotton in relation to altered plant morphology. *International Journal of Pest Management Science*, 54: 181-187. DOI: <https://doi.org/10.1080/09670870801908456>
- Panini, M., Manicardi, G. C., Moores, G. D. and Mazzoni, E. (2016). An overview of the main pathways of metabolic resistance in insects. *Invertebrate Survival Journal*, 13(1): 326-335. DOI: <https://doi.org/10.25431/1824-307X/isj.v13i1.326-335>.
- Prasanna, B. M., Huesing, J. E., Eddy, R. and Peschke, V. M. (2018). Fall armyworm in Africa: a guide for integrated pest management. Textbook, USAID and CIMMYT publisher, Mexico. DOI: <http://hdl.handle.net/10883/19204>
- Pratissoli, D., Thuler, R. T., Pereira, F. F., Reis, E. F. and Ferreira, A. T. (2004). Transovarian action of lufenuron on adults of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and its effect on the parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *Ciência e Agrotecnologia*, 28: 9–14. <https://doi.org/10.1590/S1413-70542004000100001>
- Rafiee, D. H., Hejazi, M. J., Nouri, G. G. and Saber, M. (2008). Toxicity of some biorational and conventional insecticides to cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its ectoparasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae). *Journal of Entomological Society of Iran*, 28: 27-37.
- Salem, A. R. S., Alhousini, E. M. E., Al-Amgad, Z. and Mahmoud, A. B. M. (2024). Efficiency of spinetoram on biological, biochemical, and histological parameters in the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Egypt. *Journal of Plant Diseases and Protection*, (131): 489-499. DOI: <https://doi.org/10.1007/s41348-023-00835-4>.
- Santos, K. F. A., Zanardi, O. Z., Morais, M. R., Jacob, C. R. O., Oliveira, M. B. and Yamamoto, P. T. (2017). The impact of six insecticides commonly used in control of agricultural pests on the generalist predator *Hippodamia convergens* (Coleoptera: Coccinellidae). *Chemosphere*, 186: 218-226. DOI: <https://doi.org/10.1016/j.chemosphere.2017.07.165>.
- Shenouda, M. M. S., Moawad, F. G., Ali, N. E. and El-Sherif, A. N. (2019). Biochemical and toxicological studies of some pesticides on cotton leafworm (*Spodoptera littoralis*) *Arab Universities Journal of Agricultural Sciences*, 27(5), 2489-2499. DOI: <https://doi.org/10.21608/ajs.2020.20797.1137>
- Shimokawatoko, Y., Sato, N., Yamaguchi, Y. and Tanaka, H. (2012). Development of the novel insecticide spinetoram (Diana®). *Sumitomo Chemical Co Ltd Tokyo*.

- <https://www.sumitomo-chem.co.jp/english/rd/report/files/docs/2012 E1.pdf>.
- Simpson, D. R., Bull, L. D., Lidquist, A. D. (1964). A semi-microtechnique for estimation of cholinesterase activity in boll Weevil. *Annals of Entomological Society of America*, 57(3):367–377. DOI: <https://doi.org/10.1093/aesa/57.3.367>
- Smegal, D. C. (2000). Human Health Risk Assessment Chlorpyrifos; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Health Effects Division, U.S. Government Printing Office: Washington, pp: 1-131.
- Tavares, W. S., Cruz, I., Petacci, F., Freitas, S. S., Serrão, J. E., Zanuncio, J. C. (2011). Insecticide activity of piperine: toxicity to eggs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Pyralidae) and phytotoxicity on several vegetables. *Journal of Medicinal Plants Research*, 5: 5301–5306. DOI: <https://doi.org/10.5897/JMPR.9000814>
- Torres-Vila, L. M., Rodriguez-Molina, C. M., Lacasaplasencia, A. and Bielza-Lino, P. (2002). Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. *Environmental Science -Crop Protection*, 21: 1003-1013. DOI: [http://dx.doi.org/10.1016/S0261-2194\(02\)00081-9](http://dx.doi.org/10.1016/S0261-2194(02)00081-9).
- Vojoudi, S., Saber, M., Mir, J. and Talaei-Hassanloui, R. (2011). Toxicity of chlorpyrifos, spinosad and abamectin on cotton bollworm, *Helicoverpa armigera* and their sublethal effects on fecundity and longevity. *Bulletin of Insectology*, 64(2): 189-193. DOI: <https://www.researchgate.net/publication/259951035>.
- Wakil, W., Gulzar, S., Prager, S. M., Ghazanfar, M. U. and Shapiro-Ilan, D. I. (2023). Efficacy of entomopathogenic fungi, nematodes and spinetoram combinations for integrated management of *Thrips tabaci*: a two-year onion field study. *Pest Management Science*. DOI: <https://doi.org/10.1002/ps.7503>.
- Wang, Y., Zhao, S., Shi, L., Xu, Z. and He, L. (2014). Resistance selection and biochemical mechanism of resistance against cyflumetofen in *Tetranychus cinnabarinus* (Boisduval). *Pesticide Biochemistry and Physiology*. 111, 24–30. DOI: 10.1016/j.pestbp.2014.04.004
- Ware, G. W., Whitacre, D. M. (2004). The pesticide book. Meister Publications, Willoughby, USA. DOI: 10.4236/wjet.2015.33C030.
- Xu, C., Zhang, Z., Cui, K., Zhao, Y., Han, J., Liu, F. and Mu, W. (2016). Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). *PLoS ONE*, 11(6): e0156555. DOI: <http://dx.doi.org/10.1371/journal.pone.0156555>