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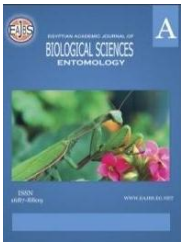

Lethal, Biological, and Biochemical Impacts of Two Insecticides on the 2nd Instar Larvae of The Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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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 LC_{50} 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 chlorpyriphos 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 LC_{50} 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 ºC 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.

B**iochemical 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 LC_{90}, LC_{75}, LC_{50}, and LC_{25} were obtained by probit analysis using LdP Line^{R} software (http://www.ehabsoft.com/ldpline).The analytical data were presented as mean± 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 LC_{50} 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 LC_{25}, LC_{75} and LC_{90} 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: LC_{25}, LC_{50}, LC_{75}, and LC_{90}values (ppm) of the two insecticides against the 2ndlarval 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

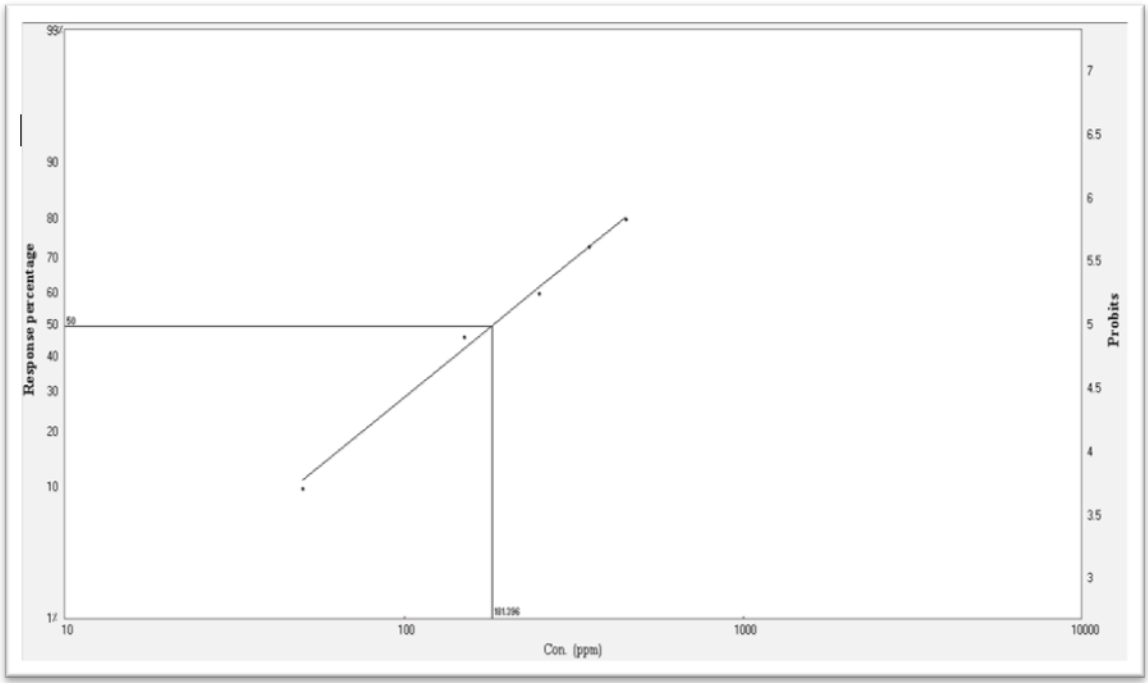

Fig. 1 Concentration-mortality regression line for the 2nd larval instar of *Spodoptera frugiperda* treated with chlorpyrifos.

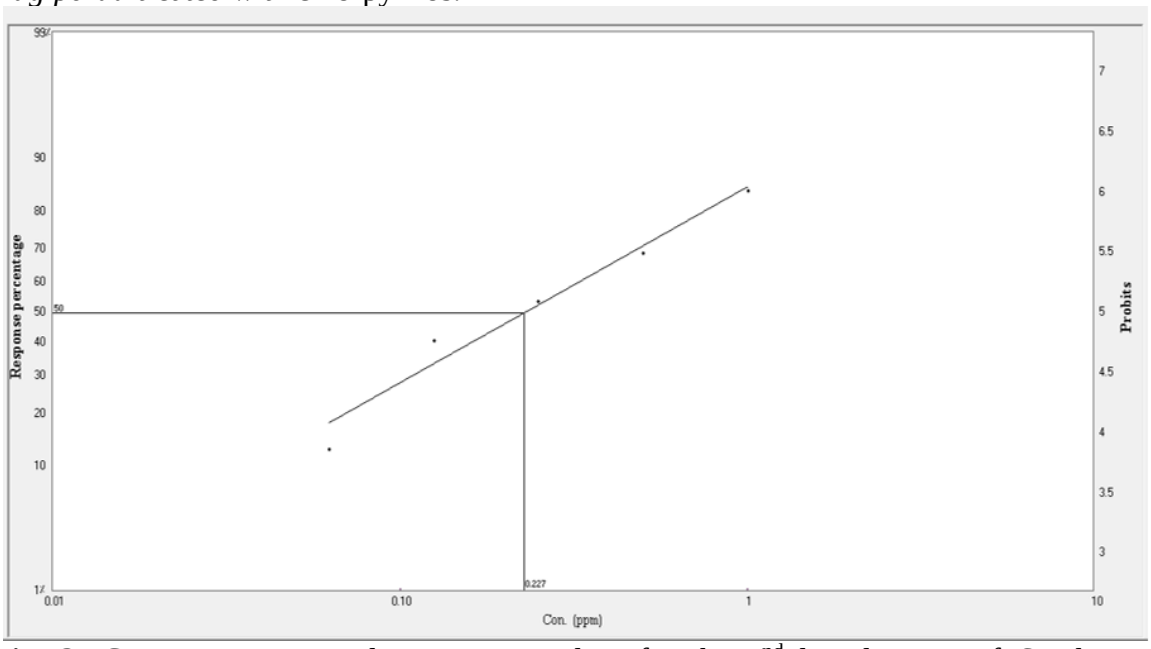

Fig. 2: Concentration-mortality regression line for the 2nd larval instar of *Spodoptera frugiperda* treated with spinetoram.

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

Treatment of the 2nd larval instar of *S. frugiperda* with LC_{50} 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 LC_{50} 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 ± 0.003g) 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 LC_{50} 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 LC_{50} 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 ± standard error.
Means have the different letters in the same row are significant (P<0.05).

Table 4 Effect of LC_{50} 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 ± 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 LC_{50} 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 LC_{50} 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± 0.16mg/g body weight "b.wt", respectively) compared with the control values (12.4 ± 0.21 and 6.20 ± 0.25mg/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 ± 0.12mg/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 ± 0.26mg/g b.wt).

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

The effects of LC_{50} 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± 4.41µg 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 ± 7.64µg AchBr/minute/g b.wt), contrary to 200.67± 6.36, the mean value of spinetoram. The LC_{50} of spinetoram significantly decreased the GST level (42.67± 1.2mmol 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 ± 2.85µg 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).

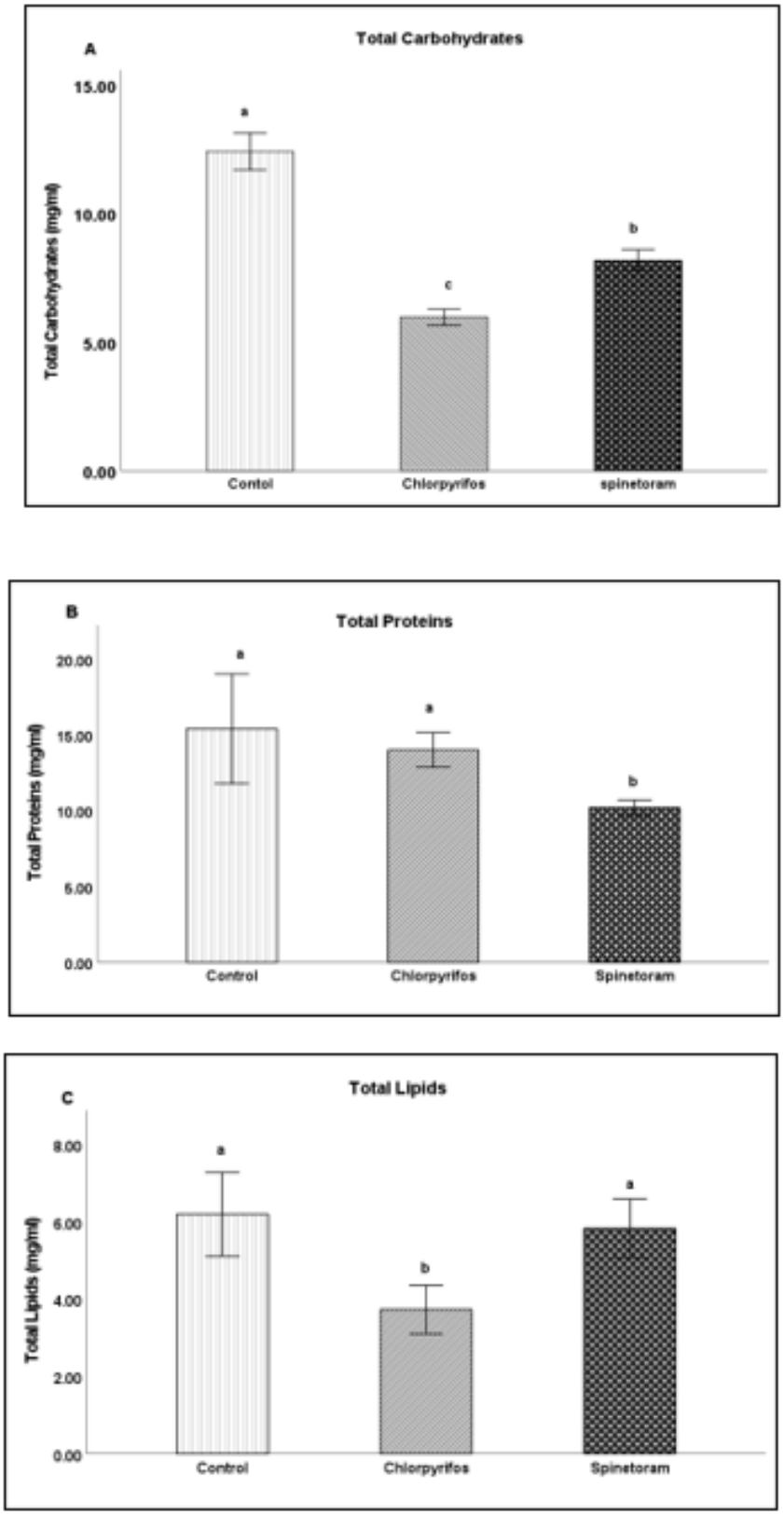

Fig. 3 (A) Total carbohydrates, (B) proteins and (C) lipids contents in the 2nd instar *Spodoptera frugiperda* larvae 24 hours post-treatment with LC_{50} of chlorpyrifos and spinetoram. Means with different letters are significantly different (Tukey's test: $P < 0.05$).

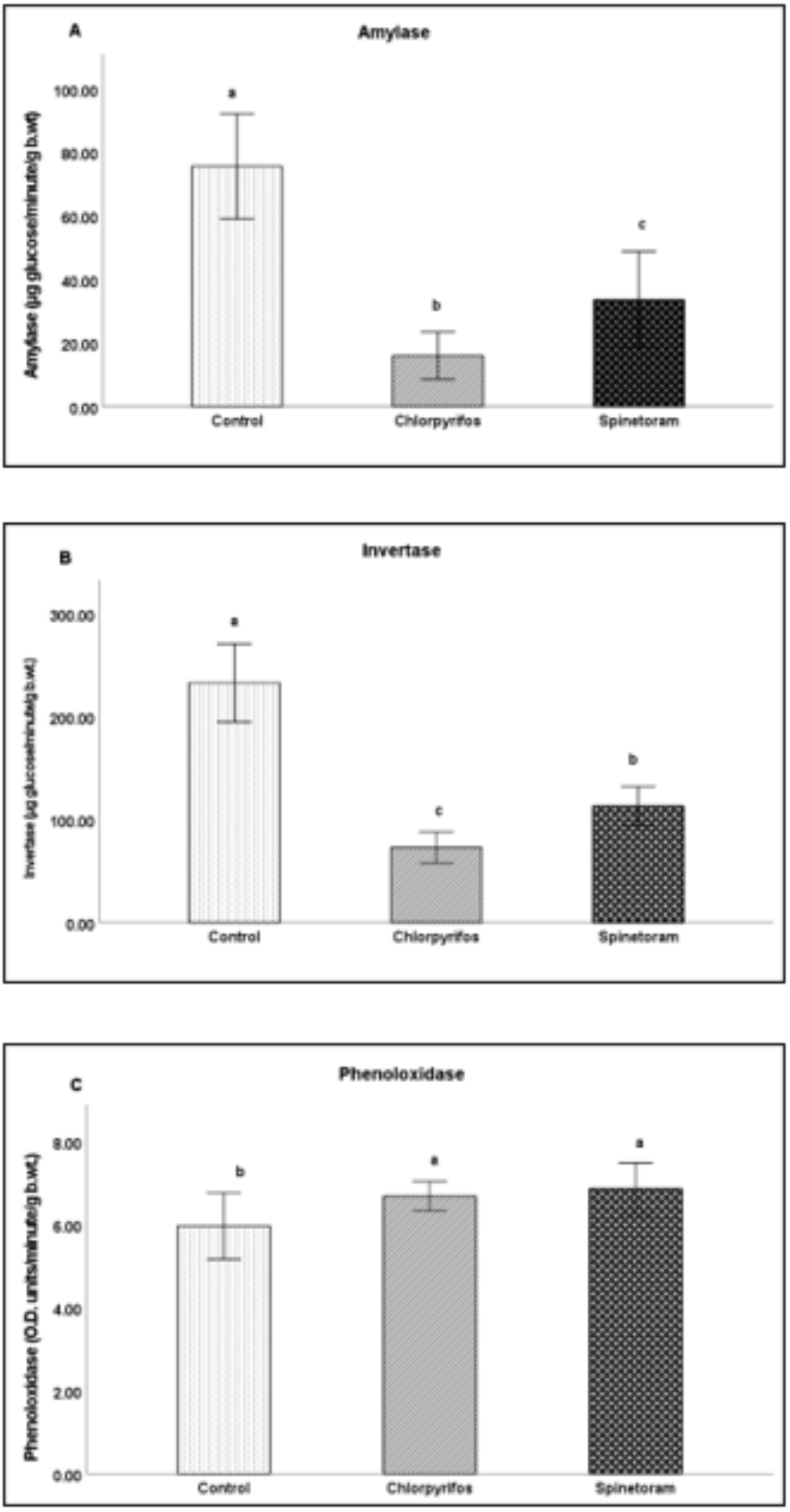

Fig. 4 (A) Amylase, (B) Invertase, and (C) Phenoloxidase activities in the 2nd instar *Spodoptera frugiperda* larvae- 24 hours post-treatment with LC_{50} of chlorpyrifos and spinetoram. Means with different letters are significantly different (Tukey's test: $P < 0.05$).

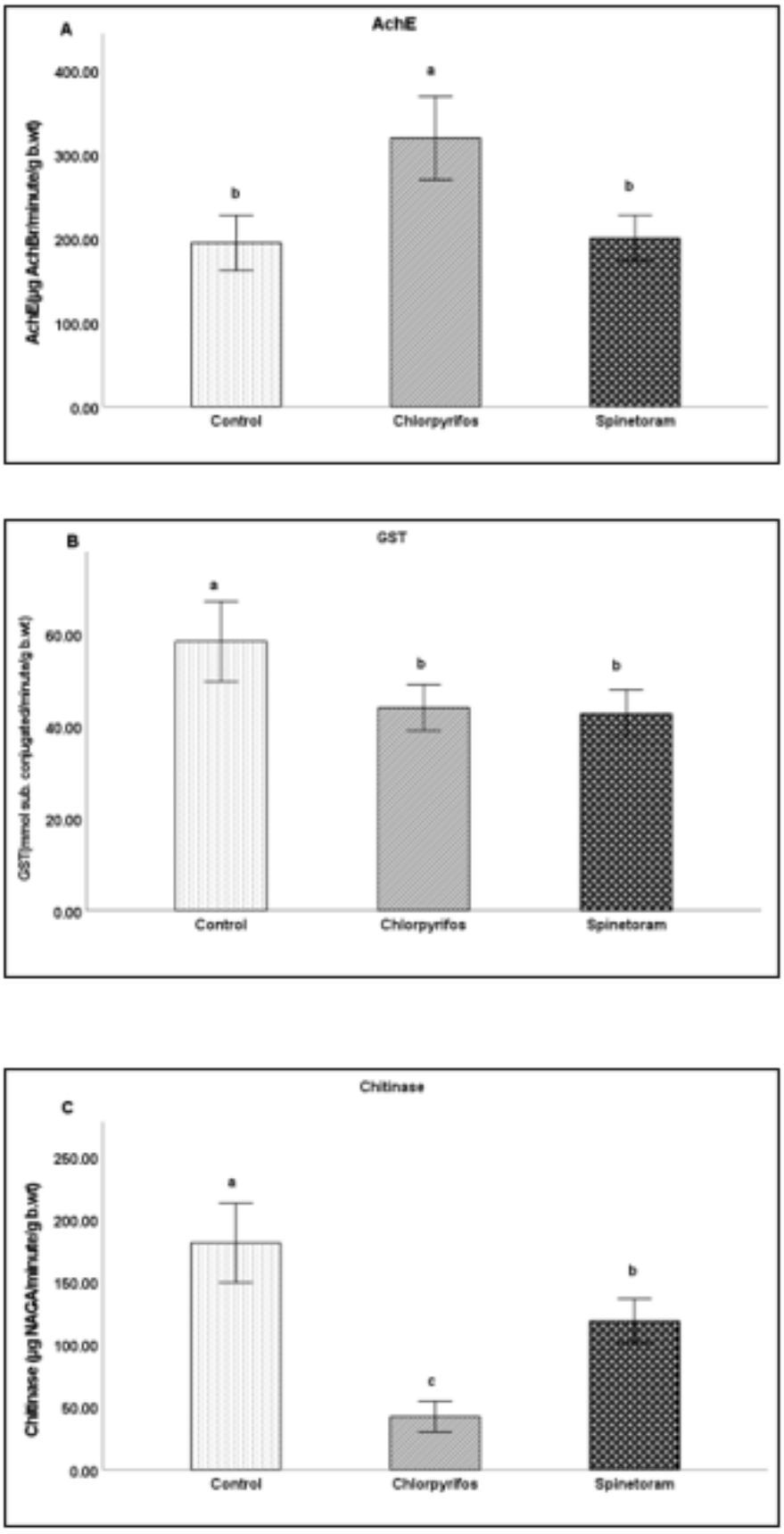

Fig. 5 (A) AchE, (B) GST, and (C) Chitinase activities in the 2nd instar *Spodoptera frugiperda* larvae- 24 hours post-treatment with LC_{50} of chlorpyrifos and spinetoram. Means with different letters are significantly different (Tukey's test: $P < 0.05$).

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 LC_{50} value is lower. The lower the LC_{50} 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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