Egyptian Journal of Zoology (EJZ)
Publisher: The Zoological Society of A. R. Egypt
DOI: 10.21608/ejz.2024.252930.1111

p-ISSN: 1110-6344
e-ISSN: 2682-3160

RESEARCH ARTICLE

COMPARATIVE BIOLOGICAL AND BIOCHEMICAL ASSESSMENT FOR TOXICOLOGICAL PROFILE OF SOME ECO-FRIENDLY INSECTICIDES APPLIED FOR CONTROLLING OF THE FOURTH INSTAR LARVAE OF *SPODOPTERA FRUGIPERDA*

Mohammed Z. Y. Aly; Heba M. Fangary; Hasnaa M. Ibrahim; Sherehan A. R. Salem*

Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

Article History:
Received: 3 December 2023
Accepted: 16 January 2024

Published Online:
19 January 2024

Keywords:
Ecofriendly insecticides
Enzymes
Fourth instar larvae
Insect growth regulator
Spodoptera frugiperda

***Correspondence:**
Sherehan Salem
Zoology Department
Faculty of Science
South Valley University
Qena, Egypt
E-mail:
sherehan.abdelkareem@sci.svu.edu.eg

ABSTRACT

The fall armyworm (*Spodoptera frugiperda*, J. E. Smith) constitutes an urgent threat to maize cultivation in Egypt. The aim of the present investigation was to evaluate the effectiveness of three insecticides: lufenuron (5% emulsifaible concentrates) as an insect growth regulator, and emamectin benzoate (5% soluble granules) and "*Bacillus thuringiensis* subspecies *kurstaki* (*Btk*, 6.4% wettable powder)" as bio-insecticides, against the 4th instar larvae of *S. frugiperda* under laboratory conditions. The larvae were collected from maize fields in Upper Egypt, Qena Governorate, and subsequently reared under laboratory conditions. Through toxicity assays, the concentration-dependent mortality rates were observed for all the three insecticides, where emamectin benzoate displayed the highest toxicity (LC_{50}: 0.0079 ppm), followed by *Btk* (LC_{50}: 1.6857 ppm), and lufenuron (LC_{50}: 3.2155 ppm). The variance in efficacy is attributed to varying in chemical compositions and modes of action. Furthermore, the impact of these insecticides on larval development was investigated. Emamectin benzoate led to prolonged larval development, while lufenuron and *Btk* induced prolonged larval development and postponed pupation. Substantially, *Btk* reduced significantly the carbohydrate level; however, both *Btk* and emamectin benzoate reduced significantly the total protein. Moreover, disparity with significance in the activity of the digestive enzymes activity of amylase and invertase, likewise detoxifying enzymes notably glutathione S-transferase (GST) and acetylcholinesterase (AChE) was apparent in insecticides-treated larvae. The present study ensures that the investigated insecticides are among the recommended pesticides in combating the armyworm.

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*, J. E. Smith) in Egypt has gained lot concerns about the potential impact on agricultural production. The fall armyworm is a destructive pest actually invaded more than 70 countries, posing a serious threat to major crops[1]. Currently, armyworm is the most damaging agricultural pest impacting maize in Egypt and other countries due to

its extensive expansion[2]. Once the fall armyworms entry in Egypt, it was indispensable to prioritize research and develop entire strategies for monitoring, early detection, and effective pest management. Contribution between researchers, farmers, and policy-makers will be crucial in mitigating the potential impact of fall armyworm on Egypt's agricultural sector[1]. Plant growth and development may stop due to a fall armyworm infestation, which may result in failed cob or tassel formation[3]. Loss of photosynthetic area, decreased reproduction, lodging (stem breakage), and structural damage to the maize plant's whorls are among the damage signs brought on by fall armyworm[4]. Insecticides are a class of chemicals that are becoming increasingly for managing crop pests and disease-associated vectors in public health. However, the use of these products is restricted due to the widespread occurrence of resistance to commercially licensed pesticides. Therefore, finding more eco-friendly and effective products is a challenge and a necessity[5,6].

Insect growth regulators could inhibit insect physiological development by inhibiting molting, the formation of new epidermis, and feeding, which lead to the death of pests. Its mode of action differs from that of conventional insecticides that act on the nervous system[7]. Low toxicity, less pollution, and little impact on natural enemies and beneficial organisms support the growth of sustainable agriculture, facilitate the production of environmentally friendly food, and improve human health. Therefore, these are known as “third generation pesticides”, “pesticides of the 21st century”, “bio-regulators”, and “novel materials for insect control[7].

Lufenuron is a member of the class of insect growth regulators known as benzoylureas, which primarily inhibits the synthesis of chitin, preventing insects from molting or pupating and ultimately leading to their death. Stomach toxicity is its primary mode of action, and it works well against a wide range of pests[8]. When present in low quantities, it can decrease insect pupation, eclosion, and egg-laying rates while also lengthening the larvae's developmental period. Additionally, it can directly harm eggs and larvae at high concentrations[8]. Lufenuron confers several advantages over conventional insecticides. These include its minimal toxicity towards non-target organisms and its prolonged residual activity[9].

Emamectin benzoate belongs to the avermectin family, a naturally produced fermentation product of the soil bacterium, *Streptomyces avermitilis*[10,11]. Emamectin benzoate is one of the most widely used biopesticides with strong antipest, antiparasitic, and anti-nematode activities with low toxicity[12]. As a chloride channel activator, emamectin benzoate increases the permeability of membrane chloride ions and disrupts nerve signals in nematodes, arthropods, and platyhelminths, and ultimately causing death[13-15]. One of the significant advantages of emamectin benzoate is its efficacy in combating *S. frugiperda*. It demonstrates low toxicity to non-target species and does not persist in the environment for prolonged periods. However, caution must be considered in applying emamectin benzoate to ensure the effective control of *S. frugiperda*, while minimizing potential adverse effects on the environment[12].

Bacillus thuringiensis (*Bt*), a bacterium discovered by Berliner, which produces toxins during the vegetative and sporulation phases. Specifically, the proteins and Cry toxins produced during the vegetative phase are toxic to insects, mites, nematodes, and protozoans[16-19]. The effectiveness of *Bt* strains against *S. frugiperda* depends on variable factors such as strain type, toxin concentration, and application timing[20]. The *Bt* proteins have low toxicity to non-target organisms, making them environmentally friendly[21].

Significantly, insecticides can affect the activity of metabolic enzymes[22]. The glutathione S-transferases (GSTs) of insects are crucial for the detoxification of toxic compounds and the alleviation of the

oxidative stress that these compounds produce[23]. Earlier studies confirmed that different insecticides can inhibit the detoxification enzymes in various pests and resulted in effective management[24].

The purpose of this study was to determine the effectiveness of three insecticides "lufenuron, emamectin benzoate, and *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*)" in the control of the 4th instar larvae of the fall armyworm (*S. frugiperda*) in order to confirm the findings of our earlier study[25] on the second instar larvae, to ensure the effectiveness of these insecticides on all instars of the pest under study, and to guarantee the most successful control of the insect. The research focused on the following key objectives:

A) Determination of the toxicity of the used insecticides against 4th instar larvae of *S. frugiperda*.
B) Assessment of the impact of the used insecticides on the biological and physiological characteristics of the fall armyworm.

MATERIAL AND METHODS

Insect rearing and tested insecticides

Fall armyworm larvae were collected from maize fields in Qena Governorate, Upper Egypt, and reared at the Faculty of Science, South Valley University, Qena. The insect rearing technique followed established protocols described by Dahi *et al.*[26]. The newly molted 4th instar of *S. frugiperda* larvae were used in this experiment. The fall armyworm larvae were reared for several generations in the laboratory to obtain the laboratory strain. Three insecticides (lufenuron, *Btk*, and emamectin benzoate) were applied for studying their efficacy and potency against the fall armyworm. As detailed in Table "1", the active ingredients, formulation type, and manufacturer for the mentioned insecticides were shown.

Table 1: Formulation type, manufacturer, and empirical formula for the mentioned insecticides.

Common name	Trade name	Manufacturer	Empirical formula
Lufenuron	Match®5% emulsifaible concentrates	Syngenta Crop Protection AG, Basel, Switzerland	$C_{17}H_8Cl_2F_8N_2O_3$
Emamectin benzoate	Proclaim®5% soluble granules	Syngenta Crop Protection AG	$C_{56}H_{81}NO_{15}$ (emamectin B_{1a} benzoate)+$C_{55}H_{79}NO_{15}$ (emamectin B_{1b} benzoate)
Btk	Dipel 6.4% wettable powder	Valent BioSciences, Libertyville, IL, USA	*Btk*

Btk: Bacillus thuringiensis subspecies kurstaki

Measuring the median lethal concentration (LC_{50})

The LC_{50} values of the three tested insecticides were determined as described previously by Aly *et al.*[25] to assess their larvicidal activity on the newly hatched 4th instar larvae of *S. frugiperda*. The following concentrations were used for each insecticide: 10, 8, 6, 5, 4, 3, 2, 1, 0.5, and 0.25 ppm for lufenuron; 0.02, 0.01, 0.005, 0.0025, 0.00125, and 0.000625 ppm for emamectin benzoate; 8, 6, 4, 2, 1.5, 1, and 0.5 ppm for *Btk*. In the bioassay, the leaf dipping technique was utilized as described previously[25].

Biological investigation

The biological impact of the calculated LC_{50} values of the three tested insecticides on *S. frugiperda* was assessed by examining several key parameters[25]. These parameters included: larval development period post-treatment, percentage of larval mortality, pupation rate, duration of the pupal stage, weight of male and female pupae, Sex ratio (♂: ♀), percentage of adult emergence, adult

longevity, fertility (proportion of hatched eggs), and fecundity (the total number of eggs deposited by a female).

Biochemical analysis

Main components (carbohydrates, proteins, and lipids) and enzymes (amylase, invertase, phenol-oxidase, acetylcholinesterase "AChE", glutathione S transferase "GST", and chitinase) were electrophoretically assayed as described previously[25].

Statistical analysis

The multiple range test of Duncan was used to conduct statistical analysis using the statistics package for social sciences (SPSS) application. When $P<0.05$, there was a significant difference in the mean. Values of LC_{90}, LC_{75}, LC_{50}, and LC_{25} obtained by probit analysis using LdP Line[R] software (http://www.ehabsoft.com/ldpline) were graphically represented as described by Finney[27].

RESULTS

Toxicity values on the 4th instar larvae of *S. frugiperda*

The summarized results in Table "2" revealed variations in the effectiveness of the tested insecticides against the 4th instar *S. frugiperda* larvae. The calculated values of LC_{50} were as follows: emamectin benzoate displaying the highest toxicity (LC_{50}: 0.0079 ppm), followed by *Btk* (LC_{50}: 1.6857 ppm), and lufenuron (LC_{50}: 3.2155 ppm). Furthermore, Table "3" displayed the values of the LC_{25}, LC_{75}, and LC_{90} of the tested insecticides on the 4th instar *S. frugiperda* larvae.

Table 2: Toxicity values of lufenuron, emamectin benzoate, and *Bacillus thuringiensis* subspecies *kurstaki* against the 4th instars larvae of *Spodoptera frugiperda*.

	Concentrations (ppm)	Mortality (%)	LC_{50}	Slope
Lufenuron	10	88	3.2155	1.8
	8	80		
	6	73		
	5	60		
	4	53		
	3	38		
	2	30		
	Control	0.0		
Emamectin benzoate	0.02	77	0.0079	1.49
	0.01	53		
	0.005	33		
	2.5×10^{-3}	23		
	1.25×10^{-3}	17		
	0.625×10^{-3}	3		
	Control	0.0		
Bacillus thuringiensis subspecies kurstaki	8	92	1.6857	1.91
	6	82		
	4	74		
	2	64		
	1.5	46		
	1	26		
	0.5	18		
	Control	0.0		

Table 3: Lethal and sublethal concentrations of lufenuron, emamectin benzoate, and *Bacillus thuringiensis* subspecies *kurstaki* against the 4th instars larvae of *Spodoptera frugiperda*..

Treatments	LC_{25}	LC_{75}	LC_{90}	r	Homogeneity LC_{90}/LC_{50} ratio
Lufenuron	1.3548	7.6315	16.6135	0.9687	5.17
Emamectin benzoate	0.0028	0.0223	0.0568	0.9841	7.19
Bacillus thuringiensis subspecies *kurstaki*	0.7460	3.8091	7.9338	0.9829	4.71

Effect of the evaluated insecticides on several of the biological aspects of the 4th instar *S. frugiperda* larval, pupal, and adult stages

The obtained results indicated that the developmental course and mortality rates of the 4th instar *S. frugiperda* larvae displayed that lufenuron, *Btk*, and emamectin benzoate prolonged significantly the larval development. The values of larval duration by lufenuron, *Btk* and emamectin benzoate were 15.72, 14.07 and 11.79 days, respectively, in comparison with the control values (8.78 days, Table 4). However, the obtained percentage of pupation recorded the highest percent of larval pupation in the treatment with lufenuron (53.0%), and the lowest value was induced by emamectin benzoate (48.0%), compared with the control value (98.0%, Table 4). The larval mortality (%) was the highest among emamectin benzoate followed by *Btk* and lufenuron as compared with the control value (Table 4). The current findings ensured that the normal larvae percentages (and the larvae malformation percentages) were 100.0 (0.0), 100.0 (0.0), and 97.0% (3.0%) after the treatment by lufenuron, emamectin benzoate, and *Btk*, respectively, compared with 100.0% (0.0%) for the control larvae (Table 4).

Table 4: Biological parameters of *Spodoptera frugiperda* larvae treated as fourth instar larvae with LC_{50s} of lufenuron, emamectin benzoate and *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*).

	Control	Lufenuron	Emamectin benzoate	*Btk*
Larval duration (days)	8.78±0.13d	15.72±0.17a	11.79±0.48c	14.07±0.41b
Pupation (%)	98.0	53.0	48.0	50.0
Larval mortality (%)	2.0	47.0	52.0	50.0
Normal larvae (%)	100.0	100.0	100.0	97.0
Malformed larvae (%)*****	0.0	0.0	0.0	3.0

The means of the same row followed by different letters are significantly different (P<0.05). *****Malformed larvae appeared with an intermediate shape between larvae and pupae, while other malformed larvae showed incomplete molting of larvae into pupae.

As expressed in Table "5", the LC_{50} doses of lufenuron, emamectin benzoate, and *Btk* prolonged significantly the pupal duration compared with the control group. The pupal duration recorded 28.04, 19.84, and 12.06 days for emamectin benzoate, lufenuron, and *Btk*, respectively, compared with 9.63 days for the control group. The LC_{50} of emamectin benzoate increased the pupal malformation percent (6.7%), followed by lufenuron (3.3%) when compared with the control value (0.0%, Table 5). The pupal

weights of females reduced significantly in lufenuron and emamectin benzoate groups compared with the control group. The pupal weights of males reduced significantly in lufenuron group only compared with the control group. The results found in Table "5" confirmed that effectiveness of the used insecticide against pupal mortality and emergence in the following order: lufenuron followed by emamectin benzoate.

Table 5: Biological parameters of *Spodoptera frugiperda* pupae treated as fourth instar larvae with LC_{50s} of lufenuron, emamectin benzoate and *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*).

	Control	Lufenuron	Emamectin benzoate	*Btk*
Pupal duration (days)	9.63±0.05d	19.84±0.19b	28.04±0.15a	12.06±0.22c
Female Pupal duration (days)	9.50±0.18c	19.94±0.24b	27.08±1.63a	12.13±0.43c
Male Pupal duration (days)	9.75±0.14c	19.75±0.16b	29.00±1.50a	12.00±0.19c
Normal pupae (%)	100.0	96.7	93.3	100.0
Malformed pupae (%)*****	0.0	3.3	6.7	0.0
Pupal weight (g)	0.22±0.00a	0.16±0.01c	0.18±0.01bc	0.20±0.01b
Female Pupal weight (g)	0.25±0.00a	0.16±0.02b	0.18±0.01b	0.22±0.01a
Male Pupal weight (g)	0.20±0.00a	0.16±0.01b	0.17±0.01ab	0.17±0.01ab
Pupal mortality (%)	0.0	12.0	3.0	0.0
Emergence (%)	100.0	88.0	97.0	100.0

The means of the same row followed by different letters are significantly different ($P<0.05$). *****Malformed pupae were observed to have humpbacks, while other deformed pupae had distinct constrictions at the head and thorax**.**

Comparing with the control female and male longevities (9.75 and 10.00 days, respectively), they were significantly extended by lufenuron and emamectin benzoate (Table 6). The emamectin benzoate increased significantly the oviposition period to 9.25 days compared with the control value. The post-oviposition period varied insignificantly after exposure to all tested insecticides compared with control value. The average numbers of eggs (fecundity) was reduced significantly after the treatment with all insecticides (Table 6). The incubation period increased significantly in emamectin benzoate group only (Table 6).

Effects of the evaluated insecticides on several biochemical aspects of the 4th instar *S. frugiperda* larvae

Biochemically, only the 4th instar *S. frugiperda* larvae treated with *Btk* insecticide exhibited a significant decline in the carbohydrates level (9.0 mg/g body weight "b.wt") compared with the control larvae (11.0 mg/g b.wt, Table 7). Regarding protein level, a significant reduction was detected among emamectin benzoate and *Btk* (12.0 and 10.0 mg/g b.wt, respectively, in comparison with the control value (15.0 mg/g b.wt, Table 7). Nevertheless, lipid profile was insignificantly changed in all insecticides as compared with the control value (Table 7).

With respect to digestive enzymes, amylase and invertase enzymes demonstrated significant elevations among emamectin benzoate (92.0 and 235.0 µg glucose/ minute/g b.wt, respectively) contrary to lufenuron (22.0 and 91.0 µg glucose/minute/ g b.wt, respectively), *Btk* (32.0 and 148.0 µg glucose/minute/g b.wt, respectively), and the control level (52.0 and 161.0 µg glucose/

Table 6: Biological parameters of *Spodoptera frugiperda* adult treated as fourth instar larvae with LC_{50s} of lufenuron, emamectin benzoate and *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*).

	Control	Lufenuron	Emamectin benzoate	*Btk*
Sex ratio (%) (♂: ♀)	1:1	0.8:1	1:1	0.9:1
Adult longevity (days)	9.88±0.13c	13.0±0.54ab	13.88±0.31a	11.88±0.38b
Female longevity (days)	9.75±0.25c	13.75±0.25a	13.75±0.25a	12.00±0.58b
Male longevity (days)	10.00±0.00c	12.25±1.03ab	14.0±0.71a	11.75±0.25bc
Pre-oviposition period (days)	2.25±0.25b	3.25±0.25a	3.00±0.41ab	2.25±0.25b
Oviposition period (days)	7.00±0.41b	7.25±0.48b	9.25±0.25a	7.25±0.48b
Post-oviposition period (days)	0.50±0.29b	2.50±0.65b	1.50±0.30ab	1.50±0.50ab
Fecundity (number eggs/female)	1305.8±63.7a	1040.3±65.2b	1066.8±21.6b	1026.5±23.0b
Hatchability (%)	93.0	83.0	76.0	82.0
Incubation period (days)	2.75±0.25a	3.25±0.25a	4.25±0.50b	2.75±0.25a

The means of the same row followed by different letters are significantly different (P<0.05).

minute/g b.wt, respectively, Table 7). The phenoloxidase enzyme was significantly increased and decreased in *S. frugiperda* larvae subjected to lufenuron and *Btk*, respectively, when compared with the control group (7.0 O.D. units/minute/g b.wt). However, the activity of AChE was significantly increased among lufenuron- and emamectin benzoate-treated groups, and increased significantly in *Btk*-treated group, in comparison with the control group (587.0 µg AchBr/minute/g b.wt). The GST activity showed a significant decline in emamectin benzoate- and *Btk*-treated groups (40.7 and 48.0 mmol sub. conjugated/ minute/g b.wt, respectively) as compared with the control group (61.0 mmol sub. conjugated/minute/g b.wt). Eventually, the chitinase enzyme was increased significantly in larvae treated with emamectin benzoate (285.0 N-acetylglucoseamine "NAGA"/ minute/g b.wt) and reduced significantly in larvae treated with *Btk* and lufenuron (78.0 and 123.0 µg NAGA/minute/g b.wt, respectively) when compared with the control group (216.0 µg NAGA/minute/g b.wt).

DISCUSSION

The current study embarked on the toxicological and subsequent physiological repercussions of employing lufenuron (an insect growth regulator), emamectin benzoate and *Btk* (bioinsecticides) against the 4th instar larvae of the *S. frugiperda*. This inquiry aimed to not only assess the toxic potential of these insecticides, but also to comprehend the subsequent consequences they instigate, considering factors such as concentration, chemical composition, and the developmental stage of the treated larvae. The study's findings underscore the varying impacts of these tested insecticides. It is evident that the toxicity of these insecticides based in a concentration-dependent manner, with

Table 7: Main components and activities of enzymes in the 4th instar *S. frugiperda* larvae, post-treatment with LC_{50s} of lufenuron, emamectin benzoate and *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*).

	Control	Lufenuron	Change (%)*	Emamectin benzoate	Change (%)*	*Btk*	Change (%)*
Carbohydrates[1]	11.0 ±0.5a	12.0 ±0.2a	11.0	12.0 ±0.4a	9.0	9.0 ±0.1b	–18.0
Proteins[1]	15.0 ±0.7a	17.0 ±0.4a	13.0	12.0 ±0.2b	–20.0	10.0 ±0.1c	–33.0
Lipid[1]	9.0 ±0.4ab	9.0 ±0.2ab	0.0	10.0 ±0.2a	11.0	8.0 ±0.1b	–11.0
Amylase[2]	52.0 ±2.1b	22.0 ±1.2d	–58.0	92.0 ±2.7a	77.0	32.0 ±2.1c	–38.0
Invertase[2]	161.0 ±4.6b	91.0 ±3.5c	–43.0	235.0 ±7.5a	46.0	148.0 ±3.5b	–8.0
Phenol-oxidase[3]	7.0 ±0.2b	9.0 ±0.2a	27.0	7.0 ±0.6b	0.0	5.8 ±0.1c	–18.0
Acetylcholinesterase[4]	587.0 ±18.8c	1004.0 ±33.6a	71.0	909.0 ±19.6b	55	429.0 ±7.6d	–27
Glutathione S transferase[5]	61.0 ±2.1a	56.0 ±2.2a	–8.0	40.7 ±1.2c	–33.0	48.0 ±1.5b	–21.0
Chitinase[6]	216.0 ±8.7b	123.0 ±3.2c	–43.0	285.0 ±10.7a	24.0	78.0 ±2.5d	–64.0

[1]mg/g body weight, [2]µg glucose/minute/g body weight, [3]optical density units/minute/g body weight, [4]µg acetylcholine bromide/minute/g body weight, [5]mmol sub. conjugated/minute/g body weight, [6]µg N-acetylglucoseamine/minute/g body weight. Means of the same row followed by different letters are significantly different (P<0.05). *Percentage of change $= \frac{\text{Treated - Control}}{\text{Control}} \times 100$

higher concentrations leading to highest mortality rates among the larvae. The LC_{50} concentrations of the evaluated insecticides led to an increase of larval mortality, a decrease in pupation percentage, and larval malformation (in the case of *Btk* only). The consequences of these insecticides extend beyond larval mortality. All treatments led to prolong larval development, reduced pupal weight, extended pupal stage duration, and increase pupal malformation (lufenuron and emamectin benzoate only). In addition, the tested insecticides led to an increase in the death rate of pupae (lufenuron and emamectin benzoate only). The tested insecticides also increased longevity; there was a concurrent decrease in egg deposition and hatchability rates, underscoring potential implications for population growth and pest management strategies. This variance in toxicity can be attributed to the distinct modes of action and target sites of the three insecticides. Lufenuron, acting as a chitin synthesis inhibitor, impedes the formation of the insect's exoskeleton Lv *et al*[28], leading to growth inhibition and eventual death. Liu *et al*.[12] investigated also the susceptibility of *S. frugiperda* to emamectin benzoate; they revealed that the LC_{20} of emamectin benzoate prolonged significantly the pupal period of male, but the oviposition period and the adult fall armyworm's longevity were significantly delayed. These outcomes aligns with and prove our previous study[25] observed alterations in larval growth, developmental timelines, and survival rates of the 2nd instar larvae of *S. frugiperda*

treated with lufenuron, emamectin benzoate, and *Btk*.

The biochemical analyses elucidated that carbohydrate level was decreased after *Btk* exposure, which may be owing to the urgent need of bacteria to glucose as major source for energy for propagation and growth. Thus, bacteria utilize carbohydrates as carbon source for energy and built a new cell; this may decrease the available carbohydrates for treated insect, especially glucose, which plays an important role in energy supply, adult maturation, and builds up a new chitin[29,30]. The decrease in the total protein content either by chlorfluazuron or *Btk* was because of the binding of protein with foreign tested compounds[29]. ElShershaby *et al.*[31] indicated also that *Btk* resulted in a great reduction in protein content of *S. littoralis* larvae, and this toxic effect of *Btk* is responsible for the inhibition of protein synthesis by forming a protein complex. Kamel *et al.*[32] observed a significant reduction in the total protein content of S. *littoralis* larvae after the treatment with *Btk*. This could be due to the breakdown of protein into amino acids, which help to supply energy for the insects.

Amylase and invertase activities tended to increase and decrease among the studied insecticides. The fluctuation in the digestive physiology may attributed to damage and destruction of gut tissues occurred under effect of different types of bioinsecticides[33]. Phenoloxidase was significantly fluctuated in *S. frugiperda* larvae after exposure to insecticides. LC_{25} lufenuron and spinosad caused a significant decrease in phenoloxidase level of *Spodoptera littoralis*[26,34]. Emamectin benzoate, *Btk*, and lufenuron altered significantly chitinase level. Badawy *et al.*[35] also examined the direct and latent effects of lufenuron, methoxyfenozide, and tetlubenzuron (the three commercial insect growth regulators) on the larvae of the susceptible strain of cotton leafworm "*S. littoralis*" in their second and fourth instars. The insecticides varied in their influences on chitinase and polyphenol oxidase activity, and these enzymes could have a relation with their toxicity against *S. littoralis* larvae. The susceptibility of the fourth larval-stage *S. littoralis* to these insecticides evidenced a similar pattern however; the activity was lower than that obtained with the second larval-stage[35]. The AChE increased significantly by lufenuron and emamectin benzoate in the current study. In addition, the instar larvae of *S. littoralis* treated with Dipel 2× bioinsecticides accompanied by a significant elevation in AChE acivity[36]. The treatment with emamectin benzoate and *Btk* lead to a significant decrease in GST. These results were different from that of Hamama and Fergani[37] who observed that 3rd instar *Spodoptera littoralis* larvae exposed to *Bacillus thuringiensis* and emamectin benzoate did not influence GST activity.

In conclusion, this laboratory investigation and our previous study ensure the important extent of selecting the tested insecticides for efficient fall armyworm management. However, it is crucial to acknowledge that while laboratory experiments provide essential insights into pesticide toxicity and effects, the translation of these findings to real-world field conditions warrants careful consideration. Therefore, further investigations conducted under authentic field conditions are imperative to validate and fine-tune laboratory observations.

ETHICS APPROVAL

All the procedures in this study were in compliance with the guidelines of the Animal Ethics Committee of the Faculty of Science, South Valley University, Qena, Egypt (approval no. 021/11/22).

FUNDING SOURCE DISCLOSURE

This research did not receive any funding.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

[1] Wang, J.; Huang, Y.; Huang L, *et al.* (2023). Migration risk of fall army-

worm (*Spodoptera frugiperda*) from North Africa to Southern Europe. Front Plant Sci, 14: 1141470 (DOI: 10.3389/ fpls.2023.1141470).

[2] Bakry, M. M. S. and Abdel-Baky, N. F. (2023). Population density of the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) and its response to some ecological phenomena in maize crop, Egypt. Braz J Biol, 83: e271354 (DOI: 10.1590/ 1519-6984.271354).

[3] Sagar, G. C.; Aastha, B. and Laxman, K. (2020). An introduction of fall armyworm (*Spodoptera frugiperda)* with management strategies: a review paper. Nipp J Environ Sci, 1(4): 1010 (DOI:10.46266/njes.1010).

[4] Anjorin, F. B.; Odeyemi, O. O.; Akinbode, O. A. *et al.* (2022). Fall armyworm (*Spodoptera frugiperda*) (J. E. Smith) (Lepidoptera: Noctuidae) infestation: maize yield depression and physiological basis of tolerance. J Plant Prot Res, 62: 12-21.

[5] 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 agric, 51: 1641902 (DOI: 10.1080/23311932. 2019.1641902).

[6] Rezende-Teixeira, P.; Dusi, R. G.; Jimenez, P. C. *et al.* (2022). What can we learn from commercial insecticides? Efficacy, toxicity, environmental impacts, and future developments. Environ Pollut, 118983 (DOI: 10.1016/j.envpol.2022.118983).

[7] Zuowen, W. (2002). On the application of insect growth regulators in control of agricultural insect pests. Pesticides, 41(4): 6-8.

[8] Sheng, D. R.; He, C.; Lu, Q. *et al.* (2018). Bioactivity and sublethal effect of benzoylurea insecticide against to *Bradysia differmis*. North Hortic, 18: 42-47.

[9] Suman, D. S.; Parashar, B. D. and Prakash, S. (2010). Efficacy of various insect growth regulators on organophosphate resistant immatures of *Culex quiquefasciatus* (Diptera: Culicidae) from different geographical areas of India. J Entomol, 7: 33-43.

[10] Stavrakaki, M.; Ilias, A.; Ioannidis, P. *et al.* (2022). Investigating mechanisms associated with emamectin benzoate resistance in the tomato borer *Tuta Absol*. J Pest Sci, 95: 1163-1177.

[11] White, S. M.; Dunbar, D. M.; Brown, R. *et al.* (1997). Emamectin benzoate: a novel avermectin derivative for control of lepidopterous pests in cotton. The Proceedings of the Beltwide Cotton Conference, National Cotton Council, Memphis, TN, USA, 2: 1078-1082.

[12] Liu, Z.-K.; Li, X.-L.; Tan, X.-F. *et al.* (2022). Sublethal effects of emamectin benzoate on fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Agriculture, 12: 959 (DOI: 10.3390/agriculture12070959).

[13] Wu, X.; Zhang, L.; Yang, C. *et al.* (2016). Detection on emamectin benzoate-induced apoptosis and DNA damage in *Spodoptera frugiperda* Sf-9 cell line. Pestic Biochem Physiol, 126: 6-12.

[14] Jansson, R. K. and Dybas, R. A. (1998). Avermectins: Biochemical Mode of Action, Biological Activity and Agricultural Importance. In: Insecticides with Novel Modes of Action. Mechanisms and Application (Ishaaya, I. and Degheele, D., eds), pp. 152-170. Springer Berlin Heidelberg, Berlin, Germany.

[15] Lu, F.; Guo, K.; Chen, A. *et al.* (2020). Transcriptomic profiling of effects of emamectin benzoate on the pine wood nematode *Bursaphelenchus xylophilus*. Pest Manag Sci, 76(2): 747-757.

[16] Frankenhuyzen, K. V. (2009). Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol, 101: 1-16.

[17] Crickmore, N. (2006). Beyond the spore–past and future developments of *Bacillus thuringiensis* as a bio-pesticide. J Appl Microbiol, 101: 616–619.

[18] Chakroun, M..; Banyuls, N.; Bel, Y. *et al.* (2016). Bacterial vegetative insecticidal proteins (Vip) from entomopathogenic bacteria. Microbiol Mol Biol Rev, 80: 329-350.

[19] Schnepf, E.; Crickmore, N.; Van Rie, J. *et al.* (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev, 62(3): 775-806.

[20] Polanczyk, R. A.; da Silva, R. F. P. and Fiuza, L. M. (2000). Effectiveness of *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Braz J Microbiol, 31: 165-167.

[21] Belousova, M. E.; Malovichko, Y. V.; Shikov, A. E. *et al.* (2021). Dissecting the environmental consequences of *Bacillus thuringiensis* application for natural ecosystems. Toxins (Basel), 13(5): 355 (DOI: 10. 3390/toxins 13050355).

[22] Wei, D.-D.; He, W.; Miao, Z.-Q. *et al.* (2020). Characterization of esterase genes involving malathion detoxification and establishment of an RNA interference method in *Liposcelis bostrychophila*. Front Physiol, 11: 274 (DOI: 10.3389/fphys.2020.00274).

[23] Xu, Z.-B.; Zou, X.-P.; Zhang, N. *et al.* (2015). Detoxification of insecticides, allechemicals and heavy metals by glutathione S-transferase $SlGSTE_1$ in the gut of *Spodoptera litura*. Insect Sci, 22(4): 503-511.

[24] Yu, Y.Y. and Ting, L.C. (2019). Synergistic effect and field control efficacy of the binary mixture of permethrin and chlorpyrifos to brown planthopper (*Nilaparvata lugens*). J Asia Pac Entomol, 22: 67-76.

[25] Aly, M. Z. Y.; Fangary, H. M.; Al-Amgad, Z. *et al.* (2023). Comparative toxicity of the bacterium *Bacillus thuringeinsis* subspecies *kurstaki*, emamectin benzoate, and lufenuron on biological and physiological aspects of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). DOI: 10.21608/ejz.2023.237079.1106.

[26] Dahi, H. F.; Salem, S. A. R.; Gamil, W. E. *et al.* (2020): Heat requirements for the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) as a new invasive pest in Egypt. Egypt Acad J Biolog Sci, A. Entomology, 13(4): 73-85.

[27] Finney, D. J. (1971). Probit Analysis. Cambridge University Press, Cambridge, UK.

[28] Lv, S. L, Xu, Z.-Y.; Li, M.-J. *et al*. (2022). Detection of chitin synthase mutation in lufenuron-resistant *Spodoptera frugiperda* in China. Insects, 13(10): 963 (DOI: 10.3390/insects13100963).

[29] El-Sheikh, T. A. A.; Rafea, H. S.; El-Aasar, A. M. *et al*. (2013). Biochemical studies of *Bacillus Thuringiensis* var. *kurstaki*, *Serratia marcescns* and teflubenzurone on cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptea: Noctuidae). Egypt Acad J Biolog Sci, C. Physiology and Molecular Biology, 5(1): 19-30.

[30] El-Sheikh, T. A. A.; Ismail, S. A.; El-Kawas, H. M. *et al*. (2005). Biological and biochemical effects of *Serratia marcescens* (Eubacteriales: Enterobacteria) as microbial agent and the chitin synthesis inhibitor lufenuron on the cotton leafworm, *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae). Egypt J Agric Res, 2(2): 785-797.

[31] El-Shershaby, M.; Farag, N. A. and Ahmed, A. A. I. (2008). Impact of *Bacillus thuringiensis* on protein content and enzymes activity of *S. littoralis*. Res J Agric & Biol Sci, 4(6): 861-865.

[32] Kamel, A. S.; Abd-EL Aziz, M. F. and El-Bakry, N. M. (2010). Bio-chemical effects of three commercial

formulations of *Bacillus thuringiensis* (Agerin, Dipel 2× and Dipel DF) on *Spodoptera littoralis* larvae. Egypt Acad J Biolog Sci, A. Entomology, 3: 21-29.

[33] Abdel Razik, M. A. A. and Abd El-Raheem, A. M. (2019). Biochemical and histological effects of lambda cyhalothrin, emamectin benzoate and indoxacarb on German cockroach, *Blattella germanica* L. Am J Biochem Mol Biol, 9, 7-16.

[34] Magdy, M., Moawad, F. G., Elsayed, N. E. *et al*. (2019). Biochemical and toxicological studies of some pesticides on cotton leafworm (*Spodoptera littoralis*). Arab Univ J Agric Sci, 27(5): 2489-2499.

[35] Badawy, M. E.; Rabea, E. I. and Nasr, H. M. (2011). Efficacy of three insect growth regulators on growth and development of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.). Alex J Agric Res, 56(1), 109-123.

[36] Lotfy, D. E. and Embaby, E. M. (2020). Field and biochemical studies of bio-and chemical pesticides on the cotton leafworm, *Spodoptera littoralis* (Boisduval) on sugar beet. Egypt Acad J Biolog Sci, F. Toxicology & Pest Control, 12(1), 197-206.

[37] Hamama, H. M.; Hussein, M. A.; Fahmy, A. R. *et al*. (2015). Toxicological and biochemical studies on use of neonicotinoids and bio-insecticides against the Egyptian cotton leaf worm. *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Egypt J Biol Pest Control, 25(3): 525-533.

تقييم بيولوجي وكيميائي حيوي مقارن للخصائص السُمية لبعض المبيدات الحشرية الصديقة للبيئة المُستخدمة في مكافحة يرقات العمر الرابع لدودة الحشد الخريفية (*Spodoptera frugiperda*)

محمد زكى يوسف على،هبة محمد فنجرى، حسناء محمد ابراهيم، شريهان عبدالكريم رفاعى سالم

قسم علم الحيوان، كلية العلوم، جامعة جنوب الوادي، قنا، جمهورية مصر العربية

تشكل دودة الحشد الخريفية (*Spodoptera frugiperda*, J. E. Smith) تهديدًا عاجلًا لزراعة الذرة في مصر. والهدف من هذه الدراسة هو تقييم فعالية ثلاثة مبيدات حشرية هي لوفينورون (5% مركزات قابلة للاستحلاب) كمنظم لنمو الحشرات، وبنزوات الإيمامكتين (5% حبيبات قابلة للذوبان) و "*Bacillus thuringiensis* subspecies *kurstaki* (*Btk*، 6.4%، مسحوق قابل للبلل)" كمبيدات حشرية حيوية، ضد يرقات العمر الرابع لدودة الحشد الخريفية في ظل الظروف المعملية. تم جمع اليرقات من حقول الذرة في صعيد مصر، محافظة قنا، وتم تربيتها بعد ذلك في ظل الظروف المعملية. ومن خلال فحوصات السُمية، تمت ملاحظة معدلات الوفيات المعتمدة على التركيز لجميع المبيدات الحشرية الثلاثة، حيث أظهرت بنزوات الإيمامكتين أعلى سُمية (LC_{50}: 0.0079 ppm)، تليها "*Btk*" (LC_{50}: 1.6857 ppm)، ولوفينورون (LC50: 3.2155 ppm). ويعزى التباين في الفعالية إلى التباين في التركيبات الكيميائية وطُرق العمل. علاوة على ذلك، تم دراسة تأثير هذه المبيدات الحشرية على نمو اليرقات. وأدى بنزوات الإيمامكتين إلى نمو اليرقات في وقت أطول، بينما أدى اللوفينورون و "*Btk*" إلى نمو اليرقات في وقت أطول وتأجيل التشرنق. بشكل كبير، أدى "*Btk*" إلى إنخفاض ملحوظ إحصائيًا في مستوى الكربوهيدرات؛ ومع ذلك، فإن كلًا من بنزوات الإيمامكتين و "*Btk*" خفضًا بشكل ملحوظ إحصائيًا من البروتين الكلي. علاوة على ذلك، فقد ظهر تباين كبير في نشاط الإنزيمات الهاضمة للأميليز والإنفرتيز، وكذلك إنزيمات إزالة السموم خاصة إنزيمات الجلوتاثيون إس ترانسفيراز والأسيتيل كولينستراز في اليرقات المعاملة بالمبيدات الحشرية. وتؤكد الدراسة الحالية أن المبيدات الحشرية المختبرة هي من بين المبيدات الموصى بها في مكافحة دودة الحشد.