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RESEARCH ARTICLE

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)

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ABSTRACT

The fall armyworm "*Spodoptera frugiperda*" is the most notorious insect pest distributed throughout the world's maize-growing areas. *S. frugiperda* has become threatening a sector of agricultural productivity attributed to its highly invasive characteristics. Nowadays, there is a trend to use eco-friendly insecticides for the management of these pests. In the present study, the 2nd instar larvae of *S. frugiperda* were exposed to series concentrations of three insecticides "*Bacillus thuringiensis* subspecies *Kurstaki* (*Btk*, 6.4% wettable powder) and emamectin benzoate (5% soluble granules) as bioinsecticides, and lufenuron (5% emulsifaible concentrates) as an insect growth regulator" to evaluate the median lethal concentration (LC_{50}) values, and to study their impacts on biological and biochemical aspects of insect in a laboratory assay. The results exhibited that LC_{50} values of *Btk*, lufenuron, and emamectin benzoate were 0.2999, 0.2722, and 0.0005 ppm, respectively. Also, exposure of *S. frugiperda* to LC_{50} values of the tested insecticides altered significantly some biological aspects. Larval mortality raised significantly in *S. frugiperda* treated with LC_{50} of all used insecticides compared with the control group. The biochemical response of *S. frugiperda* larvae against the insecticides lowered the mean values of carbohydrates and proteins compared with the control group; besides the digestive enzymes of *S. frugiperda* larvae exhibited significant reduction too. The activity of phenoloxidase, acetylcholinesterase, glutathione S-transferases, and chitinase were significantly varied at the LC_{50} levels of the insecticides. In conclusion, all used insecticides were efficient, and can be recommended to control the fall armyworm, and is a promising alternative to conventional insecticides.

INTRODUCTION

According to scientific-based economic analysis, the rapid extension of agricultural land specialized to maize (*Zea mays L.*) crops was the major source of increase the income northern Laos[1]. However, maize

yields are continuously attacked by pest insects, which are among the serious biotic stresses affecting maize crop[2]. *Spodoptera frugiperda* is a significant migratory agricultural pest, known as fall armyworm[3]. In Asia and Africa, *S. frugiperda* mainly affects maize[4]; where the young larvae damage the crop at the vegetative stage, since they have the ability to uproot young plants and tear or raggedly disfigure foliage[5]. At the filling stage they attach to destroy the grain yield and the core leaves clusters, consequently no corn grains[5]. It also threatens the agricultural productivity of wheat, sorghum, sugar cane, and other harvests[6].

Environmental stewardship and food safety are the most critical standards based on agriculture. In many cases, misapplication of insecticides caused resurgence of pesticide residues in humans and animals, and insect resistance. Concerning their effectiveness, as well as environmental advantages, biopesticides are alternate candidates for conventional insecticidal compounds for pest control[7]. The bacterium *Bacillus thuringiensis* is an emerging solution for chemical insecticidal replacement; it was proven to be a more successful weapon for fighting agricultural pests and it conferred several advantages overcome chemical-based insecticides. *B. thuringiensis* is among the most pathogenic species of bacteria, which cause larval mortality after a course of infection[8]. It has specific entomopathogenic characteristics due to Cry toxins, produced during the sporulation of bacteria[9]. In respect to emamectin benzoate (a second-generation avermectin) is produced through natural fermentation of the soil bacterium *Streptomyces avermitilis*[10]. Emamectin benzoate is highly effective against a variety of insects by interfering with central nervous system function, causing nerve paralysis and eventually killing insects[11]. Among the other promising pesticides, lufenuron (the insect-growth regulators, IGRs) is a chitin synthesis inhibitor induced inability molting or pupation process in insects. Stomach based toxicity is the specific action of lufenuron against a variety of pests[12]. It can extend the developmental period of larvae, and decrease the rate of egg laying and pupation in insects at low concentrations. Whereas, lufenuron can directly destroy eggs and larvae at higher concentrations[12].

The toxicological evaluation of the insecticides is assigned by lethal and sublethal investigations through mortality assessment and monitoring of biology, physiology, behavior, and demographic aspects of insect pests[13]. Surprisingly, a tendency of emamectin benzoate bioinsecticide to manifest high mortality synergistically correlated with biochemical dysfunction even at lower doses[14]. Correspondingly, the major advantages of IGRs are they disrupt the molting and cuticle structure of insect pests, as well as interrupt the endocrine system[15]. IGR compounds play a critical role in insect control, particularly pests in habitant urban areas. Owing to privacy in their mode of action and environmental advantages, these compounds are more appropriate for pest eradication than other synthetic insecticides[16].

The present study aimed to assess the potential response of fall armyworm "*S. frugiperda*" larvae to the median lethal concentration (LC_{50}) of two naturally-based bioinsecticides "*B. thuringiensis* and emamectin benzoate", and one of IGR insecticides "lufenuron" on the biological and biochemical points of view.

MATERIAL AND METHODS

Laboratory strain

The experiment was established on fall armyworm "*S. frugiperda*" larvae gained from the cultivated maize field in Qena Governorate inhabitant Upper Egypt. *S. frugiperda* kept under controlled conditions of 25°C temperature at well-ventilated laboratory room belonging to Faculty of Science, South Valley University, Qena, Egypt. The fall armyworm larvae were reared for several generations under the laboratory conditions to obtain the laboratory strain. Newly molted 2nd instar

of *S. frugiperda* larvae were used in this experiment.

Tested insecticides

Three insecticides were examined for their potency and efficacy on fall armyworm: *B. thuringiensis* subspecies *kurstaki* (*Btk*) strain (6.4% wettable powder, DiPel® Biological Insecticide, Valent BioSciences, Libertyville, IL, USA), emamectin benzoate (Proclaim®5% soluble granules, Syngenta Crop Protection AG, Basel, Switzerland), and lufenuron (Match®5% emulsifaible concentrates, Syngenta Crop Protection AG). The concentrations used for each insecticide were 2.0, 1.5, 1.0, 0.5, 0.25, 0.125, and 0.0625 ppm for *Bacillus thuringeinsis*; 4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625 ppm for lufenuron, and 2.5×10^{-3}, 1.25×10^{-3}, 0.625×10^{-3}, 0.3135×10^{-3}, and 0.1563×10^{-3} ppm for emamectin benzoate.

Bioassay of tested insecticides on *S. frugiperda*

The treatment against 2nd instar larvae of laboratory strain was carried out by feeding technique using immersion in fresh castor for 30 seconds for each concentration. The leaves in the control group were immersed in distilled water. The leaves were left to dry at room temperature before being offered to newly hatched 2nd instar larvae and then placed in a ventilated container. In this bioassay, forty healthy 2nd instar larvae contain four replications (one replicate = 10 larvae/ice cube packs) for each treatment exposed to each of the contaminant leaves. Emamectin benzoate treatments were lasted for 24 hours, while lufenuron and *Btk* treatments were extended to 48 hours. The control comprised similar numbers of larvae, and given castor oil leaves immersed in distilled water. The obtained results were expressive graphically and LC_{50} values were calculated using LdP Line^{R} software (http://www.ehabsoft.com/ldpline).

Biological studies

Fresh castor oil leaves were immersed in the LC_{50} of each insecticide and then left to dry at room temperature before being offered to larvae. Approximately, 50 larvae were comprised in each treatment enclosed by five times replications (10 larvae/ice cube packs). The same number of larvae were considered a control; these larvae were offered castor oil leaves immersed in distilled water. Survived individuals have undergone several biological observations. The following parameters were recorded: larval mortality percentage, larval duration, pupation percentage, pupal weight, male pupal weight, female pupal weight, pupal duration, male pupal duration, female pupal duration, pupal mortality percentage, adult emergence, sex ratio, male and female longevity, fecundity (number of eggs laid per female), and fertility percentage (egg hatchability).

Preparation of samples for physiological analysis

Following the exposure of 2nd instar *S. frugiperda* larvae to LC_{50} of such insecticides, emamectin benzoate treatments lasted for 24 hours, while lufenuron and *Btk* treatments were extended to 48 hours, and insect homogenates by pooled samples from the triplicate determinations were done. Homogenate samples were collected from larvae by homogenizing in normal saline (0.9% sodium chloride solution) and assembled in tubes under ice conditions. For getting supernatant, the collected samples were centrifuged by Allegra V-15R bench-top centrifuge (Beckman Coulter, Brea, CA, USA) at 320 rpm for 5 minutes under cooling to discard the debris. The resultant supernatants were still frozenly preserved until the detection of enzyme activity.

Biochemical assessments

Variable biochemical components were electrophoretically assayed on second instar larvae of *S. frugiperda* comprising carbohydrates, total lipids, total proteins, invertase, amylase, phenoloxidase, chitinase, acetylcholinesterase (AchE), and glutathione S-transferase (GST). Total carbohydrates were estimated in acid extract of sample

by the phenol-sulphuric acid reaction of DuBois *et al*.[17]. The level of total protein was primarily measured through method of the Bradford[18]. The level of total lipids was determined using the demonstrated method expressed by Knight *et al*.[19]. Digestive enzymes activities were determined according to the method described by Ishaaya and Swirski[20]. The phenoloxidase activity was calculated trough using a method modulated by Ishaaya and Casida[21]. For the chitinase enzyme activity assay, preparation of colloidal chitin substrate was done according to Ishaaya and Casida[21]. The activity of AchE was measured using the method expressed by Simpson *et al*.[22]. The GST level was detected by an increase in absorbance against a blank sample as described by Habig *et al*.[23].

Statistical analysis

Statistical analysis was carried out *via* statistical package for social sciences (SPSS) program by the Duncan's multiple range test (DMRT). The results were mentioned in the form of mean ± standard error. The mean was significantly changed when $P<0.05$. The LC_{90}, LC_{75}, LC_{50}, and LC_{25} values were graphically calculated by probit analysis using LdP Line^{R} software Finney[24].

RESULTS

Toxicity of the tested insecticides against the 2nd instar *S*. *frugiperda* larvae

Toxicological profile following the insecticides exposure of the 2nd larval instar of *S. frugiperda* indicated that the LC_{50} values of *Btk*, lufenuron, and emamectin benzoate were 0.2999, 0.2722, and 0.0005 ppm, respectively (Table 1 and Figure 1). Additionally, the LC_{25}, LC_{75}, and LC_{90} values were shown in Table "2".

Effects of the tested insecticides on some biological aspects of *S*. *frugiperda*

The three tested insecticides prolonged significantly the larval stage duration

Table 1: The toxicity of the tested insecticides on the 2nd larval instar of fall armyworm "*S. frugiperda*".

	Concentrations (ppm)	Mortality (%)	LC_{50} (ppm)	Slope
Bacillus thuringiensis subspecies *Kurstaki* (*Btk*)	2	95	0.2999	1.246
	1.5	80		
	1	62		
	0.5	60		
	0.25	45		
	0.125	32		
	0.0625	22.5		
	Control	0.0		
Lufenuron	4	95	0.2722	1.109
	2	82		
	1	70		
	0.5	57		
	0.25	45		
	0.125	37		
	0.0625	27		
	Control	0.0		
Emamectin benzoate	2.5×10^{-3}	77.5	0.5×10^{-3}	1.396
	1.25×10^{-3}	75		
	0.625×10^{-3}	60		
	0.3135×10^{-3}	37		
	0.1563×10^{-3}	20		
	Control	0.0		

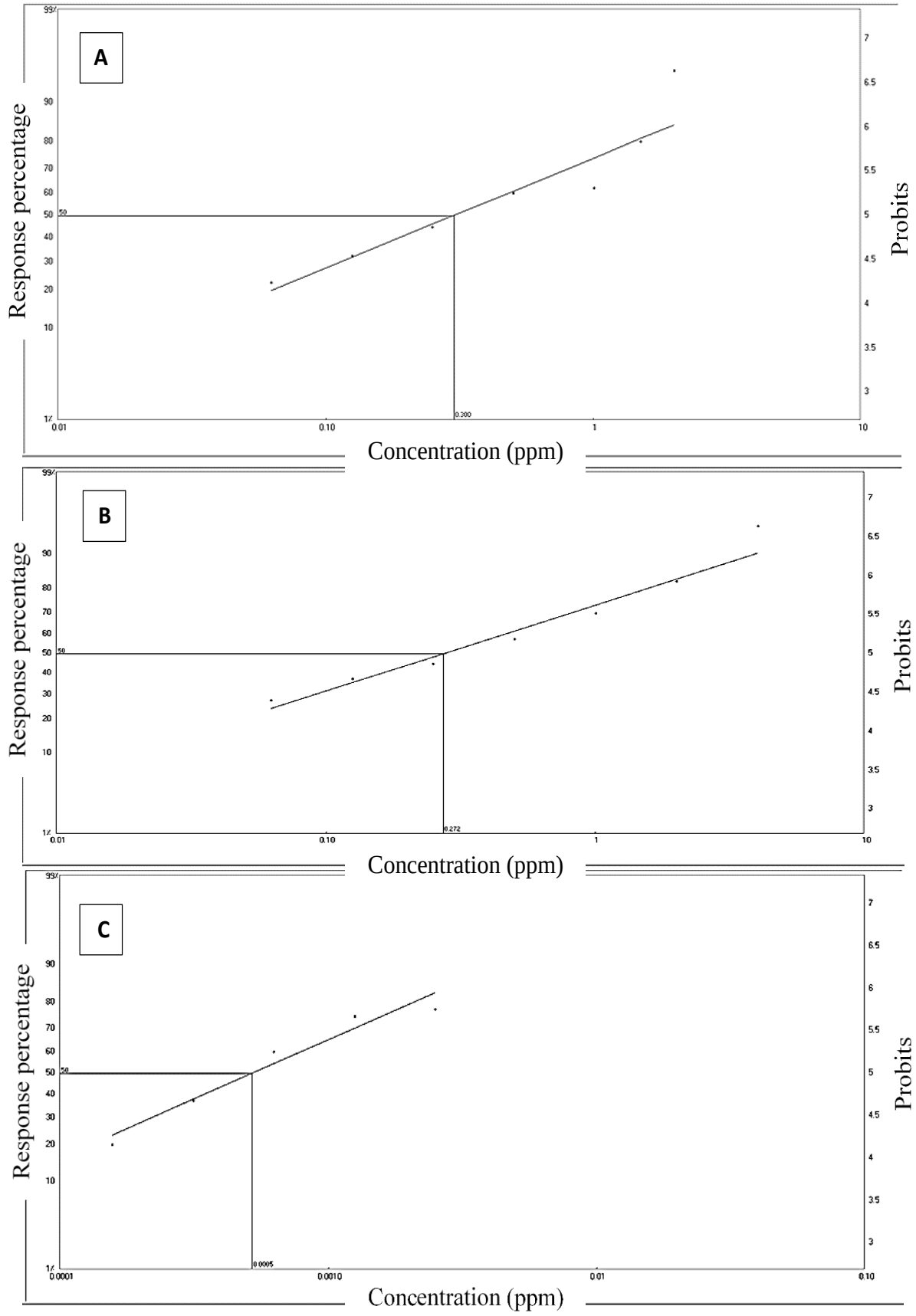

Figure 1: The toxicity LdP Line of the insecticides used on the 2nd larva instar of fall armyworm, *S. frugiperda*. **(A)** *Bacillus thuringiensis* subspecies *Kurstaki*. **(B)** lufenuron. (C) Emamectin benzoate.

Table 2: LC_{25}, LC_{75}, and LC_{90} (ppm), as well as the homogeneity LC_{90}/LC_{50} ratio, of the tested insecticides against 2nd instar *S. frugiperda* larvae.

	LC_{25}	LC_{75}	LC_{90}	r	Homogeneity LC_{90}/LC_{50} ratio
Btk	0.0862	1.0429	3.2025	0.9384	10.68
Lufenuron	0.0671	1.1052	3.9003	0.9790	4.31
Emamectin benzoate	0.0002	0.0016	0.0043	0.9749	8.60

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*

(Table 3). These periods were 16.85, 15.96, and 22.84 days for *Btk*, lufenuron, and emamectin benzoate, respectively, compared with 12.75 days in the control group. The percent of pupation recorded was 53.13 for *Btk* followed by emamectin benzoate 48.44% and lufenuron 46.88%, opposite to 96.88% for the control group (Table 3). Percent of larval mortality was higher in all insecticide groups than in untreated larvae. The percentages of larval mortality recorded 53.12%, 51.56%, and 46.87% for lufenuron, emamectin benzoate, and *Btk*, respectively, compared with 3.12% for the control group. In the same trend, normal larvae percentage manifested 100% for *Btk* and emamectin benzoate, mean-while the percentage of normal larvae was 83.33% for lufenuron in comparison with the control group, which was 96.83%. Lufenuron treat-ment detected the highest percentage of larval malformation (16.6%), while 0.0% of larval malformation was noticed in *Btk* and emamectin benzoate compared with non-treated larvae (3.17%) as shown in Table "3".

Table (3): Effects of LC_{50} concentrations of the tested insecticides on larval stage of *S. frugiperda*.

	Control	*Btk*	Lufenuron	Emamectin benzoate
Larval duration (days)	12.75±0.14d	16.85±0.11b	15.96±0.24c	22.84±0.46a
Pupation (%)	96.88	53.13	46.88	48.44
Larval mortality (%)	3.12	46.87	53.12	51.56
Normal larvae (%)	96.83	100	83.33	100
Malformed larvae (%)	3.17	0	16.6	0

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*. Means have the different letters in the same row are significant (P<0.05).

The tested compounds induced a highly significant rise in pupal duration of fall armyworm from newly treated larvae (Table 4). The *Btk*, lufenuron, and ema-mectin benzoate recorded 14.84, 18.92, and 23.35 days/pupa, respectively, when compared with 8.81 days for control. Normal pupae percentages reached 100% among *Btk* and lufenuron, the same percentage was observed also in the control group (100%), but emamectin benzoate recorded 87.09% in normal pupae. In contrast, malformed pupae in *Btk*, lufenuron, and emamectin benzoate treatments were 0, 0, and 12.90%, respectively, compared with the control group (0%). In addition, the average pupal weight revealed the highest significant decrease in lufenuron (0.12 g) followed by 0.15 g for emamectin benzoate and 0.21 g for *Btk*, while it was 0.23 g

in the control group. The percent of pupal mortality was 3.33% for lufenuron and 0.0% for both *Btk* and emamectin benzoate, as in the control group (0.0%). On contrary, the emergence was 96.67% for lufenuron and 100.0% either for *Btk* or emamectin benzoate, similarly as in the control group (Table 4).

Table (4): Effects of LC_{50} concentrations of the tested insecticides on pupal stage of *S. frugiperda*.

	Control	*Btk*	Lufenuron	Emamectin benzoate
Pupal duration (days)	8.81±0.15d	14.84±0.09c	18.92±0.05b	23.35±0.29a
Female pupal duration (days)	8.69±0.06d	14.44±0.06c	18.84±0.10b	23.19±0.52a
Male pupal duration (days)	8.92±0.29d	15.25±0.18c	19.00±0.13b	23.5±0.10a
Normal pupae (%)	100	100	100	87.09
Malformed pupae (%)	0.0	0.0	0.0	12.90
Pupal weight (g)	0.23±0.01a	0.21±0.00b	0.12±0.00d	0.15±0.01c
Female pupal weight (g)	0.25±0.01a	0.22±0.01b	0.13±0.01c	0.15±0.01c
Male pupal weight (g)	0.20±0.01a	0.19±0.01a	0.12±0.01b	0.15±0.02b
Pupal mortality (%)	0.0	0.0	3.33	0.0
Emergence (%)	100	100	96.67	100

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*. Means have the different letters in the same row are significant (P<0.05).

Table "5" displayed that the average adult longevity was insignificantly changed in all treatments in comparison with control was 11.63, 12.25, and 12.13 days for *Btk*, lufenuron, and emamectin benzoate, respectively, compared with 10.25 days in the control group. The same results were proved in the male adult longevity. Otherwise, the female adult longevity differed significantly in emamectin benzoate and *Btk* (13 and 12 days, respectively) compared with the control group (10 days), while 13.5 days were for lufenuron. All tested insecticides affected insignificantly the pre-oviposition, oviposition, and post-oviposition periods deposited from normal adult females fall armyworm compared with the control group. Data of fecundity termed average numbers of eggs was significantly lowered for all insecticides (997, 1031, and 1061.25 eggs/female for emamectin benzoate, *Btk*, and lufenuron, respectively, opposite to 1277.25 eggs/female for the control group).

Data found in Table "6" showed that the average percent of egg hatchability reached 82.5% in the case of *Btk*, while it reached 92% and 93% with emamectin benzoate and lufenuron, respectively. As for the incubation period, it extended to 3 days for *Btk*, and 2.75 and 2.25 days for treatment by emamectin benzoate and lufenuron, respectively, in comparison with 2.5 days in the control group.

Effect of tested insecticides on some physiological aspects of *S. frugiperda*

The latent impact of treatment of the 2^{nd} instar larvae with the LC_{50} of the tested insecticides on carbohydrates, total proteins, and total lipids is shown in Table "7". Treatment with the tested lufenuron decreased significantly carbohydrates (6.4±0.2 mg/g body weight "b.wt") more than *Btk* and emamectin benzoate (8.7±0.1 and 8.8±0.2 mg/g b.wt, respectively) compared with the control (12.4 mg/g b.wt). The level of total protein

exhibited a significant decrease in all treatments by insecticides (12.2, 11.6, and 12.6 mg/mL for *Btk*, emamectin benzoate, and lufenuron, respectively) as compared with the untreated larvae (15.4 mg/g b.wt). Meanwhile, total lipid level was significantly reduced only by lufenuron (4.5 mg/g b.wt) in comparison with the control group (6.2 mg/g b.wt).

Table (5): Effects of LC_{50} concentrations of the tested insecticides on adult stage of *S. frugiperda*

	Control	*Btk*	Lufenuron	Emamectin benzoate
Sex ratio (%) (♂ : ♀)	1:1	0.89:1	1.07:1	0.72:1
Adult longevity (days)	10.25±0.14a	11.63±0.38a	12.25±0.38a	12.13±0.31a
Female longevity (days)	10.0±0.00c	12.00±0.71b	13.50±0.29a	13.00±0.41ab
Male longevity (days)	10.50±0.29a	11.25±0.48a	11.25±0.75a	11.25±0.25a
Pre-oviposition period (days)	2.25±0.25a	2.50±0.29a	2.25±0.25a	2.25±0.25a
Oviposition period (days)	7.00±0.41a	7.25±0.25a	7.75±0.48a	8.50±0.65a
Post-oviposition period (days)	0.75±0.25b	1.75±0.48ab	2.25±0.63b	2.25±0.48ab
Fecundity (Number of eggs/female)	1277.25±59.98a	1031.00±24.94b	1061.25±15.97b	997.00±6.83b

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*. Means have the different letters in the same row are significant (P<0.05).

Table (6): Effects of LC_{50} concentrations of the tested insecticides on egg stage of *S. frugiperda*.

	Control	*Btk*	lufenuron	Emamectin benzoate
Hatchability (%)	96%	82.5%	93%	92%
Incubation period (days)	2.50±0.29a	3.00± 0.00a	2.25±0.63a	2.75±0.25a

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*. Means have the different letters in the same row are significant (P<0.05).

Table 7: Total carbohydrate, proteins, and lipids content (mg/g body weight) in 2nd instar *S. frugiperda* larvae 24 hours post treatment with LC_{50s} of tested insecticides.

	Control	*Btk*	Change (%)*	Lufenuron	Change (%)	Emamectin benzoate	Change (%)
Carbohydrates	12.4±0.8a	8.7±0.1b	–29.6	6.4±0.2c	–48.6	8.8±0.2b	–29.0
Proteins	15.4±0.2a	12.2±0.2b	–20.9	12.6±0.1b	–18.4	11.6±0.1b	–24.5
Lipid	6.2±0.3a	6.3±0.2a	1.1	4.5±0.1b	–27.9	6.7±0.2a	8.6

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*. Means have the different letters in the same row are significant (P<0.05). *Percentage of change $= \frac{\text{Treated - Control}}{\text{Control}} \times 100$

The data presented in Table "8" illustrated the effect of tested compounds on some digestive enzymes. Accordingly, the data explained that *Btk* and lufenuron led to a significant decrease in amylase enzyme activity (19.0 and 21.0 µg glucose/minute/g b.wt, respectively) followed by emamectin benzoate (29.3 µg glucose/minute/g b.wt) in comparison with the control group (75.7 µg glucose/minute/g b.wt). Whereas, the LC_{50} of lufenuron induced the highest significant reduction in the invertase activity (53.0 µg glucose/minute/g b.wt) followed by *Btk* (103.3 µg glucose/minute/g b.wt) and then emamectin benzoate (126.7 µg glucose/ minute/g b.wt) in comparison with control larvae (233.3 µg glucose/minute/g b.wt).

Table "8" exhibited the effect of three insecticides on the activities of phenol-oxidase, AchE, GST, and chitinase of 2nd instar larvae homogenates. The data expressed that LC_{50} of lufenuron tended to decrease significantly the phenoloxidase enzyme activity (4.6 optical density "O.D." units/minute/g b.wt) more than emamectin benzoate (5.2 O.D. units/minute/g b.wt), despite *Btk* recorded a non-significant decrease (5.7 O.D. units/minute/g b.wt) when compared with the control group (6.0 O.D. units/minute/g b.wt). The mean activity of AchE recorded significant obvious reduction following treatment with *Btk* (85.0 µg AchBr/minute/g b.wt) when compared with the control level (195.0 µg AchBr/minute/g b.wt). Whereas, after exposure to lufenuron insecticide, AchE was significantly increased (420.7 µg AchBr/minute/g b.wt), but emamectin benzoate didn't significantly affect AchE activity (170.7 µg AchBr/minute/g b.wt). GST enzyme exhibited a significant decrease among *Btk* and lufenuron insecticides (48.7 and 50.3 mmol sub. conjugated/minute/g b.wt, respectively) when compared with the control level (58.3 mmol sub. conjugated/minute/g b.wt), with non-significant variation in emamectin benzoate group in the treated larvae (53.7 mmol sub. conjugated/minute/g b.wt). The data in Table "8" confirmed that *Btk* induced more significantly decrease in the chitinase levels (76.7 µg N-acetylglucoseamine "NAGA"/ minute/g b.wt) compared with the control group (181.0 µg NAGA/minute/g b.wt), followed by lufenuron (127.3 µg NAGA/ minute/g b.wt) and emamectin benzoate (161.0 µg NAGA/minute/g b.wt).

Table 8: Enzymes activities in 2nd instar *S. frugiperda* larvae 24 hours post treatment with LC_{50s} of tested insecticides.

	Control	*Btk*	Change (%)*	Lufenuron	Change (%)*	Emamectin benzoate	Change (%)*
Amylase[1]	75.7±3.8a	19.0±2.1c	–74.9	21.0±2.1bc	–72.3	29.3±3.2b	–61.2
Invertase[1]	233.3±8.8a	103.3±2.9c	–55.7	53.0±1.7d	–77.3	126.7±4.4b	–45.7
Phenol-oxidase[2]	6.0±0.2a	5.7±0.1a	–5.0	4.6±0.1c	–23.1	5.2±0.1b	–13.4
AchE[3]	195.0±7.6b	85.0±3.6c	–56.4	420.7±16.4a	115.7	170.7±4.6b	–12.5
GST[4]	58.3±2.0a	48.7±1.9b	–16.6	50.3±2.0b	–13.7	53.7±1.2a	–8.0
Chitinase[5]	181.0±7.4a	76.7±2.6d	–57.6	127.3±3.9c	–29.7	161.0±4.9b	–11.1

Btk: *Bacillus thuringiensis* subspecies *Kurstaki*, AchE: acetylcholinesterase; GST: glutathione S transferase. Means have the different letters in the same row are significant (P<0.05). *Percentage of change $= \frac{\text{Treated - Control}}{\text{Control}} \times 100$. [1]µg glucose/minute/g body weight, [2]optical density units/minute/g body weight, [3]µg acetylcholine bromide/minute/g body weight, [4]mmol sub. conjugated/minute/g body weight, [5]µg N-acetylglucoseamine/minute/g body weight.

DISCUSSION

Investigation on sublethal toxicity of insecticides might be correlated with variations in life history criteria like developmental growth stages of the insects[25], in addition to behavioral and physiological alterations[15]. In context, bioassay findings demonstrated that LC_{50} concentrations of some bioinsecticides led to considerable impacts on the developmental stages and parameters of *S. frugiperda*. The LC_{50} of the tested insecticides increased the larval mortalities, larval malformations (lufenuron only), and pupal durations in *S. frugiperda*. Besides, the reduction in hatchability (especially with *Btk*), fecundity, and pupal weight was detected. Sublethal toxicity with emamectin benzoate, and other microbial pesticides such as *Btk* and chlorfluazuron induced prolongation in the larval stage, decrease in larval weight, increased larvae mortality and malformations[26]. Edomwande *et al*.[27] found also that adult emergence of *Phthorimaea operculella* (Zeller) was < 2%; most of the emerged adults had morphological deformities such as reduced wing size, and they were unable to free themselves from the pupal sacs when treated with lufenuron. Our results were consistent with López *et al*.[28] who discovered that *Helicoverpa zea* treated with sub-lethal dosages of emamectin benzoate lowered significantly the larval survival stage. Also, Salem *et al*.[29] found that the mortality percentage markedly increased when *Spodoptera frugiperda* larvae treated with other insecticides "methomyl, chlorpyrifos, and spinosad". El-Barkey[30] recorded a significant decrease in the adult emergence obtained by treatment of the larval instar of *P. gossypiella* with chlorfluazuron at different concentrations. Also, adults of *P. gossypiella* when exposed to diflubenzuron resulted in a reduction in female and fecundity fertility[31]. Sammour *et al*.[32] also reported that a significant reduction in the hatchability with a number of deposited eggs and a reduction in fecundity might be owing to the toxicity of *S. littoralis* by insecticides residues, which adversely affected the embryonic cuticle synthesis.

Our results explained that the sublethal insecticidal concentrations could disturb the carbohydrates, lipids (lufenuron only), and proteins of the insects; consequently, reducing their contents in the fall armyworm. This result was in accordance with Abd El-Kareem *et al*.[33] who displayed that the treatment of the 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* with the LC_{50} of emamectin benzoate and lufenuron led to a decrease in carbohydrate, total protein, and lipids levels. The reduction in carbohydrates might be because of the increased metabolism to generate extra energy under toxicant stress conditions[34]. In addition, a deficiency in protein level was associated with a compensatory mechanism for lower energy under insecticide stress[35]. The reduction in total lipids may be due to a large amount of the energy consumed in larvae in response to the detoxification process[36].

The results of the digestive enzymes function indicated that the tested insecticides altered significantly the digestive enzymes in fall army worm. Polenogova *et al*.[37] showed that *Btk* and the natural complex of avermectins reduced significantly the activity of digestive enzymes notably amylase activity in the gut of the Colorado potato beetle larvae attributed to the destructive changes provoked in the gut structure. Also, the occurrence of a decrease in amylase activity was found in *Spodoptera littoralis* when exposed to half-lethal doses of *Btk*[38]. Under the effect of lufenuron and emamectin benzoate, phenoloxidase enzyme in the treated larvae was significantly reduced. The phenoloxidase, an oxidative enzyme, can diminish oxygen in insecticides producing (–OH) group, consequently inhibition of the phenoloxidase enzyme activity occurred. Fetoh and Asiry[39] estimated the activity of phenoloxidase toward the 4th instar larvae of *S. littoralis* treated with lufenuron, spinosad, and chlorpyrifos. These insecticides lessened significantly the activity of phenoloxidase enzyme[39].

AchE and GST are the detoxifying enzymes used as contaminant biomarkers for monitoring environmental pollution in insects[40]. At the same time and under the influence of *Btk* on *S. frugiperda*; a significant reduction in the AchE activity was resultant oppositely to a significant increase promoted by lufenuron compared with the control values. Furthermore, it is well illustrated by Abdel Aziz[41] that lufenuron induced the highest increase in AchE activity of the 2nd and 4th *S. littoralis* larvae. The activated AchE enzyme was an immune response resulting from insecticide resistance or tolerance[42].

Chitinase enzyme is a main structural component of chitin containing pathogens like fungi and arthropods, and confers a contributory part of the defense mechanism of the host[43]. Depending on our findings, a significant decrease in the chitinase activity of *S. frugiperda* larvae treated was noticed with LC_{50} of lufenuron, *Btk*, and emamectin benzoate insecticides. According to previous study[39], exposure to insecticides may affect chitinase activity. A similar result was recorded for *E. insulana* larvae treated with LC_{50} of uphold and closer insecticides[44], also a decrease in chitinase activity was observed in *S. littoralis* treated with tebufenozide and lufenuron insecticides[45].

In conclusion the three insecticides exert a unique approach to the control of pest populations; consequently, they induce developmental, and biochemical modifications in the insect. Bioinsecticides are based on the principle that is to bring safety to the environment and human. Finally, our study showed that the bioinsecticides *Btk* and emamectin benzoate, and the IGR insecticide "lufenuron" were efficient and recorded significant changes in the most studied parameters of *S. frugiperda* larvae at the biological and biochemical levels. Therefore, these tested insecticides can be recommended to control the fall armyworm and considered a promising alternative to conventional insecticides.

THE ETHICAL APPROVAL

The experimental procedure concerning this work was conducted and approved by the Institutional Review Board for Animal Experiments of South Valley University according to the ethical guidelines for the animals handling in laboratory experiments of the Faculty of Science, South Valley University, Qena, Egypt (approval number: 021/11/22).

COMPETING INTERESTS

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

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مقارنة سُمية البكتيرة "*Bacillus thuringiensi* تحت نوع *Kurstaki*"، وبنزوات الإمامكتين، واللوفينورون، على الجوانب البيولوجية والفيزيولوجية لدودة الحشد الخريفية "*Spodoptera frugiperda* (Lepidoptera: Noctuidae)"

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دودة الحشد الخريفية "*Spodoptera frugiperda*" هي من أكثر الآفات الحشرية شهرة المنتشرة في جميع مناطق زراعة الذرة في العالم. وأصبحت دودة الحشد الخريفية تهدد قطاعًا من الإنتاجية الزراعية بسبب خصائصها الغازية للغاية. وفي الوقت الحاضر، هناك اتجاه لاستخدام فئات من المبيدات الحشرية الصديقة للبيئة لمكافحة هذه الآفات. في هذه الدراسة، تم تعريض يرقات الطور الثاني لحشرة دودة الحشد الخريفية لسلسلة من التركيزات لثلاثة مبيدات حشرية "*Bacillus thuringiensis* subspecies *Kurstaki* (*Btk*، 6.4% مسحوق قابل للبلل) وبنزوات الإمامكتين (5% حبيبات قابل للذوبان) كمبيدات حشرية حيوية، واللوفينورون (5% مركزات قابلة للاستحلاب) كأحد منظمات النمو الحشرية" لتقييم "LC_{50}" ودراسة تأثيرها على الجوانب البيولوجية والكيميائية الحيوية للحشرة في المعمل. أظهرت النتائج أن قيم "LC_{50}" لكل من "*Btk*"، واللوفينورون، وبنزوات الإمامكتين كانت 0.2999، 0.2722، و 0.0005 جزء في المليون، على التوالي. أيضًا، أدى تعرض دودة الحشد الخريفية إلى "LC_{50}" للمبيدات الحشرية المختبرة إلى تغيير ملحوظ إحصائيًا في بعض الجوانب البيولوجية. وارتفع معدل وفيات اليرقات بشكل ملحوظ إحصائيًا في دودة الحشد الخريفية المعاملة بـجرعة "LC_{50}" لجميع المبيدات الحشرية المستخدمة مقارنة بالمجموعة الضابطة. وأدت الاستجابة البيوكيميائية ليرقات دودة الحشد الخريفية ضد المبيدات الحشرية إلى خفض متوسط قيم الكربوهيدرات والبروتينات مقارنة بالمجموعة الضابطة، بالإضافة إلى أن الإنزيمات الهضمية لليرقات أظهرت انخفاضًا ملحوظًا أيضًا. وتباين نشاط الفينولوكسيديز، والأسيتيل كولينستريز، والجلوتاثيون S-ترانسفيريز والكيتينيز بمقادير ملحوظة إحصائيًا عند مستويات "LC_{50}" للمبيدات الحشرية. نستنتج من ذلك أن جميع المبيدات الحشرية المستخدمة فعالة ويمكن التوصية بها في مكافحة دودة الحشد الخريفية وتعتبر بديلًا واعدًا للمبيدات الحشرية التقليدية.