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The potency of the green algae *Chlorella vulgaris* and *Bacillus thuringiensis* as biocides against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT

Background: Nowadays, the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) FAW, is the most *destructive crop pest invading maize in different countries.

Objective: This study holds significant implications for the field of pest management. It aims to assess the efficacy of crude algal extract of Chlorella vulgaris and Bacillus thuringiensis strain against the 2nd and 4th larval stages of the fall armyworm, a pest currently causing significant damage to maize crops.

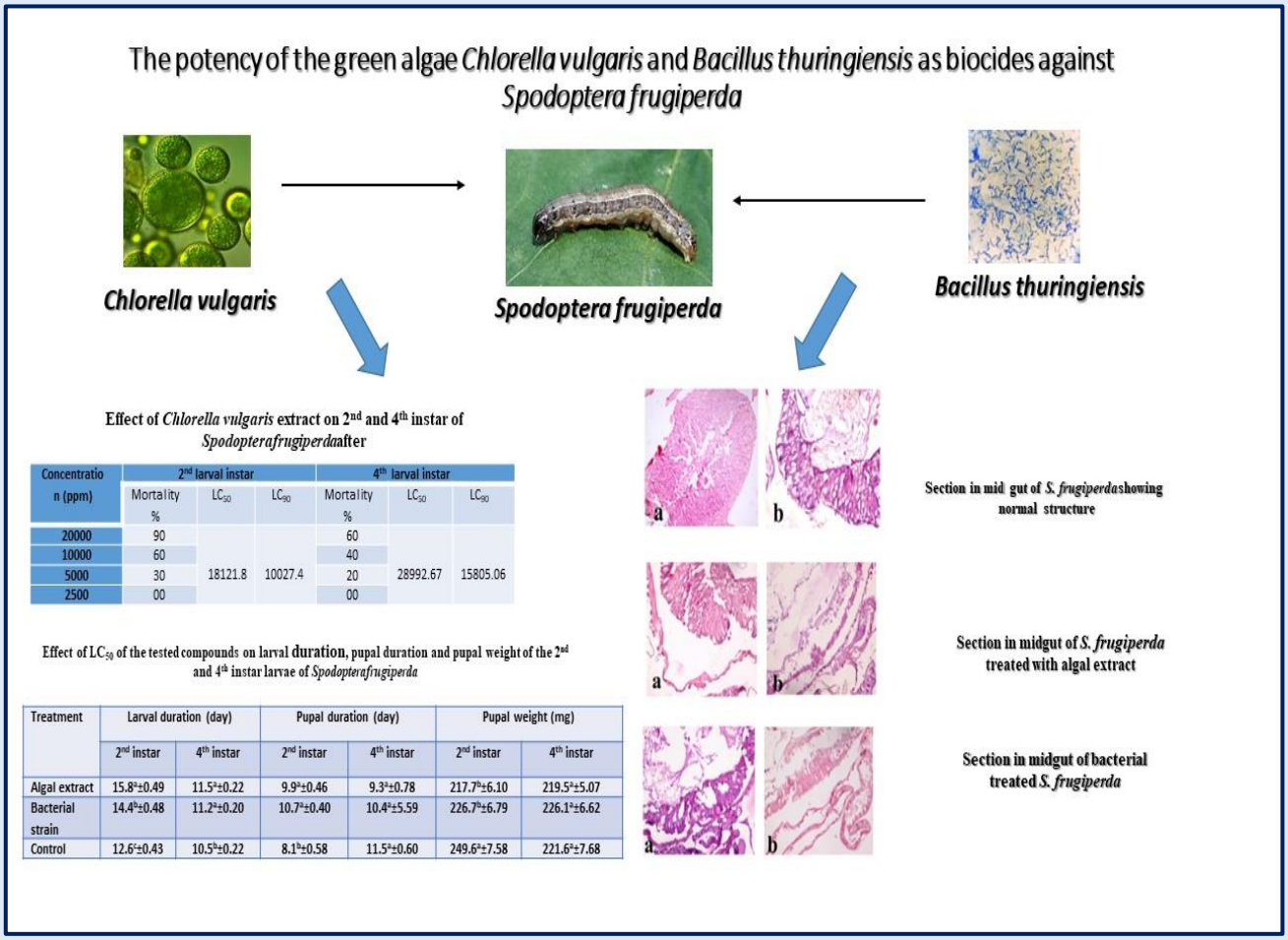
Methods: A rigorous research process was followed, using serial concentrations of bioagents to determine the mortality percent and LC50 values via a feeding technique.

Results: Regarding the effect on the biological aspects, LC_{50} values were fed to the 2nd and 4th larval instar. In addition, histological investigations were checked. The results indicated that the high mortality percents were 90% and 60 % at the concentrations of 20000 ppm of algal extract, recording LC_{50} values of 10027.42 and 15805.06 ppm for the 2nd and 4th larval instar, respectively. Furthermore, bacterial strains induced 100% and 60 % mortality at the concentrations of

1000 ppm with LC_{50} values of 232.50 and 883.46 ppm for the 2nd and 4th larval instar, respectively. Moreover, feeding these compounds caused alterations in the mid-gut tissue and disturbances in the life cycle of the fall armyworm.

Conclusions: The algal extract and the bacterial strain have demonstrated a significant toxic impact on the fall armyworm and could be involved in the integrated management protocol of this pest.

Keywords: *Chlorella vulgaris*, *Bacillus thuringiensis,* fall armyworm, Biological control.



INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is regarded as one of the most injured pests causing economic losses in maize and several other crops of the family Gramineae [1-3]. Chemical pesticides have played a central role in protecting crops from destruction caused by insect pests. The overuse of these pesticides causes disorders in the ecosystem's stability and increases pests' resistance towards them; there is an imperative necessity for safe control methods [4]. Biocides perform promising alternatives to the severe usage of ordinary insecticides for *S. frugiperda* control. In this correlation, *Bacillus*

thuringiensis (Bt) is known as the most broadly used biocontrol agent [5]. Crystalline proteins known as Cry toxins are produced by *Bacillus thuringiensis,* making it a valuable method for pest management [6]. Additionally, Bt formulations have low toxicity to other organisms [7]. Recently, many researchers assessed the efficiency of some algae and cyanobacteria as biopesticides [8-9]. This research aims to study the insecticidal efficacy of crude algal extract of *Chlorella vulgaris* against various larval stages of *Spodoptera frugiperda* (2^{nd} and 4^{th} larval instars) compared with *Bacillus thuringiensis* strain.

MATERIALS AND METHODS

***Spodoptera frugiperda* Rearing:** *S. frugiperda* field strain was collected from various regions of Sids Village, Beba district, and Bani-Suef Governorate. Rearing occurred in the laboratory (27 ± 2 °C, RH 60 ± 5%) [10-11] for the 3 generations. The larvae of the 2^{nd} and 4^{th} instar were chosen for tests in this study and supplied with fresh castor leaves.

Tested compounds 1- *Chlorella vulgaris* Extract

Extraction technique: Soaking 350 dried fine algal powder in n-hexane-isopropanol (in the percent of 3:2 (v/v)) overnight. Then filter under vacuum over silica gel 60, then evaporate using a rotary evaporator at 85°c and wash by solvent under vacuum over silica gel 60. Fine filtration 45°C circulate oven. Then, it is treated with anhydrous sodium sulfate (Na_2SO_4) to remove the accompanying moisture. Re-run over silica gel 60 more than once to obtain the clear yellow oil [12].

2- Protecto: *Bacillus thuringiensis var. kurstaki,* (32000 I.U. /mg) WP were obtained from the Bioinsecticide Production Unit, Plant Protection Research Institute, Agriculture Research Centre, Giza, Egypt

Experimental procedures

Toxicity studies: Lethal concentration tests of *C. vulgaris* and *B. thuringiensis* extracts were evaluated against the 2^{nd} and 4^{th} instar larvae using different concentrations *via* feeding technique on castor leaves. Serial concentrations (2500, 5000, 10000, and 20000 ppm) of algal extract and (31.25, 62.5, 125, 250, 500, 750, 1000, and 1250 ppm) of Bacillus strain were tested with constant factor 2. Castor leaves were immersed in each concentration of each compound, and distilled water was used for the control. Every concentration was used in three replicates. After 48 h of exposure, LC_{50}, $LC_{90,}$ and mortality percentages were estimated.

Biological Aspects Investigation: The 2^{nd} and 4^{th} instar larvae of *S. frugiperda* were exposed to the LC_{50} values of *C. vulgaris* and *B. thuringiensis* for 48 h then the biological aspects were tested. Castor leaves were dipped in the LC_{50} values of every bioagent and then left to dry. Distilled water was used for the control. Three replicates were used (each of 40 identical larvae of either 2^{nd} or 4^{th} instars). After 48 h of feeding, survival larvae were taken and fed with untreated leaves. They were checked for the biological aspects (larval and pupal duration, pupal weight, adult longevity, adult fecundity, and egg hatching percent) [13]**.**

Histopathological studies: According to Banchroft *et al.* (1996) [14] technique**,** the midgut of the treated and control larvae was removed and placed in buffered formalin (10%). Using tap water, the specimens were washed and then dehydrated with alcohol. Tissues were cleared with xylene and then put in paraffin. After that, the blocks were ready, and microtome sectioning at 4-micron thickness occurred. Sections were deparaffinized and stained using hematoxylin and eosin (H&E) stain, which were detected *via* light microscopy.

Statistical analysis: The present data was analyzed by one-way analysis of variance (ANOVA)using the SPSS (version 20) statistical program (SPSS Inc., Chicago, IL,

USA). Differences between the means of different groups were analyzed using Duncan's multiple range and t-test. The lethal concentration values of LC_{50} were calculated by Probit analysis [15].

RESULTS

Toxicity test: Data in Table (1) presented the toxic impact of the *C. vulgaris* extract against the 2nd and 4th larval instars of *S. frugiperda*. The results indicated that mortality percentages were raised progressively with increasing algal extract concentration. Regarding 2nd instar, the extract concentrations were 2500, 5000, 10000, and 20000 ppm, inducing 0, 30, 60, and 90% mortality, respectively. Moreover, the extract concentrations were 2500, 5000, 10000, 15000, and 20000 ppm, achieving 0, 20, 40, and 60% mortality of the 4th instar consecutively. The calculated LC_{50} of extract was 10027.42 ppm for the 2nd and 15805.06 for the 4th instar. On the other hand, the *B. thuringiensis* strain showed larvicidal activity against *S. frugiperda* in a concentration-dependent manner. The highest used concentrations (1000 ppm) caused the mortality rate to reach 100 and 60% for the 2nd and 4th instars, respectively. LC_{50} values were achieved at 232.50 and 883.46 ppm concentrations for the 2nd and 4th instars, respectively (Table 2).

Table 1: Effect of *Chlorella vulgaris* extract on 2nd and 4th instar of *Spodoptera frugiperda* after feeding for 48 h.

Treatment	Larval duration (day)		Pupal duration (day)		Pupal weight (mg)	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
Algal extract	15.8^{a}±0.49	11.5^{a}±0.22	9.9^{a}±0.46	9.3^{a}±0.78	217.7^{b}±6.10	219.5^{a}±5.07
Bacterial strain	14.4^{b}±0.48	11.2^{a}±0.20	10.7^{a}±0.40	10.4^{a}±5.59	226.7^{b}±6.79	226.1^{a}±6.62
Control	12.6^{c}±0.43	10.5^{b}±0.22	8.1^{b}±0.58	11.5^{a}±0.60	249.6^{a}±7.58	221.6^{a}±7.68

Effect on Biological Aspects, Effect on larval duration, and pupal duration, pupal weight

Table 2: Effect of *Bacillus thuringiensis* strain on 2nd and 4th instar of *Spodoptera frugiperda* after feeding for 48 h.

Concentration (ppm)	2nd larval instar			4th larval instar		
	Mortality %	LC_{50}	LC_{90}	Mortality %	LC_{50}	LC_{90}
20000	90	18121.80	10027.42	60	28992.67	15805.06
10000	60			40		
5000	30			20		
2500	00			00		

Table 3: Effect of LC_{50} of the tested compounds on larval duration, pupal duration, and pupal weight of the 2nd and 4th instar larvae of *Spodoptera frugiperda*.

Concentration (ppm)	2nd larval instar			4th larval instar		
	Mortality %	LC_{50}	LC_{90}	Mortality %	LC_{50}	LC_{90}
1250	100	654.40	232.50	60	1741.81	883.46
1000	100			60		
750	90			50		
500	80			40		
250	60			20		
125	40			0		
62.5	30			0		
31.25	20			0		

* Data are expressed as mean ± SE * Means with different letters in column are significant (P<0.05)

Data in Table (3) shows the effect of feeding the 2nd and 4th instars of *S. frugiperda* on LC_{50} of algal extract or bacterial strain. Algal extract feeding significantly ($p<0.05$) increased the larval duration of each of the 2nd and 4th instar larvae (15.8 and 11.5 days, respectively) compared to controls (12.6 and 10.5 days). In the same pattern, bacterial treatment significantly ($p<0.05$) increased the larvae duration. The most potent effect was with algal extract feeding. 2nd instar larvae were more susceptible than the 4th ones with both treatments. Regarding pupal duration, feeding on algal extract or bacterial strain significantly ($p<0.05$) increased pupal duration for the 2nd instar. At the same time, feeding the 4th instar on each tested compound did not change the pupation duration. Regarding pupal weight, feeding of each tested compound significantly ($p<0.05$) decreased the weight of pupae, except feeding of the 4th instar on bacterial strain increased the weight insignificantly ($p>0.05$).

Effect on adult longevity and fecundity: As presented in Table 4, adult longevity significantly decreased with each of the tested compounds' feeding. Regarding adult fecundity, feeding the 2nd instar markedly reduced the number of eggs laid, while feeding the 4th instar to a bacterial strain showed an insignificant decrease ($P>0.05$).

Effect on egg hatching percent: The impacts of the tested compounds are illustrated in Table 4. Feeding of the 2nd instar caused a significant reduction ($p<0.05$) in the percent of egg hatching to 16.67 and 38.80 % compared with controls at 78.48 %. Likewise, the percent of egg hatching decreased significantly ($p<0.05$) after the feeding of the 4th instar on the algal extract while decreasing insignificantly ($p>0.05$) after bacterial treatment.

Table 4: Effect of LC_{50} of the tested compounds on Adult longevity, adult fecundity, and egg hatching percent of the 2nd and 4th instar larvae of *Spodoptera frugiperda.*

Treatment	Adult longevity (day)		Adult fecundity		Egg hatching %	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
Algal extract	4.1^{c}±0.41	4.8^{b}±0.29	60^{c}±20.63	350.0^{b}±34.98	16.67^{c}±1.83	31.00^{c}±3.40
Bacterial strain	6.1^{b}±0.48	6.1^{b}±0.48	450^{b}±21.10	560.0^{a}±17.96	38.80^{b}±5.16	66.37^{b}±4.00
Control	7.8^{a}±0.33	8.3^{a}±0.59	571^{a}±21.76	602.5^{a}±12.51	78.48^{a}±2.09	79.09^{a}±2.37

* Data are expressed as mean ± SE * Means with different letters in column are significant (P<0.05)

Histopathological studies

The control section of the midgut of S. frugiperda shows typical structures of a pseudo-stratified epithelium and a simple column type, which consists of 4 layers. One layer covers the connective tissue membrane, the other two are muscular fibers, and the rest of the layers are epithelial cells located in the basement membrane. This epithelial layer contains three cell types: columnar cells, caliciform cells, and interstitial or regenerative cells. The peritrophic matrix could be seen as a transparent membrane that separates the food from the epithelial cells of the gut. After treatment with the algal extract, several alterations in the midgut were observed, including separation and destruction of the basement membrane, stratified epithelium, and columnar epithelium suffered significant alteration, mainly with the loss of the apical cytoplasm and necrosis along the midgut cells becoming flattened, and the digestive tube appeared empty and thinner. The peritrophic matrix was thickened, degraded, and folded. Also, necrosis and degeneration of epithelial lining malpegian tubules were observed. Moreover, the larvae fed on bacteria showed severe changes in the midgut involving separation and destruction of the basement membrane and severe vacuolar degeneration of the epithelial lining of both midgut and malpegian tubules with degraded and folded of the peritrophic matrix. (Figs. 1-3).

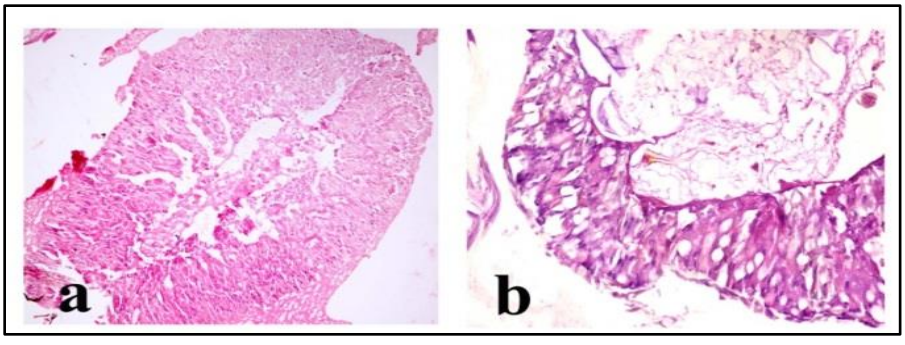


Fig. 1: Section in the midgut of S. frugiperda showing the typical structure of cells: a pseudo-stratified epithelium, simple column type composed of four layers with one layer covering the connective tissue membrane, two layers of muscular fiber, and a single layer of epithelial cells resting on the basement membrane. This epithelial layer is composed of three distinct cell types: columnar, which is the largest cell group; calceiform cells; and interstitial or regenerative cells. Beneath this and separated by the ectoperitrophic space, the peritrophic matrix could be seen as a transparent membrane that separates the food from the epithelial cells of the gut. (a): x 200, (b): x 400

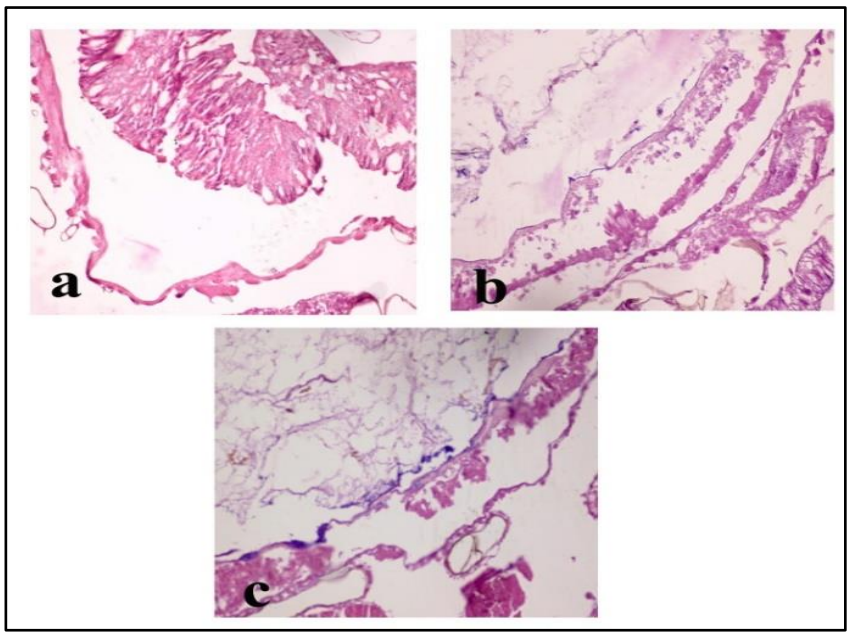


Fig. 2: Section in the midgut of *S. frugiperda* treated with algal extract showing separation and destruction of the basement membrane, stratified epithelium, and columnar epithelium suffered significant alteration mainly with the loss of the apical cytoplasm and necrosis, along the midgut cells become flattened and the digestive tube appeared empty and thinner (a, b, c). The peritrophic matrix was thickened, degraded, and folded. Also, necrosis and degeneration of epithelial lining malpegian tubules were observed (b, c). x 400

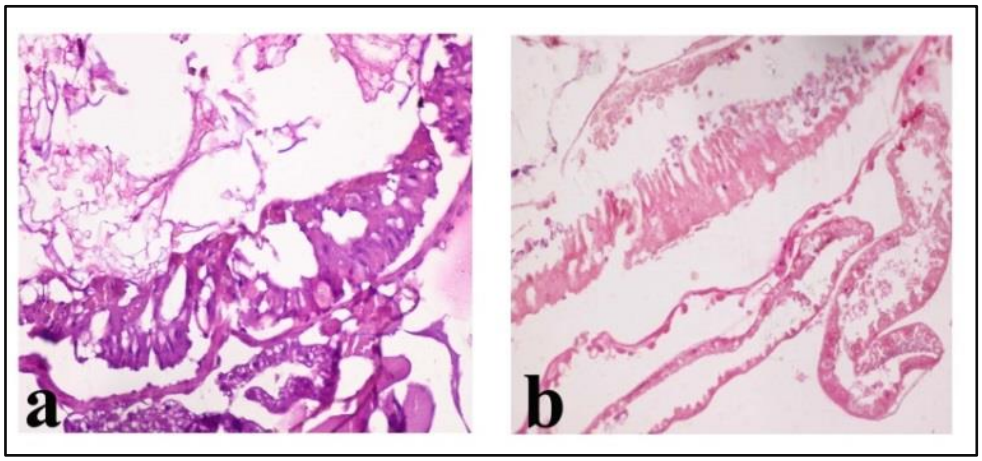


Fig. 3: Section in the midgut of bacterial-treated *S. frugiperda* showing separation and destruction of the basement membrane, vacuolar severe vacuolar degeneration (a), and necrosis (b) of the epithelial lining of both midgut and allegiant tubules with degraded and folded of the peritrophic matrix.x 400

DISCUSSION

In recent years, functional foods have gained significant importance because they are linked to many health benefits, such as protecting against chronic diseases, treating nutrient deficiencies, and promoting healthy growth and development. This study is considered a path to strengthening public health by presenting alternative methods to synthetic pesticides that cause general environmental pollution and harmful effects on human health. Thus producing healthy foods free of toxic contaminants that cause chronic diseases such as kidney and liver failure.

Treatment with *C. vulgaris* extract and *B. thuringiensis* induced toxic impacts on *S. frugiperda* larvae in a dose-dependent manner. This toxic action may be returned to the presence of active constituents in both tested compounds. The fatty acids in the algal extract facilitate the penetration and the accumulation in larvae, as reported by [12 and 16]. So, the larvicidal activity of the algal extract may be ascribed to the fatty acids, which have an insecticidal effect [17-18]. It was stated that *C. vulgaris* can be considered a new origin of a biocide that can efficiently control *Aedesaegypti* larvae; hence, it can inhibit the trypsin-like enzymes that cause the larvicidal mechanism [19]. On the other hand, *B. thuringiensis* toxicity may return to the Cry proteins produced by bacteria, causing the death of larvae. Moreover, *C. vulgaris* extract exhibited higher toxicity and mortality percent against second and fourth larval instars than *B. thuringiensis*. 2nd larval instar was more sensitive in all treatments than fourth instar. The extracts included in this study substantially affected and disordered different life stages of *S. frugiperda,* including adult fecundity, longevity, and egg hatching. Moreover, the second larval instars were more susceptible than the 4th larval instars. The algal extract and *B. thuringiensis* significantly delayed pupae formation prolonged their life durations and reduced their weights and moth emergence. Our results are in line with those of [9]. The toxicant could bind to the epithelium of the midgut, affecting the digestive system, causing larval and pupal duration to rise and suppressing the conversion efficiency of ingested food. This consequently may also decrease pupal weight and the pupation percent [9 and 20]. The histopathological observations in our study can support this illustration of treatments that caused various alterations in the cells of the midgut. Previous studies reported that treatment of the 4th instar of cotton leaf worm with the coccoid green alga *P. kessleri* and the cyanobacterium *N. carneum* caused an increase in the larval duration and a decrease in the adult longevity and fecundity compared with controls [21]. The reduction in adult longevity in this study may result in lowering sugar, carbohydrate, and fat contents in the insect's body, which are very important for adult survival and consequently cause suppression of the production of eggs, as illustrated by [22]. It is also essential to provide agricultural products free of chemical pesticides to serve human health, especially individuals who suffer from diseases caused by an improper nutritional system. It is also essential to provide agricultural products free of chemical pesticides to serve human health, especially individuals who suffer from diseases caused by improper nutrition. Healthy food plays a vital role in managing disease and the symptoms of many chronic diseases. [23-24]. The results of the current research are also consistent with what was mentioned by [25], which recommended the need to grow agricultural products with good specifications to produce health-enhancing and protective foods.

Novelty: This is where the importance of current research becomes clear, as it presents modern and environmentally friendly pest control methods that serve sustainable development goals. Using *Chlorella vulgaris* and *Bacillus thuringiensis* as bioagents proved a toxic action against *Spodoptera frugiperda.* These agents are considered modern, environmentally friendly alternatives that promote public health.

CONCLUSION

Treatment with *Chlorella vulgaris* and *Bacillus thuringiensis* has a toxic impact on *Spodoptera frugiperda* by increasing the larval and pupal duration and affecting the adult longevity and egg-hatching percentage.

Declaration of competing interest: The authors declare that they have no competing interests

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Author contributions: AM and AK designed the experiments, AE and HA explained the results, AE, HA, and AM statistically analyzed the data, and all authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

Conflict of Interest: All authors declare no conflict of interest

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