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Feeding deterrence and larvicidal effects of latex serum and latex-synthesized nanoparticles of *Calotropis procera* against the cotton leafworm, *Spodoptera littoralis*

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Abstract

Background: *Spodoptera littoralis* is an important agricultural pest; thus, knowledge about the effect of latex serum and latex-synthesized nanoparticles of *Calotropis procera* on this species can assist in its management.. Nanotechnology is currently taking root in the agriculture economy as a substitute for pest management that is targeted, safe, and effective. The repellent and antifeedant efficacy of *C. procera* latex serum and its nanoparticles against *S. littoralis* fourth instar larvae was investigated in this work.

Methods: The process of synthesizing silver nanoparticles has been carried out, followed by their subsequent characterization. The objective of this study was to assess the antifeedant properties of the latex serum and its nanoparticles against the 4th instar larvae of *S. littoralis* through the implementation of a bioassay.

Results: With rising latex serum content, the proportion of repellency and hunger rose. In comparison to insects injected with latex serum, utilizing nanoparticles of LAgNPs with this larva via injection resulted in much increased mortality.

Conclusion: According to the findings of this study, insects injected with LAgNPs died at a substantially higher rate than insects injected with latex serum. LAgNPs was efficient against *S. littoralis* larvae and can thus be utilized to specifically control the pest.

Introduction

The cotton leaf worm, *Spodoptera littoralis*, is one of the most serious agricultural pests in the Middle East, tropical and subtropical Africa [1]. It is a very harmful pest of many crops including cotton, alfalfa, peanut, potato, lettuce, celery, pepper and tomato. Consequently, several researchers have looked into the biology of *S. littoralis* and the impact of various host plants on its development and reproduction [2,3]. Several chemical research have been conducted to manage *S. littoralis*, but these synthetic pesticides have resulted in a slew of issues, including pollution, pesticide resistance, and negative impacts on beneficial species like honey bees and birds. As a result, we are applying natural repellents and antifeedants against *S. littoralis* larvae, such as latex from *Calotropis procera*.

C. procera (Ait) (family Asclepiadaceae), also known as Sodom apple, usher, and milk weed, is found in tropical and subtropical Africa, Asia, and America. It is a hardy, pubescent, evergreen, erect, compact shrub that grows to a height of 4.5 meters, with a simple, rarely branching stem that is woody at the base and covered with cracks. When the leaves, stem, and flower of this plant are cut or damaged, they can generate a huge amount of latex [4-6].

The latex of numerus plants contains a variety of physiologically active chemicals [7]. The existence of various types of diterpenes, triterpenes, cardiac glycoside, alkaloids, glucosinolates, tannins, saponins, phenolics, and flavonoids has been linked to biological activity in many situations [8]. *C. procera* latex possesses a variety of biological properties, including antibacterial activity [9], anticancer [10], anti-inflammatory [11-13], anthelmintic [14,15], antidiarrheal [16], analgesic [17], antipyretic [18], insecticidal [19-22], antidiabetic [17], hepato-protective [23], schizonticidal [15,17] and antioxidant activities [6,24]. The latex of this plant includes a number of poisonous chemicals called as cardiac glycosides (CGs), which act as particular inhibitors of Na, K-ATpase, and boost intracellular sodium levels. The increase in cardiac contractility is related to an increase in calcium ions in cardiac myocytes [25,26].

Different latex proteins appear to be involved in insect defense strategies [27]. Latex has also been proved to repel insects [28].

In recent years, the world has shifted swiftly to nanotechnology to give answers in a variety of industries, including cosmetics, agriculture, pharmaceuticals, food, and paint. Nanoparticles have an atomic or molecular size of fewer than 100 nanometers [29]. Nanoparticles have attracted a lot of attention because of their unique and intriguing qualities compared to their rivals in terms of applicability. Plant infections, weeds, and insect pests have all been found to be susceptible to nanoparticles. Insecticides and insect repellents have also included them in their compositions. Fortunately, unlike traditional chemical insecticides, nanoparticles offer no health risks to the environment or the general population [30,31]. However, the exact mechanics of nanoparticles have yet to be fully understood, necessitating in-depth examination or experiments of their interaction with biological systems prior to the development of edible goods.

The purpose of this study is to investigate the potential of utilizing latex serum and latex-synthesized nanoparticles of *C. procera* to manage *S. littoralis*, which is a significant agricultural pest. The study aims to assess the repellent and antifeedant properties of these substances against fourth instar larvae of *S. littoralis*. Additionally, the study contributes to the broader field of nanotechnology in agriculture by exploring the use of nanoparticles as a targeted, safe, and effective means of pest management.

Methods

Insect rearing

The establishment of a stock colony of *S. littoralis* was began using eggs sourced from the Research Division of the Cotton Leafworm at the Plant Protection Research Institute in Assiut, Egypt. Prior to commencing the studies, the larvae were cultivated in the insectaries located within the Zoology Department of the Faculty of Science at Assiut University. This rearing process spanned 10 generations and involved the utilization of castor-bean leaves, which belongs to the Euphorbiaceae family. The adult participants were provided with a sucrose solution of a concentration of 10%. The insects were kept under controlled conditions, with a temperature of 25 ± 2°C, relative humidity of 65 ± 5%, and a photoperiod of 16 hours of light followed by 8 hours of darkness. An oleander branch, namely from the species *Nerium oleander* L. (Apocynaceae), was introduced inside the cage to serve as a site for oviposition. The egg masses were collected on a daily basis and thereafter stored in 90-ml plastic cups at a temperature of 95 degrees till the hatching process occurred.

Sampling and fractionation of crude latex

The latex of *C. procera* plant was obtained by taping method and fractionated into three layers: top layer containing natural rubber; clear middle layer of serum, and the bottom of the lipids [32].

Synthesis and characterization of latex silver nanoparticles (LAg-NPs)

The synthesis and characterization of the synthesized silver nanoparticles has been described before [5].

Antifeeding assay

The antifeeding properties of the latex serum and its nanoparticles (LAg-NPs) was evaluated against the 4th instar larvae of *S. littoralis* using leaf- dip technique. For latex serum, one hundred and eighty larvae were starved overnight, divided into 3 groups each contained 60 larvae, two groups for the treatments one group latex serum (5, 2.5, 1.25, 0.75 ml) and second group for control. Fresh castor bean leaves of almost equal size were dipped in each treatment and control for 20 seconds before being placed in the shade to dry. The dried leaves were placed in plastic cups one by one. For three days, ten larvae were placed in each cup and allowed to feed on the treated and untreated leaves. For each treatment, six duplicates were carried out. For LAg-NPs, a total of 120 larvae were subjected to an overnight period of starvation. These larvae were then separated into three groups, with each group consisting of 40 larvae. Two of these groups were assigned for experimental treatments, while the remaining group served as the control. Two concentrations of LAg-NPs were used. The weight of each larva and leaf was measured both before and after a 24-hour eating period. The quantification of food consumption and larval mass was determined.

The antifeedant index (AFI) was calculated according to [33].

$$\%AFI = [(C-T) / (C + T)] X 100$$

Where: C: the amount of food consumed (leaves) in the control

T: the amount of food consumed (leaves) in the treatment

Starvation percentage assay

This bioassay was designed to check the starvation percentage of the 4th instar larvae of *S. littoralis* fed on latex serum and its nanoparticles using leaf- dip technique. For latex serum, a total of 240 larvae were subjected to an overnight period of starvation. These larvae were then separated into four groups, with each group consisting of 60 larvae. Two of the groups were assigned for experimental treatments, one group served as a control, and the remaining group consisted of starved larvae. Fresh castor bean leaves of equal size were dipped in each treatment and control for 20 seconds before being placed in the shade to dry. Each dried leaf was placed in its own plastic cup. The treated and untreated leaves were fed to ten larvae in each cup for 24 hours, whereas the starved larvae went without food for the same amount of time. For each treatment, six duplicates were carried out. All larvae were weighed before to treatment.

For LAg-NPs, one hundred and sixty larvae were starved overnight and were separated into four groups, each with 40 larvae: two for the treatments, one for control, and one for starvation. The latex nanoparticles were utilized in two concentrations. The larvae were subsequently subjected to reweighing, and the starving percentages of the larvae under investigation were determined using the equation provided by reference [1].

$$\text{Starvation (\%)} = C - E/C - S \text{ X } 100$$

Where:

C = Mean weight gain of untreated larvae after 24 h

E = Mean weight gain of larvae treated with latex serum after 24 h

S = Mean weight gain of untreated starved larvae after 24 h.

Topical and injection application of *S. littoralis* with latex serum or latex nanoparticles

All treatments for each experiment were performed on 4th instar larvae from the same batch. Four batches of ten fourth instar larvae were obtained, each containing 40 individuals. On the dorsal side of the abdomen, the larvae were given a single dosage of latex serum or latex nanoparticles (LAgNPs) (5 percent or 10 percent). In the case of latex serum treatment, control insects were given a single dosage of 5 µl, 10 µl, or 20 µl of silver nitrate, whereas untreated insects were used as controls. Another experiment involved injecting 5 µl or 10 µl of latex serum or LAgNPs (5 percent or 10%) between the second and third abdominal sternites. As a positive control, control larvae were given 5 µl or 10 µl of silver nitrate, whereas untreated larvae were used as a negative control.

Statistical analysis

Percent corrected control was calculated using Abbott's formula [34]. The results were analyzed by ANOVA and Least Significant Difference test (LSD) at $P < 0.05$ using SPSS.

Results

Synthesis and Characterization of latex silver nanoparticles (LAg-NPs)

The latex silver nanoparticles (LAG-NPs) was synthesized.

UV-vis spectral analysis

The synthesis of LAg-NPs was validated by UV-vis spectroscopic investigations of the colored solution, which revealed distinct surface plasmon resonance bands with a peak centered at roughly 400 nm (Fig. 1). Silver ion reduction was indicated by this color.

Repellent and antifeedant activity of latex serum

In laboratory tests, the repellent and antifeedant activity of *C. procera* latex serum was tested against *S. littoralis* 4th instar larvae during a 24-hour period (Tables 1 and 2). At high concentrations, *C. procera* latex

serum had substantial repellent activity ranging from 99.3 to 68.1 %, with starvation percentages of 94.1 and 78.43 %, but at lower concentrations, repellency varied from 8.8 to 37.9%, with starvation percentages of 94.1 and 78.43 % (21.56 & 47.0 %). In the present study, Data in table 2 indicated that the mean % repellency and starvation was increased with increasing in concentration of latex serum (Fig 2).

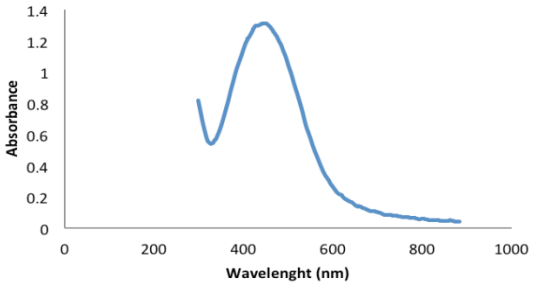

Figure 1: Absorption spectrum solution of silver nanoparticles synthesized by using *C. procera* latex serum after 1 h.

Repellent and antifeedant properties of latex (LAg-NPs)

After 24 hours of feeding on treated castor leaves, the antifeedant activity of the 2 % and 5 % concentrations of LAg-NPs against 4th instar *S. littoralis* larvae is shown in Table 3. Both concentrations had antifeedant characteristics against 4th instar larvae, according to the data. The antifeedant impact of a 5 % concentration was more strong than that of a 2 % concentration, with 5 % having four times the antifeedant activity of 2 %. The starving percentage of the 4th instar larvae treated with the aforementioned concentrations is shown in table (4). The starvation percentage of larvae treated with a 5 % concentration was 17,75 %, whereas it was 9.66 % with a 2 % concentration. As a result, the greater concentration proved to be more effective than the lower one as an antifeedant. (Fig. 3).

Latex serum Conc.(ml)	Antifeedant index (%) Days post-treatments			Mean*
	1st day	2nd day	3rd day	
5	100.0 ± 0.00 a	97.90 ± 0.69 a	100.0 ± 0.00 a	99.3
2.5	76.92 ± 0.89 b	61.42 ± 1.01 b	65.76 ± 1.20 b	68.6
1.25	39.39 ± 0.49 c	30.49 ± 0.86 c	43.75 ± 1.24 c	37.9
0.75	6.350 ± 0.19 d	6.370 ± 0.20 d	13.58 ± 0.73 d	8.80
LSD	0.738	1.075	1.328	
P	0.0000	0.0000	0.0000	
df	3,20	3,20	3,20	

Table 1: Antifeedant activity of latex serum against 4th instar larvae of *S. littoralis* fed on castor leaves treated with (*C. procera)* different latex concentrations. Data are expressed as mean ± SE (n= 6). * General mean of each treatment at different time intervals. Data are statistically analyzed by one-way ANOVA. Means within each column followed by different letters are significantly different based on LSD (P < 0.05).

The effects of topical application of latex serum or its nanoparticles (LAgNPs)

Topical application of different concentrations (5 µl, 10 µl or 20 µl) of latex serum or latex synthesized nanoparticles (LAgNPs) (5 % or 10%) on 4th instar larvae of *S. littoralis* had no significant effect in mortality in comparison to control insects.

Treatments Conc.	Average weight at zero time (mg/larva)	Average weight after 24 h (mg/larva)	Difference* (mg/larva)	Starvation (%)
5	43	43	0	94.1%
2.5	46	54	+ 8	78.43%
1.25	33	57	+ 24	47.0%
0.75	40	77	+ 37	21.56%
Control larvae	43	91	+ 48	------
Starved larvae	42.0	39.0	- 3	------

*Average weight after 24 h – Average weight at zero time.

Table 2: Starvation percentage (%) of the 4th larval instar of *S. littoralis* as affected by *C. procera* latex serum.

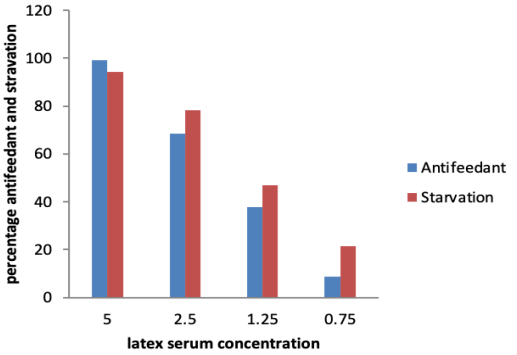

Figure 2. Antifeedant and starvation percentage (%) activity of *C. procera* latex serum against 4th instar larvae of *S. littoralis* fed on castor leaves treated with different latex concentrations.

Latex nanoparticles Conc.(per mg)	Antifeedant index (%) Days post-treatments			Mean*
	1st day	2nd day	3rd day	
2	2.920 ± 0.19 b	5.190 ± 0.38 b	9.832 ± 0.41 b	6.00
5	28.50 ± 0.87 a	28.50 ± 1.24 a	27.33 ± 0.73 a	28.5
LSD	0.894	1.306	0.845	
P	0.0000	0.0000	0.0000	
df	1,6	1,6	1,6	

Table 3: Antifeedant activity of LAg-NPs (*C. procera)* against 4th instar larvae of *S. littoralis* fed on castor leaves treated with different concentrations. Data are expressed as mean ± SE (n= 4). * General mean of each treatment at different time intervals. Data are statistically analyzed by one-way ANOVA. Means within each column followed by different letters are significantly different based on LSD (P < 0.05).

Treatments Conc.	Average weight at zero time (mg/larva)	Average weight after 24h (mg/larva)	Difference* (mg/larva)	Starvation (%)
2 %	54.33	86.26	+ 31.93	9.66 %
5 %	47	75.52	+ 28.52	17.75 %
Control larvae	54	90	+ 36	------
Starved larvae	40.13	34	- 6.13	------

*Average weight after 24h – Average weight at zero time.

Table 4: Starvation percentage (%) of the 4th larval instar of *S. littoralis* as affected by LAg-NPs (*C. procera*).

The effects of injection of latex serum or its nanoparticles (LAgNPs)

The efficacy of *C. procera* latex serum and LAgNPs produced from this latex serum against *S. littoralis* 4th

instar larvae were tested. Figure (4) shows the findings of a serum larvicidal bioassay, while Figure 5 A and B shows the results of latex-synthesized AgNPs. A single dosage of 5 µl of latex serum injected into ^{4}th instar larvae was unsuccessful (P = 0.267), but a single dose of 10 µl of latex serum injected into ^{4}th instar larvae resulted in a minor influence (P = 0.077) in mortality percentage on day 2 following treatment when compared to control insects (Fig. 4). Furthermore, on day 3 following treatment, there was a significant difference (P = 0.002) in mortality percentage when compared to control insects (Fig. 4).

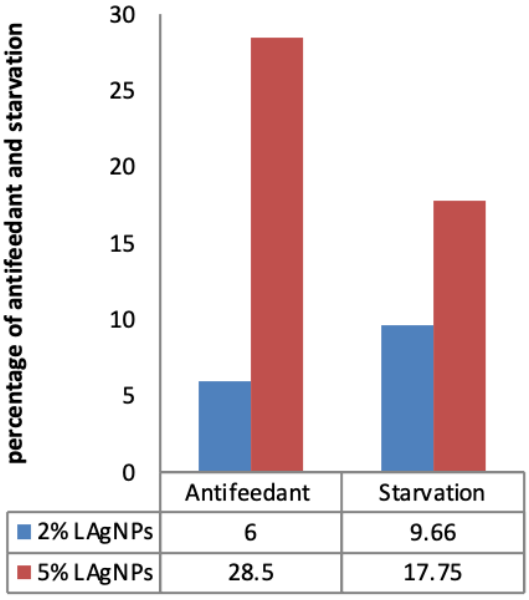

Figure 3: Antifeedant and starvation percentage (%) activity of *C. procera* latex nanoparticles against 4th instar larvae of *S. littoralis* fed on castor leaves treated with different concentrations.

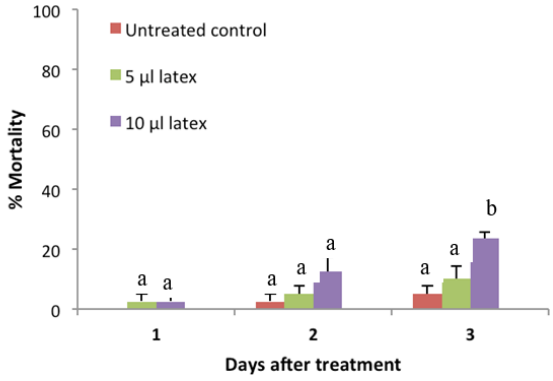

Figure 4: Mortality percentage (mean ± SE) of *S. littoralis* larvae caused by different doses of latex serum. Bars with different letters were significantly different ($P < 0.05$) by LSD after ANOVA.

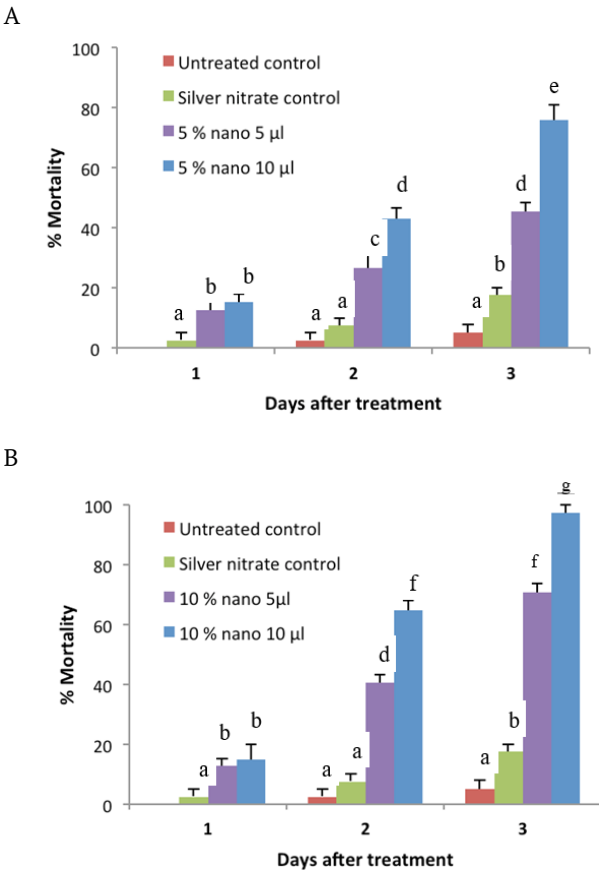

Figure 5: Mortality percentage (mean ± SE) of *S. littoralis* larvae caused by different doses of 5 % LAgNPs (A) and 10 % LAgNPs (B). Bars with different letters were significantly different ($P < 0.05$) by LSD after ANOVA.

Injection of a single dosage of 5% (5 µl, or 10 µl) or 10% (5 µl, or 10 µl) of LAgNPs, on the other hand, had a significant influence on mortality when compared to controls (Fig. 5 A and B). In general, the mortality rate has risen steadily over time. With rising LAgNPs concentrations, the percentage mortality rose, showing a direct link between dosage and percent mortality (Fig. 5 A and B). Furthermore, on days 1 and 2, there was no statistically significant difference observed in the percentage of mortality between the untreated control (negative control) and the silver nitrate-treated control (positive control), but there was a significant difference (P = 0.009) on day 3 of treatment (Fig. 5 A and B).

Discussion

Insects injected with LAgNPs had much greater mortality than insects injected with latex serum, according to the findings of this study. This might be due to the larvae's improved protection system against injected latex serum due to more efficient detoxifying. This, on the other hand, is supported by the way AgNPs behave as larvicidal agents. The high surface area-to-

volume ratio of AgNPs confers a wide range of biocidal and catalytic properties. In latex-mediated nanosynthesis, encapsulating latex metabolites on the surface of AgNPs improves their larvicidal activity in addition to providing stability [35].

C. procera is a well-known medicinal plant, with several physiologically active chemicals in its latex [5] used Transmission Electron Microscopy (TEM) analysis, laser diffraction particle size analyzer, X-ray diffraction (XRD) analysis, and FTIR spectrophotometer to characterize latex nanoparticles.

The repellent and antifeedant activity of *C. procera* latex serum has been recorded in *C. procera* latex with high concentrations of active compounds such as Terpenes, phenolic compounds, and cardiac glycoside, and these compounds play a role in cotton leaf worm repellent and antifeedant [6].

Plants have a multitude of compounds that each have their own biological action. *C. procera* latex was discovered to contain a variety of chemicals, including cardenolides, proteolytic enzymes, alkaloids, and carbohydrates, according to many studies [35].

C. procera latex has also been found to work as repellents and oviposition deterrents by a number of studies [36]. Moreover, [37] showed that the latex of *C. procera* possessed high repellent activity against larvae of *Culex quinquefasciatus*.

In compared to control insects, topical administration of latex serum or its nanoparticles (LAgNPs) showed no significant influence on mortality. This might be due to improved peripheral latex or LAgNP protection mechanisms, such as more effective metabolic detoxification, faster excretion rates, and reduced integument penetration rates. In contrast to the current findings, *C. procera* latex had insecticidal action against *Musca domestica* ^{3}rd instar larvae at a topically dosage of 3 µl (5 %of the latex) [35].

In general, the 4th instar larvae of *S. littoralis* showed a greater sensitivity to LAgNP injection than to latex serum injection. In this respect, [38] reported that latex materials from different plants (*Euphorbia milii, E. hirta, Ficus racemosa* and *Jatropha curcas*) were less toxic than the synthesized AgNPs to the 2nd and 4th instar larvae of *Aedes aegypti* and *Anopheles stephensi*. In addition, [39,40] reported larvicidal activity of silver nanoparticles synthesized by *Pergularia daemia* latex against *Aedes aegypti* and *Anopheles stephensi*.

The latex serum utilized in this investigation had significant levels of terpenes, phenolic compounds, and cardiac glycoside, as previously reported [6], so a preliminarily conclusion could be addressed that there is an interaction between these compounds in AgNPs formation. Furthermore, *C. procera* Latex serum includes "cardiac glycoside," one of the most bioactive substances, which predominantly affects the cardiovascular, neurologic, and gastrointestinal systems [41].

Cellular depolarization and the loss of the negative membrane potential essential for proper cell activity would result if these ion gradients were lost. More research on the mechanism of silver nanoparticles' larvicidal effect is needed. Additionally, more studies should evaluate nanoparticles that are naturally occurring and easily available for IPM.

Food security concerns may benefit from nanotechnology, such as targeted pesticide delivery and control of pestiferous insects that destroy crops and their products in the field. The current study demonstrated that the insects injected with LAgNPs died at a significantly greater rate than insects injected with latex serum, according to the findings of this study. LAgNPs were effective against *S. littoralis* larvae and can thus be used to control the pest specifically.

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Author Contributions

Amer Tawfik: contributed to conceptualization, Nadia Mohamed and Ahmed Ali: contributed to methodology, Ahmed Ali and Mady Ismail: contributed to statistical analysis writing, Amer Tawfik and Ahmed Ali: contributed to writing, review and editing, Wael. M. Abdel-Mageed and Ahmed Shoreit: contributed to project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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