Toxicity of *Zingiber officinale* Nanoparticles Against the Spiny Bollworm, *Earias insulana* and Their Effects on Some Biological and Histological Aspects

Rania M. El-Shennawy
Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.
Email of the corresponding author: raniashennawy2017@yahoo.com

Abstract

El-Shennawy, R.M. 2024. Toxicity of *Zingiber officinale* Nanoparticles Against the Spiny Bollworm, *Earias insulana* and Their Effects on Some Biological and Histological Aspects. Arab Journal of Plant Protection, 42(2): 202-207.
https://doi.org/10.22268/AJPP-001237

The toxicity of Ginger extract nano-sized particles (Ginger AgNPs) against the spiny bollworm (*Earias insulana*) larvae, and its adverse effects on some biological and histological aspects were studied under controlled conditions of 26±1°C and 75±5% RH. LC_{50} treatment of newly hatched larvae produced larval and pupal mortality rates of 60.00 and 47.06%, respectively, compared to the control treatment (6.33 and 2.00%). Treatment resulted in significant larval and pupal deformity rates (11.00 and 13.51%, respectively) compared to the control (1 and 3%). Ginger AgNPs greatly reduced the adult emergence rate (52.94 %) with a high rate of malformation (11.11%) compared to 89.0 and 0.0 % for the control, respectively. Treatment sharply affected females' fecundity (69.67%) with an acute decrease in oviposition periods and hatchability rate (66.67%) in comparison to the untreated control. The LC_{50} of Ginger AgNPs resulted in different histological alternations in the cuticle and midgut compared to the normal structure of the control.

Keywords: Ginger, nanoparticles, spiny bollworm, biological changes, histological changes.

Introduction

One of the most severe pests affecting several crops in Egypt is the spiny bollworm (SBW), *Earias insulana* (Lepidoptera: Nolidae). It attacks a few Tiliaceae species and the majority of Malvaceae species. It damages cotton bolls causing a significant reduction in the amount and quality of cotton yield, (Kandil *et al.,* 2013). Biopesticides originating from plant extracts are considered valid alternative control method to traditional synthetic insecticides. These plant extracts have many bioactive ingredients with insecticidal effect (El-Bokl, 2016). Ginger, *Zingiber officinale* (L.) Rosc has been used for many years as a vital component in various alternative control methods (Ahmed *et al*., 1984; Bartley & Jacobs, 2000; El-Sayed *et al*., 2013). Nanotechnology is currently a rapidly growing science, and nanoparticles are widely used in various agricultural applications (Nagarajan, 2008). Green synthesized nanoparticles are one of the foremost effective and environment friendly biopesticides (Benelli, 2016; Mousavi & Rezaei, 2011). The objective of this study is to determine the toxicological effect of Ginger AgNPs in treating spiny bollworm 1st instar larvae and to study the effect Ginger AgNPs on some biological and histological pest characteristics.

Materials and Methods

Preparation of Ginger leaves extract

Fresh Ginger leaves were collected in September 2022 from the Agricultural Research Center El-Quanater El-Khairya, Kaliobia, Egypt, and identified by the Horticulture Department, Faculty of Agriculture, Menofia University. Plant samples were washed, air-dried, and ground into a fine powder (Kulkarni *et al*., 2012). The powder was stored at 4ºC for further use. A sample of 50 grams of Ginger powders was soaked in 300 ml of distilled water and the solution was then kept in a shaker incubator at room temperature. 24 hrs later, the prepared solution was filtered with Whatman filter paper No.1. A rotary evaporator apparatus was used to remove the solvent from the filtrate. The dried plant crude extracts were kept in a refrigerator for further use (Dorman *et al*., 2003).

Biosynthesis of silver nanoparticles

Silver nanoparticles were prepared by mixing silver nitrate ($AgNo_3$), distilled water, and an aqueous extract of Ginger leaves. Silver nanoparticles were prepared from aqueous silver nitrate using a simple green route and Ginger leaves extract as a reducing and capping agent. Silver nitrate (0.034 g) was added to 200 ml of double distilled water to make 1 ml silver nitrate solution. 200 ml of $AgNO_3$ solution was put in a 500 ml beaker, then, 100 ml of Ginger extract solution was dropped from the burette and heated at room temperature for 24 hrs using a hot plate with a magnetic stirrer (1000 rpm). As soon as the extract solution was added, a dark brown precipitate was formed in the solution. Enough precipitate was obtained after the addition of 100 ml solution. After the solution reached room temperature it was separated by centrifugation at 8000 rpm. Finally, the pellet was dried in an oven at 70 °C as a black-colored material (30 mg), which was transformed into powder by using a mortar and a pestle and used for characterization.

Characterization of silver nanoparticles

UV-Visible spectrophotometry - The periodic scans of the optical absorbance between 300 and 700 nm with a double-beam UV-visible spectrophotometer (Carry 100 with tungsten halogen light sources) were performed to

https://doi.org/10.22268/AJPP-001237

investigate the reduction of silver ions by the aqueous extract. The biosynthesized Ag NPs solution was collected at room temperature, after various time intervals (15, 30, 45, 60 and 90 min.). UV-visible spectra were obtained using a Lambda 25 UV-visible spectrometer, Perkin Elmer, Inc.

Transmission electron microscopy (TEM) - TEM analysis was performed at the National Research Center (NRC), Giza, Egypt, using JOEL JEM-1230 electron microscope equipped with tungsten source and operating at 120kv. TEM analysis was done using Philips (Technai 10). A small droplet of the sample solution was placed on a carbon-coated copper grid, excess solution was removed by blotting paper, and the film on the TEM grid was allowed to dry in an incubator. The first instar larvae of *E. insulana* used in this experiment were obtained from a susceptible strain established in the Bollworms Research Department, Plant Protection Research Institute, reared on an artificial diet for several generations without any exposure to insecticides' contamination under constant conditions of 26±1°C and 75±5% RH) as described by Amer (2015).

Toxicological evaluation of tested compounds - Serial aqueous concentrations of Ginger AgNPs were freshly prepared (80, 40, 20, 10, 5 and 2.5%) to estimate LC values. 1 ml of the tested concentration was spread evenly on the surface of five grams of artificial diet poured into a Petri-dish (5 cm in diameter). The control dishes were sprayed only with water (untreated diet). Three replicates were prepared for each concentration as well as the control. One hour after drying, thirty newly hatched larvae of the spiny bollworm were transferred to the surface of the treated and untreated diet. Following a day of feeding, live larvae were transferred individually to glass tubes (2×7 cm), and allowed to feed on untreated diet where they were incubated under constant conditions of 26±1°C and 75±5% RH. After Three days after incubation, larval mortality rate was recorded. The LC_{90}, $LC_{50,}$ and LC_{25} values were calculated according to Finney (1971).

Biological studies

E. insulana newly hatched larvae were treated with LC_{50} of Ginger AgNPs as per the method above. Three replicates, each of 50 larvae, were used for treatment and the same was used for untreated control. After one day of feeding on the treated diet, surviving larvae were transferred individually into glass tubes (2×7 cm) with untreated food, where they were kept under the same conditions until pupation. Sex of spiny bollworm newly emerged moths was identified, placed into rearing cages (five pairs/cage), and allowed to feed on a 10% sugar solution. Three replicates of rearing cages were prepared for treated as well as untreated controls, and several biological characteristics were observed.

Histological studies

Autopsy samples of SBW full-grown larvae were fixed in 10% formal saline for 24 hrs. After washing with tap water, serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for sample dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24 hrs. Paraffin beeswax tissue blocks were prepared for sectioning at 4 microns thickness by sled microtome. The tissue sections obtained were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin-stain for examination through the light electron microscope (Banchroft *et al*., 1996).

Statistical analysis

The data was analyzed using Costat program, COHORT software (CoStat, 2005).

Results

Ginger nanoparticles (AgNPs) composition

Due to the excitation of surface plasmon vibrations in nano-silver, a brown color appearance suggested the successive production of AgNPs. A quick interaction occurred on blending the solution of silver nitrate and aqueous extract as proved by the immediate color alteration that demonstrates the occurrence of a redox reaction, whereby Ag+ ions were reduced to Ag0 by the extract components, which are oxidized to different species (Halawani, 2017).

Ultraviolet-visible (UV-visible) spectrophotometric characterization of AgNPs

Since AgNPs have a strong absorption peak as a result of the surface plasmon excitation, which represents the collective excitation of conductive electrons in a metal, UV visible spectra were sensitive to the production of silver colloids. The characteristic absorption peak of AgNPs in the UV visible spectra around 300 nm was generated due to the surface plasmon resonance (SPR) of Ag-NPs (Liz-Marzan, 2006) (Figure 1).

Transmission Electron Microscopy (TEM)

Based on the TEM image obtained, the prepared AgNPs are shown to be sphere-shaped and well dissolved in the polymer matrix with a mean particle size of 10.77 nm found in several arbitrarily chosen areas in enlarged microphotographs (Figure 2).

Toxicity of Ginger AgNPs on *E. insulana*

The susceptibility of *E. insulana* larvae to Ginger AgNPs is shown in Table 1. The corresponding LC_{25}, LC_{50} and LC_{90} values were 8.85, 20.48, and 100.87%, respectively. The slope value was 1.851±0.548.

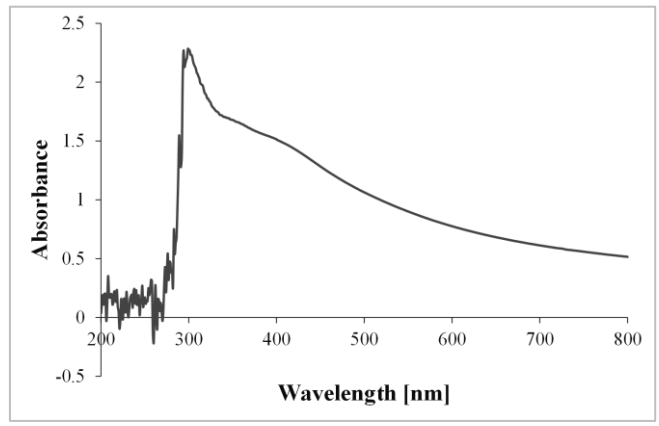

Figure 1. UV-Visible spectrum of the prepared AgNPs from Ginger aqueous extract.

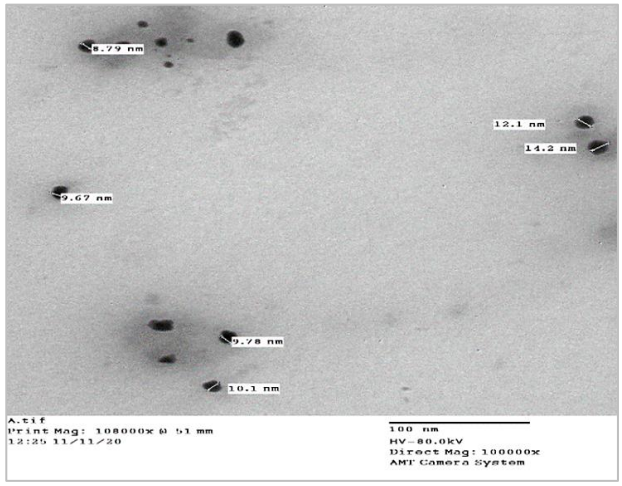

Figure 2. Transmission electron microscopic image showing particles' size of prepared Ginger AgNPs.

Table 1. Toxicity of Ginger AgNPs against newly hatched larvae of *E. insulana*.

Concentration (%)	Mortality rate (%)	Lethal concentrations			Slope
		LC_{25}	LC_{50}	LC_{90}	
80	93.33	8.85 (1.74-15.05)	20.48 (10.33-31.89)	100.87 (54.89-734.61)	1.851+/-0.548
40	93.33				
20	93.33				
10	93.33				

Effect of Ginger AgNPs on some biological aspects of *E. insulana*

Immature stages - Ginger AgNPs treatments resulted in accumulative larval and pupal mortality rates of 60.0 and 47.06%, respectively, compared to 6.33 and 2.00 % for the untreated control, respectively (Table 2). However, no significant difference was detected for both larval and pupal durations in response to Ginger AgNPs treatment, as compared to the control. The average larval and pupal durations were 14.33 and 9.00 days for the treated compared to 15.27 and 7.60 days for the untreated control, respectively.

In addition, Ginger AgNPs treatment insignificantly reduced larval and pupal weight to 0.0267 and 0.0243 g, respectively, for treated individuals. Whereas, the weight of untreated insects were 0.0443 and 0.0307 g, respectively (Table 2). Additionally, the treatment produced some deformed larvae, pupae, and adult stages (Figure 3).

Adult stage - The effect of treating newly hatched larvae of *E. insulana* with Ginger AgNPs was extended to the emerged adults, (Table 3). The percentage of adult emergence was reduced to 52.94% compared to 89% for the untreated control.

In addition, a marked adults malformation rate (11.11%) was recorded compared to no malformation in the control adults. Ginger AgNPs treatment intensely reduced female fecundity (Table 3). The mean number of deposited eggs/female was significantly decreased to 69.67 eggs/female in treated females compared to 249.33 eggs/female in the untreated control. Besides, treatment significantly elongated female pre- and post-oviposition periods compared with the untreated control, and the oviposition period was reduced. Consequently, noticeable reductions in females and males longevity in response to treatment. Furthermore, the hatching rate was greatly reduced to 66.67% in response to treatment compared to 95.94 % in the untreated control (Table 3).

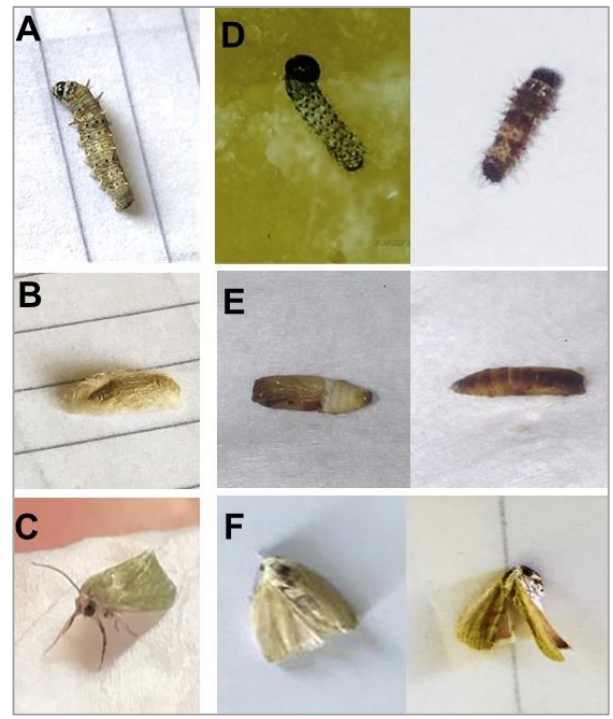

Figure 3. (A) *Earias insulana* normal larva, (B) normal pupa, (C) normal adult, (D) malformed small dwarfed larvae with darkened colored bodies and reduced or absent spins, (E) malformed stripper and trampled pupae, (F) malformed adults.

Table 2. Effect of Ginger AgNPs on some biological aspects of *E. insulana* immature (larval and pupal) stages.

	Larval stage				Pupal stage			
LC_{50} treatment	Mortality rate (%)	Malformation rate (%)	Duration (days)	Weight (g)	Mortality rate (%)	Malformation rate (%)	Duration (days)	Weight (g)
Ginger AgNPs	60.00 a	11.00 a	14.33	0.0267	47.06 a	13.51 a	9.00	0.0243
Control	6.33 b	1.00 b	15.27	0.0443	2.00 b	3.00 b	7.60	0.0307
$LSD_{0.05}$	11.75	7.30	-	-	8.60	6.45	-	-
p	0.0000	0.0032	ns	ns	0.0000	0.0006	ns	ns

Values followed by the same letters in the same column are not significantly different at P=0.05.

Table 3. Effect of Ginger AgNPs on the *E. insulana* adult stage.

Treatment (LC_{50})	Emergence rate (%)	Malformation rate (%)	Female longevity (days)				Male longevity (days) ±SE	Fecundity		
			Pre-Oviposition	Oviposition	Post-oviposition	Total longevity		Total eggs/female	No. eggs daily/ female	Hatching rate (%)
Ginger AgNPs	52.94 b	11.11 a	5.00 a	4.33 b	5.67 a	15.00 b	7.00 b	69.67 b	16.24 a	66.67 b
Control	89.00 a	0.00 b	2.77 b	13.5 a	1.9 b	18.17 a	15.30 a	249.33 a	18.33 a	95.94 a

Values followed by the same letters in the same column are not significantly different at P=0.05.

Histological Studies

Different histological alternations were observed after treating newly hatched larvae of *E. insulana* with Ginger AgNPs at the level of LC_{50}. In the untreated larvae, the integument displays a normal cellular structure of epidermis, buds, and spines (Figure 4-A). Moreover, insect fore- and mid-guts were of normal glandular and cellular structures with no histological modification (Figure 4-B, 4-C, 4-D).

Furthermore, different histological alternations were detected in larval integument and gut when newly hatched larvae of *E. insulana* were treated with the tested Ginger AgNPs. The cuticle was compressed with loss of spines and buds. In addition, treated larva showed degeneration and necrobiosis of the gut epithelial cells (Figure 5).

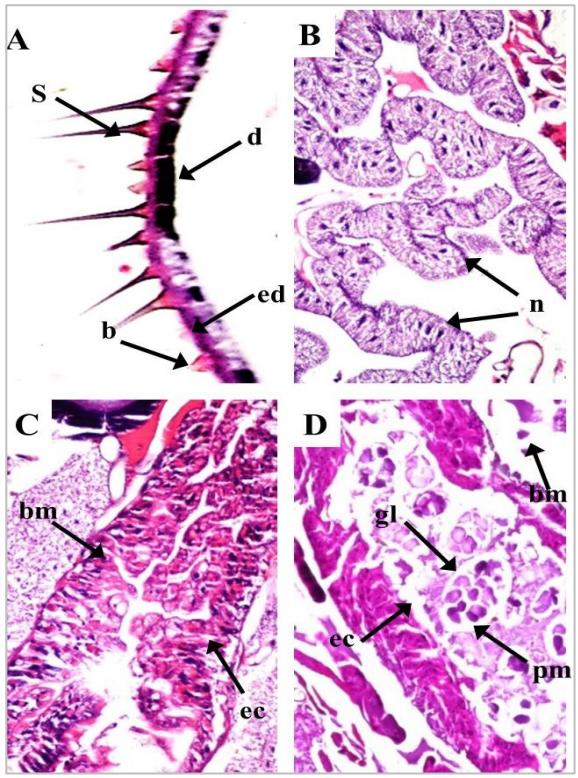

Figure 4. (A) Cuticle of *Earias insulana* control larva showing normal dermis and epidermis with outer spines, (B) glandular structure of untreated larva showing the normal histological structure of the cells, (C) normal histological structure of the foregut and mucosal lining epithelium in control larva, (D) normal histological structure of the midgut of the untreated larva. d= dermis, ed= epidermis, b= buds, S= spines, n= nuclei, ec= epithelium, bm= basement membrane, gl= gut lumen, and pm= peritrophic membrane.

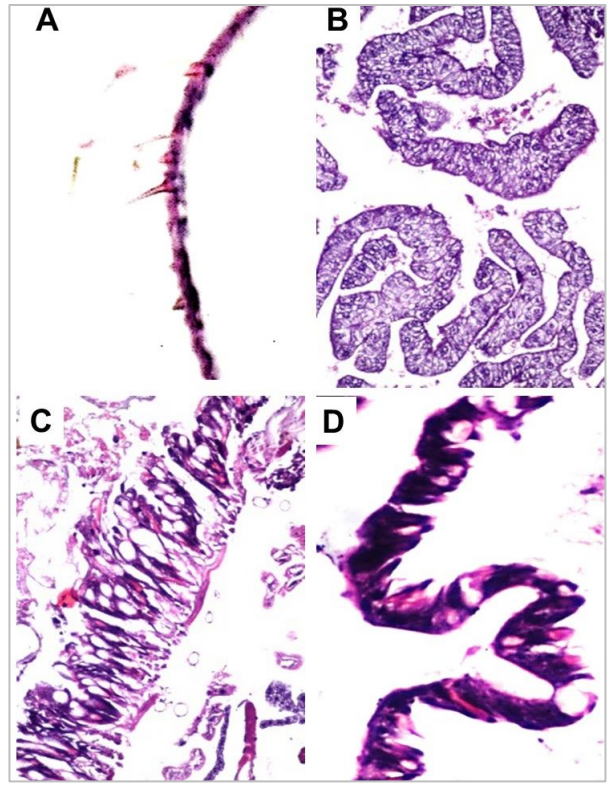

Figure 5. Compresed cuticle of GingerAgNPs treated *E. insulana* larva, with loss of spines and buds (A), Glandular structure of GingerAgNPs treated larva showing degeneration of the epithelial cells (B), The foregut of Ginger AgNPs treated larva showing diffuse goblet cell formation (C), and the midgut of Ginger AgNPs treated larva showing necrobiosis of lining epithelial cells (D).

Discussion

Results indicated a toxic effect of Ginger AgNPs against the 1st instar larvae of *E. insulana* at the level of LC_{50} level, which is in agreement with the findings of Elumalai *et al.* (2010) who showed a moderate toxic effect of *Zingiber officinale* against the larvae of *S. lituralis*, 24 hr after exposure. In addition, Farhad *et al*. (2019) indicated that *Zingiber officinale* nanoparticles as a botanical insecticide with a good toxic effect against the 3rd instar larvae of diamondback moth *Plutella xylostella*. The toxic effects of NPs can be attributed to their small size and large surface area, thereby increasing chemical reactivity and penetration in the living cells (Medina *et al*., 2007).

Treating the newly hatched larvae of the spiny bollworm with LC_{50} concentration of Ginger AgNPs resulted in larval and pupal mortality and malformation. Moreover, the effects extended to include the emerged adults producing a clear reduction in adults emergence rate and high malformation rate. In addition to the effect on female fecundity, the treatment had an acute decrease in oviposition periods and a severe reduction in the number of deposited eggs and hatchability rate.

Our results are consistent with previous findings. Mahdavi *et al*. (2018) demonstrated that *Z. officinale* nano-formulation played an important role as a natural pesticide for the management of different developmental stages of potato tuber moth *Phthorimaea operculella* (Zeller). Hamada *et al*, (2018) showed significant prolongations of larval and pupal durations in contrast to the significant reduction in hatchability rates of deposited eggs when *S. littoralis* was treated as neonate larvae with LC_{50} of Ginger oil (*Zingiber officinale*). Furthermore, Said & Abdelaal (2020) indicated a prolongation in the pink bollworm *Pectinophora gossypiella* larval and pupal duration in response to eggs treatment with Ginger AgNPs.

At the level of cellular structure, different histological alternations were detected at the larval integument and gut when newly hatched larvae of *E. insulana* were treated with the tested Ginger AgNPs. Similar to our results, Knaak *et al.* (2010) showed changes in the histology of the midgut of *S. frugiperda* larvae, 3 hours after *Z. officinale* application. Moreover, our results are in agreement with that of Abdullah (2009) who indicated some histological effects on the midgut of palm weevil *Rnchophorus ferrugineus* due to Boxes chinensis oil treatments. In addition, many histological changes were demonstrated at the level of cuticle and gut for different insects; Atwa *et al*. (2010) on *Agrotis ipsilon* larvae and Neelima *et al*. (2015) on *Helicoverpa armigera* larvae, as a result of Neemazal and *Artemisia annua* extracts treatments, respectively.

It can be concluded from this study that the Ginger AgNPs treatment significantly affected different biological parameters of *E. insulana* newly hatched larvae as compared to the untreated control. Variation in larval and pupal duration, adult longevity in addition to reduction in pupation, adult emergence, and female fecundity rates were clearly observed. Results also clearly showed that treatment caused different histopathological alternations for larval integument, showing the abnormal structure of the epidermis and dermis with destructive spines. Furthermore, degenerative changes in the glandular structure and necrobiosis in the lining epithelial cells of both foregut and midgut cells were also observed.

Acknowledgment

The author wishes to express her thanks to Prof. Dr. Mervat A. Kandil for her unlimited cooperation and help during this study.

الملخص

الشناوي، رانيا. 2024 سمية جزيئات النانو لمستخلص الزنجبيل المائي ضدّ دودة لوز القطن الشوكية (*Earias insulana*) وتأثيرها في بعض الصفات الحيوية والنسيجية للحشرة. مجلة وقاية النبات العربية، 42(2): 202–207. https://doi.org/10.22268/AJPP-001237

تمّت دراسة سمية جزيئات النانو لمستخلص الزنجبيل المائي (Ginger AgNPs) ضدّ دودة اللوز الشوكية (*Earias insulana*) وتأثيراتها الضارة في بعض الجوانب الحيوية والنسيجية تحت ظروف ثابتة (حرارة 26±1°س ورطوبة نسبية 75±5%). نتج عن معاملة التركيز المميت النصفي (LC_{50}) لليرقات حديثة الفقس نسب موت لليرقات والعذارى بلغت 60.0 و 47.06%، على التوالي، مقارنة باليرقات غير المعاملة التي بلغت 6.33 و 2.0%، على التوالي. كما نتج عن المعاملة نسب معنوية من تشوه اليرقات والعذارى (11 و 13.51%، على التوالي) مقارنة بغير المعاملة (1 و 3%، على التوالي). كذلك قلل استخدام جزيئات النانو لمستخلص الزنجبيل (AgNPs) بشكل كبير من نسبة خروج الفراشات إلى 52.94% مقارنة بـ 89.0% في الشاهد غير المعامل. نتج عن معاملة التركيز المميت النصفي (LC_{50}) للزنجبيل (AgNPs) تغيرات نسيجية مختلفة على مستوى خلايا البشرة والمعي المتوسط مقارنة بالبنية الطبيعية غير المعاملة.

كلمات مفتاحية: الزنجبيل، جزيئات النانو، دودة لوز القطن الشوكية، التغيرات الحيوية، التغيرات النسيجية.

عناوين الباحثين: رانيا الشناوي، معهد بحوث وقاية النبات، مركز البحوث الزراعية، الدقي، الجيزة، مصر. البريد الإلكتروني للباحث المراسل: raniashennawy2017@yahoo.com

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Received: April 4, 2023; Accepted: July 19, 2023

تاريخ الاستلام: 2023/4/4؛ تاريخ الموافقة على النشر: 2023/7/19