Egyptian Journal of Zoology (EJZ), Vol. 80: 1-17 (December, 2023) p-ISSN: 1110-6344
Publisher: The Zoological Society of A. R. Egypt e-ISSN: 2682-3160
DOI: 10.21608/ejz.2022.174453.1090

RESEARCH ARTICLE

THE PROFICIENCY OF SILVER NANOPARTICLES IN CONTROLLING COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.), UNDER THE LABORATORY CONDITIONS

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Article History:
Received: 13 November 2022
Revised: 4 December 2022
Accepted: 5 December 2022

Published Online:
2 January 2023

Keywords:
AgNPs
Detoxification system
Insecticides
SDS-PAGE
Spodoptera littoralis

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ABSTRACT

A new control strategy is required to face the resistance of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), to chemical insecticides, and to decrease the pollution of the environment. Silver nanoparticles (AgNPs) were used to overcome these problems and to control *S. littoralis* at the immature stage. AgNPs were prepared by the reduction method. The prepared AgNPs were sphere-shaped with an average diameter of 20.73 nm. Several concentrations of AgNPs (50, 100, 200, 400, and 800 ppm) were used as insecticides against the third instar larvae of *S. littoralis* under laboratory conditions. The feeding treatments and adsorption *via* the integument produced malformations and morphological changes in the treated *S. littoralis* larvae. The larvicidal effect of AgNPs recorded LC_{50} equal to 224.8 ppm after 14 days of the application. Weight loss in the larvae and the pupae after treatment was significant with averages 47.13% and 60.41%, respectively. The treatment produced malformations in the shape and size of larvae and pupae. Some metal-detoxifying enzymes were significantly affected due to the treatment. There were quantitative and qualitative changes in the protein contents, in addition to a significant reduction in the total lipids and total carbohydrates of the treated larvae compared with the untreated larvae. The application of AgNPs against *S. littoralis* larva, as demonstrated by our findings, paves the way for new pest control options.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.), is a member of the Lepidopteran family called Noctuidae and feeds on a variety of crops and is one of the most damaging insects in tropical and global subtropical regions[1]. To control the several forms of this insect's violent attacks, a variety of pesticides including synthetic organophosphates and pyrethroids have been used. The widespread use of these pesticides has resulted in the development of resistant insect strains and severe health issues in both the environment and people[1].

Interdisciplinary research in the field of nanotechnology looks promising. It opens up numerous opportunities in agriculture, pharmaceuticals, electronics, insecticides, and other industries. Nanotechnology has a vast array of potential applications and benefits. These include the formulation of insecticides based on nanomaterial for the

control of pest insects[2]. In addition to reducing soil fertility, the application of chemical pesticides has negative effects on humans and animals, and traditional agricultural strategies like integrated pest management are insufficient. As a result, nanotechnology would offer environment friendly, effective, and non-toxic alternatives for the control of insect pests in agriculture[2].

Since silver has an antibacterial effect against pathogens yet is harmless to people, it has been used in many applications, either as pure free metal or as a composite. Nanoparticles declare a new era of natural remedy improvements that might offer feasible solutions to the most challenging ecological clean-up problems[3]. Nano-technology has turned out to be a standout amongst the most encouraging new methodologies for pest control[4]. Silver nanoparticles (AgNPs) were reported to be toxic and induce high mortalities in several insects[5]. Yasur and Pathipati[6] found a reduction in the weight of Asian armyworm, *Spodoptera litura* (Fabricius), larvae and pupae when treated by AgNPs. Also, they found positive physiological effects of AgNPs on the target larvae through the alteration in the activities of detoxifying enzymes. Shaker *et al.*[7] found that titanium dioxide nanoparticles were toxic to *S. littoralis* and caused mal-formations in the larvae and pupae. In addition, AgNPs were tested by Rouhani *et al.*[8] against the cowpea seed beetle, *Callosobruchus maculatus* (Fabricius), and they reported that AgNPs have strong toxicity on adults and larvae. Furthermore, previous research has recommended that AgNPs can be used as a precious tool in pest management programs for the oleander aphid, *Aphis nerii*[9]. Silica nanoparticles were also found to have very toxic effects on *S. littoralis* when tested under semi-field conditions[10]. In addition to the lethal direct effects of AgNPs on the larvae, some other indirect effects can alter and adversely affect the physiology of the target pests. Accordingly, the present work aimed at the usage of AgNPs as a new alternative strategy to control larval stages of *S. littoralis* for a modern approach to nanotechnology.

MATERIAL AND METHODS

Experimental insect

S. littoralis pupae were acquired from the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. The culture was raised at controlled conditions (27ºC, 65% relative humidity) in the laboratory. The rearing protocol was carried out according to Bakr *et al.*[11]. Fresh castor leaves (*Ricinus communis*) were daily provided to the larvae. A sugar solution (10%) was provided to the newly emerged adults on a piece of cotton, as a food source. After allowing females to lay eggs on clean filter papers, eggs were collected daily and stored in clean jars for a subsequent generation.

Synthesis of AgNPs

The AgNPs were synthesized by the reduction method according to Malassis[12]. In order to prepare a 10000 ppm AgNPs stock, a solution mixture of 10^{-2} mol $AgNO_3$ (0.169 g in 100 mL) and 0.5 g of poly [N-vinylpyrolidone] (PVP) was allowed to stir on ice for 2 hours. Then 15 mL of 10^{-2} mol ascorbic acid was added over a period of three hours (about 1.0 mL every 10 minutes). The reduction of Ag^+ by these reducing agents results in the formation of metallic silver (Ag^0), which then aggregates into oligomeric clusters. Metallic colloidal silver particles eventually emerge as a result of these clusters' formation. The produced silver NPs were stabilized and protected by PVP. The observed color was changed in the aqueous solution matrix from a faint yellow color to brown and later gray color qualitatively, which confirmed the synthesis of AgNPs as proved before. The size and shape of samples were characterized by transmission electron microscope (TEM).

TEM analysis

The shape and size of AgNPs were examined by the TEM. Part of the synthesized AgNPs was sonicated and

a drop of this solution was applied to a copper-coated grid as a thin film. The TEM analysis was carried out in the Central Laboratory, Faculty of Science, Ain Shams University.

Larval treatments with AgNPs

The larvae that reached third instar were used for the AgNPs insecticidal assay; as the third instar larvae have a high feeding consumption, they were chosen for the experiment. To study the toxic effects of AgNPs, third instar larvae were fed on castor leaves treated with different concentrations (50, 100, 200, 400, and 800 ppm) of AgNPs and compared with insects fed on untreated castor leaves. Six replications were main-tained with twenty larvae per replicate.

Healthy third instar larvae were starved for 3-4 hours prior to the bioassay and placed in plastic containers. Leaf discs were dipped for a few seconds in the corresponding concentration or in distilled water for the control and allowed to dry for a few minutes before being placing in the corresponding cups.

Starved larvae were introduced gently using a small brush into the leaf discs and allowed to feed. After larvae finished the feeding on the treated leaf discs (48 hours), fresh castor leaves were regularly provided. The accumulated mortalities were recorded after 14 days. The aberrations or the malformations that occurred in the treated larvae or after pupation were photo-graphed.

Larval and pupal weight loss after treatments with AgNPs

Three concentrations (LC_{25}, LC_{50}, and LC_{95}) of AgNPs were used to monitor the weight loss of the treated larvae and pupae compared with the control ones. In this test, large containers were used as six replicates with fifty third instar larvae in each one. After 14 days, ten larvae from each treatment were weighed separately. The same was done after the pupation.

Biochemical studies

To monitor the biochemical changes as adverse effects of AgNPs, third instar larvae were treated with three concentrations (LC_{25}, LC_{50}, and LC_{95}) of AgNPs as previously described. After 48 hours, treated larvae were homogenized in distilled water (50 mg/mL). The homogenates were centrifuged in a cooling centrifuge at a speed of 4000 ×*g* for fifteen minutes at 5°C. The supernatants were kept in a deep freezer at −20°C to be used for biochemical assays later.

The assessment of the total proteins was achieved according to the methods described by Bradford[13], the total protein contents of the whole body were measured in treated larvae and control ones. The assessment of the total lipids was achieved according to the original method described by Knight *et al.*[14] by using phospho-vanillin reagent. The assessment of the total carbohydrates was achieved according to the phenol-sulfuric acid reaction of Dubois *et al.*[15] in an acid extract of the sample. According to Crompton and Birt[16], the total carbohydrates were extracted from the sample.

Quantification of the metal-detoxifying enzymes

After 48 hours, insect guts from larvae treated with three concentrations (LC_{25}, LC_{50}, and LC_{95}) of AgNPs and from control larvae were grinded in 0.1 mol potassium phosphate buffer (pH 7.0), and the obtained supernatant from the homo-genate was collected to be used in the subsequent experiments. The gut extracts were kept at −20°C until they were used.

The β-glucosidase (Glu) activity was quantified according to the method of Low *et al.*[17]. They used p-nitro phenyl gluco-pyranoside as a substrate, and the optical density was colorimetrically read at 400 nm against water as a blank. The carboxyl esterase (CaE) activity was measured by using the described method by Govindappa *et al.*[18], where the α-naphthyl acetate was used as a substrate and the optical density

was detected at 600 nm. The glutathione-S-transferase (GST) activity was done by following Habig *et al.*[19] methodology, where the 1-chloro-2,4-dinitrobenzene was used as a substrate and the colorimetric test was read at 340 nm.

Qualitative analysis of the total protein contents

Three concentrations (LC_{25}, LC_{50}, and LC_{95}) of AgNPs were used to investigate the changes in the protein profile in treated larvae compared with the normal larvae. Protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the protocol of Laemmli[20]. The amount of protein loaded on each well in the gel was 100 µg.

Statistical analysis

The bioassay results were processed by computer software Ldp to produce the regression line and for the detection of LC_{25}, LC_{50}, and LC_{95} values based on the described method by Finney[21]. Using SPSS version 19 for Windows (IBM, Armonk, NY, USA), the achieved data were subjected to analysis of variance (ANOVA) and post-hoc analysis (LSD test) using the equation of the standard deviation, standard errors, and probabilities (P). The terms "significant" ($P<0.05$) and "non-significant" ($P\geq0.05$) were used to indicate the level of significance.

RESULTS

Characterization of nano-scale silver particles

The high-resolution study of the nano-particles using TEM revealed that AgNPs are polydisperse and spherical in shape. The diameter of AgNPs ranged from 5.43 to 58.38 nm and the recorded average diameter of AgNPs was 20.73 ± 2.44 nm. As identified by the international organization for standardization, the nanoparticle is a particle with a dimension ranging from 1-100 nm. According to our synthesized AgNPs, the size of the particles obtained was not greater than 58.38 nm (Figure 1).

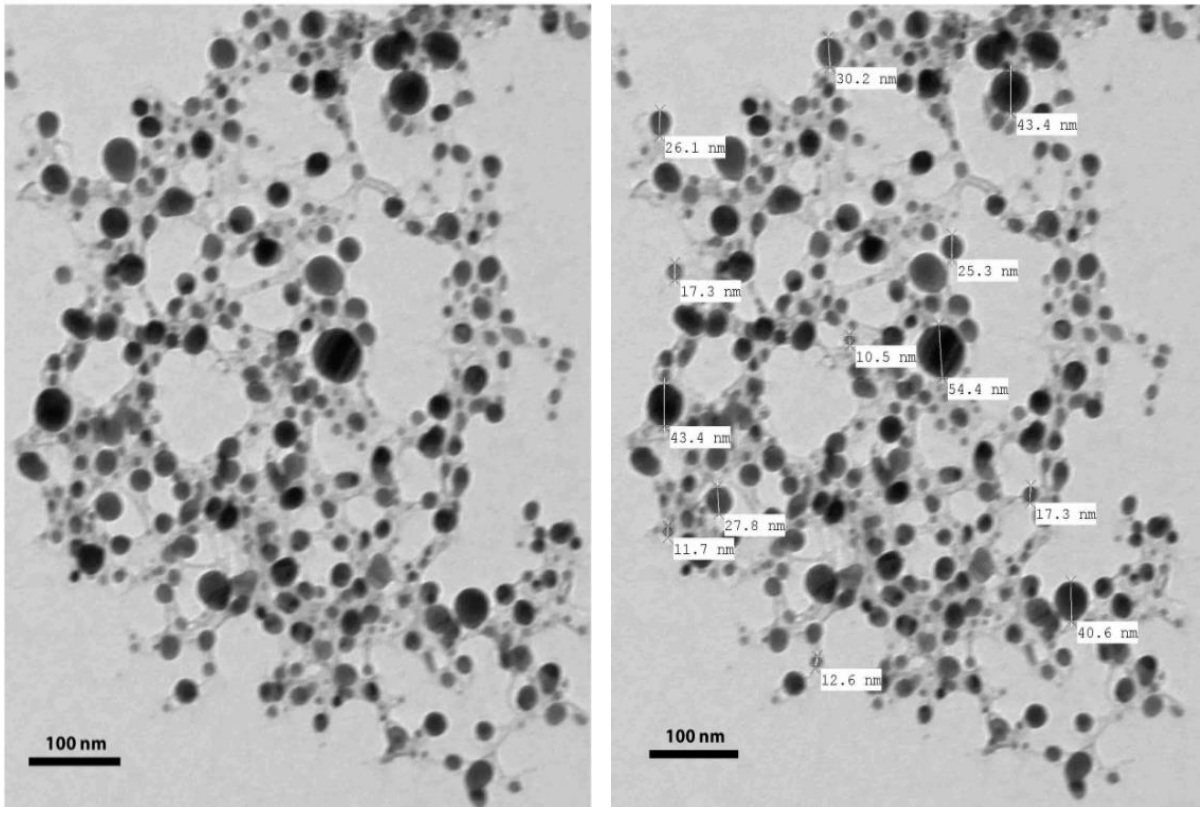

Figure 1: Transmission electron microscopy images of silver nanoparticles that were synthesized by the chemical reduction method. The scale bar is 100 nm.

Toxic effects of AgNPs on *S. littoralis* larvae

Data in Figure "2" showed the larval mortalities at different concentrations of AgNPs. The larval mortality values increased significantly as the concentration of AgNPs was increased. The mean mortality percentages were 0.0, 10.83±1.67, 16.67±2.13, 35.83±0.67, 73.33±2.76, and 96.67±1.23 at treatment concentrations 0, 50, 100, 200, 400, and 800 ppm, respectively. From the regression line, the values of LC_{25}, LC_{50}, and LC_{95} were determined as 121.61, 224.79, and 1005.65 ppm, respectively.

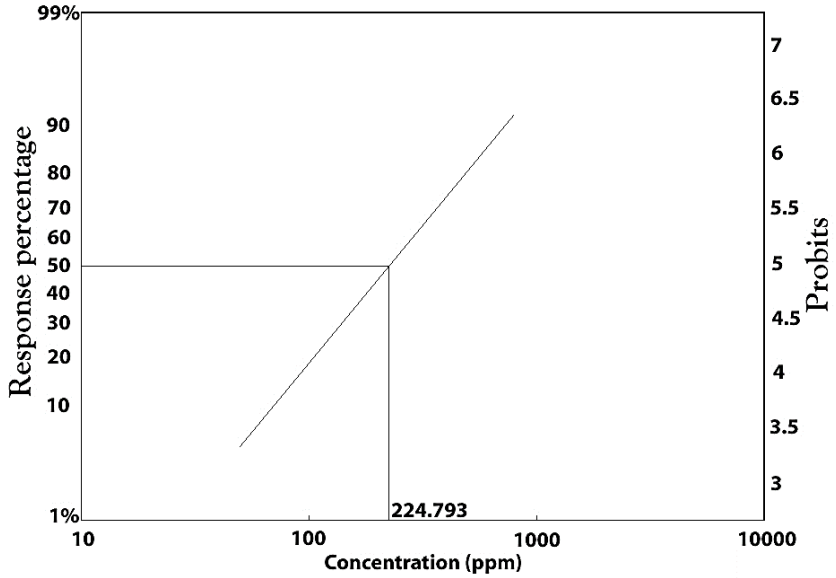

Figure 2: The regression line of the relationship between the concentration of silver nanoparticles and the percentage mortalities of *Spodoptera littoralis* larvae.

Morphological changes and weight loss in larvae and pupae after AgNPs treatments

Photos in Figure "3" showed the toxic effects on the morphology of larvae fed on AgNPs. Before larval death, some mal-formations and aberrations were noticed in affected larvae. Treated larvae showed body shrinkage, deformed morphology, and disappeared segmentation. Some larvae fail to shed the old exuviae during the larval molt and eventually die (Figure 4). In addition, the size of the treated larvae was smaller as compared with the control.

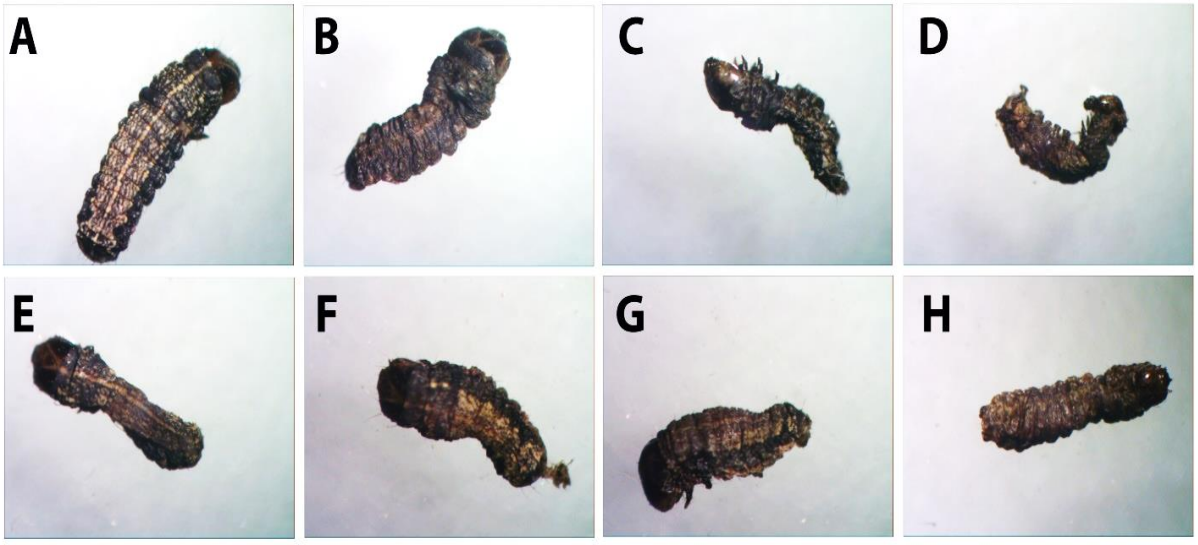

Figure 3: Photographs of control (A) and AgNPs treated *Spodoptera littoralis* larvae (B-H). The treated insects became small and shrunk.

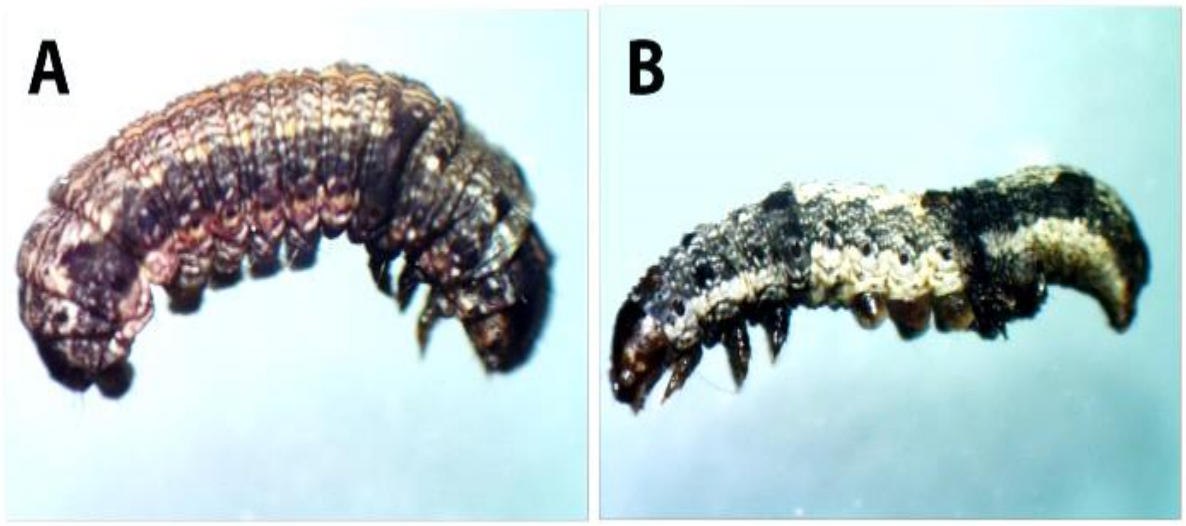

Figure 4: Photograph of control (A) and AgNPs treated ***Spodoptera littoralis*** larva (B). The treated larva could not molt.

Accordingly, the weights of the treated larvae were detected after three different treatments (LC_{25}, LC_{50}, and LC_{95}) of AgNPs, and the values were presented in Table "1". Significant reductions in the larval weights were observed in larvae treated with 121.61, 224.79, and 1005.65 ppm of AgNPs as −45.41, −47.06, and −48.93%, respectively. The survival treated larvae with AgNPs molted at the end of the 6th larval instar into small pupae compared with the untreated pupae (Figure 5). The weights of the pupae were decreased significantly by values of −59.11, −62.64, and −58.49% compared with normal pupae in the treatment with 121.61, 224.79, and 1005.65 ppm of AgNPs, respectively (Table 1).

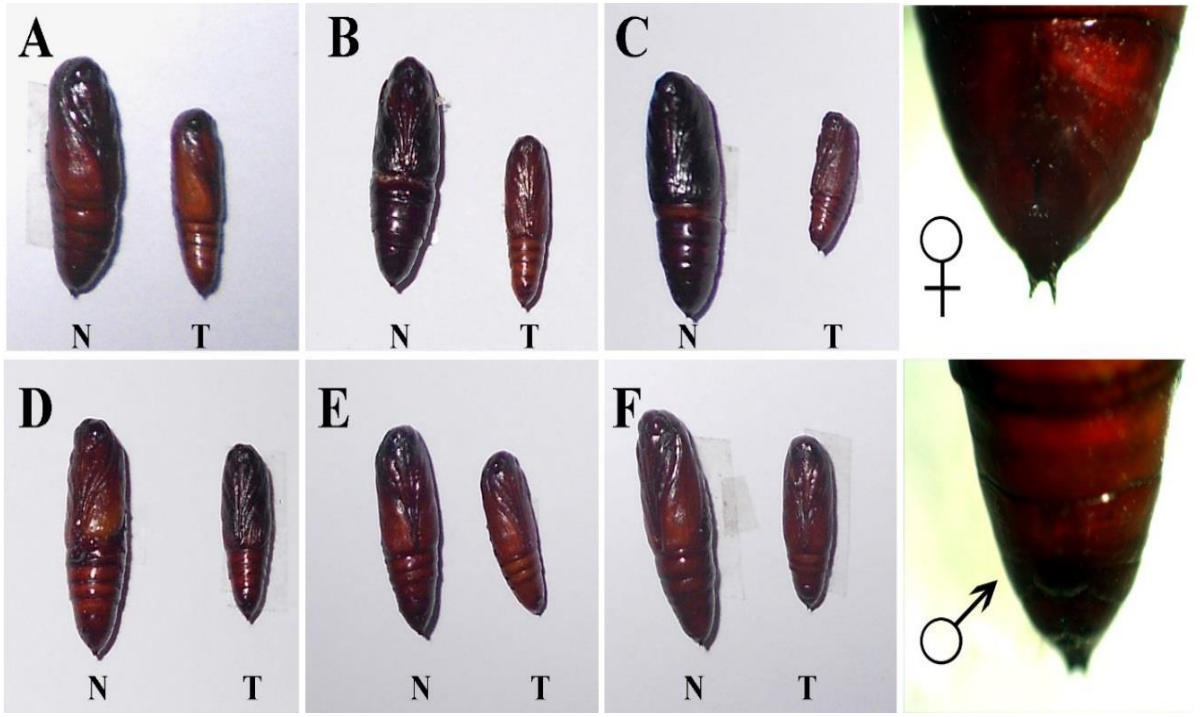

Figure 5: Photographs of female pupae (A-C) and male pupae (D-F) showing normal-sized pupae (N) and treated small pupae (T). Magnified female (♀) and male (♂) genital parts.

Table 1: The effects of silver nanoparticles on the larval and pupal mean weight of *Spodoptera littoralis*.

Concentrations	Larval mean weight (mg/larva) ± SE	Pupal mean weight (mg/pupa) ± SE
Control	597.67 ± 9.17[a]	313.86 ± 2.52[a]
LC_{25}	326.25 ± 10.11[b]	128.34 ± 3.61[b]
LC_{50}	316.41 ± 8.13[b]	117.25 ± 4.24[b]
LC_{95}	305.23 ± 12.67[b]	130.27 ± 2.67[b]

Means with different letters in the same column are significantly different (P<0.05), SE: standard error.

Effects of AgNPs on the biochemical measurements of *S. littoralis* larvae

The main insect metabolites as total proteins, lipids, and carbohydrates of *S. littoralis* treated larvae with 121.61, 224.79, and 1005.65 ppm of AgNPs were reported in Table "2". Results indicated that the treatments induced a significant reduction in the total protein, carbohydrates, and lipid contents in treated larvae compared with the control (P<0.05). The reductions in the total proteins were −47.16, −50.71, and −52.88% in the treatment with 121.61, 224.79, and 1005.65 ppm of AgNPs, respectively. The values of the total lipids in treated larvae with 121.61, 224.79, and 1005.65 ppm of AgNPs were reduced by −53.74, −56.34, and −60.46%, respectively. In addition, the recorded amounts of total carbohydrates were decreased by −50.06, −53.65, and −59.81% in treated larvae with 121.61, 224.79, and 1005.65 ppm of AgNPs, respectively.

Table 2: Total proteins, lipids, and carbohydrates of *Spodoptera littoralis* larvae treated with different concentrations of AgNPs.

Concentrations	Total proteins	Total lipids	Total carbohydrates
	Mean mg/g body weight ± SE		
Control	27.65±0.03[a]	9.23±0.32[a]	17.54±0.28[a]
LC_{25}	14.61±0.05[b]	4.27±0.51[b]	8.76±0.32[b]
LC_{50}	13.63±0.09[b]	4.03±0.91[b]	8.13±0.13[b]
LC_{95}	13.03±0.11[b]	3.65±0.43[b]	7.05±0.21[b]

Means with different letters in the same column are significantly different (P<0.05), SE: standard error.

The activities of Glu, CaE, and GST were measured in the control and the treated larvae with 121.61, 224.79, and 1005.65 ppm of AgNPs. The activity of the Glu enzyme was significantly decreased by an average of −45.18% in the treated larvae compared with the control. The level of CaE enzyme was significantly reduced, with an average value of −39.23% in the treated larvae over the control ones. Contrarily, the GST enzyme activity was increased by an average of 39.57% in the treated larvae over the control larvae with statistical significance (Table 3).

The total proteins extracted from normal and treated larvae with 121.61, 224.79, and 1005.65 ppm of AgNPs were separated by SDS-PAGE. The molecular weight of the

Table 3: The activities of the metal-detoxifying enzymes in *Spodoptera littoralis* larvae treated with different concentrations of AgNPs.

Concentrations	*β-glucosidase*	Carboxyl esterase	Glutathione-S-transferase
	Mean µ*mol/min/mg of protein* ± SE		
Control	7.38±0.03[a]	19.93±0.92[a]	14.11±0.23[a]
LC_{25}	4.13±0.17[b]	12.91±0.52[b]	17.98±0.18[b]
LC_{50}	4.27±0.31[b]	12.31±0.18[b]	19.17±0.42[b]
LC_{95}	3.75±0.02[b]	11.11±0.21[b]	21.93±0.35[b]

Means with different letters in the same column are significantly different (P<0.05), SE: standard error.

separated proteins from normal and treated larvae can be determined by comparing their electrophoretic mobility with known protein markers (Figure 6). Data in Table "4" showed the comparison between protein bands of the normal and treated larvae according to the retardation factor (Rf), which represented the relative mobility of the protein bands. The results showed that bands numbers 10, 11, 13, and 14 were the common bands between the control sample and treated samples with molecular weights of 61.78, 53.05, 39.52, and 31.77 KDa, respectively. Bands numbers 1, 5, and 7 were present only in the control sample and these three bands disappeared in the treated samples, where their weights were 166.34, 125.58, and 93.33 KDa, respectively. In each of the three treated samples, two new bands appeared compared with the control sample, which were bands numbers 8 and 12 with a molecular weight of 80.47 and 49.56 KDa, respectively. The bands numbers 2, 4, and 17 were unique bands in the treated sample with the LC_{95} with molecular weights of 155.28, 131.57, and 19.17 KDa, respectively.

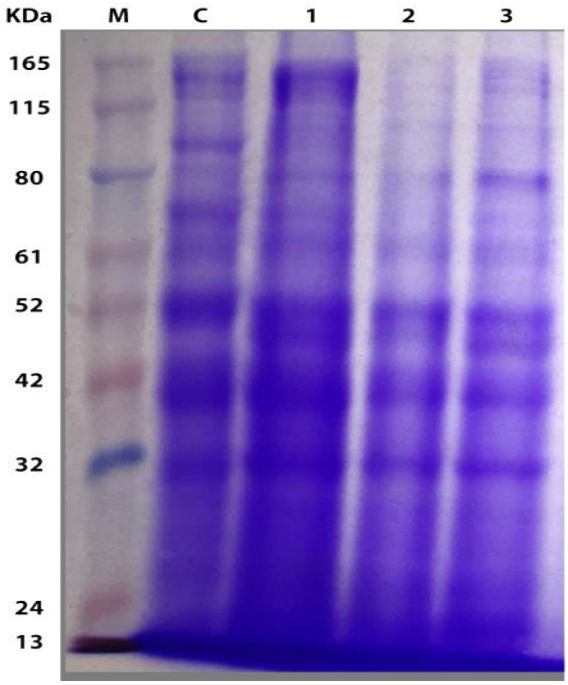

Figure 6: Photograph of electrophoretic protein pattern of *Spodoptera littoralis* larvae as a control and treated sample with three concentrations of AgNPs. M: Marker, C: control larvae, 1, 2, and 3: larvae treated with LC_{25}, LC_{50}, and LC_{95} AgNPs, respectively.

Table 4: Retardation factor (Rf) and concentration of different fractionated protein bands detected in *Spodoptera littoralis* larvae as a control and treated sample with three concentrations of AgNPs.

Band Number	Rf	Molecular weight	Band (%)			
			Control	LC_{25}	LC_{50}	LC_{95}
1	0.05	166.34	6.61	-	-	-
2	0.06	155.28	-	-	-	2.86
3	0.08	145.24	2.31	2.65	16.70	-
4	0.10	131.57	-	-	-	3.50
5	0.11	125.58	18.09	-	-	-
6	0.15	105.61	-	-	13.23	21.03
7	0.18	93.33	1.47	-	-	-
8	0.23	80.47	-	37.86	17.75	0.83
9	0.29	69.18	1.32	9.75	-	15.60
10*	0.35	61.78	19.59	16.09	13.36	14.26
11*	0.44	53.05	0.71	2.13	1.77	0.97
12	0.48	49.56	-	3.16	10.19	12.03
13*	0.59	39.52	14.99	0.63	8.62	9.76
14*	0.69	31.77	10.40	27.03	0.92	0.66
15	0.77	30.03	-	-	13.81	13.85
16	0.88	26.05	23.91	-	2.77	-
17	0.94	19.17	-	-	-	4.64
18	0.98	12.69	0.60	0.71	0.88	-
Total number of bands			11	9	11	12

*Common bands in all samples

DISCUSSION

The AgNPs were prepared by using the chemical reduction method with the protection of PVP. This method produces a high yield of AgNPs. The produced AgNPs were fit in uniform shape and in the size (<100 nm) of the effective nano-particles, as previously proved[4,22,23]. In this study, *S. littoralis* third instar larvae were treated by feeding with different concentrations of the synthesized AgNPs. During feeding, larvae crawl over the treated leaves, which allows direct contact with AgNPs. Our results proved that AgNPs are toxic to *S. littoralis* larvae and induced a statistically significant increase in the mortalities according to the elevation in the used concentrations. The small spherical shaped particles of AgNPs can pass through the cellular membrane barriers without any difficulties and, as a result, induce efficient mortality[24].

Several previous studies presented AgNPs as a candidate insecticide. Jayaseelan *et al.*[25] tested the larvicidal and pediculocidal effects of AgNPs against *Culex quinquefasciatus* (filariasis vector), *Anopheles subpictus* (malaria vector), and *Pediculus humanus capitis* (head louse). They reported that AgNPs can be used as an ultimate eco-friendly approach for the control of the head louse and vectors of diseases. Similarly, the toxic efficacy of silica and zinc oxide nanoparticles was estimated by Osman *et al.*[26] against *S. littoralis* larvae in the laboratory. They recorded the cumulative mortalities in larvae after twelve days of exposure. The mortalities were 83.33% and 86.67 after treatment with nanosilica and nanozinc

oxide, respectively. When different concentrations of nanosilica were applied on *S. littoralis* larvae, high mortality as 95.33% was obtained at 425 ppm after 15 days of treatment[27]. In another study, Pavunraj *et al.*[28] mentioned that AgNPs are toxic to the spiny bollworm (*Earias vittella*) and mosquito vectors (*Cx. quinquefasciatus* and *Aedes aegypti*). In addition, Abd El-Rahman *et al.*[29] applied two nanocomposites prepared by combining Ag and graphene oxide (GO) nanomaterials with magnesium chlorophillin (Mg-Chl/Ag and Mg-Chl/GO, respectively) on *S. littoralis* larvae. These two nanocomposites induced high mortality of 91.6% after 15 days of treatment. Abou El-Ela *et al.*[30] applied AgNPs against the brown planthopper (*Nilaparvata lugens*) and the results showed that the mortality rates were increased by increasing concentrations over time.

During the bioassay tests, several morphological changes in the treated larvae were observed. In addition, larvae and pupae appeared smaller in size and weight compared with the control. These findings are in accordance with those of Yasur and Pathipati[6], who used the synthesized AgNPs which were stabilized by PVP on castor leaf against the larvae of Asian armyworm, *Spodoptera litura*. They recorded a reduction in the weight of both larvae and pupae due to the physiological changes in the insects' bodies caused by the treatment of AgNPs. During early developmental stages of the wild strain of *Drosophila melanogaster*, cuticular formation was impacted by the dietary supplement of AgNPs at sublethal dosages over a prolonged period. All adult animals had a discolored appearance because of the lack of melanin pigments in their cuticles, which was a result of their exposure to AgNPs[31]. Osman *et al.*[26] verified that the treated second instar larvae of *S. littoralis* with nano zinc oxide and nano silica produced a high decrease in the weight of the pupae. In agreement with our findings, Abd El-Hamid *et al.*[32] noticed several morphological abnormalities and a change in the size of both larvae and pupae of the housefly (*Musca domestica*) after treatment with AgNPs by feeding. On the other hand, Abd El-Rahman *et al.*[29] found a significant reduction in the pupal weight after treatment of *S. littoralis* larvae with other two nanocomposites (Mg-Chl/Ag and Mg-Chl/GO)

When nanosilver particles are applied to insect species, it alters the morphology, physiology, and biochemistry of their bodies, as well as several aspects of their life cycle including development, growth, and fecundity. Nanomaterial toxicity mechanisms include membrane disruption, disruption of energy transduction, genotoxicity, protein oxidation, the formation of reactive oxygen species, and even the release of toxic constituents. When AgNPs are applied, they adhere to the cellular surface, changing the properties of the membrane, and affecting permeability and cell respiration[33].

Since total proteins, total lipids, and total carbohydrates reflect the health status in insects' bodies, the estimation of these three key parameters will lead to investigating the role of AgNPs in insect physiology. Significant reductions were observed in these three components compared with the control larvae. These reductions explain the observed reductions in the weights (weight loss) of both larvae and pupae after treatment. The reductions in these three biochemical parameters were observed by several authors when they applied different kinds of nanoparticles on insects. Osman *et al.*[26] revealed that silica and zinc oxide nanoparticles caused declines in total carbohydrates and total proteins in treated *S. littoralis* larvae. Kos *et al.*[34] reported reductions in the protein levels in the head, thorax, and in the hemolymph of the honeybee (*Apis mellifera*) when treated by cerium oxide nanoparticles. According to research by Fouad *et al.*[35], exposure to AgNPs caused a decrease in total protein levels in *Aedes albopictus* larvae. According to Assar[36], protein loss occurs during intoxication as a result of reduced body weight, protein breakdown to

release energy, or a substance's direct impact on the transport of amino acids by cells. Therefore, it is possible to conclude that nanosilver significantly contributed to the decrease in total protein content. This may be because protein is broken down into amino acids, which the insect uses to produce energy. Because the protein was bound with foreign substances like the tested chemical, the protein content of the current study may have decreased.

In nanomaterial-stressed organisms, it was suggested that a mechanism for meeting energy needs might be affected by the depletion of proteins and other nutrients caused by nanomaterials[37]. Part of the cell membrane and cuticle are made of lipids as essential components. In general, lipids provided a high supply of metabolic energy. In our results, AgNPs induced a significant reduction in the total lipids compared with the normal insects. The significant decrease in total lipids may be attributable to the breakdown of lipids into simpler forms that can be utilized for growth as a carbon source. Bennett and Shotwell[38] suggested that for energy requirements, the treated larvae would produce an enzyme that utilizes lipids. El-Aasar *et al.*[39] declared that the reduction in total carbohydrate content of the treated larva might be related to metamorphic changes in the larva. During this stage, the organism receives glucose from the carbohydrate content, which serves as an energy source for the synthesis of larval and adult tissues, notably the cuticle. Carbohydrates are important for the regular functioning of the male and female reproductive systems, in addition to the development of the embryo. In agreement with our results, Abd El-Rahman *et al.*[29] reported significant reductions in total proteins, total lipids, and total carbohydrates in treated *S. littoralis* larvae with two nanocomposites (Mg-Chl/Ag and Mg-Chl/GO). They stated that the soluble carbohydrate matter is affected by the metamorphic variations induced in larvae under stress. This could explain why *S. littoralis* larvae treated with nano-composite had a high reduction in total carbohydrates. Morsy *et al.*[40] used AgNPs against the cutworm, (*Agrotis ipsilon*) and they found significant reductions in the total proteins and total lipids after all tested periods, and the highest reduced values were noticed after 7 days of treatment.

It has also been reported that metal nanoparticles can bind to sulfur in proteins and phosphorus in DNA, causing a decline in membrane permeability and, as a result, organelle and enzyme denaturation, followed by cell death. AgNPs are known to reduce protein synthesis and gonadotrophin release, resulting in developmental and reproductive failure[41]. The detoxifying enzymes are supposed to be key biomarkers in evaluating tissue injury due to AgNPs treatments in insects. Accordingly, the activities of Glu, CaE, and GST were measured after the treatment of three different concentrations (LC_{25}, LC_{50}, and LC_{95}) of AgNPs. The data in the current work showed that the levels of the Glu and CaE enzymes decreased significantly in the treated larvae regardless of the used concentrations of AgNPs. This finding is in agreement with Yasur and Pathipati[17] who found a reduction in the Glu and CaE in the Asian armyworm (*Spodoptera litura*) treated larvae with AgNPs. In addition, Parthiban *et al.*[42] stated that the insect's physiological system relies heavily on the detoxification enzyme carboxylesterase to protect itself from various allelochemicals. They recorded reduction in the level of carboxylesterase enzyme in *Aedes aegypti* treated larvae by AgNPs. The elevation in these enzymes represents the first defensive mechanism in insects due to the stress induced by the AgNPs treatment. Some authors[43] clarified the antioxidant defensive mechanism in insects and demonstrated that the ingested pro-oxidant allelochemicals increase the activity of antioxidant enzymes, protecting the body from both endogenous and exogenous oxidative radicals. Glutathione is regarded as the most significant low

molecular-weight antioxidant, and it is crucial to the removal of damage from oxidative stress.

When insects are under abiotic or biotic stress, many defense mechanisms and metabolic responses are activated, resulting in detoxification processes. They are typically brought on by antioxidant enzymes or metabolic detoxification. Detoxifying enzymes (Glu, CaE, and GST) and antioxidant enzymes are all involved in metabolic resistance mechanisms. Most cases of metabolic resistance in insects could be recognized by elevated levels of these enzymes in comparison to susceptible equivalents[44]. The GST, as a detoxifying enzyme, is implicated in the resistance developed in insects by decreasing detoxifying lipid superoxide metabolites and oxidative injury[45]. In this study, the level of GST was increased in the treated larvae with AgNPs. This finding is in accordance with Yasur and Pathipati[6] who found an elevation in the GST in the Asian armyworm (*Spodoptera litura*) treated larvae with AgNPs. Chinnaperumal *et al.*[46] applied titanium dioxide nanoparticles against cotton bollworm (*Helicoverpa armigera*) larvae. They recorded a reduction in carboxylesterase level and an elevation in GST level. As a result, the carboxylesterase enzyme level decreased when exposed to the synthesized AgNPs, supporting the development of new insecticides against various insect pests, including the cotton leafworm, *S. littoralis*.

For the separation of protein, lipoprotein, and glycoprotein from animal sources, SDS-PAGE has been applied as an appropriate technique. The electrophoretic analysis of SDS-PAGE protein was performed for untreated and treated larvae of *S. littoralis*. The obtained results showed differences in the protein patterns between treated and untreated larvae. The treatment with AgNPs caused the disappearance of normal bands and/or the appearance of abnormal bands as compared with the control samples. The appearance of new protein bands might be due to an increase in protein synthesis; these findings were in line with Hassan *et al.*[47], who looked at differences in the protein pattern of the untreated and treated *S. littoralis* larvae with indoxacarb, spinetoram, and methoxyfenozide. They found that some proteins were either lost or expressed with different densities. In addition, in treated larvae of *Tuta absoluta* with phenthoate, imidacloprid, and dinotefuran, Radwan and Taha[48] found a correlation between the loss of various proteins and the reduction of band intensity when using SDS PAGE. In addition, Farag *et al.*[49] observed the disappearance of normal protein bands in the treated *Cx. pipiens* larvae with pomegranate peel extracts.

In conclusion, a physical strategy was utilized to change and enhance the efficacy of various forms of synthetic chemical pesticides using particulate systems, such as nanoparticles. Nanotechnology-based methods should be used in intergraded pest management in place of conventional methods based on chemical insecticides. Nanotechnology reduces the need for chemical insecticides, making agriculture more economic and environment friendly. Our research showed that relatively low concentrations of synthetic nanoparticles could potentially suppress *S. littoralis* and remarkably reduce larval and pupal body weight. Also, it has a serious effect on biochemical parameters such as total protein, total lipid, and total carbohydrate. Additionally, the stress caused by AgNPs changes the metal-detoxifying enzymatic levels in the midgut tissues of *S. littorlais* larvae. These results also point to a potential role for AgNPs in defending numerous agricultural products against pest attacks. As a result, it is also possible to derive the conclusion that AgNPs function as an effective tool for offering environmentally beneficial and green options. This research recommended that AgNPs be further investigated under field conditions to be involved in the Integrated Pest Management approach for controlling *S. littorals*.

COMPLIANCE WITH ETHICAL STANDARDS

This research paper was approved by the research ethics committee of Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2022/11/5).

FUNDING SOURCE DISCLOSURE

This work did not receive any fund.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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How to cite this article:
Abdou, M. A. and Zyaan, O. H. (2023). The proficiency of silver nanoparticles in controlling cotton leafworm, *Spodoptera littoralis* (BOISD.), under the laboratory conditions. Egyptian Journal of Zoology, 80: 1-17 (DOI: 10.21608/ejz.2022.174453.1090).

كفاءة جسيمات الفضة النانوية في مكافحة دودة ورق القطن *Spodoptera littoralis* (Boisd.) تحت الظروف المعملية

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استجابة لإيجاد حل لمشكلة مقاومة دودة ورق القطن – (Lepidoptera: Noctuidae) *Spodoptera littoralis* (Boisd.) – للمبيدات الحشرية الكيميائية وللحد من تلوث البيئة، أصبحت هناك ضرورة ملحة لاستحداث استراتيجية جديده للمكافحة. ولتحقيق ذلك تم في هذا البحث استخدام جسيمات الفضة النانوية لمقاومة المراحل غير الناضجة لدودة ورق القطن. وقد تم تحضير الجسيمات النانوية الفضية بطريقة الاختزال، وكانت على شكل كروي بمتوسط قطر يبلغ 20.73 نانومتر. وقد تم استخدام تركيزات مختلفة من جسيمات الفضة النانوية (50، 100، 200، 400، و 800 جزء في المليون) كمبيد حشري ضد يرقات الطور الثالث من دودة ورق القطن تحت الظروف المعملية. وقد نتج عن المعاملة نتيجة التغذية والامتزاز من خلال جليد الحشرة تشوهات وتغيرات في شكل اليرقات المعاملة. وقد سجل تأثير استخدام التركيز نصف المميت (224.8 جزء من المليون) نقص في وزن اليرقات ووزن العذارى بمتوسط 47.13% و 60.41%، علي التوالي، بعد 14 يوم من المعاملة. وقد تأثرت بعض الإنزيمات المسؤولة عن إزالة السموم في الجسم بشكل كبير نتيجة المعاملة. وقد كانت هناك تغيرات كمية ونوعية في محتوي البروتين، بالإضافة إلى انخفاض ذو دلالة إحصائية في الدهون والكربوهيدرات لليرقات المعاملة مقارنة بتلك غير المعاملة. إن تطبيق مركب جسيمات الفضة النانوية ضد يرقات دودة ورق القطن – كما يتضح من النتائج التي توصلنا إليها – يمهد الطريق لخيارات جديدة لمكافحة الآفات.