Insecticidal effects of pure and silver-doped copper oxide nanosheets on *Spodoptera littoralis* (Lepidoptera: Noctuidae)

Atwa A. Atwa,[1] Numan A. Salah, Wedad E. Khafagi, Ahmed A. Al-Ghamdi

Abstract—*Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most destructive agricultural pests. We investigated the cumulative lethal effects of two nanostructure materials by feeding nanostructural form of copper oxide (CuO) or copper oxide doped with silver (CuO:Ag), nanosheets-treated diet to third and/or fifth instars of *S. littoralis* until pupation. Nanosheet concentrations were individually incorporated into 150 mL of artificial diet and offered as treated food. Both nanosheets had no immediate effect but caused cumulative larval mortality. At high concentrations and longer exposure times, nanosheet had a significant insecticidal effect against *S. littoralis* larvae. Nanosheets at 300 mg had the highest insect mortality effect, reaching 97.6% and 100% among CuO-treated third and fifth instars compared with 57.6% and 48.2% among the same instar treated with CuO:Ag. Treated larvae exhibited higher pupal mortality, and pupal and moth deformities compared with untreated larvae. There were more deformities among those fed CuO:Ag. Incorporation of nanosheets into the diet had a significant effect on the timing of larval development. Results showed that CuO was more influential than CuO:Ag at all concentrations and with similar exposure durations. Our results suggest that nanosheets may have important implications on the population dynamics of *S. littoralis*.

Introduction

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae), commonly known as the Egyptian cotton leafworm, causes severe damage to various crops because of its polyphagous nature and intricacies involved in its management. *Spodoptera littoralis* has significant importance in agriculture, particularly in Egypt, where cotton leaves and truck crops are most affected by *S. littoralis*. *Spodoptera littoralis* has a wide range of hosts, belonging to more than 87 species and 40 families (Salama *et al*. 1970).

Cotton leafworm is mostly reported from the Mediterranean and North African regions (Commonwealth Institute of Entomology 1967; European and Mediterranean Plant Protection Organization 2014). As the result of feeding by *S. littoralis*, crop shows disease symptoms in the form of irregularly large and irregular shaped holes, leaving aside only larger veins. The insect larvae bore through the bud or young boll to consume leaf contents, causing them to dry and shed off the tree (Bishara 1934). In Egypt, population of *S. littoralis* on cotton is successfully managed by the application of chemicals, such as synthetic pyrethroids and organophosphorus compounds; however, in many cases resistance to these chemicals has also been reported (Issa *et al*. 1984; Abo-El-Ghar *et al*. 1986). Yadav (2010) reported the occurrence of biological imbalance as a consequence of developing resistance in insects and residual effects to non-target organisms. As the result of such adverse effects of chemical pesticides on the environment and non-target organisms, the focus has been shifted to evaluate, use, and apply more ecofriendly and less hazardous products for the pest management (Bulmer *et al*. 2009; Cloyd and Bethke 2011).

Nanotechnology is a rapidly growing technique that has been successfully applied against microbial

Received 8 January 2017. Accepted 18 June 2017. First published online 24 August 2017.

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Subject editor: Susan Bjornson
doi:10.4039/tce.2017.36

pests and parasites (Feng *et al.* 2000; Samuel and Guggenbichler 2004; Elchiguerra *et al.* 2005; Reddy *et al.* 2007). Similar studies on nanoparticles and their effects on insect pests are not widely known (Chinnamuthu and Murugesa-Boopathi 2009), whose application in the management of insect pests may result in the discovery of insect repellents or growth regulators derived from indigenously available products (Owolade *et al.* 2008; Bhattacharyya *et al.* 2010). The advantages of using nanoparticles in the insect pest management programmes are manifold, since the strategy is aimed to introduce and apply ecofriendly, economical, and effective measures in pest management (Vinutha *et al.* 2013). In order to obtain biologically active compounds, nanoparticles are extracted from various sources by using method of extraction precipitation in the water (Levy-Ruso and Toledano 2005). Margulis-Goshen and Magdassi (2012) suggested that nanoparticles are effective and successful alternatives to chemical control of insect pests, since they circumvent disturbing biological balance and reduce pollution. During the present study *S. littoralis*, a common pest on many crops, was selected as the model for screening and evaluating effectiveness of copper nanoparticles. In this paper, we have focussed on insecticidal properties of two copper nano sheets on *S. littoralis* and their potential in the insect pest management.

Materials and methods

Rearing the cotton leafworm

The cotton leafworm, *S. littoralis*, was reared in mass culture on a semi-artificial diet using the same procedure followed by Hegazi *et al.* (1979). This diet is modified from Shorey (1963). The diet comprises kidney beans (160 g), medical dried yeast (35 g), methyl-p-hydroxybenzoate (3.5 g), ascorbic acid (3.5 g), agar (13 g), formaldehyde (2.5 ml), and water to a total of 700 mL. The cotton leaf worm *S. littoralis* was mass-reared on the above semi-artificial diet. Larvae were the only bioassay test organisms used in the present investigation. Larval instars were determined by checking the shed head capsules (Mironidis and Savopoulou-Soultani 2008). Second and fourth instars in the pre-moult stage from the above cultures were held overnight without food, and those that moulted into the third and fifth instars within 12 hours were used in the experiments (6 × 20 larvae/experiment), which continued until the pupal stage was reached. Thus, all larvae were similar in a physiological condition, and of similar size and age. The gut of the newly moulted larvae was almost free of residual fecal material. The advantages of using the starved, newly moulted insects in growth and feeding studies were first described by Waldbauer (1962).

Nanomaterial synthesis and characterisation

Pure and silver (Ag)-doped copper oxide (CuO) nanosheets were synthesised using a research designed microwave oven (Milestone, Sorisole, Italy). The concentration of Ag as a dopant was 0.2 mol%. In this experiment, the procedure used for pure samples is similar to that described previously (Nahas *et al.* 2016), but with potassium hydroxide (KOH) replaced by sodium hydroxide (NaOH). Briefly, in this method, highly pure (99.99%) copper nitrate ($Cu(NO_3)_26H_2O$) and NaOH obtained from Sigma Aldrich (Steinheim, Germany) were used without further purification. They were used at a molar ratio of 1:20 by dissolving them in 200 mL of distilled water. The flask containing this solution was placed into a microwave oven and exposed to microwave irradiation at 750 W power for 20 minutes. The precipitate was collected and washed several times with distilled water and absolute ethanol. The clean material was then dried in an oven. For the Ag-doped samples, highly pure silver nitrate ($AgNO_3$) was used as a precursor. It was initially dissolved in 10 mL of distilled water and then added to the desired nitrate solution before adding NaOH.

The morphology of the synthesised pure and Ag-doped samples was studied using a field emission scanning electron microscopy (FESEM), JSM-7500 F (JEOL Ltd., Tokyo, Japan) operated at 15 kV. The presence of Ag dopant in the produced CuO material was estimated using the energy dispersive spectroscopy systems (EDS/EDX; Oxford Instrument, Abingdon, United Kingdom) attached to the FESEM. The samples were characterised by X-ray diffraction, using an Ultima-IV (Rigaku, Japan) diffractometer with Cu Kα radiation. The absorption spectrum for this sample was measured using a ultraviolet light–visible computerised spectrophotometer (model UV-1650PC; Shimadzu, Japan) in the wavelength region of 200–1100 nm.

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Raman spectra for the pure and Ag-doped CuO was recorded using a Raman Microscope (DXR model; ThermoFisher Scientific, Waltham, Massachusetts, United States of America) was used in this experiment. This system has a laser with a wavelength of 532 nm, which can be was used at an excitation power of 8 mW.

Experiments

Two materials (copper oxide (CuO) and copper oxide doped with silver (CuO:Ag)) were synthesised in their nonstructural forms. Preliminary tests were performed to determine the suitable series of test material concentrations. For each preparation, concentrations of the nanostructure materials (CuO and CuO:Ag) of 50, 100, 200, and 300 mg/150 mL diet were tested.

Each concentration was dissolved in 3 mL of distilled water and stirred for about six hours using an electric stirrer. The solution was then mixed with the hot diet to obtain an end volume of 150 mL of diet. The latter mixture was stirred for about five minutes using an electric stirrer while the diet was still soft at 50–55 °C and left for about two hours to solidify and become suitable for the larvae to eat (Kares 1978). The solidified diet was cut to small cubic pieces, ~ 2 cm^3, and placed into small clear plastic cups. Only newly moulted third and fifth instars (0–5 hours) were used in the experiments. For each concentration, five sets of test instars were used. The first four sets were offered the treated diet for 24, 48, 72, or more than 144 hours and were then left to feed on the untreated diet until pupation. Larvae in the fifth set were fed an untreated diet daily as a control treatment. The larvae were placed inside the previous cups (20 larvae/cup) and six replicates were used to investigate the effect of each tested concentration. The larvae were first starved for ~ 12 hours, as described above, to obtain rapid simultaneous ingestion of the diet, and were then continuously fed for a limited feeding period using the bioassay feeding diets. The experiments involved returning the larvae to the untreated diet after exposure. After treatment, each of the 20 larvae/replicate were returned to the five plastic cups (diameter, 9 cm; height, 5 cm) and fresh, untreated diet was provided daily until pupation. Larvae from the fifth set were fed daily on an untreated diet as a control. After treatment, all tested larvae were provided fresh diet cubes daily until they died or until they successfully developed into the adult stage.

All larvae were observed daily to record larval abnormalities and mortality. All malformed larvae in all treatments died and they were counted in the per cent mortality, as well as shown as a separate ratio. Assays were performed under standard laboratory conditions of 25 ± 1 °C and at 60 ± 5% relative humidity, with a 12-hour light photophase. The data are presented as the mean ± standard error of the mean of six replicates that included 20 larvae each.

Data analysis

The data, presented as mean ± standard error, were transformed into arcsine when necessary. The cumulative mortality (defined as the total number of larvae killed after treatment) and larval data were analysed using one-way analysis of variance (ANOVA) by using SAS software package 9.2. (SAS 2008). The assumptions of ANOVA were tested by homogeneity of variance (Levene's test) and normality of residuals (Shapiro–Wilk) tests. The SPSS 18.0 software (SPSS, Chicago, Illinois, United States of America) was used to analysis data for deformation of pupae to adults and Student *t*-test.

Results and discussion

Nanomaterial characterisation

The scanning electron microscopy images at different magnifications of the synthesised pure CuO nanostructures are shown in Figure 1A, D. These nanostructures have a dendrite shape and some sheet structures can also be seen. The lengths and widths of these structures are in the micro/submicro size range, while the thickness is much smaller in the nanoscale range (*i.e.*, ~ 10 nm). They formed small clusters and have an almost-uniform shape. This morphology in the present sample differs than that observed earlier (Nahas *et al*. 2016), where well-defined sheets could be formed. This might be because NaOH was used instead of KOH. These compounds are used as capping agents/modifiers and might have different strengths to control the growth and shape of this material. The produced nanostructures have an ultra-thin thickness and can be dispersed easily, which is a good indicator

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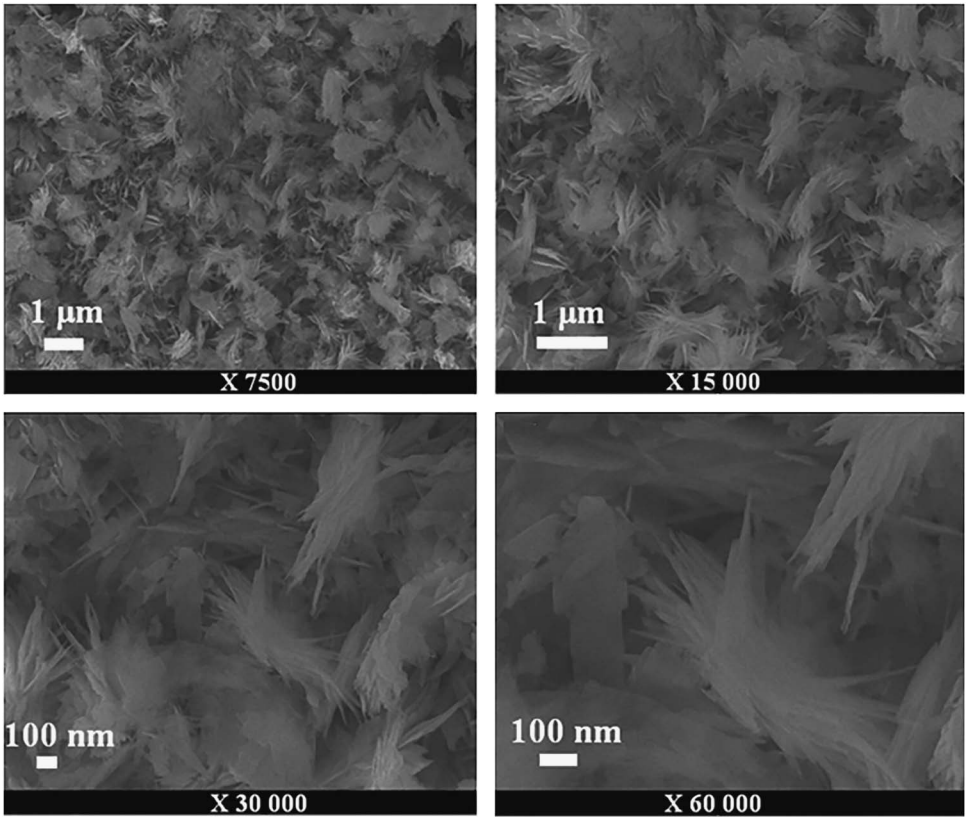
Fig. 1. Scanning electron microscope images of pure CuO nanostructures at different magnifications.



for this material to be used for different applications. Other groups described closer results, but different routes/precursors were used (Liu *et al*. 2013; Shahmiri *et al*. 2013; Lei *et al*. 2015; Maddinedi *et al*. 2015). The molar ratio of NaOH to $Cu(NO_3)_26H_2O$ was high (20:1), and therefore it is expected to create high pressure as a capping agent/modifier in the reaction. This pressure could form ultra-thin nanostructures. Zhi-Ang *et al*. (2009) used the hydrothermal process to produce CuO nanosheets. They dissolved Cu foil and NaOH in diluted hydrogen peroxide (H_2O_2, 30%). They showed large sheet structures compared with the present CuO nanosheets/dendrites. This might be because of the high NaOH concentration that was used in the present experiments. The scanning electron microscopy images of Ag-doped CuO nanostructures are shown in Figure 2. The observed structures are similar to the pure sample shown in Figure 1, with the only difference being a tendency to form non-uniform, larger clusters. The nanostructure produced also seems to have smaller dendrite structures. The Ag impurity may induce a surface charge in the doped powder samples, which could form larger clusters.

Qualitative energy dispersive spectroscopy results are shown in Figure 3A, and have been included in this study to confirm the presence of Ag dopant inside the CuO host. The element signals are clearly shown, indicating that the Ag precursor selection, along with the use of the microwave synthesis route, is the best choice for producing the Ag-doped CuO nanostructure. The X-ray diffraction patterns are used to examine the crystallinity and phase of the pure and Ag-doped CuO nanostructures. The obtained results are illustrated in Figure 3B. For the pure sample (curve i), all the diffracted peaks indexed as the monoclinic phase of CuO with JCPDS (International Centre for Diffraction Data, http://id.loc.gov/authorities/names/n78034812) reference code 98-001-7500.

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Fig. 2. Scanning electron microscope images of Ag-doped CuO nanostructures at different magnifications.

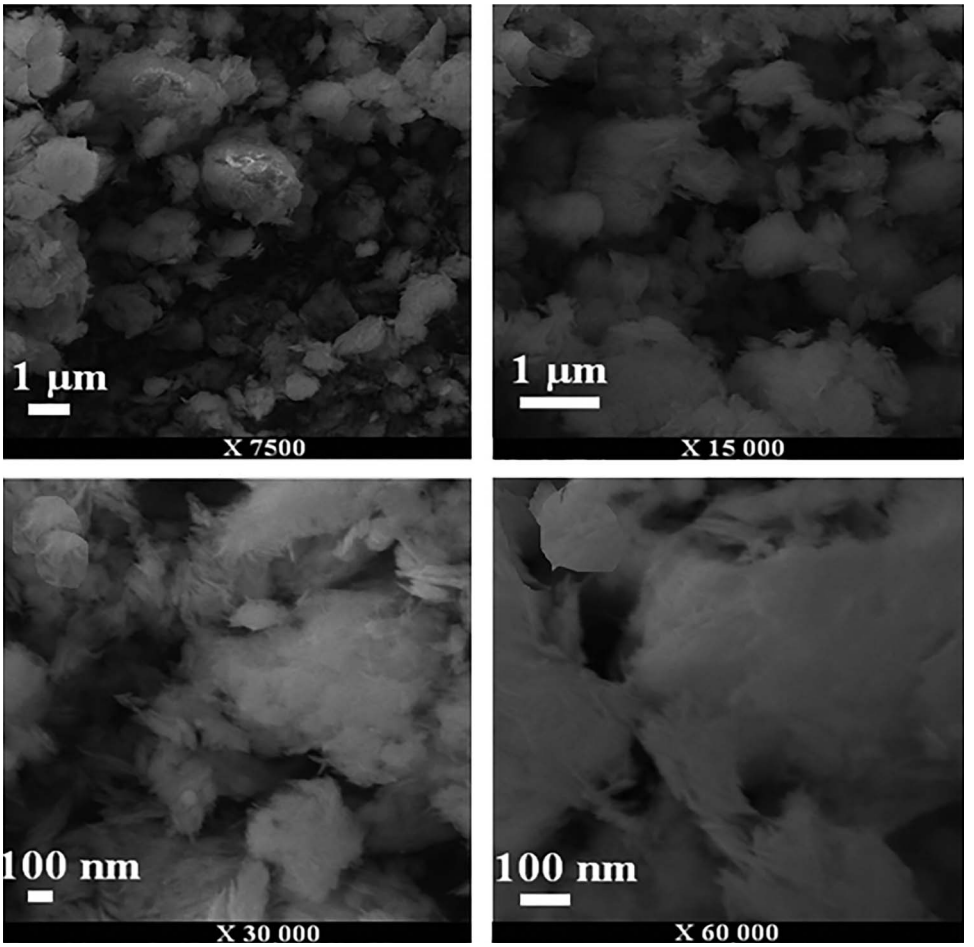
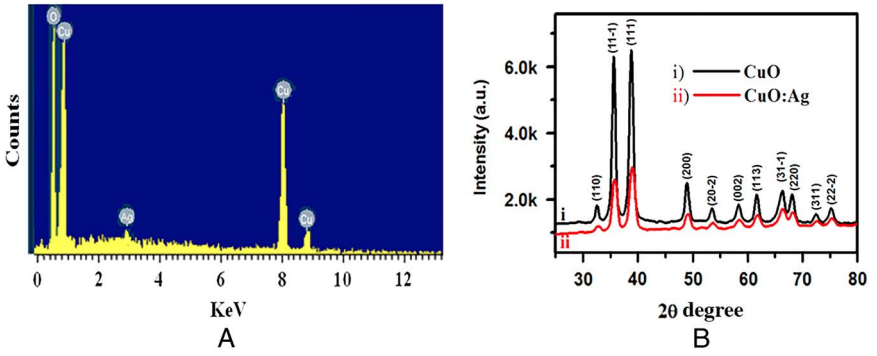


Fig. 3. (A) Qualitative energy dispersive spectroscopy results of Ag-doped CuO nanosheets; (B) X-ray diffraction patterns of pure and Ag-doped CuO nanosheets.



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This result is almost identical to previously reported results (Nahas *et al.* 2016). Similar results were also observed by other authors, who produced CuO nanosheets using other methods (Lei *et al.* 2015; Maddinedi *et al.* 2015). The Ag-doped sample (curve ii) has similar diffracted peaks that have lesser intensity. These peaks have a small shift by ~0.30. The reduction in the intensity of the diffracted peaks indicates a reduction in the crystallinity of the sample. No other peaks related to other phases are observed. This means that Ag ions could interstitially substitute Cu sites with no other defect in formation.

Figure 4A shows the ultraviolet–visible absorption of pure and Ag-doped CuO nanosheets (curves i and ii, respectively). The curve of the pure sample has a broad absorption range of 250–400 nm. The maximum intensity is located at ~295 nm. This result is similar to that reported by Felix *et al.* (2015), for microwave-synthesised CuO (using Cu acetate as the precursor and polyethylene glycol as a stabiliser in ethanol medium). They also estimated the value of the energy band gaps to be ~2.24 eV. The present CuO seems to have a similar value, which is higher than that of the bulk CuO (1.85 eV). This difference might be attributed to the decrease in size of the nanomaterial (Wang *et al.* 2010). The Ag-doped samples have a similar absorption spectrum, but the intensity is enhanced. This means that the energy band gap of CuO has not been affected by the Ag dopants.

Raman spectra of pure and Ag-doped CuO nanosheets are shown in Figure 4B (curves i and ii, respectively). Both the pure and Ag-doped samples have the regular Ag and Bg Raman vibration modes of CuO (Yu *et al.* 2004; Nahas *et al.* 2016). The Ag vibration mode is observed at 284/cm, while the Bg(1) and Bg(2) modes are located at 336/cm and 618/cm. The bands of the Ag-doped sample are shifted to the higher side by around 2/cm. This might be because of the reduction in nanostructure size in the Ag-doped CuO nanosheets. Similar results were also reported in the literature (Pal *et al.* 2012).

Larval mortality

Copper oxide: effect of varying exposure duration and concentration. Mortality of third-instar and fifth-instar *S. littoralis* was influenced by being fed diet containing CuO concentrations (in mg/150 mL diet) at varying exposure durations (Fig. 5). For the one-day fed larvae, the percentage of cumulative mortality significantly increased from third instars ($F = 260.3$, df = 4.25, $P < 0.05$) to the fifth instars ($F = 322.9$, df = 4.25, $P < 0.05$; Fig. 5A, 5B). Third instar mortality reached to $34.9 \pm 1.5\%$ and $48.13 \pm 0.43\%$ in the lowest and highest concentrations, and to $34.8 \pm 0.6\%$ and $46.6 \pm 1.0\%$ for the fifth-instar group. Additional differences in larval mortality were observed at the highest concentration when the larvae were fed for a period longer than two days for third instars (Fig. 5A: $F = 236.8$, df = 4.25, $P < 0.05$) and for fifth instars (Fig. 5B: $F = 444.7$, df = 4.25, $P < 0.05$), where larval mortality derived from the third or fifth instar was 10.0-times higher than the control. Similar results were obtained when larvae were fed a diet containing different concentrations

Fig. 4. (A) Ultraviolet–visible absorption and; (B) Raman spectra of pure and Ag-doped CuO nanosheets.

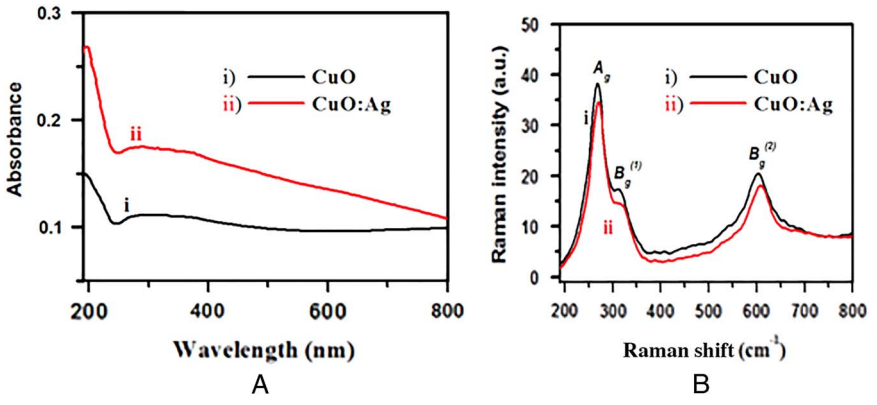

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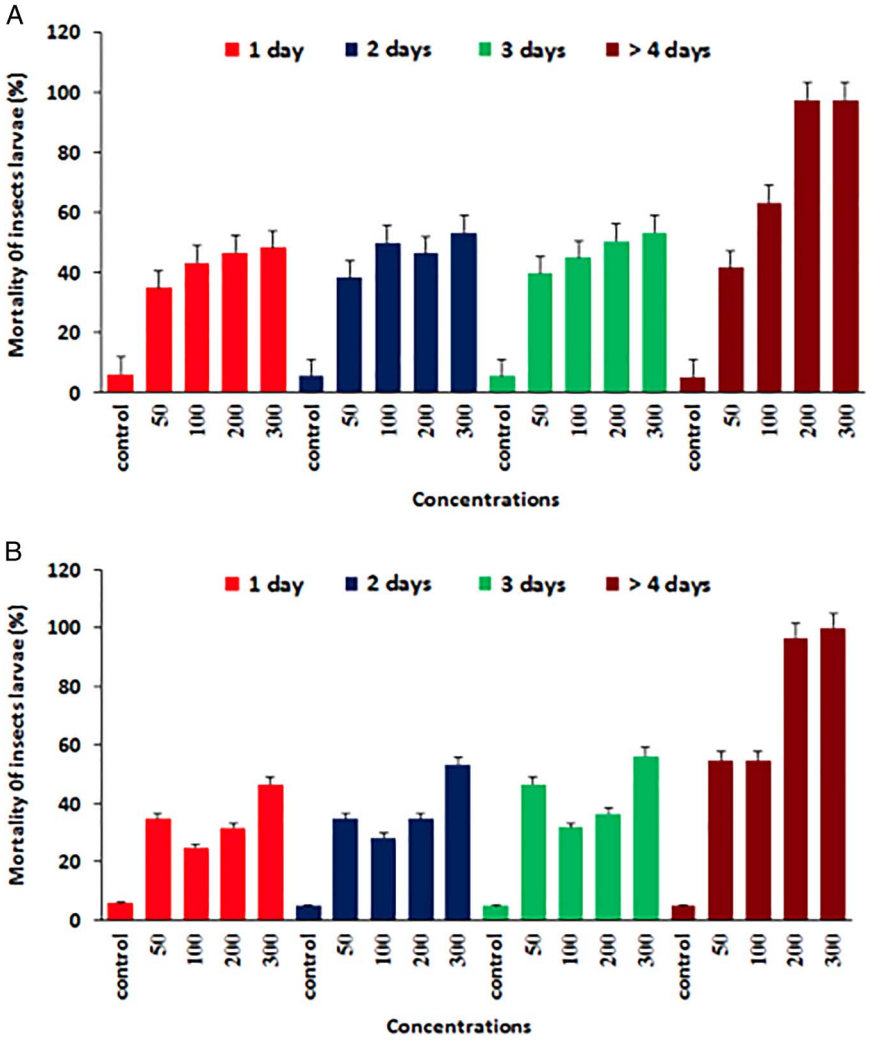
Fig. 5. Effect of different concentrations (mL) and varying exposure duration to CuO incorporated into the *Spodoptera littoralis* cumulative larval mortality when fed on the treated diet: (A) third instars; (B) fifth instars.



of CuO for three days (Fig. 5A: for third instars – $F = 775.9$, df = 4.25, $P < 0.05$; Fig. 5B: for fifth instars – $F = 604.9$, df = 4.25, $P < 0.05$). When the larvae were exposed for a longer feeding period (*i.e.*, for more than four days (four to six days; Fig. 5A–B)) the cumulative larval mortality increased significantly (Fig. 5A: for third instars – $F = 1679.7$, df = 4.25, $P < 0.05$; Fig. 5B: for fifth instars – $F = 4334.8$, df = 4.25, $P < 0.05$). We observed a progressive larval mortality from 41.6 ± 1.3% for larvae fed a diet containing 50 mL to 97.6 ± 1.3% for larvae fed a diet containing 200 or 300 mg among larvae derived from the third instar (Fig. 5A) compared with a mortality of 54.9 ± 0.6% to 100% for larvae derived from the fifth instar that were fed diets containing the same concentrations (Fig. 5B). For a diet containing 300 mg, the larval mortality derived from the third and fifth instars was 18.5 and 17.2 times higher than the control (Fig. 5A–B). In all test sets, higher mortality was observed at the higher concentration (300 mg) compared with the lower concentration (50 mg). Mortality in the control groups did not exceed 5.8 ± 0.2%.

Figure 5A–B shows the effect of a single concentration of 50, 100, 200, or 300 mg of CuO

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incorporated into the diet in relation to the length of the feeding period on the per cent mortality of *S. littoralis* larvae when the third or fifth instar was fed the treated diet. At each concentration, when the larvae were fed for longer feeding periods, the CuO had a greater insecticidal effect against *S. littoralis*. Significant differences were observed among larvae that were fed a 50-mg diet for one, two, three, or more than four days (Fig. 5A: for third instars – $F = 110.2$, df = 4.25, $P < 0.05$; Fig. 5B: for fifth instars – $F = 322.9$, df = 4.25, $P < 0.05$). For larvae fed the lowest concentration (50 mg), larval mortality among third-instar and fifth-instar groups reached $34.9 \pm 1.5\%$ and $34.8 \pm 0.6\%$ when fed for one day, and it increased to $41.6 \pm 1.3\%$ and $54.9 \pm 0.6\%$ when fed for more than four days (Fig. 5A–B). A significant increase was recorded when the CuO in the diet increased to 100 mg (Fig. 5A: for third instars – $F = 352.9$, df = 4.25, $P < 0.05$; Fig. 5B: for fifth instars – $F = 544.2$, df = 4.25, $P < 0.05$). Cumulative larval mortality among the third-instar and fifth-instar groups reached $43.3 \pm 1.8\%$ and $24.9 \pm 0.9\%$ when fed for one day, and it increased to $63.2 \pm 0.8\%$ and $54.9 \pm 0.6\%$ when fed for more than four days (Fig. 5A–B). When 200 mg CuO was incorporated into the diet, higher larval mortality was observed (Fig. 5A: for third instars – $F = 2322.7$, df = 4.25, $P < 0.05$; Fig. 5B: for fifth instars – $F = 2360.5$, df = 4.25, $P < 0.05$). Larval mortality among third-instar and fifth-instar groups reached $46.6 \pm 0.5\%$ and $31.6 \pm 1.0\%$ when fed for one day, and increased to $97.6 \pm 1.3\%$ and $96.6 \pm 0.7\%$ when fed for more than four days (Fig. 5A–B). There was a significantly higher effect when the CuO in the diet was increased to 300 mg (Fig. 5A: for third instars – $F = 274.6$, df = 4.25, $P < 0.05$; Fig. 5B: for fifth instars – $F = 1860.5$, df = 4.25, $P < 0.05$). Cumulative larval mortality in the third-instar and fifth-instar groups reached $48.3 \pm 0.43\%$ and $46.6 \pm 1.0\%$ when fed for one day, and it increased to $97.6 \pm 1.3\%$ and 100% when fed for more than four days (Fig. 5A–B). Mortality in the control groups did not exceed $5.8 \pm 0.2\%$. Thus, a longer feeding duration with the treated diet resulted in higher larval mortality.

Copper oxide doped with silver

Effect of varying exposure duration and concentration. Figure 6A–B shows the response of *S. littoralis* larvae when fed a semi-artificial diet containing a range of concentrations (50, 100, 200, or 300 mg Ag-doped CuO in a 150-mL diet) from the beginning of the third or fifth instar. For larvae fed for one day (Fig. 6A–B), the percentage of the cumulative mortality significantly increased with the increase of concentration in the diet (for third instars: $F = 245.2$, df = 4.25, $P < 0.05$; for fifth instars: $F = 294.1$, df = 4.25, $P < 0.05$). Larval mortality reached $31.6 \pm 1.1\%$ and $42.7 \pm 1.8\%$ for the low and high concentration in third-instar group (Fig. 6A), and $26.8 \pm 0.5\%$ and $39.6 \pm 0.9\%$ in fifth-instar group (Fig. 6B). Small differences in larval mortality were observed at the highest concentration when the larvae were fed for a longer period of two days using the same range of concentrations (Fig. 6A: for third instars – $F = 282.1$, df = 4.25, $P < 0.05$; Fig. 6B: for fifth instars – $F = 233.0$, df = 4.25, $P < 0.05$), where larval mortality derived from the third or fifth instar was 7.3 times higher than that of the control. Similar results were obtained when the larvae were fed a diet containing the above concentrations of Ag-doped CuO for three days (Fig. 6A: for third instars – $F = 568.7$, df = 4.25, $P < 0.05$; Fig. 6B: for fifth instars – $F = 627.6$, df = 4.25, $P < 0.05$). When the larvae were exposed for more than four days (four to six days), larval mortality increased significantly (Fig. 6A: for third instars – $F = 1238.7$, df = 4.25, $P < 0.05$; Fig. 6B: for fifth instars – $F = 238.1$, df = 4.25, $P < 0.05$). Larval mortality did not differ significantly. Mortality ranged from $53.5.6 \pm 0.7\%$ for a diet containing 50 mL to $57.6 \pm 0.4\%$ for a diet containing 300 mg among larvae derived from third instars (Fig. 6A) compared with $46.6 \pm 1.0\%$ to $48.2 \pm 0.8\%$ for larvae derived from fifth instars fed diets containing the same concentrations (Fig. 6B). In all test sets, higher mortality was observed among third-instar groups compared with fifth-instar groups. For larvae that were fed diets containing the highest concentration for the longest duration, the percentage and mortality were 10.8 and 7.6-times higher among the third and fifth-instar groups compared with control. Mortality in the control groups did not exceed $5.9 \pm 0.4\%$.

Figure 6A–B shows the effect of varying exposure durations to different concentrations of CuO doped with Ag, which was incorporated into the *S. littoralis* diet, on cumulative larval mortality

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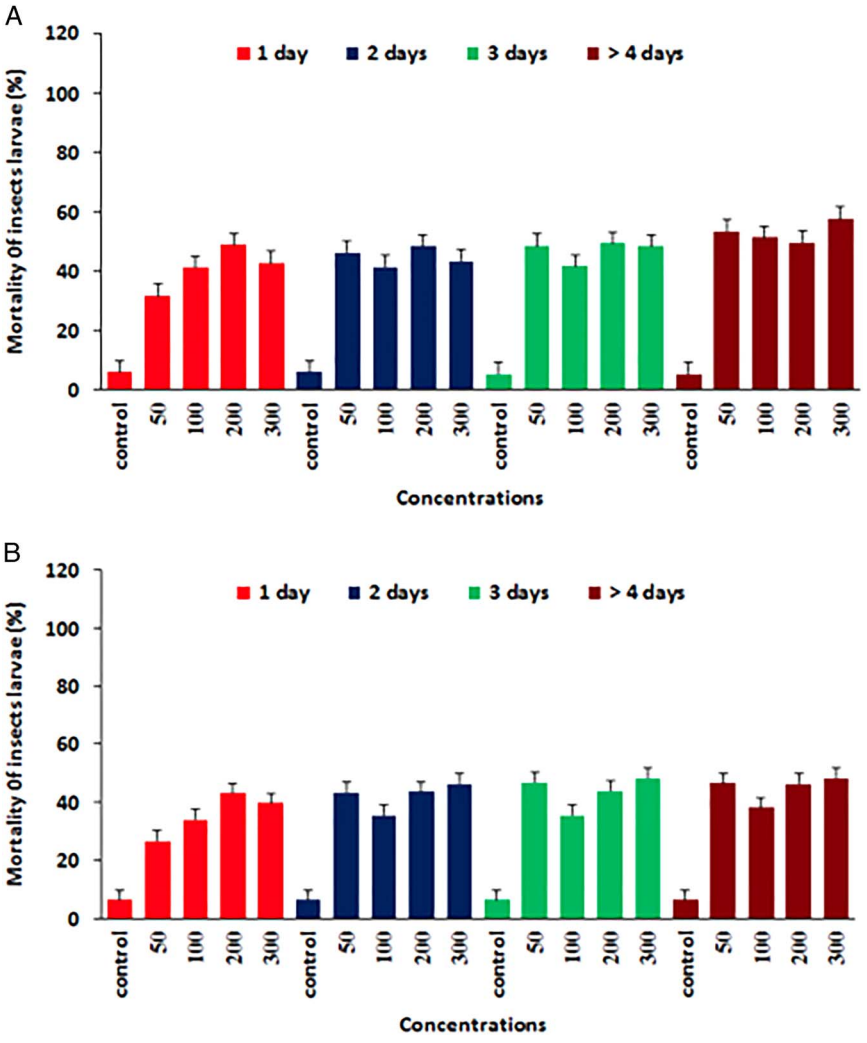
Fig. 6. Effect of different concentrations (mL) and varying exposure duration to CuO doped with Ag incorporated into the *Spodoptera littoralis* cumulative larval mortality when fed on the treated diet: (A) third instars; (B) fifth instars.



when fed a treated diet from the beginning of third or fifth instar. At each concentration, when the larvae fed for longer periods (four to six days), the CuO doped with Ag had an insecticidal effect against *S. littoralis*. Significant differences were recorded among larvae that were fed a 50-mg diet for one, two, three, or more than four days (Fig. 6A: for third instars – $F = 680$, df = 4.25, $P < 0.05$; Fig. 6B: for fifth instars – $F = 3292.0$, df = 4.25, $P < 0.05$). For larvae fed the lowest concentration (50 mg), cumulative larval mortality among the third-instar and fifth-instar groups was $31.6 \pm 1.1\%$ and $26.8 \pm 0.5\%$ when fed for one day, which increased to $53.5 \pm 0.7\%$ and $46.6 \pm 1.0\%$ when fed for more than four days (Fig. 6A–B). A moderate effect was recorded when the CuO doped with Ag in the diet was increased to 100 mg (Fig. 6A: for third instars – $F = 552.4$, df = 4.25, $P < 0.05$; Fig. 6B: for fifth instars – $F = 130.1$, df = 4.25, $P < 0.05$). Cumulative larval mortality among the third-instar and fifth-instar groups was $41.2 \pm 0.8\%$ and $34.0 \pm 1.1\%$ when fed for one day, and this increased to $51.3 \pm 0.8\%$ and $38.2 \pm 2.2\%$ when fed for more than four days (Fig. 6A–B). When 200 mg CuO doped with Ag was incorporated into

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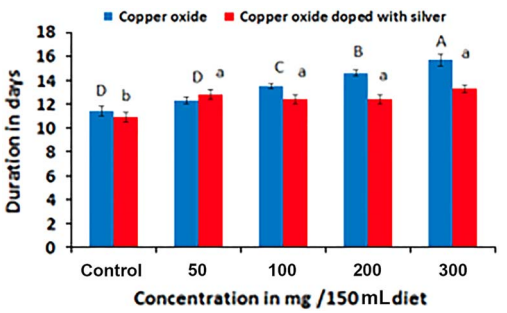
the diet, higher larval mortality was observed (Fig. 6A: for third instars – $F = 411.1$, df = 4.25, $P < 0.05$; Fig. 6B: for fifth instars – $F = 786.2$, df = 4.25, $P < 0.05$). Cumulative larval mortality among the third-instar and fifth-instar groups was 48.9 ± 0.9% and 43.1 ± 0.8% when fed for one day, and it changed to 49.6 ± 0.8% and 46.3 ± 0.5% when fed for more than four days (Fig. 6A, 6B). Significantly better efficiency against third instars was observed when the CuO doped with Ag in the diet increased to 300 mg (Fig. 6A: for third instars – $F = 725.5$, df = 4.25; $P < 0.05$; Fig. 6B: for fifth instars – $F = 250$, df = 4.25, $P < 0.05$). Cumulative larval mortality among the third-instar and fifth-instar groups was 42.7 ± 1.8% and 39.6 ± 0.9% when fed for one day, and increased to 57.6 ± 0.4% and 48.2 ± 0.8% when fed for more than four days (Fig. 6A, 6B). Mortality in the control groups did not exceed 6.3 ± 0.2%. Thus, feeding a treated diet for a longer period caused higher larval mortality.

Duration of larval development

Figure 7 shows the effect of incorporating different concentrations of CuO or CuO doped with Ag into the larval diet on the timing of larval development (in days) of *S. littoralis* when fed a treated diet from the beginning of the third instar. The incorporation of CuO into the diet had a significant effect on the timing of living larval development ($F = 6.4$, df = 4.25, $P < 0.05$), and this effect was concentration dependent. A maximum larval duration of 15.7 ± 0.5 days was found when the larvae were fed a diet containing 300 mg, compared with 14.6 ± 0.25, 13.5 ± 0.2, 12.3 ± 0.25, and 11.4 ± 0.4 days when they were fed diets containing 200, 100, 50, or 0 mg nanosheets, respectively (Fig. 7). Incorporating CuO doped with Ag resulted in a significant effect between all treated diets and control ($F = 6.3$, df = 4.25, $P < 0.05$). The timing of living larval development lasted 12.8 ± 0.4, 12.4 ± 0.4, 12.4 ± 0.4, and 13.3 ± 0.3 days at 50, 100, 200, and 300 mg, respectively compared with 10.9 ± 0.4 days for the controls (Fig. 7). Thus, incorporating the nanostructures prolongs the duration of living larvae stage.

Fig. 7. Mortality of third and fifth instars of *Spodoptera littoralis* exposed to various concentrations of CuO or CuO doped with Ag on the development of *S. littoralis*. Bars with the same uppercase or lowercase letter are not significantly different ($P < 0.05$).



Deformations of pupae and adult malformations

Deformations of pupae and malformations of adults resulted from the third and fifth instars of *S. littoralis* fed CuO and CuO doped with Ag are shown in Figure 8A, 8B ($n = 24$/concentration × 4 treatments). Both nanostructure products caused deformations in the *S. littoralis* pupae and adults. Feeding *S. littoralis* third larvae a diet treated with CuO produced 23.3 ± 1.2% and 6.6 ± 1.1% deformation between pupae and adults (moths), respectively (Fig. 8A), while as feeding *S. littoralis* fifth larvae a diet treated with CuO produced 15.0 ± 1.1% and 5.0 ± 0.7% deformation between pupae and adults (moths), respectively (Fig. 8A). The difference was significant ($t = 6.5$). The incorporation of CuO doped with Ag into the diet had a significant effect ($t = 2.23$) on the mean number of resulting deformed pupae (33.6 ± 2.1% and 21.4 ± 2.0%) and adult moths (5.0 ± 0.4% and 2.8 ± 0.6%), between third and fifth larvae, respectively as shown in Figure 8B.

Pest management in agriculture has often relied on toxic, broad-spectrum insecticides with negative impacts on insect pollinators and natural enemies. While the concept of integrated pest management was subsequently expanded to include the integration of biological, cultural, and chemical tactics in a compatible manner to achieve favourable economic and environmental consequences (Zhu *et al*. 2016). Continuous use of insecticides has frequently resulted in the development of resistance in insect pests that are targeted for population suppression. In recent years, increasing information on the hazardous

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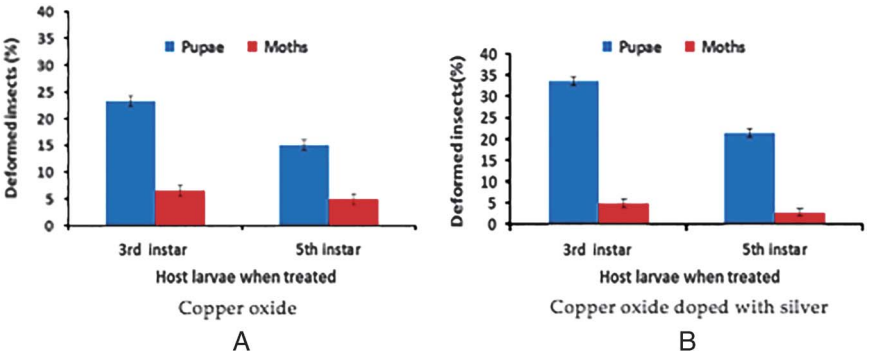
Fig. 8. (A) Effects of incorporating CuO or (B) CuO doped with Ag in the larval diet of *Spodoptera littoralis* when fed a treated diet from the beginning of the third or fifth instar (50–300 mg/150 mL diet) on percentages (mean ± standard error) of deformities among its pupae and moths. Deformities in control insects were nearly zero.



effects of synthetic insecticides on plant and animal health has prompted scientists to seek alternative methods that are ecofriendly. About 450 pest species of insects and mites have now developed resistance to synthetic pesticides (Georghiou 1986; Karunamoorthi and Sabesan 2012). The concept of using nanoparticles as crop protectants shows great potential. Although there have been numerous studies on the toxic effects of nanoparticles on bacteria, fungi, and animal pathogens (Bragg and Rannie 1974; Feng *et al.* 2000; Samuel and Guggenbichler *et al.* 2004; Elchiguerra *et al.* 2005; Reddy *et al.* 2007), little research has been performed to investigate the toxicity of nanoparticles on insects.

Nanotechnology is a promising field of research that has evolved in the present decade, and there are wide arrays of opportunities that are expected to provide strong support to technical innovations in many industrial sectors (Bhattacharyya *et al.* 2010). Previous research has confirmed that metal nanoparticles are effective against plant pathogens, insects, and pests. Thus, nanoparticles can be used in the preparation of new formulations such as pesticides, insecticides, and insect repellants (Barik *et al.* 2008; Owolade *et al.* 2008; Gajbhiye *et al.* 2009; Abo-Arab *et al.* 2014). *Spodoptera littoralis* is a common pest in many crops in different parts of the world, and it was, therefore, selected in the present study as a model to evaluate the insecticidal effect of both CuO and CuO doped with Ag at concentrations of 50, 100, 200, and 300 mg/150 mL diet by ingestion. Experiments involving both nanostructure products were conducted using six replicates, and each containing 60 larvae. Both of these nanostructure products had no immediate impact. Significantly better efficiency in larval mortality was observed when CuO in the diet was increased to 300 mg. A higher concentration and a longer exposure time to the nanostructures had an insecticidal effect against *S. littoralis* larvae (larvicidal effect). Mortality increased over time to 100% for a concentration of 300 mg CuO nanosheets. A higher cumulative larval mortality was observed at a higher concentration (300 mg) compared with the lower concentration (50 mg). Intermediate mortality rates corresponded to the intermediate concentrations (100–200 mg). Larval–pupal intermediates that were closer in shape to the larval features than to the pupal features, and larvae that expanded, did not move, and died were observed when intermediate concentrations were used (Fig. 9).

The cumulative larval mortality was higher in CuO diets (higher mortality was 100%) than on Ag-doped CuO diets (higher mortality was 57.6 ± 0.4%). The incorporation of nanostructures into the diet had a significant effect on the timing of living larval development. In addition, both nanostructure products caused deformations in the *S. littoralis* pupae (pupicidal effect) and in adults (adulticidal effect). Adding Ag produced more deformation compared with the CuO nanosheet treatment only. These deformations may be associated with hormonal balance of the insect.

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Fig. 9. Deformations of *Spodoptera littoralis* larvae required to fed a treated diet of CuO and CuO:Ag nanosheets.

Food intake and, to a greater degree, larval weight gain (although not quantified) were reduced soon after the third instars began feeding on a diet treated with 300 mg CuO (the antifeedant effect) (Margulis-Goshen and Magdassi 2012). CuO seems to have decreased the ability of the larvae to convert ingested food into growth. Thus, the antifeedant effect and the cumulative larval mortality were concentration dependent. Results showed that CuO was more influential than CuO doped with Ag at the all concentrations and for similar exposed periods. Our results suggest that the effects of nanosheets may have important implications for population dynamics of the cotton leafworm. Further research is required to test nanostructures for management of the pest in the field.

Conclusions

The continuing increase in resistance to insecticides against insect pests led to the discovery of new technology or new nanomaterials to use as effective and safer insecticides. Nanosheets of CuO and CuO doped with Ag provide a good platform to overcome insect resistance to insecticides. The cumulative larval mortality was higher in CuO diets compared with Ag-doped CuO diets. The incorporation of nanostructures into the diet had a significant effect on the timing of living larval development. In addition, both nanostructure products caused deformations in the *S. littoralis* pupae and adults. Adding Ag produces more deformation than treatment with CuO nanosheets only. These deformations may be associated with the hormonal balance of the insect. Our results suggest that the effects of nanosheets may have important implications for the population dynamics of the cotton leaf worm *S. littoralis*. Further research is required to test the effect of the nanostructures in managing the pest in the field.

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (grant number G-570-305-36). The authors thank the Deanship of Scientific Research for the technical and financial support. The authors also thank Professor Esmat Hegazi (Faculty of Agriculture, Alexandria University, Egypt) for assisting with the statistical analysis and for technical advice.

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