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Research Article
Toxicity and Growth-Disruptive Effects of Silica, Zinc and Aluminum Oxide Nanoparticles on Spiny Bollworm, *Earias insulana*

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Abstract

Background and Objective: Nanoparticles (NPs) are the most efficient technique of pest control without disturbing the potential environmental balance. This study was carried out to compare the toxicity effects of Silica, Zinc and Aluminum oxide NPs on growth, development, malformation and chitin formation parameters of *Earias insulana*. **Materials and Methods:** Newly hatched larvae fed on the treated diet for 48 hrs following by the untreated one until pupation. The toxicity impacts of LC_{50}values for each tested NPs were calculated. The fourth instar larvae prepared for scanning electron microscopy and also for defining the chitin formation. **Results:** The ZnONPs was the highest toxicity, followed by SiO_2 and Al_2O_3 NPs. The treatment with ZnONPs has scored an extreme reduction in the weight compared to other tested NPs. The growth rate inhibition decreased gradually until the fourth instars and then increased slightly in the prepupae and pupae in favor of ZnONPs, while Al_2O_3 NPs was the least effective one. Under-tested NPs, the increase in the larval and pupal periods led to a sharp reduction in the developmental rate and a significant increase in larval deformations. Besides, the least chitin formation ratio was registered in response to ZnONPs. By using electron microscopy, distinct differences were observed in the distribution of NPs on the cuticle surface of the fourth-instars body parts. **Conclusion:** This study recommends the practical use of ZnO, SiO_2 and Al_2O_3 NPs, respectively, in pest control programs of *E. insulana* as safe alternative materials.

Key words: Metal oxide, toxicity effects, growth, development, malformation, chitin formation, cotton bollworms, pest control

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egypt is still one of the leading countries interested in cotton cultivation due to its economic importance. Therefore, the government strived to increase its competitiveness in producing high-quality cotton in various international markets[1]. Reports have proven that many pests attack cotton plants during their growth period. Cotton bollworms are prevalent among various insects, causing many economic losses in the rate of cotton production[2]. The Spiny Boll Worm, *Earias insulana* (Boisd.) Lepidoptera: Noctuidae is one of the most dangerous pests, not only on the cotton plants but also many other crops, in Egypt and other North African countries. Its larvae cause damage to seeds, fibers, flowers and buds, particularly at the final growing stage of the cotton plants. It induces a decrease in the quantity and quality of the lint and the oil extracted from yield[3]. The extensive spread of pesticides has increased the serious response associated with environmental pollution such as toxic waste, natural enemies and pest resistance. Therefore, it is crucial to look for other alternatives to avoid using conventional pesticides in the control program of the cotton bollworms[4]. In recent years, pest control strategies are designed towards eco-friendly agriculture to maintain environmental balance[5].

Nanoparticles have become a new generation of pest control techniques and an alternative strategy to the traditional pesticides that have provided effective resolutions to most environmental pollution problems[6]. Pest control with NPs depends on the penetration rate of the active substance through the target living cells, according to the size and properties of the outer surface of the particles compared to the volume rate[7]. The size of the NPs ranges between 10 and 1000 nanometers. A group of materials is useful for making NPs, such as silicates, metal oxides, lipids, emulsions and polymers[8,9]. Metal NPs such as ZnO, Al_2O_3, Ag and TiO_2 released the active substance for a relatively long period, causing slow degradation of the living cells as a result of the toxic effects on insects[10,11].

Various studies have tested the toxicity of NPs in controlling many insects, but they have not revealed the most potent compound in protecting cotton plants from *E. insulana*. El-Ghohary and El-Samahy[12] showed statistical differences toward SiO_2 NPs in reducing cotton bollworms *Pectinophora gossypiella* (Saunders) and *E. insulana* (Bois d.) compared to other insecticides. Haroun *et al.*[13] proved that ZnO and SiO_2 NPs showed a toxic influence against some stored seed insects at the highest concentration. El-Shennawy and Kandil[14] cleared that treatment with ZnO NPs against *E. insulana* increased the larval and pupal duration and decreased their weight more than the chitin inhibitor and bio-insecticides that were used. Debnath *et al.*[15] evidenced that Al_2O_3 and SiO_2 were achieved to be highly toxic pesticides compared to other Nano pesticides against *Lipaphis pseudo brassicae*. The present study planned to compare the entomotoxic efficacy of tested NPs (SiO_2, ZnO and Al_2O_3) against the larval and pupal stage of *E. insulana* when treated as newly hatched larvae and its toxicological impacts on growth and developmental parameters. Also, malformation and chitin formation rate induced by tested NPs were studied.

MATERIALS AND METHODS

Study area: This study was carried out in the Entomology Lab, Department of Zoology, Faculty of Science, Zagazig University, Egypt, from August, 2019 to October, 2020.

Tested insects: Newly hatched larvae of *E. insulana* were obtained from Plant Protection Research Institute, Sharkia Branch, Agricultural Research Center (ARC). The colony was reared on an artificial diet for several consecutive generations without any contamination with insecticides under constant laboratory conditions at 26±2°C, 65±5% RH and 12 hrs (L:D) photoperiod according to Amer[16].

Tested nanoparticles: Silica (SiO_2), Zinc oxide (ZnO) and Aluminum oxide (Al_2O_3) NPs have an average size of 50 nm. These particles have a purity of more than 99%. The SiO_2 NPs and ZnONPs were purchased from Egypt Nanotech Co. limited, 6th October, Giza, Egypt, while Al_2O_3 NPs were purchased from Lab Chemical Trading Co., Cairo, Egypt.

Toxicity values of the tested nanoparticles: To evaluate the lethal concentration LC_{50} of each tested nanoparticles; five gradient concentrations (1000, 500, 250, 125 and 62.5 ppm) were prepared for each compound by dilution in distilled water. An artificial diet (5 g/ replicate) was made in a petri dish and sprayed with different concentrations for each nanoparticle that was left to dry. Distilled water was used alone to treat the control sample. Newly hatched larvae of *E. insulana* (10 larvae/replicate) were directly fed on the treated diet for 48 hrs and then replaced with the untreated one until pupation. The diet of the control sample replaced when needed. Each concentration of ZnO, SiO_2 and Al_2O_3 NPs replicated four times. The number of larvae used in the control

sample and the number of the replicates equaled with each tested NPs concentration. Mortalities were counted daily until the 18th day to estimate the percentage of the accumulative larval mortality at indicated days after the exposure of newly hatched larvae of *E. insulana* to different concentrations of the tested NPs consequently; all mortalities were corrected by using Abbott's Eq.[17]:

$$\text{Corrected mortality (\%)} = \frac{\text{Test mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

To avoid the harmful effects of NPs overdoses by the toxicity impacts of LC_{50}values for each tested nanoparticle were calculated to determine the growth rate inhibition and the developmental rate of the immature stages of *E. insulana* when treated as newly hatched larvae. Linear regression variables were also analyzed to predict mortality according to the various potential concentrations. Toxicity index studied according to Sun's Eq.[18]:

$$\text{Toxicity index (\%)} = \frac{LC_{50} \text{ of most toxic material}}{LC_{50} \text{ of the tested material}} \times 100$$

Growth and developmental parameters: One hundred larvae per nanoparticle were treated using lethal concentration LC_{50} pre-calculated for SiO_2, ZnO and Al_2O_3 NPs with values of 168.94, 89.41 and 707.85 ppm, respectively. The same numbers of the control sample larvae were studied using distilled water instead of LC_{50} of the NPs. Each treatment was replicated four times (25 larvae/replicate), where treated larvae were fed on a previously treated diet with LC_{50} values for each nanoparticle. Additionally, live control larvae were transferred individually to glass tubes 20 mm in diameter, which including 2 g of the untreated diet that was kept under previous laboratory conditions. Larvae and pupae weights were recorded daily using a digital scale from 1st instar larvae until the end of the pupal stage. The pupae were individually kept in Glass jars (250 mL) covered with a white mesh. The growth rate inhibition was calculated as follows:

$$\text{Growth inhibition (\%)} = \frac{\text{Maximal weight of (treated sample} - \text{control sample)}}{\text{Maximal weight of the control sample}} \times 100$$

The developmental rate induced by tested NPs calculated using Dempster's equation[19]. Besides, the growth index of the larval and pupal stages of *E. insulana* was calculated using Pretorius's equations[20], as follows:

$$\text{Developmental rate (\%)} = \frac{100}{\text{Mean duration (days)}}$$

$$\text{Larval growth index (\%)} = \frac{\text{Pupation (\%)}}{\text{Laval period (days)}}$$

$$\text{Pupal growth index (\%)} = \frac{\text{Emergence (\%)}}{\text{Pupal period (days)}}$$

Larvae preparation for SEM observation: Fourth instar larvae of *E. insulana* were transferred in primary fixed with 2.5% glutaraldehyde+2% formaldehyde and washed 3×15 min in 0.1 M sodium phosphate buffer pH 7.4+0.1 M Sucrose, post-fixed with 2% sodium phosphate buffer, osmium tetroxide pH 7.4 and washed 3×15 min in 0.1 M sodium phosphate buffer pH 7.4, dehydrated sequentially with ethanol (50, 80, 90, 96, 100%/2×15 min each), contrasted overnight using 70% acetone+0.5% uranyl acetate+1% phosphotungstic acid, after dehydrated with ethanol (in distilled water), at 4°C. Larvae were then coated with gold-palladium membranes and observed in a Jeol JSM-6510 L.V SEM. The microscope was operated at 30 KV at SEM Unit, Mansoura University, Egypt.

Chitin formation of the body wall: The tested NPs with LC_{50} values were placed in the diet of 10 newly molted of the fourth instar larvae (4 replicates/treatment), none treatment used for the control larvae. Deformed and freshly larvae were weighed after 48 hrs from treatment. The larvae were anesthetized by cooling and dissected longitudinally from the ventral side after removing the head. The gut, various internal tissues and fat bodies were extracted. The body wall was placed in 3 mL of 10% potassium hydroxide (KOH) per each gram of the larval weight to 4 hrs at 100°C and left at room temperature overnight. Chitin extracts were dried at 80°C overnight and then weighed for each larva and compared with chitin larval fresh weight. The chitin formation ratio induced by tested NPs against fourth instar larvae of *E. insulana* was determined according to equation of Hughes *et al.*[21]:

$$\text{Chitin formation ratio (\%)} = \frac{\text{Chitin dry weight (mg)}}{\text{Larval fresh weight (mg)}} \times 1000$$

Statistical analysis: The toxicity data under LC_{50} values of the tested NPs were estimated using probit analysis[22]. Data were used one-way ANOVA, followed by LSD's test to check the significant differences between means by SPSS software.

RESULTS

Toxicity values of the tested NPs against *E. insulana*: The obtained data in (Table 1) summarized that the LC_{50} values for SiO_2, ZnO and Al_2O_3 NPs after the treatment of the newly hatched larvae of *E. insulana* by different concentrations were (168.94, 89.41 and 707.85 ppm, respectively). Moreover, the current results revealed that the highest tested NPs concentrations induced the larval mortality rate (83.78, 86.49 and 54.05%, respectively). Based on these values, *E. insulana* larvae were more sensitive to ZnO NPs than other tested NPs. The displayed data in (Fig. 1) indicated that the ZnO NPs ratio was the highest toxicity followed by SiO_2 and Al_2O_3 NPs against the newly hatched larvae of *E. insulana* at (100, 52.92 and 12.63%, respectively), according to the Sun's toxicity index and based on the LC_{50} values of the most toxic compound among the various tested NPs.

Accumulative larval mortality induced by tested NPs against *E. insulana*: The data in Fig. 2a-c showed the cumulative mortality rate of *E. insulana* larvae (3, 6, 9, 12, 15 and 18 days) after the exposure of the newly hatched larvae to different concentrations of the tested NPs for 48 hrs. The results also showed that the highest ratio of larval mortality, at a concentration (1000 ppm), associated with an increase in the time after treatment (18^{th} day), where it recorded 85, 87.5 and 57.5% mortalities, while the lowest mortality was 32.5, 35 and 17.5% at 62.5 ppm concentration of SiO_2 (Fig. 2a), ZnO (Fig.2b) and Al_2O_3 NPs (Fig. 2c), respectively. The data in Fig. 2a indicated gradual larval mortality (32.5, 47.5, 65, 75 and 85%, respectively) in ascending order at the various concentrations of SiO_2 NPs. Meanwhile, the mortality was ranged from 35-87.5% under ZnO NPs treatment (Fig. 2b). The results also scored 17.5% at 62.5 ppm and graduated up to 57.5% at 1000 ppm when larvae were treated with Al_2O_3 Nps. The results also showed that the larval mortality rate reached its highest value at a concentration of 1000 ppm on the 18th day after treatment with the tested nanoparticles, where 32.5, 47.5, 65, 75 and 85% mortalities were recorded when treated with various concentrations of SiO_2 NPs, respectively (Fig. 2a). The data in Fig. 2b indicated that the cumulative larval mortality was 35, 62.5, 70, 85 and 87.5%, respectively, according to the ascending order of concentrations in ZnO NP. Also, overall mortality under the various prior concentrations of Al_2O_3 NPs in Fig. 2c was 17.5, 27.5, 40, 47.5 and 57.5%, respectively.

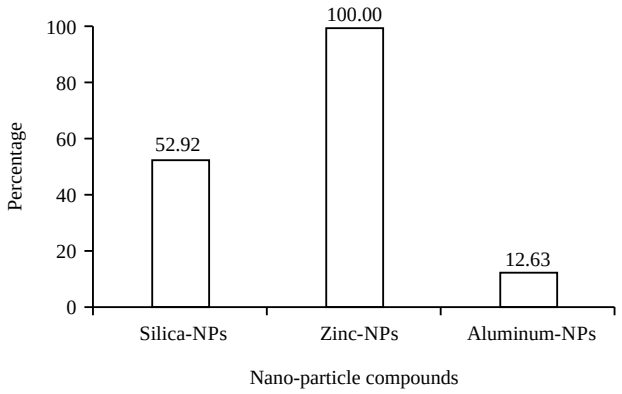

Fig. 1: Toxicity indexes of SiO_2, ZnO and Al_2O_3 NPs against the newly hatched larvae of *E. insulana* relative to zinc oxide NPs the most effective compound

Table 1: Toxicity values of the tested NPs after the treatment of newly hatched larvae of *E. insulana* under laboratory conditions

Treatments	Concentration (ppm)	Larval mortality (%) Total	Corrected	LC_{50} (ppm) (95% CL)	Intercept	R^2	Slope±SE
Silica-NPs	1000	85.00	83.78	168.94 (88.62-322.06)	2.055	0.994	1.321±0.143
	500	75.00	72.97				
	250	65.00	62.16				
	125	47.50	43.24				
	62.5	32.50	27.03				
Zinc-NPs	1000	87.50	86.49	89.41 (41.69-191.74)	2.780	0.968	1.139±0.169
	500	85.00	83.78				
	250	70.00	67.57				
	125	62.50	59.46				
	62.5	45.00	40.54				
Aluminum-NPs	1000	57.50	54.05	707.85 (321.12-1560.31)	1.883	0.977	1.093±0.201
	500	47.50	43.24				
	250	40.00	35.14				
	125	27.50	21.62				
	62.5	17.50	10.81				

LC_{50}: Lethal concentration that killed 50% of the larvae, CL: Fiducial confidence limits, R^2: Regression coefficient, SE: Standard error

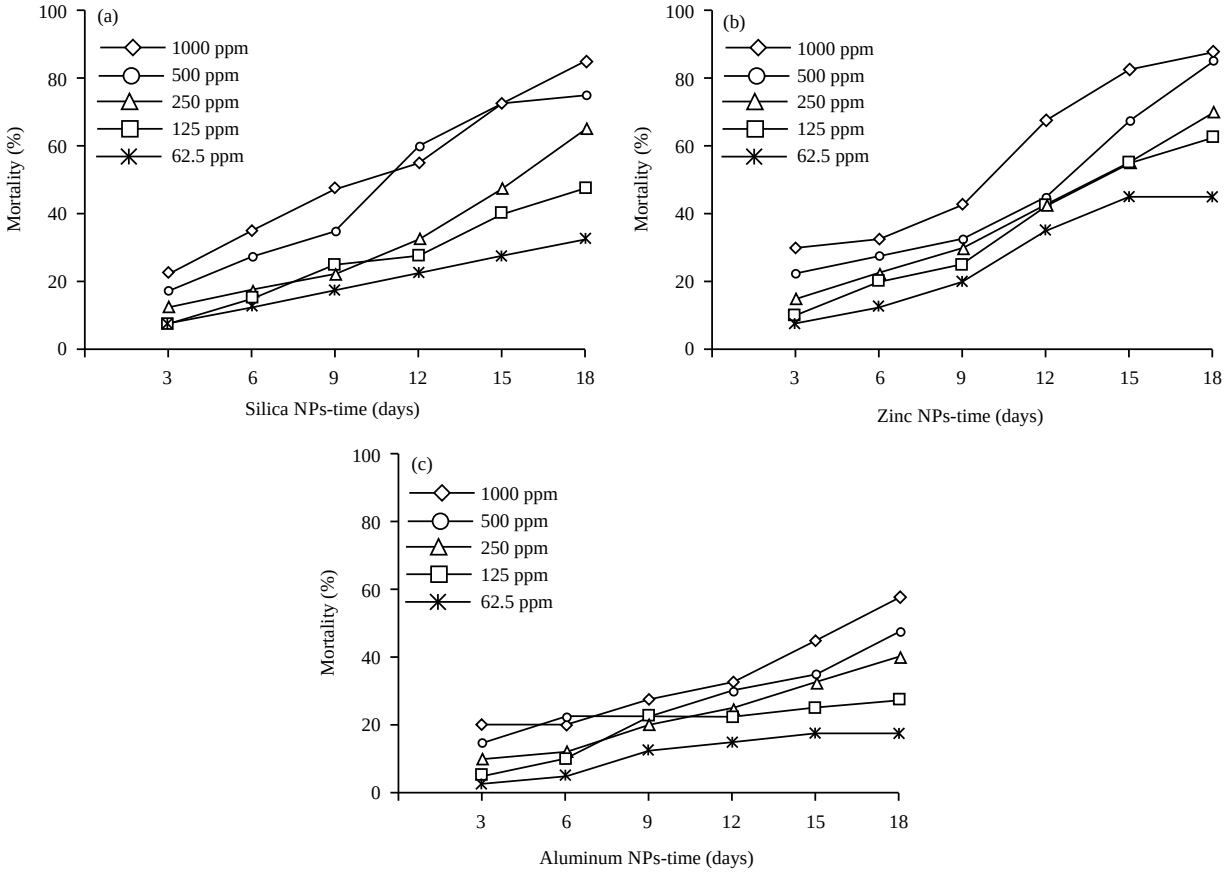

Fig. 2(a-c): Percentage of accumulative larval mortality at indicated days after the exposure of the newly hatched larvae to different concentrations of NPs, (a) SiO_2, (b) ZnO and (c) Al_2O_3

Table 2: Toxicity impacts of LC_{50} of the tested NPs on the weight and duration of immature stages of *E. insulana* when treated as newly hatched larvae

	Nano-particle compounds				
Parameters	Silica-NPs	Zinc-NPs	Aluminum-NPs	Control	$LSD_{0.05}$
1st instars weight	3.67±0.45^{c}	3.29±0.50^{c}	5.92±0.75^{b}	13.21±1.25^{a}	0.667
2nd instars weight	8.93±0.90^{c}	4.55±0.71^{d}	14.54±1.75^{b}	29.65±3.05^{a}	1.680
3rd instars weight	15.35±1.75^{c}	12.97±1.35^{c}	29.27±2.88^{b}	47.80±5.22^{a}	3.111
4th instars weight	31.50±3.84^{c}	17.85±1.90^{d}	54.95±6.49^{b}	78.45±9.63^{a}	6.772
Pre pupae weight	19.33±2.25^{c}	11.08±1.25^{d}	35.84±4.15^{b}	65.70±7.44^{a}	4.869
Pupae weight	14.45±1.90^{c}	10.75±0.95^{c}	22.33±2.50^{b}	50.90±6.06^{a}	3.770
Larval duration	22.30±2.90^{b}	27.25±1.95^{a}	18.50±1.75^{c}	13.90±1.50^{d}	1.324
Pupal duration	12.35±1.45^{b}	14.40±1.50^{a}	11.83±1.25^{b}	8.85±1.00^{c}	0.832

Average weight in mg±SD, Mean duration in days±SD, LSD: Least significant difference, No significant differences at p>0.05 when repeating the same alphabet above the means in each row

Growth, developmental and malformation parameters induced by the tested NPs against *E. insulana*: The current results in (Table 2) showed significant differences between the treated larvae with the tested NPs and untreated larvae in all treatments. The comparison data between tested NPs indicated statistical differences in larval weights in the direction of ZnO NPs at 4.55, 17.85 and 11.08 mg for the 2nd, 4th instars and prepupae, respectively. Moreover, SiO_2 topped Al_2O_3 NPs with values 8.93, 31.50 and 19.33 mg at the same previously mentioned ages, respectively. Otherwise, there were no significant differences between SiO_2 and ZnO NPs in the weights of 2nd, 3rd instars and pupae at 3.67, 15.35 and 14.45 mg compared to 3.29, 12.97 and 10.75 mg, respectively.

Table 3: Toxicity impacts of the LC_{50} of tested NPs on the growth rate inhibition of the immature stages of *E. insulana* when treated as newly hatched larvae

	Nano-particle compounds					
	Silica-NPs		Zinc-NPs		Aluminum-NPs	
Parameters	Reduction	Growth	Reduction	Growth	Reduction	Growth
1st instar larvae	9.54±0.85	72.22	9.92±0.87	75.09	7.29±1.02	55.19
2nd instar larvae	20.72±1.95	69.88	25.10±1.92	84.65	14.71±2.38	49.61
3rd instar larvae	32.45±3.45	67.89	34.83±3.25	72.87	18.53±3.99	38.77
4th instar larvae	46.95±6.75	59.85	60.60±5.75	77.25	23.50±7.95	29.96
Pre pupae	46.37±4.85	70.58	54.62±4.85	83.14	29.86±5.78	45.45
Pupae	40.15±3.98	71.61	40.15±3.98	78.88	28.57±4.25	56.13

Mean reduction in mg±SD, Growth in percent (%)

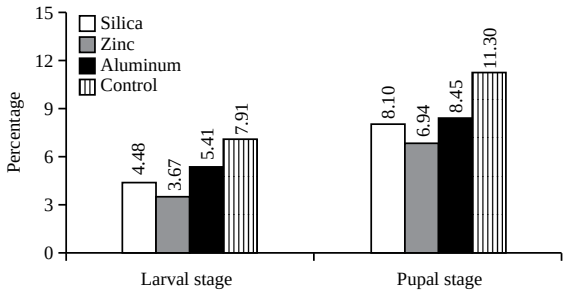

Fig. 3: Larval and pupal developmental rate of *E. insulana* when treated by LC_{50} values of the NPs

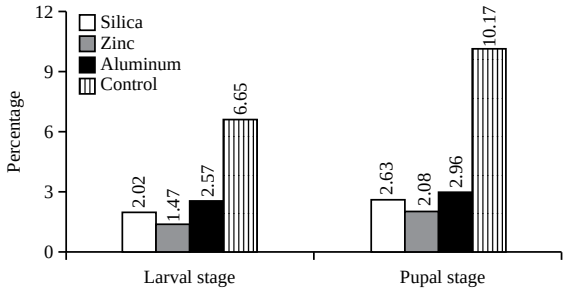

Fig. 4: Larval and pupal growth index of *E. insulana* when treated by LC_{50} values of the tested NPs

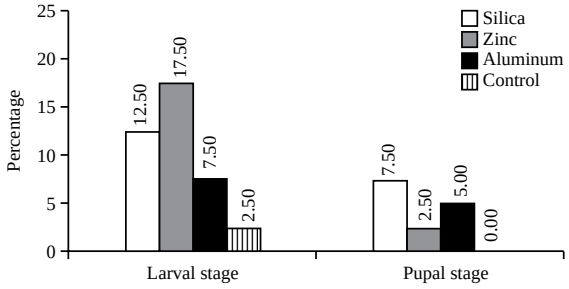

Fig. 5: Larval and pupal malformation rate of *E. insulana* induced by the tested NPs and untreated samples

The growth rate inhibition values in (Table 3) decreased gradually until the fourth instar larvae at 59.85, 77.25 and 29.96% for SiO_2, ZnO and Al_2O_3 NPs, respectively, then these values increased slightly in the prepupae and pupae. The results also indicated that ZnO NPs induced the growth rate inhibition during the various instar larvae and pupae at 75.09, 84.65, 72.87, 77.25, 83.14 and 78.88%, respectively, while Al_2O_3 NPs was the least effective one at 55.19, 49.61, 38.77, 29.96, 45.45 and 56.13%, respectively.

Data in (Fig. 3) revealed that tested NPs affected the developmental rate of the larval and pupal stages of *E. insulana* when treated as newly hatched larvae, where it severely reduced in the larval stage at 4.48, 3.67 and 5.41%, for the tested NPs, respectively, vs. 7.19% of the untreated larvae. Furthermore, the pupal developmental rate prolonged to 8.10, 6.94 and 8.45% for the tested NPs, respectively, vs. 11.30% of the untreated pupae. Moreover, data in (Fig. 4) showed that the growth index in the larval stage was 1.47, 2.05 and 2.57% on ZnO, SiO_2 and Al_2O_3 NPs, respectively, vs. 6.65% of the untreated larvae. The highest pupal growth index was 2.96% on Al_2O_3 NPs, while the lowest was 2.08% on ZnONPs, vs. 10.17% of the untreated pupae. The larval and pupal malformation rates are displayed in (Fig. 5), where the larval deformation significantly was increased at (12.5, 17.5 and 7.5% for SiO_2, ZnO and Al_2O_3 NPs, respectively) compared to 2.5% of control larvae. These ratios were not associated with the larval mortality rate, but actually, they were related to the morphological changes in the lived larvae. The current results exhibited an evident decrease in the pupal malformation percentages with 7.5, 2.5 and 5% for the tested NPs, respectively, without any malformed pupae in the control sample.

Under the electron microscopy (SEM), distinct differences were showed in the distribution of NPs on the epicuticle surface of different body parts (head, thorax and abdomen) of the spiny bollworm fourth-instars, (Fig. 6a-h). Larval dwarfism, severe malformed thorax cuticle and head capsule were displayed in (Fig. 6a) creating due to the treatment with SiO_2 NPs also, abnormal abdominal cuticle induced by a high density of SiO_2 NPs (Fig. 6b). The highest deformations were represented in (Fig. 6c-d) by the stiffness of the epidermal wax

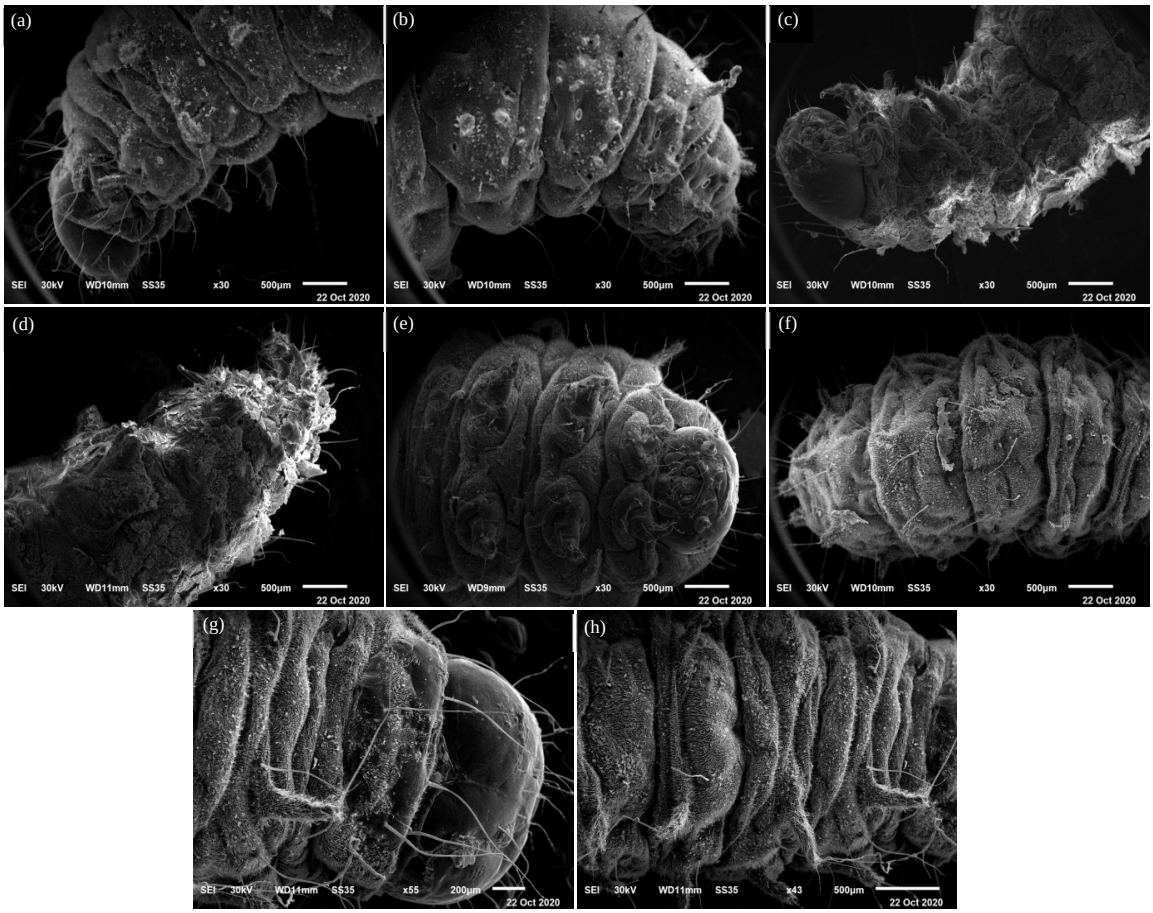

Fig. 6(a-h): SEM of epicuticle surface in the head, thorax and abdomen region of the fourth instar larvae *E. insulana* induced by the tested NPs, (a) Larval deformation induced by SiO_2 NPs, causing sever malformed thorax cuticle and head capsule, (b) Abnormal abdominal cuticle produced by a high density of SiO_2 NPs, (c) Stiffness of the epidermal wax layer resulting from ZnO NPs, in the thorax, (d) Abdomen, (e) A low density of Al_2O_3 NPs on the cuticle of the thorax, (f) Abdomen caused less larval dwarfism, (f) At a magnification of 30× and scale bar 500 µm and (h) Normal head and thorax cuticle (g) at 55× and 200 µm, abdomen of the fourth instar larvae, at 43× and 500 µm

Table 4: Toxicity impacts of LC_{50} values of the tested NPs on chitin weight of *E. insulana* larvae when treated as newly molted of the fourth instars

	Nano-particle compounds				
Parameters	Silica-NPs	Zinc-NPs	Aluminum-NPs	Control	$LSD_{0.05}$
Larval fresh weight	47.85±5.04^{c}	38.44±3.50^{d}	55.69±5.45^{b}	78.45±9.63^{a}	8.482
Chitin dry weight	1.05±0.20^{c}	0.65±0.10^{d}	1.62±0.27^{b}	3.06±0.45^{a}	0.382

Average weight in mg±SD, LSD: Least significant difference, No significant differences at p>0.05 when repeating the same alphabet above the means in each row

layer resulting from ZnO NPs, in the thorax and abdomen, which has caused the death of the larvae. However, a low density of Al_2O_3 NPs showed on the thorax and abdomen cuticles (Fig. 6e-f), causing less larval dwarfism than other tested NPs. Moreover, the untreated 4th instar larvae appeared without any abnormalities with typical head, thorax (Fig. 6g) and abdomen cuticles (Fig. 6h).

Chitin of the body wall induced by the tested NPs against the fourth instars: The fresh weight of the 4th instar larvae was measured before the viscera have removed, as shown in (Table 4). The results recorded significant differences in the dry chitin weight between the tested NPs at 1.05, 0.65 and 1.62 mg, respectively, compared to 3.06 mg of the control larvae. The ratio of chitin formation was calculated according to the fresh weight of the fourth instar larvae. The obtained

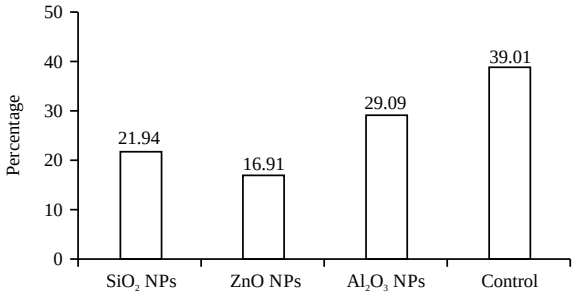

Fig. 7: Chitin formation ratio of E. insulana larvae induced by the tested NPs and untreated samples

results in (Fig. 7), indicating that the tested ZnO NPs could be considered the least chitin formation ratio in the fourth instar followed by SiO_2 NPs and finally Al_2O_3 NPs, were recorded 16.91, 21.94 and 29.09%, respectively, compared to 39.01% of control larvae.

DISCUSSION

Results of the toxicity index of NPs against the newly hatched larvae of *E. insulana* showed that ZnO NPs were the most potent compound, followed by SiO_2 and Al_2O_3 NPs. These results agreed with the findings of Derballah *et al.*[4] confirmed that ZnO NPs were the highest toxicity against the first instar larvae of *P. gossypiella,* followed by silica, with LC_{50} values at 11.29 and 37.78 ppm, respectively. Also, Haroun *et al.*[13] proved that ZnO and SiO_2 NPs were the most efficient treatments on the *C. maculatus* and *S. oryzae* at the highest concentration while *T. castaneum* displayed high defense against the tested components; they have recorded mortality at 98, 98,3 and 57% of SiO_2 NPs for the previously mentioned insects, respectively, moreover 88.3, 100 and 36% of ZnO NPs, respectively. In the same direction, El-Shennawy and Kandil[14] indicated that ZnO NPs was the most toxic compound against *E. insulana* larvae when treated by it in the first instar larvae, compared to other traditional insecticides. The current results disagreed with those of Debnath *et al.*[15] who reported that Al_2O_3 NPs were more toxic than ZnO NPs against *L. pseudo brassicae*.

The ratio of the cumulative larval mortality of *E. insulana* scored the highest mortalities when larvae were treated with various concentrations of ZnO then SiO_2 NPs. The current results are due to the absorption of ZnO and SiO_2 NPs through the larval cuticle lipid, which is considered lethal due to desiccation. As NPs have a massive surface area, they caused deformation and abrasion in the water barrier of the insects' cuticle. The bodies of insects began to lose water and finally died. The dead larvae became excessively dried and shrunken compared to the live ones. These results concurred with Rahman *et al.*[23] indicated the absorption of ZnO and SiO_2 NPs into the cuticular lipids and the penetration of the water barrier of *E. insulana.* Causing an imbalance of the protective wax layer resulted in the desiccation and death of these insects. Goswami *et al.*[10] investigated the insecticidal features of ZnO and Al_2O_3 NPs to control many insect pests. These results also agreed with Osman *et al.*[24] showed that the highest larval mortalities scored 86 and 83% at the maximum concentration of ZnO and SiO_2 NPs, respectively. These results confirmed with El-Ghoharyand El-Samahy[12] illustrated that SiO_2 NPs were the most operative treatment against *E. insulana* compared to other bio-insecticides after 15 days post-treatment. Additionally, Benelli[25] hypothesized that the mortality rate induced by the treatment of ZnO NPs could be caused by the passage of these ultra-small particles through the cuticle of mosquito larvae and intervene in molting processes.

There are no significant differences between the weights of the first, third instar larvae and pupae of *E. insulana* when treated with ZnO and SiO_2 NPs. Although most of the results showed significant differences in the weight of the larvae and pupae when treated with ZnO NPs compared to SiO_2 and Al_2O_3 NPs, at the same time, when compared to the untreated larvae. This result was due to the small size, surface area of zinc particles and their high density compared to other NPs. Several studies managed to clarify the structural properties of ZnO NPs, as it characterized by high density and sharp peaks that reflected the greater crystal size and high crystallinity[26,27]. Furthermore, SiO_2 NPs were less crystalline and intensive than ZnO NPs. The current results agreed with Farnaz *et al.*[28] indicated that Al_2O_3 NPs were the low density and less diversified in crystallization and it has spherical shapes compared to the hexagonal forms of ZnO NPs. According to the previously listed data, El-Samahy *et al.*[29] showed that one of the most significant characteristics of SiO_2 NPs is their capacity to prevent insects from feeding directly after food treatment with these particles. In the same context, El-Shennawy and Kandil[14] emphasized that when using ZnO NPs against newly hatched larvae of *E. insulana*, it caused highly decrease in larval and pupal weight by approximately 10 and 13.6 times, respectively, compared to the control. The reduction in larval and pupal weight recorded 91 and 93.7%, respectively.

The current results also indicated that the growth rate inhibition values decreased gradually until the fourth instar larvae. In addition, ZnO NPs caused the highest growth rate inhibition during the various instar larvae and pupae of *E. insulana*, while Al_2O_3 NPs was the least effective one. These

outcomes are similar to those recorded by Massey[30] who observed a reduction of feeding efficacy and growth rates of the *Spodoptera* by treating them with SiO_2 NPs. The results of many previous studies are consistent with the current data in the growth rate inhibition when treated with different types of NPs against some insects, such as ZnO and Al_2O_3 NPs by Goswami *et al.*[10] and Salem *et al.*[31]; SiO_2NPs by Debnath *et al.*[32]; ZnO by Kadarkarai *et al.*[33].

The present results showed significant differences between the larval and pupal periods of *E. insulana* for different treatments; meanwhile, these differences did not appear between SiO_2 and Al_2O_3 NPs treatments in the pupal period. These results are attributed to the increase in the developmental periods when dealing with NPs more than the control samples. At the same time, they recorded a remarkable decrease in the developmental rate of larval and pupal stages. These results agreed with previous reports of development duration by El-Shennawy and Kandil[14] recorded that the larval and pupal periods were exceptionally prolonged post-treatment with ZnO NPs against newly hatched larvae of *E. insulana*. Whereas, El-Helaly *et al.*[34], Shaker *et al.*[35] revealed that the newly hatched larvae of *S. littoralis* were treated with SiO_2 NPs at different concentrations gave prolonged larval and pupal durations. Furthermore, the treatment of the second instar larvae of *S. littoralis* with tested NPs at LC_{50} values significantly increased the larval duration, as compared with the control. In this regard, the results of Al-Bandari and EL-Healy[36] also indicated that a highly toxic activity of SiO_2NPs after 15 days post-treatment against *S. littoralis* negatively affected the developmental rate of larvae and pupae.

Results of the larval and pupal malformations rate agreed with El-Shennawy and Kandil[14] showed that the highest ratio of malformations in *E. insulana* larvae, when treated with ZnO NPs was 12% compared to untreated larvae at 1%. The deformations of the treated larvae appeared as the abnormal color of larval bodies, resulting from cuticle pigment and disability to shedding out the previous cuticle, which causes the failure of molting or metamorphosis and then death. Likewise, the pupa couldn't fully form its cocoon and thus the pupation process has not been completed. The toxic effects of NPs were attributed to their small size and large surface area, which helped to penetrate the cuticle cells and increase the interaction with them, according to Medina *et al.*[7].

Treatment with ZnO NPs scored the least ratio of chitin formation in the fourth instar larvae, followed by SiO_2 NPs and finally Al_2O_3 NPs, compared to control larvae. These interpretations were in agreement with the previous findings of many authors, such as Martinez and Van Emden[37], El-Sabrout *et al.*[38], Abdelgaleil and El-Sabrout[39]. They studied the insecticidal effects on cuticle stretching and inhibiting the larval growth rate of the cotton leaf worm, consequently on chitin formation. Furthermore, nanoparticle action is based on drying the insect's cuticle by absorbing the lipids, which produces damage in the cell membrane, causing cell breakdown and insect dead[40]. Previous studies have confirmed that NPs of metal oxides are efficient against insects. Hence, NPs have been applied in the novel formulations of insecticides[31,41,42].

CONCLUSION

To sum up, the effectiveness of zinc, silica and aluminum nanoparticles was tested as insecticides against the Spiny Bollworm, causing growth disturbances and abnormalities in the larvae and pupae. The possibility of using these unconventional agents to control the Spiny Bollworm is possible if commercial production at a low cost is taken into consideration. Hence, the tested nanoparticles find a promised place in pest control strategies due to their low environmental risks.

SIGNIFICANCE STATEMENT

This study discovers three nanoparticle oxides that can be used in the control of agricultural pests, particularly those cause losses in cotton production in Egypt. Also, Low doses of nanoparticles, as environmentally friendly tools, give positive results. This study will help the researchers to discover other nanoparticles that many researchers have not been able to explore, which could have an actual effect on integrated pest management programs. This research can also be considered a new point for integrating nanoparticles with other chemical pesticides to reduce their toxicity to humans and other non-target organisms.

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