



Insecticidal Activity of ZnO NPs Synthesized by Green

Method Using Pomegranate Peels Extract on Stored Product Insects

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Abstract

Green synthesis of nanomaterials is the most recent trend in nanotechnology since it takes into consideration the sustainable development and waste recycling, and it produces high value materials. Furthermore, the green synthesis excludes the use or production of toxic and hazardous chemicals to the environment. This work aimed to the green synthesis of zinc oxide nanoparticles (ZnO NPs) using pomegranate peels extract as a reducing and capping reagent instead of chemicals. Structural and morphological characterization of ZnO NPs was studied using X-ray diffraction (XRD), Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR), High-Resolution Transmission Electron Microscopy (HRTEM) and Energy Dispersive X-Ray Spectroscopy (EDX). In addition to the application of ZnO NPs as a green insecticide to control two destructive stored product insect pests; the rice weevil *Sitophilus oryzae* (L.) and the Angoumois grain moth *Sitotroga cerealella* (Olivier). The results of XRD, FTIR-ATR and EDX confirmed the formation of ZnO NPs. Also, XRD and HRTEM affirmed that the size of synthesized ZnO NPs have very small size around 4nm. The toxicity studies showed that green synthesized ZnO NPs exhibited a progressive increase in mortality of *S. oryzae* and *S. cerealella* adults subjected to treated wheat grains by increasing exposure intervals and concentration. A post toxic effect on progeny counts of *S. oryzae* and *S. cerealella* adults exposed to three concentrations of ZnO NPs was observed. Adherence of ZnO NPs to the body surface of treated adults was visualized using Scanning Electron Microscopy (SEM). Images showed strong uptake and massively attachment of nanoparticles through different parts of insect body. The present work suggests that eco-friendly synthesized ZnO NPs are promising green insecticide to control *S. oryzae* and *S. cerealella* stored product insect pests.

Keywords: Green synthesis; ZnO NPs; Plant wastes extract; progeny production; *Sitophilus oryzae*; *Sitotroga cerealella*; Toxicity.

1. Introduction

Stored legumes and grains such as maize, rice, wheat and others play a predominant role in the world economy and trade as food, feed and industrial grain crops [1]. Due to the high increase in population particularly in developing countries, the demand for cereal grains will be increased dramatically. Stored products mainly cereals and legumes are subjected to considerable quantitative and qualitative losses due to insect infestation [1-2]. The rice weevil *Sitophilus oryzae* (L.) (Coleoptera:

Curculionidae) is considered as one of the most destructive pests of stored products. The adult and larval stages are feeding on whole grains and attacking many products in storage mainly rice, maize, oats, dried beans and sorghum [3]. Likewise, the Angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is a serious worldwide pest of wide variety of stored cereal grains such as maize, rice, wheat, barley and sorghum. The larval development is completed in a single grain, and the internal feeding resulted in serious grain loss [4]. Both insects have been recognized as major threats to production of maize and other stored

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products in Africa [5]. The current used methods to control insects in storage depend mainly on the use of residual insecticides and fumigants [6]. The resistance to pesticides and pesticide residues are major problems in controlling pests in stored grain and field crops. Furthermore, the world demand for pesticide free food is necessitated the search for eco-friendly methods of pest control. Hence, many countries are replacing chemical-based agriculture to green agriculture, where the utilization of biopesticides and biological nanomaterials has lots of role to play in pest control.

Nanotechnology is playing a significant role via its unique properties in many fields like engineering, medicine, food science, animal nutrition and pest control [7-9]. Nanoparticles (NPs) have large surface area, which leads to increased activity, reactivity, effectiveness and absorption [10]. Thus, nanomaterials could help to produce new pesticides and insect repellants. It is expected that nanopesticides could be effectively used with less dose/application times in insect pest control. Recently, nanomaterials are considered as one of the most studied alternatives to traditional neurotoxic grain protectants. For example, many researchers evaluated the toxicity of diatomaceous earths composed mainly of amorphous hydrated silica against a wide range of stored product insect pests, such as beetles and moths [11-12]. It was reported that silica NPs caused 100% mortality in *S. oryzae* [13]. Badawy et al. [14] reported that bio-synthesized copper oxide nanoparticles (CuO-NPs) showed significant toxicity against *S. granaries* and *Rhyzopertha dominica* in a size and dose dependent manner. However, the use of nanomaterials in agriculture, especially for plant protection and production, is under-explored research area [15].

Zinc oxide has been known as food and feed additive, and it was entitled by US FDA as GRAS (generally recognized as safe) [16]. Also, Zinc oxide NPs are used in a wide range of applications such as nutrition, drug delivery, antibacterial, batteries and fertilizers [10, 17-19]. Keratum et al. [20] and Salem et al. [21] evaluated the toxicity of zinc oxide (ZnO) and aluminum oxide (Al₂O₃) nanoparticles against *S. oryzae* and *T. castaneum* adults. Their results showed that both nanomaterials have moderate to strong toxic effect against the tested insects and significantly inhibited the progeny production. Green synthesis of nanomaterials not only reducing the usage and

production of hazardous materials, but also it produces highly valuable compounds from wastes. The agro-industrial wastes such as extracts of fruit peels could be used as both reducing and capping reagent to synthesis nanomaterials [10, 18-19]. Pomegranate (*Punica granatum* L.) peel which represents about 50% of fruit weight, contains bioactive compounds such as phenolics (flavonoids, ellagitannins and proanthocyanidin compounds) and complex polysaccharides [22]. The most abundant polyphenols and antioxidant compounds in pomegranates are hydrolysable tannins including gallotannins, ellagitannins and gallagyl esters such as punicalagin and punicalin more than condense tannins [23]. These compounds exhibit various activities in the synthesis of nanomaterials [19, 24]. The present work aims to green synthesis and characterize ZnO NPs using pomegranate peels extract as reducing and capping reagent. The insecticidal efficacy of produced ZnO NPs was evaluated against two stored product insects; *S. oryzae* and *S. cerealella* under laboratory conditions. The post effect of ZnO NPs on the progeny production of treated adults was studied. Also, the adherence of synthesized ZnO NPs to adults of *S. oryzae* and *S. cerealella* was examined

2. Materials and methods

2.1. Materials

Pomegranate peels obtained from juice factories wastes. Zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O, 98%) as zinc precursor purchased from Sigma-Aldrich, and deionized water.

2.2. Preparation of peel extract

Pomegranate peels were washed then placed overnight in an oven at 60°C, and then after the complete dryness the peels grounded to get fine powder. Afterwards, 1:5 (w/v) powder to deionized water was stirred for 2h at 40°C in water bath. Finally, the mixture was centrifuged at 5000 rpm for 15 min at 4°C. The resulting extract was stored in an argon atmosphere for later use.

2.3. Green sol-gel method for the synthesis of ZnO NPs

ZnO NPs were green synthesized by mixing zinc precursor (zinc nitrate) (1M) with stoichiometric pomegranate peels extract as reducing and surfactant reagent under vigorous stirring for 2h., and then the white precipitate was obtained. In order to obtain the

powder, centrifugation at 3000g for 15 min has been made then the precipitate was dried at 60°C overnight. Finally, produced nano zinc oxide calcinated at 350°C, and the samples were then ready to be used for further characterization and toxicity studies.

2.3. Measurement techniques

X-ray diffraction (XRD) was performed by PANalyticalX'Pert Pro target Cu-K α with secondary monochromator Holland radiation, the tube operating at 45 kV with 0.1540 nm wavelength over a 2 θ range of (5°–80°). FTIR-ATR spectral data were collected using Vertex 80 (Bruker, Germany) at room temperature in the range of 4000-400 cm⁻¹. High-Resolution Transmission Electron Microscopy (HRTEM) was performed by JEM-2100F electron microscope with accelerating voltage of 200 kV. Energy Dispersive X-Ray Spectroscopy (EDX) using scanning electron microscope (JEOL-JSM 6100) with the OXFORD X-ray microanalysis software used for the identification of the elemental analysis of the powder components. All characteristics of the nanomaterial were conducted at laboratories of National Research Centre, Cairo, Egypt.

2.4. Insects and commodity

Adults of *S. oryzae* and *S. cerealella* were obtained from laboratory colonies that have not exposed to insecticides and established in Pests and Plant Protection Department, National Research Centre, Egypt. Both species; *S. oryzae* and *S. cerealella* were reared on intact hard wheat grains in glass jars (2L capacity) covered with muslin cloth for ventilation and were kept at 28±1°C, 60-65% relative humidity and 13:11 light:dark photoperiod. New adult insects 1-7, and 1-3 days old of mixed sex of *S. oryzae* and *S. cerealella* respectively were used for the experiments. Wheat grains used for insects feeding and bioassays were purchased from a local market and stored for about 5 days prior to be used at -18°C to kill any insects or parasites living in the product, and then equilibrated before use for additional one week to the mentioned laboratory conditions where the culture was kept.

2.5. Toxicity study

Three concentrations of ZnO NPs were used; 50, 100 and 150 ppm to evaluate the insecticidal effect of green synthesized ZnO NPs on the tested adult insects in hard wheat using dry dust

application methods. The wheat grains were dusted with selected concentrations of ZnO NPs in the rate of 50, 100 and 150 mgkg⁻¹ in separate plastic vials (120 ml), and were shaken manually for approximately 2 min to achieve equal distribution of nanomaterial. Another set was supplied with untreated wheat grains and served as control. Each treatment was replicated thrice, each one was provided separately with 40 g of wheat grains and 20 adult insects of *S. oryzae* and *S. cerealella*. All sets treated and untreated were kept at the same previous conditions. Dead and survived insects were counted after 1, 2, 3, 7 and 14 days of exposure to determine the percentage of mortality for each observed interval. Adults of both insects were considered dead when gently probed with sharp tip forceps and showed no response.

2.6. Progeny production

The post effect of ZnO NPs was studied by recording the number of emerged individuals. All alive and dead *S. oryzae* and *S. cerealella* adults were removed from the plastic vials after the final count of mortality of all treatments. The treated and untreated sets were kept under the same previously mentioned conditions and the emerging adults were counted thrice after 20, 30 and 60 days. The total sum was calculated to indicate the number of adults emerged in each vial in order to determine the progeny production in F1. The inhibition rate percentage (%IR) was calculated according to Tapondju et al. [25] as following:

$$\%IR = \frac{\text{Mean No. of individuals in control} - \text{Mean No. of individuals in treated}}{\text{Mean No. of individuals in control}} \times 100.$$

2.7. Adherence of ZnO NPs to *S. oryzae* and *S. cerealella*

Scanning Electron Microscopy (SEM) examination was carried out to observe the adherence of green synthesized ZnO NPs to the adults of *S. oryzae* and *S. cerealella*. Before this examination adult insects of both species were exposed for 7 days exposure interval to wheat grains treated with 150 ppm of ZnO NPs. The dead insects collected after exposure were air-dried for approximately 5 days and placed onto adhesive stubs and coated with gold. Adult insects were then observed under low vacuum SEM (TESCAN, Vega III/Czech Republic) in the Electronic Microscope Unite, Central Laboratory, National Research Centre, Cairo.

2.8. Data analysis

Obtained results of mortality and progeny production were analyzed using one way ANOVA and significant differences between treatments were determined by Duncan's test ($P < 0.05$). SPSS version 14.0 (SPSS, Inc., Chicago IL) was used for data analysis.

3. Results and discussion

3.1. X-ray Diffraction (XRD)

In this work, green sol-gel method was used successfully to synthesis ZnO NPs using pomegranate peel extract as reducing and capping reagent. Synthesis of ZnO NPs was confirmed by XRD analysis. The XRD is a technique used to identify the chemical compounds contents in the powder fingerprint and to estimate the purity of the powder i.e., the quantity of each compound in the powder. The XRD pattern of ZnO NPs in Figure 1 shows the diffraction peaks which have a crystalline structure at $2\theta = 31.6^\circ, 34.4^\circ, 36.2^\circ, 47.5^\circ, 56.6^\circ, 62.8^\circ$ and 67.9° referred to lattice planes (100), (002), (101), (102), (110), (103) and (112), respectively which corresponds to reference code number card No. 89-1397 that indicated the purity of ZnO NPs without any observed impurities [10, 18-19, 26].

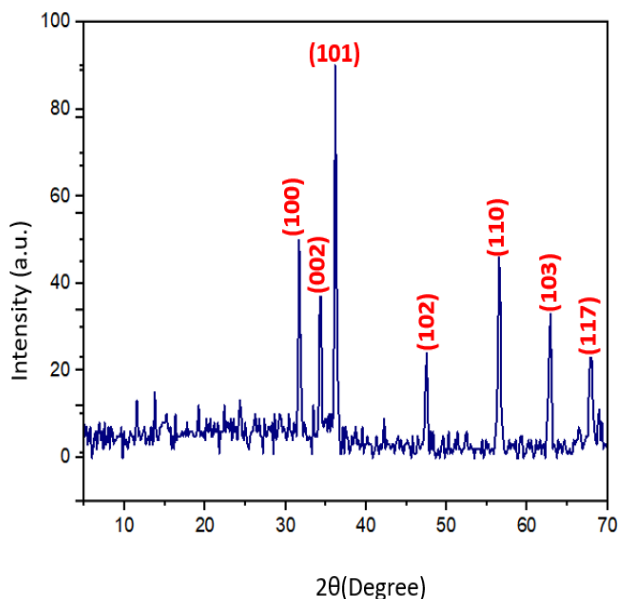


Figure 1. The XRD analysis of ZnO NPs

3.2. Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)

FTIR-ATR analysis was studied to determine the functional groups in green synthesized

nanoparticles. Figure 2 showed the FTIR-ATR of the ZnO NPs, bands at 3445 cm^{-1} and 1394 cm^{-1} are related to O-H stretching and bending vibration, respectively due to water adsorbed from air and atmospheric moisture [17-18, 26-27]. The absorption at 2922 cm^{-1} and 2852 cm^{-1} indicates C-H stretching vibration. The bands at 1647 cm^{-1} and 1437 cm^{-1} were corresponded to the stretching vibration of carbonyl and carboxylate group, respectively. The C-O stretching vibration was seen at 1115 cm^{-1} , and the C-C bond at 1028 cm^{-1} . The absorption at 687 cm^{-1} is attributed to the formation of tetrahedral coordination of Zn. The bands at 547 cm^{-1} , 424 cm^{-1} and 400 cm^{-1} were assigned to metal-oxygen bond, which is Zn-O bond. The obtained results of FTIR-ATR confirmed the chemical composition of produced ZnO [10, 18-19, 29].

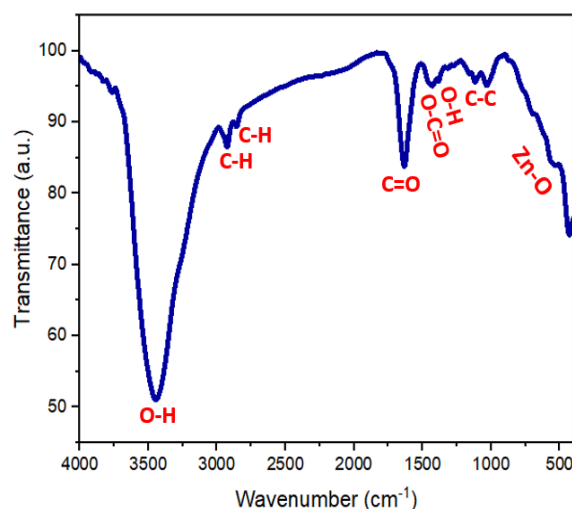


Figure 2. The FT-IR pattern of ZnO NPs

3.3. High Resolution Transmission Electron Microscopy (HRTEM)

The morphology of synthesized ZnO NPs was examined using HRTEM. As illustrated in Figure 3, HRTEM image shows that ZnO NPs have infinitesimal very small size ranged from 2-8 nm. In addition, this image indicated that most of the ZnO NPs are spherical in shape with average particle size of 4nm. The diffraction rings of ZnO NPs exhibited Debye Scherrer rings assigned (010), (002), (011), (012), (110) and (103) respectively. Sukri et al. [24] reported that TEM imaging of ZnO NPs fabricated using pomegranate showed the formation of spherical and hexagonal-shaped nanoparticles with mean size of 32.98 nm and 81.84 nm at $600\text{ }^\circ\text{C}$ and $700\text{ }^\circ\text{C}$

respectively. Adding peel extract of pomegranate decreased the size of iron oxide NPs with the average particle size less than 11 nm as confirmed by morphological studies [30].

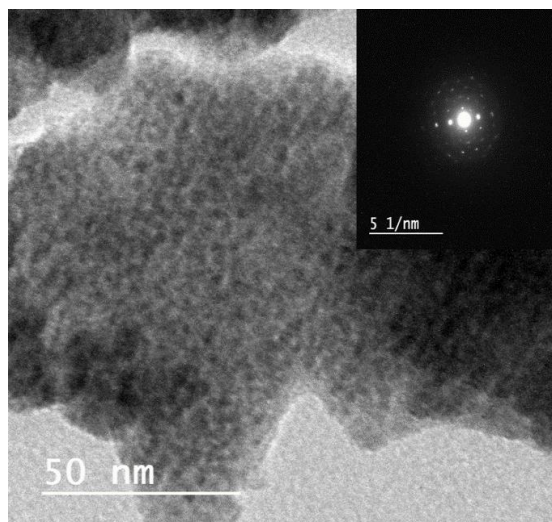


Figure 3. The HRTEM of ZnO NPs

3.4. Energy Dispersive X-Ray Spectroscopy (EDX)

Figure 4 demonstrate the EDX of green synthesized ZnO NPs. The results show the presence of Zn and O in stoichiometric ratios. Also, the presence of C refers to the carbon from the pomegranate waste extract. The obtained result of EDX was agreed with the IR and XRD results indicated that ZnO NPs produced without any impurities [18-19, 29].

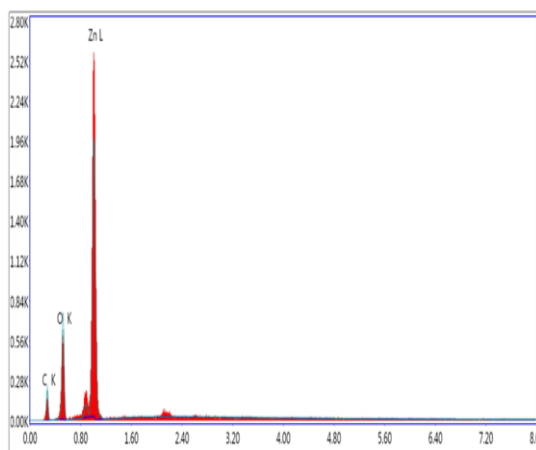


Figure 4. EDX pattern of ZnO NPs

3.5. Insecticidal effect of ZnO NPs on *S. oryzae* and *S. cerealella*

The rice weevil *S. oryzae* and the Angoumois grain moth *S. cerealella* are considered

as the most dominant primary pests of cereal grains such as maize, paddy, oats, sorghum, wheat and other cereals. In the present work the toxicity effect of green synthesized ZnO NPs was examined against both insect species. Results of toxicity experiment presented in Figure 5A showed that mortality of *S. oryzae* adults treated with green synthesized ZnO NPs generally increased as concentration and exposure interval increased. Data demonstrate that mortality of *S. oryzae* exposed for 1 day did not exceed 21.67 ± 1.67 with highest tested concentration i.e., 150 ppm. Nevertheless, as the exposure interval increased to 2 and 3 days, mortality increased by increasing concentration to 33.24 ± 1.83 , 27.89 ± 2.98 and 16.94 ± 4.03 after exposure to 150, 100, and 50 ppm of ZnO NPs for 3 days respectively. After 1 week exposure to treated wheat grains the mortality significantly ($P < 0.05$) increased by increasing concentration and ranged from 31.28 ± 5.85 to 49.40 ± 10.53 . While, adult mortality of *S. oryzae* after 14 days of exposure to treated wheat grains increased significantly and it was greater than the initial mortality recorded after 1 day exposure interval. This result proved that the adult insects continued to be affected by exposure to ZnO NPs even after exposure to treated wheat for maximum time interval. After 14 days of exposure to treated wheat grains, the highest mortality (88.89 ± 11.11) recorded after application with 150 ppm compared to control (zero).

Data presented in Figure 5B showed the progressive increase in mortality of *S. cerealella* adult moths subjected to wheat grains treated with ZnO NPs with increasing exposure interval and concentration. Mortality of *S. cerealella* adults after 1 day of exposure was lower than 32.39 ± 5.37 , and 3 tested concentrations exhibited similar toxicity. After 2 days exposure interval the toxicity of ZnO NPs increased greatly in concentration-dependent manner. The observed mortality values were 71.32 ± 9.79 , 50.94 ± 8.06 and 40.11 ± 1.54 after treatment with 150, 100 and 50 ppm of ZnO NPs respectively. The less tested concentration of ZnO NPs (50 ppm) has resulted in 67.22 ± 4.33 mortality after 3 days exposure interval. While, the application with highest concentration of 150 ppm has resulted in highest mortality of 93.33 ± 6.66 under the same exposure time. The data revealed that the severe toxicity of ZnO NPs caused the death of all tested adult moths after 7 days exposure interval, thus the mortality reached 100% after application with 3 tested concentrations (150, 100 and 50 ppm).

Based on our knowledge, this is the first published work on investigating the insecticidal efficacy of ZnO NPs green synthesized by using pomegranate peel extract against *S. oryzae* and *S. cerealella* adults. However, previous studies are found in the literature regarding the susceptibility of stored product insect pests to different formulations of inert dusts in bulk form and nano size range. For example, studying the insecticidal action of commercial and modified zeolite and silica nanoparticles against *S. oryzae* [13, 31-32], diatomaceous earth against *S. cerealella* [33] and nanoalumina against *S. oryzae* and *R. dominica* [34].

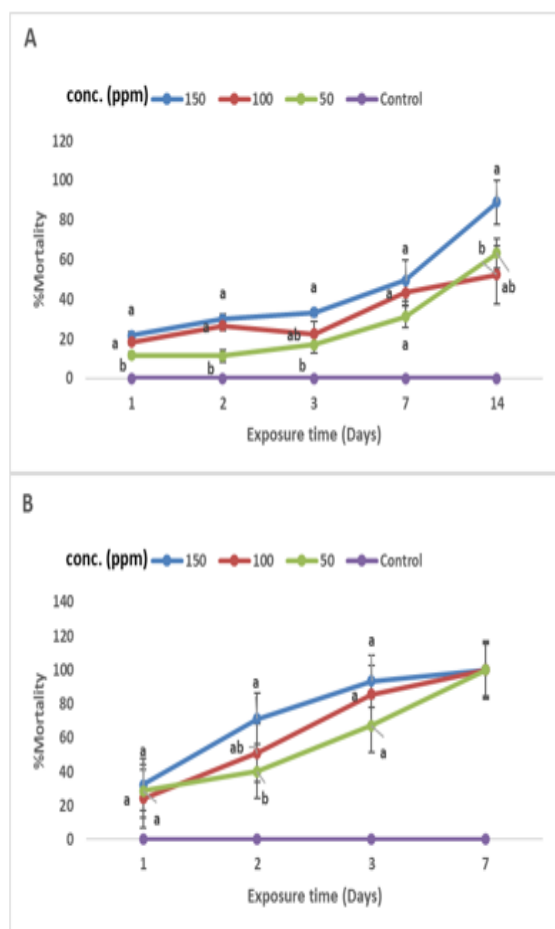


Figure 5. Toxicity of ZnO NPs against A) *S. oryzae* adults and B) *S. cerealella* at three concentration rates (ppm) and different exposure intervals (days). Mean (\pm SE) values with different letters within the same exposure interval are significantly different ($P < 0.05$) (ANOVA) (Duncan test).

In the present work *S. cerealella* adult moths showed more sensitivity than *S. oryzae* adults. Generally, stored product insects exhibit a wide range of susceptibility to inert dusts. It has been reported that secondary insect pests of stored grains are more

susceptible to diatomaceous earth than internal feeders such as *S. oryzae* or *R. dominica* [35]. Also, Korunic [36] mentioned that *Sitophilus spp.* are less sensitive to diatomaceous earth. The variations in insect's susceptibility to inert dusts could be dependent on the variations of insect behavior and cuticle thickness, as different insect species possess a different composition of epicuticular lipid [37]. High susceptibility of *S. cerealella* may be also attributed to the fact that species with a rough or hairy body surface showed to collect more particles of applied dust per unit of area causing greater cuticle damage and increasing sensitivity of insects. Rumbos et al. [6] reported that *S. oryzae* was moderately susceptible to the tested formulations of zeolite even at the highest concentration and maximum exposure time. Furthermore, sensitivity of different species is believed to depend on other factors such as size (volume to surface area ratio), differences in cuticular lipids and the rate of insect movement through grains [38]. In the current study ZnO NPs synthesized with very small particle size (2-8 nm) that could explain the high toxicity against adult insects of both species even at very low concentrations i.e., 50-150 ppm. Smaller particle size of applied dust increases the contact with insect cuticle, which subsequently increases the insecticidal action.

3.6. Post toxic effect of ZnO NPs

Progeny count of *S. oryzae* exposed to 3 concentrations of ZnO NPs was affected significantly ($P < 0.05$) in comparison to untreated group (Table 1). The mean number of emerged adults decreased by increasing the concentration but did not differ significantly with application with 3 concentrations. The offspring counts were 27.33 ± 7.53 , 36.0 ± 7.02 and 66.67 ± 20.20 after application with 150, 100 and 50 ppm respectively. While, these values were significantly different with regard to progeny count of control group (112.33 ± 28.89). Accordingly, the percent of inhibition rate was also affected. The highest inhibition percent was 75.66%, which noted for application with 150 ppm of ZnO NPs, and followed by 67.95% and 40.64%, which observed after application with 100 and 50 ppm of ZnO NPs respectively.

Table 1. Progeny counts and inhibition rate (%) of *S. oryzae* exposed for 14 days to three concentration rates of ZnO NPs treated wheat grains

ZnO NPs concentrations (ppm)	Mean No. of emerged individuals	% Inhibition rate
150	27.33±7.53 b	75.66%
100	36.0±7.02 b	67.95%
50	66.67±20.20 ab	40.64%
Control	112.33±28.89 a	-
F-Value	4.373*	-

Mean (\pm SE) values with different letters within the same column are significantly different ($P < 0.05$) (ANOVA) (Duncan test).

*= Significant

Due to the dominant toxicity effect of ZnO NPs against adult moths of *S. cerealella*, the progeny production was also affected significantly after 7 days exposure to the tested nanoparticles (Table 2). The number of emerged offspring was significantly ($P < 0.05$) low after treatment with 3 concentrations of ZnO NPs in comparison to untreated group. However, the progeny count was not significantly affected by ZnO NPs concentration, and recorded as 15.33 ± 7.83 , 11.0 ± 4.50 and 37.67 ± 15.77 after treatment with 150, 100 and 50 ppm respectively compared to the progeny count of control group (78.67 ± 14.67). Likewise, it was observed that the percent of inhibition in adult moths of *S. cerealella* ranged from 52.11 to 86.01% with the 3 concentrations tested.

Table 2. Progeny counts and inhibition rate (%) of *S. cerealella* exposed for 7 days to three concentration rates of ZnO NPs treated wheat grains

ZnO NPs concentrations (ppm)	Mean No. of emerged individuals	% Inhibition rate
150	11.0±4.50 b	86.01%
100	15.33±7.83 b	80.51%
50	37.67±15.77 b	52.11%
Control	78.67±14.67 a	-
F-Value	7.020*	-

Mean (\pm SE) values with different letters within the same column are significantly different ($P < 0.05$) (ANOVA) (Duncan test).

*= Significant

Our findings are in agreement with previous study when *S. cerealella* adults exposed to DE and rice husk ash for 14 days, the progeny emergence (F1) was significantly reduced (< 20 individuals) [39].

Also, Al_2O_3 and ZnO nanoparticles applied to wheat grains significantly inhibited the number of *T. castaneum* progeny [21]. In the present work, ZnO NPs inhibited the reproduction of *S. oryzae* and *S. cerealella* after 14 days exposure interval to treated wheat grains particularly at higher concentration, which might due to that most of individuals died and fewer survived the treatment.

3.7. Adherence of ZnO NPs to *S. oryzae* and *S. cerealella*

Images obtained from SEM showed that ZnO NPs are strongly attached on the body surface of both insects; *S. oryzae* and *S. cerealella* as shown in Figure (6 a,b,c,d and 7 a,b,c) compared to untreated insects (Figure 6 e,f,g,h and 7 d,e,f). Adults of *S. oryzae* and *S. cerealella* exposed to wheat grains treated with green synthesized ZnO NPs appeared coated densely and uniformly with NPs. The images in Figure (6 a,b,c,d) showed the strong adhesion of ZnO NPs on dorsal and ventral surfaces of *S. oryzae* particularly on joints and intersegmental areas. Similarly, ZnO NPs were adhering to different parts of *S. cerealella* adult moth, the NPs attached in high density and resulted in losing most of scales on insect's wing (Figure 7 a,b,c).

In our research it was observed (personal observation) that the applied ZnO NPs dust adhered to body of *S. oryzae* and *S. cerealella* adult insects. This observation confirmed by examining the treated insects using SEM. The obtained images showed the massive covering of insects' body by nanoparticles on dorsal and ventral surfaces compared to untreated insects. Debnath et al. [13] and Rumbos et al. [6] suggested that the mode of action of inert dusts is based not only on the absorption but also on the abrasion caused by the particles. So, as the water barrier is damaged, the insects found to lose water leading to their death from desiccation [40]. The physical mode of action of such inert dusts and nanocides is making them favorable to be used as dust protectants against different storage insect pests. Nanocides such as ZnO NPs have a lower risk of resistance in insects to these nanoparticles on long term application comparing to commercial insecticides. Our result is in accordance with that of Ibrahim and Salem [41] who observed under SEM zeolite nanoparticles (< 45 nm) strongly attached to the cuticle of *T. confusum* and *C. maculatus* adults,

and reported that the nanoparticles aggregated between thorax and abdomen joints of treated *T. confusum*, and adhered to the whole body of *C. maculatus*. Similarly, Stephou et al. [42] and Rumbos et al. [6] reported the uptake of Entostat (an electrostatically chargeable powder), DE added to *Beauveria bassiana* and zeolite to adults of *O. surinamensis*, *S. oryzae* and *T. confusum* using SEM and fluorescence indicators.

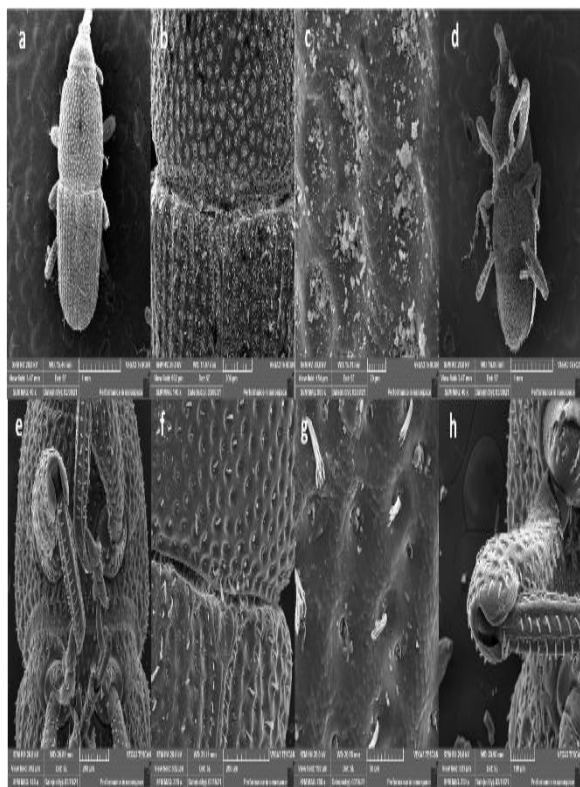


Figure 6. (a,b,c,d) Scanning electron microscopic photographs of *Sitophilus oryzae* adults treated with green synthesized ZnO NPs showing deposition and aggregation of NPs; a) on the dorsal surface of insect, b) between thorax and abdomen, c) on the elytra and d) on the ventral surface. SEM photographs (e,f,g,h) showing untreated adults of *S. oryzae*.

Nanomaterials such as ZnO NPs are known to be electrically charged, they aggregate and showed to be attached strongly to the cuticular wax layer of insects by sorption process. Subsequently disruption in wax layer occurs, leading to water loss and dehydration of insects and finally death. In the present experiment, as adult insects of *S. oryzae* and *S. cerealella* are moving on ZnO NPs treated wheat, the dust of nanoparticles get trapped and attached on the insect cuticle layer. The mode of action of inert dust could be summarized as follows; (1) spiracles of insects are blocked leading to insect death by

asphyxiation, (2) inert dusts cause cuticular abrasion as they settled between cuticular segments and increasing the loss of water, (3) the water from insects' cuticle gets absorbed, and (4) finally excessive water loss occurs as the dusts absorb the epicuticular lipids.

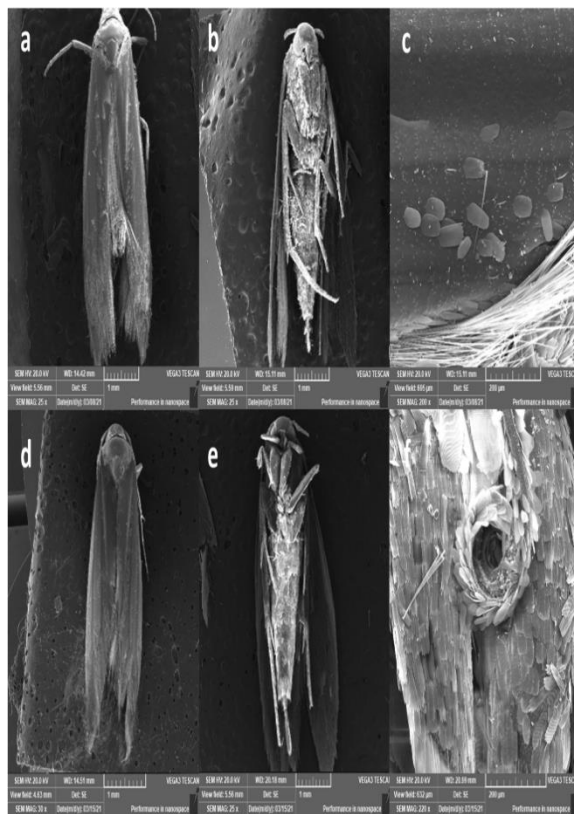


Figure 7. (a,b,c) Scanning electron microscopic photographs of *Sitotroga cerealella* adult moths treated with green synthesized ZnO NPs showing deposition and aggregation of NPs; a) on the dorsal surface of insect, b) on the ventral surface and c) on the insect's wing, most of scales are lost. SEM photographs (d,e,f) showing untreated adult moth of *S. cerealella*.

4. Conclusion

The current work was performed to synthesize ZnO NPs by green sol-gel method. Pomegranate peels extract was used successfully as reducing and surfactant reagent. The produced nanoparticles were analyzed for their structural and morphological characterizations. Nanoparticles of ZnO were produced in very small size of particles ranged 2-8 nm. The insecticidal potential of ZnO NPs as an effective eco-friendly control agent against the most important pests of stored products *S. oryzae* and *S. cerealella* was investigated. In this work insecticidal effect of ZnO NPs against *S. oryzae* and

S. cerealella adults was depending on concentration and exposure interval i.e., the insect mortality increased by increasing the concentration and time of exposure to applied nanomaterials. It was also shown that ZnO NPs had a post effect on the progeny production of both *S. oryzae* and *S. cerealella* adults. The number of emerged individuals significantly decreased with high percent of inhibition rate particularly at tested higher concentration. Nanomaterials such as ZnO NPs exerted their toxicity against stored product insects through physical mode of action. Therefore, insects are unlikely to develop physiological resistance to applied nanoparticles. Additional advantage is that dusts in the form of nanomaterials can be removed easily by conventional milling process, while spraying of conventional pesticides usually leaving residues on the stored products. In the current study, although ZnO NPs applied to wheat grains in very small amount, but it exhibited high toxicity against *S. oryzae* and *S. cerealella* adults that could minimize the adverse effects, if any, of inert dusts. Further research regarding the assessment of nanomaterials potential toxic effects on human health and environment is required.

5. Conflicts of interest

“There are no conflicts to declare”.

6. Acknowledgment

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Graphical Abstract

