ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Evaluating the Toxicity of Synthetic Hydroxyapatite Nanoparticles (HAPNPs) against Pulse Beetle, *Callosobruchus maculatus* (Insecta: Coleoptera)

Nawal Abdulaziz Alfuhaid, Samar Adel, Ahmed M. A. Ibrahim,* Mohamed A. Amro, Mohamed H. A. Hassan, Ali Mohamed Ali, and Mohamed Abd El-Aal

Cite This: *ACS Omega* 2025, 10, 10724−10732

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: The pulse beetle *Callosobruchus maculatus* is a serious insect pest of stored legumes. Therefore, the management of such pests has become a necessity as it causes great economic loss to its plant host. Unfortunately, pest management programs against *C. maculatus* encounter several obstacles, such as the generation of insecticide resistance and environmental hazards of traditional insecticides. The current study was designed to overcome these obstacles by using synthetic nanoparticles as alternative insecticides. In this study, a synthetic form of eggshell-based hydroxyapatite nanoparticles (HAPNPs) was used as a control agent against *C. maculatus*. This material was selected because of its environmental safety, which is ensured due to its wide spectrum of applications in our daily activities. HAPNPs originating from eggshells were characterized by XRD, FTIR, and TEM. The obtained results revealed a lack of impurities in the synthesized particles and that the average plate size is ∼62.8 nm, while the rod structure has a length and width of ∼91 nm and ∼22.7 nm, respectively. A comparative study on the toxicity of HAPNPs and Malathion insecticide against *C. maculatus* showed a significant impact of NPs originating from eggshells than the positive control insecticide. Based on this finding, further analyses were performed to understand its subsequent effects. Eggshell-based HAPNPs disrupted *C. maculatus* fecundity and adult emergence rate. In the meantime, it highly reduced the negative effects of *C. maculatus* on cowpea seeds. Scanning electron microscopy showed clear disruption of the insect integument wax layer and the aggregation of HAPNPs on the beetle's spiracles, leading to respiratory failure and hence the death of the insects. Interestingly, there was no impact of HAPNP application on total antioxidants and H_2O_2 levels in *C. maculatus*. These results introduce a novel management tool using a safer nanopesticide against the cowpea beetle.

1. INTRODUCTION

The world's population is continuously increasing in number and needs a food supply. Grain production is an important source of diet for humans.[1] Unfortunately, damage to stored grain has reached 40% in developing countries. In general, stored grains are considered the primary host of the pulse beetle pest *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae).[2] This small insect is considered a primary pest of *Vigna unguiculata* (L.) Walp. and *Phaseolus vulgaris* L. grains.[1] These grains are rich in protein (20−25%), carbs (50−60%), and have a moderate fat content (1−2%), making them an ideal dietary source around the world.[3] Cowpea is a versatile food legume with significant nutritional value, containing proteins, carbs, vitamins, minerals, dietary fibers, antioxidants, polyunsaturated fatty acids, and polyphenols.[4,5] The cowpea seed beetle (popularly known as the cowpea weevil) is a major storage pest that attacks cowpea (*V. unguiculata*) and many other legumes, primarily in warm regions of the planet.[2] Larvae of this beetle feed on the seeds, resulting in qualitative and quantitative losses to the stored grains.[6,7] Prevention of this pest in stored grain, foodstuffs, and harvestable crops has attracted critical attention, specifically in territories depending on these crops for nutrition. Faster pest control usually relies on the use of chemical pesticides in agricultural lands and storage areas. Typically, methyl bromide and phosphine pesticides are used to suppress coleopteran pests in stored grains.[8] Excessive use of these chemical pesticides results in the onset of many undesired effects, including toxicity to nontarget

Received: January 29, 2025
Revised: February 20, 2025
Accepted: February 21, 2025
Published: March 3, 2025

Table 1. Toxicity of Hydroxyapatite Nanoparticles (HAPNPs) Prepared Using the Eggshell as a Substrate against the Cowpea Beetle *C. maculatus* Adults[a]

NP source[a]	(LC_{20} (mg/6 g)[b]) - (LC_{50} (mg/6 g)) - (LC_{99} (mg/6 g))	Probit equation $Y = a + bx$	Chi-Square	*p*-Value
Hydroxyapatite nanoparticles from the eggshell	(0.001) - (0.003) - (0.016)	$Y = 5.67 + 55.43x$	0.002	0.999
Malathion	0.001) - (0.003) - (0.056)	$Y = 4.75 + 65.04x$	0.095	0.954

[a]Data of toxicity were collected 24 h post treatment. [b]6 g refers to the quantity of cowpea seeds used in the experiment.

species, negative environmental impacts, and the appearance of pest resistance.[9] The common method for controlling *C. maculatus* relies on using fumigants; nevertheless, this approach has led to many adverse effects on human health.[10]

Hydroxyapatite (HAP), with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$, like other calcium phosphate biocrystals, has proven exceptional bone integration and ingrowth properties. HAP is harmless and biodegradable, and readily binds to the surfaces of bioactive compounds. These properties make it suitable for tissue engineering (TE) and drug delivery applications,[11−17] thanks to its safety. The application of HAPNPs to stored grains can be done by directly mixing HAPNPs with the grains in the storage areas, thanks to its insolubility in water, which allows for the removal of HAPNPs by washing.

Several studies in the literature have addressed the possible effects of HAP nanoparticles on living organisms of the ecosystem, specifically on soil microorganisms and higher animals in the ecosystem. Nano-HAP did not show any negative effects on the soil microbial communities and fortunately increased the number of beneficial bacteria involved in the solubility of phosphorus.[18] Additionally, a previous study evaluated the safety of the synthesized nanoneedle HAP administered orally or subcutaneously in rats and confirmed normal function in both the kidney and liver of the treated individuals.[19] Unfortunately, no reports were found discussing its possible effects on beneficial insects in the ecosystem.

The priority of using HAPNPs rather than other forms of metal and metal oxide NPs was reviewed.[20,21] This study concluded that exposure to metal and metal oxide nanoparticles can induce immunotoxic effects via various mechanisms such as inflammation, oxidative stress (OS), autophagy, and apoptosis. These effects are not present in BM mesenchymal SCs treated with HAPNPs up to 800 μg/mL.[22]

There are numerous ways for synthesizing HAPNPs, including wet chemical deposition, biomimetic approaches, sol−gel, and hydrothermal methods.[23] The structure, shape, and size of the synthesized calcium apatite nanopowders vary according to various synthesis processes. Therefore, it was concluded that any change in the synthesis process will influence the final powder shape and characteristics.[24]

This study aimed to determine the toxicity of synthetic HAP nanoparticles as a nanobased pesticide against the cowpea beetle, *C. maculatus*, one of the main damaging pests of cowpea grains. Furthermore, it addressed the impact of this substance on some physiological and developmental characteristics of the cowpea beetles. Additionally, the protective effect of HAPNPs on cowpea seeds against the infestation of *C. maculatus* immature stages was also investigated.

2. MATERIALS AND METHODS

2.1. Insect Rearing. The initial strain of the cowpea beetle was obtained from the Horticulture Research Institute, Agriculture Research Center (ARC), in Dokki, Egypt. For mass rearing, male and female adults were reared in a 1 L container jar containing seeds of cowpea (8 cm in diameter, 19 cm in depth). Adults were removed from the jars after 1 week to ensure mating and oviposition. Adults emerging from these seeds were collected and used in subsequent experiments. During the experimentation, the temperature was maintained at 29 ± 2 °C and the relative humidity was 60 ± 5% R.H., with about 12 h of light. According to international guidelines, research on insects does not require ethical approval.

2.2. Synthesis of Hydroxyapatite Nanoparticles (HAPNPs). A modified method from ref 20 was used to synthesize eggshell HAPNPs. Briefly, the chicken eggshells were collected, washed in distilled water, dried, and ground in an agate mortar to produce a fine powder. The obtained powder was boiled in a mixture of distilled water and ethanol to remove impurities, followed by a drying step at 100 °C for 24 h and calcination at 500 °C for 3 h to remove the volatile organic compounds. About 22 g of the calcined powder was dissolved in 500 mL of 1 M HCl (36−38%, ADWIC, Egypt) and stirred at room temperature for 1 h. After that, the solution was filtered three times to separate the undissolved material. Then, the filtrate was stirred and heated at 70 °C for 30 min. A solution of NH_4HPO_4 (assay: 98.1%, ADWIC, Egypt) (14.28 g in 200 mL of bidistilled water) was added dropwise with vigorous stirring. The solution's pH was adjusted to 10 using NH_4OH solution (ADWIC, Egypt). After that, the formed precipitate was aged at 70 °C for 2 h. Next, the resulting HAPNP precipitate was filtered and rinsed three times with bidistilled water and twice with ethanol, followed by drying at 80 °C for 24 h. Then, the HAPNP powder was calcined at 500 °C for 3 h under a static air atmosphere.

2.3. Characterization of HAPNPs. The prepared HAPNP sample was analyzed by X-ray diffraction (XRD) using a Philips PW 2103 diffractometer (Netherlands, Cu Kα = 1.54056 Å radiation source). Fourier transform infrared spectroscopy (FTIR, Nicolet spectrophotometer, model 6700, with a wavenumber range of 4000−400 cm^{-1}) was conducted using the KBr technique.[25] The size and shape of the prepared particles were visualized using a transmission electron microscope (TEM, JEOL, Model 100 CX II; Tokyo, Japan).

2.4. Contact Toxicity of HAPNPs against *C. maculatus*. A dose-mortality bioassay was conducted to determine the lethal doses of nanohydroxyapatite (HAPNPs) synthesized from eggshells (Table 1) on adult *C. maculatus*. Briefly, synthesized HAP nanoparticles were applied to 6 g of cowpea seeds in 0.1 L glass jars. Four doses of HAPNPs were tested in the bioassays (0.005, 0.01, 0.05, and 0.1 mg of NPs/6 g of cowpea seeds). After application, the jars were manually shaken to ensure a complete distribution of the nanoparticles. Ten unsexed 0−2-day-old *C. maculatus* adults were placed in each jar, and the jars were sealed with a fine porous cloth to allow ventilation. The jars were maintained under controlled

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732

conditions: 29 ± 2 °C temperature, 65 ± 5% relative humidity, and a 12 h photoperiod. The insect mortality was recorded after 24 h of the exposure period. Insects were considered dead if they did not respond to fine needle stimuli (i.e., several subsequent touches). Ten replications were used per dose, and the control treatment did not receive any application. As a positive control, we used malathion, an organophosphate insecticide, under the same experimental conditions.

2.5. Ovipositional Deterrent Activity of Eggshell-Based HAPNPs on *C. maculatus*. In order to address the impact of HAPNPs on *C. maculatus*, an experiment was conducted to determine the ovipositional deterrence of eggshell-based HAPNPs on *C. maculatus*. Cowpea seeds were treated with sublethal concentrations LC_{20} (0.001 mg NPs/6 g cowpea seeds) and LC_{50} (0.003 mg/6 g cowpea seeds), respectively. For each treatment, cowpea seeds were placed in a 100 mL glass jar (5 cm in diameter, 6.5 cm in depth) mixed with HAPNPs, and then ten adults (0−2 days old) (5 males and 5 females) were released. Jars were covered well with muslin cloth, tightened and fastened with rubber bands, and incubated at 29 ± 2 °C and 65 ± 5% R.H. For the control, cowpea seeds were not exposed to any particles. The experiment was replicated three times. After 24 h, the adults were removed, and the numbers of eggs laid on the cowpea seeds were counted.

2.6. Alterations Caused by HAPNPs on *C. maculatus* and Cowpea Seeds. The infested seeds from the ovipositional deterrence experiment were maintained for about 38 days to record the adult emergence, average development time, and seed weight loss. The average development time (T) is the time needed for the emergence of 50% of adults and was calculated as:[26]

T (days)= D1A1 + D2A2 + D3A3 + ... + DnAn/Total no. of adults emerged

where D1 is the first day on which adults began to emerge, and A1 is the number of adults that emerged on D1.

The seed weight loss was calculated as[27]

Weight loss (%) = (Initial weight of grains − Final weight of grains)/Initial weight of grains × 100

2.7. SEM Analysis of HAPNP-Treated *C. maculatus*. Treatment (HAPNPs treated) and untreated control insects were collected to visualize the morphological changes on *C. maculatus* body following treatment with sublethal concentrations (LC_{20} and LC_{50}) of HAPNPs. Briefly, adults were collected from samples treated for 24 h with HAPNPs and stuck on metallic blocks with double carbon tape immediately, without washing with cacodylate buffer, and fixed in 70% ethanol or glutaraldehyde (Sigma-Aldrich, CAS No. G5882, Darmstadt, Germany) as usual to observe changes on the insect cuticle following nanoparticle treatment. Using gold sputter coating apparatus, samples were evenly gold-coated to a thickness of 15 nm. Samples were examined by using a JEOL JSM 5400 LV scanning electron microscope. Images were taken at different magnifications in different body regions to visualize any changes on the body surfaces of adults.

2.8. Biochemical Assays. Homogenates of control insects or those treated with LC_{20} HAPNPs were used for the biochemical assays. Briefly, insects were homogenized in liquid nitrogen and suspended in potassium phosphate-buffered saline (PBS), pH 7.4 in a 1:10 ratio, and centrifuged (9000 rpm, 10 min, 4 °C). The clear supernatants were stored at −80 °C for biochemical analyses. Hydrogen peroxide and total antioxidant content were measured by commercial kits (Biodiagnostic, Cairo, Egypt) according to the manufacturer's instructions. All assays were performed based on 3 independent replicates using 20 insects.

2.9. Statistical Analyses. The toxicity of HAPNP powder on *C. maculatus* was calculated by probit analysis using SPSS (2025) statistical software. Log transformations of concentrations were chosen for data analysis. Data of fecundity, adult emergence, biochemical studies and seed weight loss were subjected to Student's t-test or one-way ANOVA, and when applicable, means were compared with Tukey's post-hoc test at a probability level of 5% to assess significant differences between treatments. The development time data did not pass the test of normality and were subjected to the H test.

3. RESULTS

3.1. Characterization of the HAPNPs. The XRD pattern of CaHAP from the eggshell is depicted in Figure 1. It was

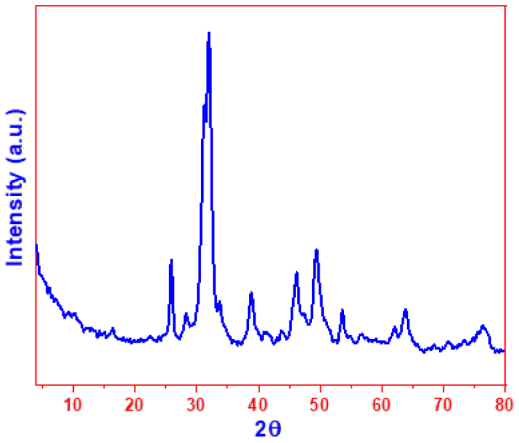


Figure 1. XRD patterns of the prepared hydroxyapatite nanoparticles (HAPNPs).

observed that the CaHAP synthesized from the eggshell showed reflections at 2θ values of 25.8°, 28.3°, 31.1°, 31.9°, 33.7°, 38.8°, 46.2°, 49.4°, 53.6°, and 63.8°, corresponding to the (002), (210), (211), (112), (300), (310), (222), (213), (004), and (304) crystal planes of the hexagonal HAP (PDF card number 01−072−1243),[24,28] respectively. No extraneous peaks related to any impurities were detected in the examined 2θ range, which indicated the high purity of the prepared HAP. Moreover, the average crystallite size for the HAP sample was evaluated using the Scherrer equation[29,30] and was found to be ∼16 nm.

The Fourier transform infrared (FTIR) spectra of the prepared HAPNPs are shown in Figure 2. It shows bands that can be characterized as follows: 3441 cm^{-1} (O−H stretching vibration), 1636 cm^{-1} (O−H bending vibration), 1088 and 1039 cm^{-1} (PO_4^{3-} group asymmetrical stretching, ν_3 of the P−O bonds), 961 cm^{-1} (PO_4^{3-} group symmetrical stretching), and 605 and 564 cm^{-1} (asymmetric vibration mode, ν_4 of the O−P−O bond).

The transmission electron microscopy (TEM) image of the prepared HAP is shown in Figure 3. It displays a mixture of rod and plate particles. The average plate size is ∼62.8 nm, while the rod structure has a length and width of ∼91 nm and ∼22.7 nm, respectively.

3.2. Contact Toxicity of HAP against *C. maculatus*. The contact toxicity of HAP nanoparticles synthesized from

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732

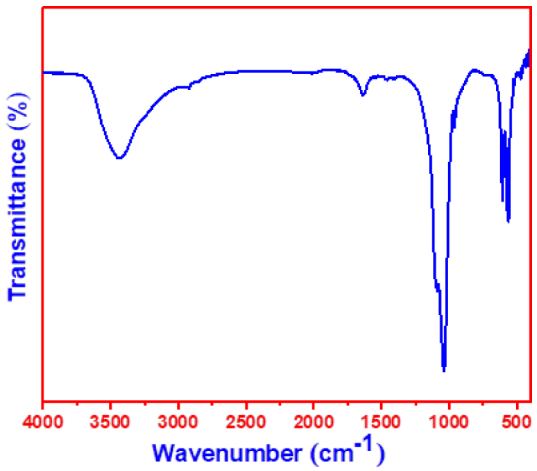


Figure 2. FTIR spectrum of the prepared HAP nanoparticles.

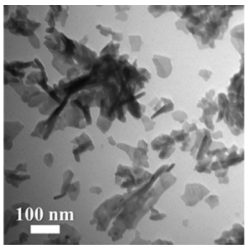


Figure 3. TEM image of the prepared HAP nanoparticles.

eggshells against the cowpea beetle *C. maculatus* is presented in Table 1. Eggshell-based HAPNPs have shown promising toxicity against *C. maculatus* (LC_{50} = 0.003, LC_{90} = 0.016 mg/6 g cowpea seeds) after 24 h, and these results were similar or higher than the toxicity of the positive control (malathion insecticide).

3.3. Physiological and Developmental Effects of Eggshell-Based HAPNPs on *C. maculatus*. The impacts of LC_{20} and LC_{50} of eggshell-based HAPNPs on *C. maculatus* were assessed via monitoring several parameters, including fecundity, adult emergence rate, and developmental time of immature stages. Fecundity of the cowpea beetle *C. maculatus* was reduced by about 90% in LC_{50}-treated *C. maculatus* when compared to the control (Figure 4a). The mean number of eggs in control insects, those insects treated with LC_{20} and LC_{50} was 98.33, 61.00, and 9.33, respectively (Figure 4a) (F = 7.02; df = 8; p = 0.01). The number of eggs laid by females for 24 h was significantly reduced in the insects treated with LC_{50}. The fecundity rate in insects treated with LC_{20} was not significantly different compared to control insects. The eggshell-based HAP nanoparticles interfered with the growth and development of the young stages. The number of emerged adults showed the same pattern as the number of eggs, where LC_{50} caused a significant reduction in the number of emerged adults (Figure 4b) (F = 6.54; df = 8; p = 0.02). Although the developmental period of the immature stage varied in average number among the treatments, there were no significant differences found in the insects treated either with LC_{20} or LC_{50} compared to the untreated control (Figure 4c) (H = 4.42; df = 2; p = 0.11).

3.4. Preventive Effects of HAPNPs on the Seeds of Cowpea Following *C. maculatus* Infestation. In order to address the preventive effect of HAPNPs on cowpea seeds, we

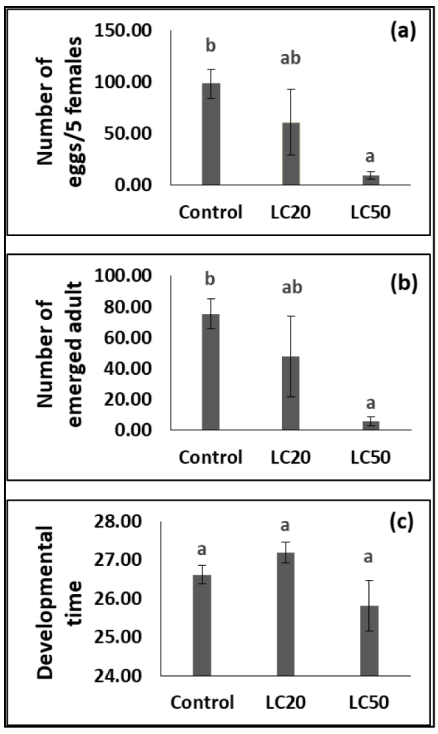


Figure 4. Effect of LC_{20} and LC_{50} of eggshell-based hydroxyapatite (HAP) nanoparticles on *C. maculatus*. Number of eggs laid by five females (a), adult emergence rate (%) (b), and developmental time (days) (c). Data are presented as (mean ± SE) based on 3 replicates. Different letters above error bars represent statistical difference at $p \leq 0.05$.

monitored the average number of holes per 6 g of cowpea seeds as well as their weight loss (Figure 5). Interestingly, the LC_{50} of HAPNPs showed about 6- and 10-fold reduction in the number of holes/6 g seeds (Figure 5a) (F = 5.11; df = 8; p = 0.05) and the seed weight loss percentage (Figure 5b) (F =

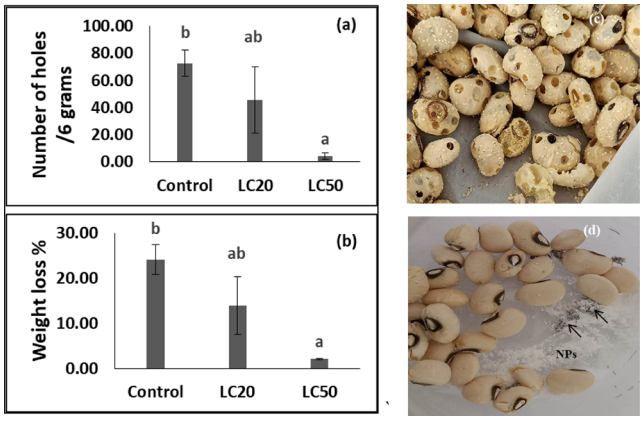


Figure 5. Effect of LC_{20} and LC_{50} of eggshell-based hydroxyapatite (HAP) nanoparticles on *C. maculatus*. Number of holes representing the destruction caused by *C. maculatus* in each 6 g seeds (a), seed weight loss due to *C. maculatus* immature stage infestation (b). Photographs of the protective effect of HAPNPs on cowpea seeds in untreated control (c) and LC_{50}-treated adults of *C. maculatus* (d). Data are presented as (mean ± SE) based on 3 replicates. Different letters above error bars represent statistical differences at $p \leq 0.05$. NPs (nanoparticles) are represented by arrows in black showing dead adult insects.

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732

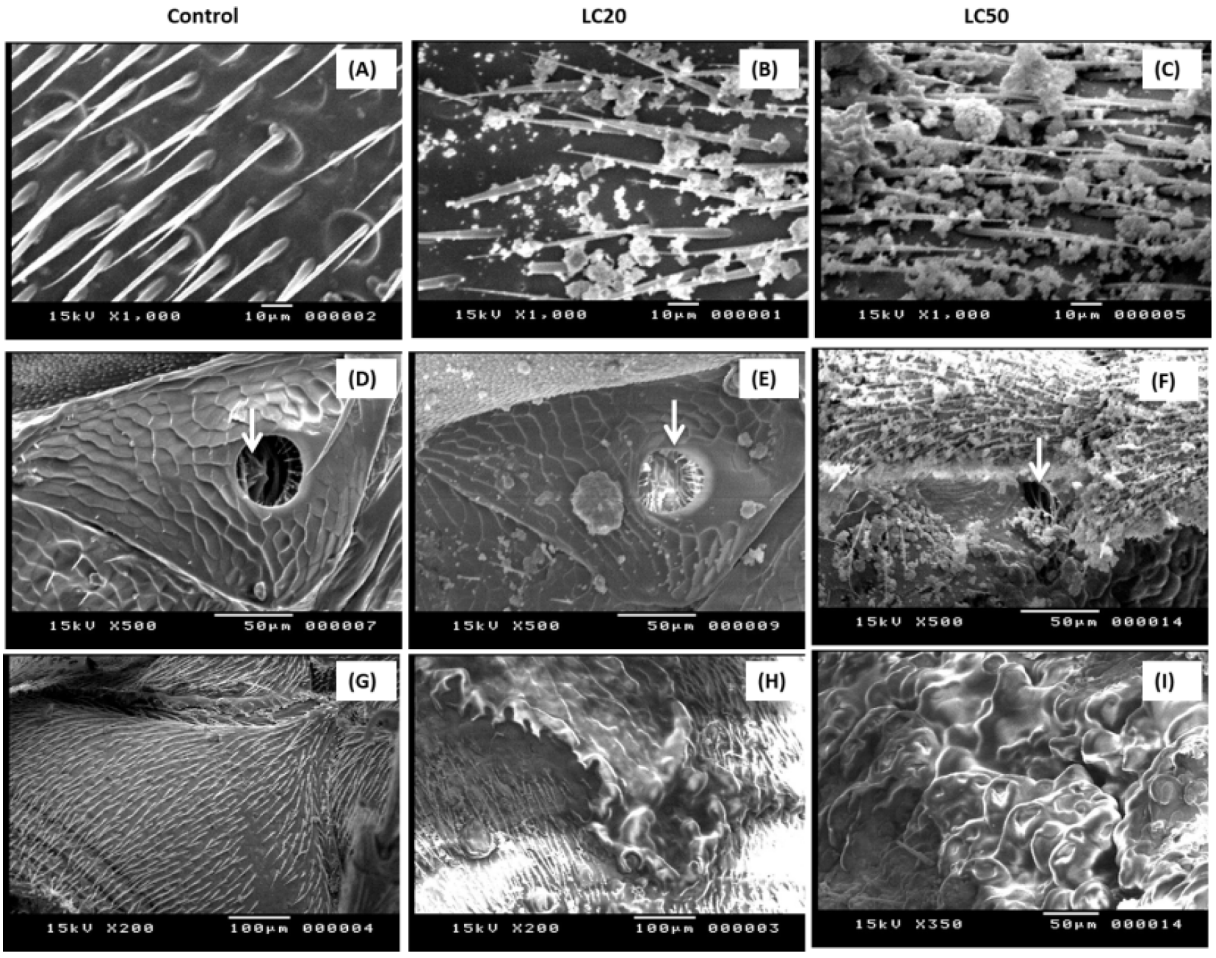


Figure 6. Scanning electron micrographs of control, LC_{20}, and LC_{50} HAPNP-treated *C. maculatus*. Sensilla of control (A) and HAPNPs treated with LC_{20} (B) and LC_{50} (C) *C. maculatus*. Spiracles of control (D) and HAPNP-treated with LC_{20} (E) and LC_{50} (F) *C. maculatus*. Arrows in white refer to spiracles. Changes in the wax layer of the cuticle were monitored in Control (G) and HAPNP-treated with LC_{20} (H) and LC_{50} (I) *C. maculatus*. The wax layer of HAPNP-treated insects is seriously damaged in both LC_{20} and LC_{50}.

9.75; df = 8; $p = 0.01$), indicating a significant inhibition in the destructive effects of *C. maculatus* on cowpea seeds.

3.5. Scanning Electron Microscopy of HAPNP-Treated *C. maculatus*. Based on the fact that adults are not feeding on cowpea, the destructive effects arise mainly from immature stages. We designed our ultrastructural analysis using SEM, assuming external manipulation of *C. maculatus* adults. Images obtained from SEM showed normal appearance of the external body cuticle with normal appearance of sensillae. Additionally, it showed that HAPNPs were strongly attached to the body surface of the insects. The adults of *C. maculatus* exposed to cowpea seeds treated with LC_{20} (0.001 mg/6 g cowpea seeds) and LC_{50} (0.003 mg/6 g cowpea seeds) of HAPNPs appeared densely and uniformly coated with particles. HAPNPs seem to be attached strongly to the sensilla of the adult insect (Figure 6a−c).

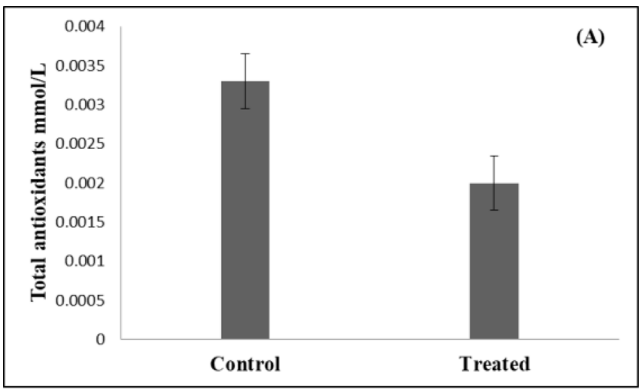
Applying HAP nanoparticles led to some morphological changes in spiracle shape (Figure 6d−f). Unlike the control (Figure 6d), it seems that the spiracle opening was contracted to prevent particle entry (Figure 6e,f). This appeared from the decreased atrium space size and clear appearance of the inner valve-like structures that allow them to close, leading to respiration prevention. The images in Figure 6h,i show that HAP attached strongly to the cuticular wax layer of insects by a sorption process, leading to disruption of the wax layer.

3.6. Effect of HAPNPs on Antioxidant-Free Radical Balance in *C. maculatus*. In order to understand whether the physiological and developmental changes in adult *C. maculatus* are associated with oxidative stress, the total antioxidant capacity and the free radical indicator, hydrogen peroxide, were measured in control and LC_{20} HAPNP-treated *C. maculatus*. Treatments did not affect either the total antioxidant content (Figure 7A) ($t = 2.623$, df = 4; $p = 0.059$) or H_2O_2 (Figure 7B) ($t = 0.85$, df = 4; $p = 0.443$).

4. DISCUSSION

The current study highlights the negative effects of the immature stages of the serious stored grain pest *C. maculatus* while attacking the seeds of cowpea. It also evaluates the possible use of a chemical compound, hydroxyapatite nanoparticles, in controlling such pests via targeting their adult stage, leading to a reduced population. Generally, 17% of food production is lost during the storage step of food resources, with insects causing more than 60% of this loss.[31] Legumes and cereal grains supply about 80% of plant-originated food.[32] This situation is pushing toward finding the optimum control method for pests infesting these food sources. Control of these pests depends mainly on mechanical and chemical methods. Chemical control necessarily requires finding highly safe chemical compounds. Hydroxyapatite is present naturally as

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732



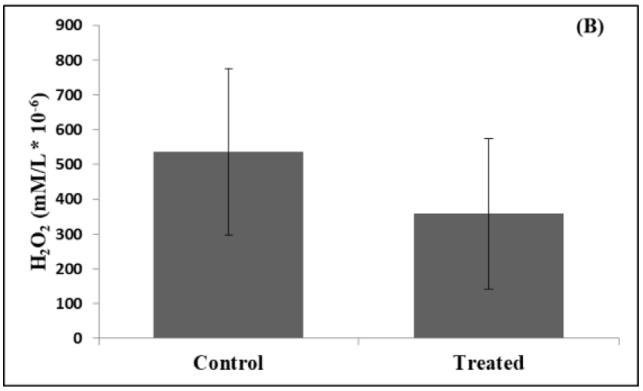


Figure 7. Effect of HAPNP treatment on the antioxidant-free radical balance in *C. maculatus*. Adults were treated with LC_{20} of HAPNPs and their effect was monitored on untreated adults (Control) or treated adults (Treated). (A) total antioxidants and (B) hydrogen peroxide. Data are presented as (mean ± SE) based on 3 replicates. Data retrieved from statistical analysis showed no statistical difference, $p \leq 0$.

calcium apatite.[33] Its nanoform has many applications in our daily life activities,[34−37] making it an ideal candidate against stored grain pests, as it can be easily washed from the grains before use.

Based on the abovementioned facts, the present study demonstrated the efficacy of hydroxyapatite nanoparticles in protecting cowpea seeds from the infestation of the insect pest *C. maculatus* in its immature stages.

The prepared HAPNPs were characterized by XRD, FTIR, and electron microscopy. X-ray diffraction (XRD) data suggested highly pure crystal planes of hexagonal HAPNPs. Fourier transform infrared (FTIR) spectroscopy was also used to identify the chemical bonding of the prepared HAPNPs.[38] The FTIR pattern showed bands corresponding to orthophosphate (PO_4^{3-}), O−H stretching, and vibrational mode of the structural hydroxyl group.[20,39] Data retrieved from EM characterization showed an average plate size of ∼62.8 nm and a rod structure with a length and width of ∼91 nm and ∼22.7 nm, respectively. These fluctuations in the size and shape of the prepared particles were expected to introduce a broader range of effects on treated *C. maculatus*. In general, size and shape are the detrimental factors in nanoparticle toxicity.[40−42] For instance, the growth index of *S. litura* and *Achaea janata* was retarded in nanoparticle treatments with a size range of up to 100 nm.[43] Additionally, three different shapes of carbon nanomaterials led to varied effects on *Daphnia magna*.[44]

HAP nanoparticles prepared from eggshell precursors showed high toxicity against *C. maculatus*. As a general phenomenon, the ways in which nanostructures act against their target organisms are governed by their chemical and physical characteristics, such as their size, shape, and concentration.[42] Generally, several forms of nanoparticles have been used in the control of several agricultural pests and insect vectors.[42−45] After a careful review of the literature, most of the trials using nanoparticles in pest management relied on metals and metal oxides.[42−45] However, the insecticidal activity of HAP nanoparticles has not been previously reported in the literature, regardless that this material was used previously as a carrier molecule to deliver Cu ions for the purposes of pest management.[46]

This study clearly showed a significant impact of eggshell-based HAPNPs on the fecundity and emergence rate of *C. maculatus* on cowpea. Several forms of nanostructures affected the developmental success and the fecundity rate in insects. Zinc oxide and silver nanoparticles negatively affected the oviposition rate of *Spodoptera frugiperda* and *Drosophila*.[47,48] It was proposed that the reduced fecundity is caused by the quick distribution of the nanoparticles within an organism's reproductive organs, which in turn led to the production of reactive oxygen species.[49] Additionally, HAPNPs showed protective effects on cowpea seeds, which appeared in the form of a reduced number of holes caused by the immature stages of the beetle and reduced seed weight loss. It was reported that uncontrolled infestation of *C. maculatus* on cowpea seeds leads to the appearance of quantitative and qualitative losses in their value.[50] The current study revealed protective effects of HAPNPs on cowpea seeds, suggesting a promising future for their use against *C. maculatus* infestation.

Although adult *C. maculatus* do not feed on cowpea seeds, as the feeding stage is restricted to the larvae, it was interesting to find the results obtained from toxicity studies reporting high mortality rates in adult *C. maculatus*. This finding suggests a need to understand how this unfed stage (unfed on cowpea seeds) was affected by HAPNP treatment.

It was observed from the results that adult *C. maculatus* suffered from desiccation and spiracular blockage following exposure to HAP nanoparticles. In most cases, the modes by which chemical insecticides reach the internal structures of the insect body can be categorized into 3 routes: penetration via the integument, oral, and spiracular inhalations.[51] The scanning electron microscopic studies of adult *C. maculatus* exposed to HAP nanoparticles showed the attachment of nanoparticles all over the body of *C. maculatus* with scratches and abrasions on the cuticle. This subsequently led to the loss of water through dehydration and finally the death of the insects.[52] Damage could have occurred to the protective wax coat on the cuticle of insects, both by sorption and abrasion.[53] It is believed that this hypothesis for the physical mode of action strengthens the use of nanocides for the control of storage pests.[54] As a result, it is assumed that there would not be any genetic selection of resistance or physiological resistance to such a mechanism of action of nanoparticles.[54] Furthermore, it has been shown that finely split particles improve the dust's insecticidal efficiency. The physical properties of the seeds are also critical for the adhesion of nanoparticles to their surface, in addition to insect species and their developmental stage.[55]

Hydroxyapatite nanoparticles did not affect the content of antioxidants and H_2O_2 in *C. maculatus*. Insecticides, as

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732

mentioned before, reach the insect body through 3 routes: penetration via the integument and oral and spiracular inhalations. We excluded the oral inhalation route based on the fact that *C. maculatus* adult is a nonfeeding stage. Scanning electron microscopy showed signs of cuticular damage and spiracular blockage. Induction of toxic effects via cuticle abrasion by metal oxide nanostructures was reviewed in several reports.[56,57] Acting over the insect cuticle by insecticides is achieved through abrasion, leading to damage of the cuticular water barrier; therefore, insects begin to lose water from their bodies and finally die.[58,59] This route relies on external effects on the cuticle of the insects, leading to slight changes in the free radical and antioxidant levels. On the contrary, spiracular blockage decreases the respiratory rate in insect bodies, leading to oxidative stress and increased levels of free radicals and subsequently the antioxidant content to overwhelm the impact of free radicals. For instance, the accumulation of carbaryl on the spiracular openings of *S. littoralis* led to increased levels of antioxidant enzymes and free radicals due to a lack of sufficient oxygen.[60] Our findings showed the unaffected antioxidant-free radical balance in *C. maculatus*. This might support the speculation that HAPNPs are likely to act against *C. maculatus* via cuticular abrasion rather than other routes of toxicity.

5. CONCLUSIONS

The current study showed that HAPNPs can be used to control *C. maculatus* beetles. The hydroxyapatite nanoparticles (HAPNPs) showed toxic effects against *C. maculatus* adults and disrupted the beetle's biology. The signs of effects appeared as a reduction in fecundity, emergence rates, and seed weight loss. Additionally, HAPNPs interfered with beetle respiration and cuticular architecture, leading to dehydration of the adult cuticle and consequently death. These results suggested that HAP nanoparticles can be used in a promising IPM program to control the cowpea beetle, *C. maculatus*, thanks to their mode of action and their safety as a chemical pesticide.

AUTHOR INFORMATION

Corresponding Author

Ahmed M. A. Ibrahim − *Department of Zoology and Entomology, Faculty of Science, Assiut University, Assiut 71516, Egypt*; orcid.org/0000-0003-1292-9979; Phone: + 201119640956; Email: ahmedmai1982@aun.edu.eg; Fax: + 20882080209

Authors

Nawal Abdulaziz Alfuhaid − *Department of Biology, College of Science and Humanities in Al-kharj, Prince Sattam Bin Abdulaziz University, Al-kharj 16326, Saudi Arabia*

Samar Adel − *Department of Zoology and Entomology, Faculty of Science, Assiut University, Assiut 71516, Egypt*

Mohamed A. Amro − *Plant Protection Research Institute, ARC, Dokki 12611, Egypt*

Mohamed H. A. Hassan − *Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut 71516, Egypt*

Ali Mohamed Ali − *Department of Zoology and Entomology, Faculty of Science, Assiut University, Assiut 71516, Egypt*

Mohamed Abd El-Aal − *Catalysis and Surface Chemistry Lab*Chemistry Department, Faculty of Science, Assiut University, Assiut 71516, Egypt*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.5c00882

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Mssillou, I.; Saghrouchni, H.; Saber, M.; Zannou, A. J.; Balahbib, A.; Bouyahya, A.; Allali, A.; Lyoussi, B.; Derwich, E. Efficacy and role of essential oils as bio-insecticide against the pulse beetle Callosobruchus maculatus (F.) in post-harvest crops. *Indust Crops Prod.* **2022**, *189*, 115786.

(2) Tuda, M.; Chou, L. Y.; Niyomdham, C.; Buranapanichpan, S.; Tateishi, Y. Ecological factors associated with pest status in Callosobruchus (Coleoptera: Bruchidae): High host specificity of non-pests to Cajaninae (Fabaceae). *J. Stored Prod Re.* **2005**, *41* (1), 31−45.

(3) França, A. F. J.; Araújo, J. N.; Santos, Y. Q.; Carelli, G. S. C.; Silva, D. A.; Amorim, T. M. L.; Migliolo, A. F.; Santos, E. A.; Oliveira, A. S.; Uchôa, A. F. Vicilin from Anadenanthera colubrina Seeds: An alternative tool to combat Callosobruchus maculatus. *Saudi J. Biol. Sci.* **2021**, *28* (9), 5229−5237.

(4) Jayathilake, C.; Visvanathan, R.; Deen, A.; Bangamuwage, R.; Jayawardana, B. C.; Nammi, S.; Liyanage, R. Cowpea: An overview on its nutritional facts and health benefits. *J. Sci. Food Agri.* **2018**, *98* (13), 4793−4806.

(5) Owade, J. O.; Abong', G.; Okoth, M.; Mwang'ombe, A. W. A review of the contribution of cowpea leaves to food and nutrition security in East Africa. *Food Sci. Nut.* **2020**, *8* (1), 36−47.

(6) Lima, M. P. L. D.; Oliveira, J. V. D.; Barros, R.; Torres, J. B. Alternation of cowpea genotypes affects the biology of Callosobruchus maculatus (Fabr.)(Coleoptera: Bruchidae). *Scientia Agricola.* **2004**, *61*, 27−31.

(7) Masoumi, Z.; Noghabi, S. S.; Izadi, H. Trehalose and proline failed to enhance cold tolerance of the cowpea weevil, Callosobruchus maculatus (F.)(Col.: Bruchidae). *J. Stored Prod. Res.* **2021**, *93*, 101853.

(8) Murugesan, R.; Vasuki, K.; Kaleeswaran, B.; Santhanam, P.; Ravikumar, S.; Alwahibi, M. S.; Saliman, D. A.; Almunqedhi, B. M. A.; Alkahtani, J. Insecticidal and repellent activities of Solanum torvum (Sw.) leaf extract against stored grain pest, Callosobruchus maculatus (F.)(Coleoptera: Bruchidae). *J. King Saud Uni-Sci.* **2021**, *33* (3), 101390.

(9) Okonkwo, E. U.; Okoye, W. I. The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by Callosobruchus maculatus (Fabricus)-(Coleoptera: Bruchidae) and Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. *Int. J. Pest Manage.* **1996**, *42* (3), 143−146.

(10) Garry, V. F.; Griffith, J.; Danzl, T. J.; Nelson, R. L.; Whorton, E. B.; Krueger, L. A.; Cervenka, J. Human genotoxicity: Pesticide applicators and phosphine. *Science* **1989**, *246* (4927), 251−255.

(11) Whited, B. M.; Whitney, J. R.; Hofmann, M. C.; Xu, Y.; Rylander, M. N. Pre-osteoblast infiltration and differentiation in highly porous apatite-coated PLLA electrospun scaffolds. *Biomaterials* **2011**, *32* (9), 2294−2304.

(12) Zhao, R.; Xie, P.; Zhang, K.; Tang, Z.; Chen, X.; Zhu, X.; Fan, Y.; Yang, X.; Zhang, X. Selective effect of hydroxyapatite nanoparticles on osteoporotic and healthy bone formation correlates with intracellular calcium homeostasis regulation. *Acta Biomater.* **2017**, *59*, 338−350.

(13) Abd El-Aziz, A.; El Backly, R. M.; Taha, N. A.; El-Maghraby, A.; Kandil, S. H. Preparation and characterization of carbon nanofibrous/hydroxyapatite sheets for bone tissue engineering. *Mater. Sci. Eng., C* **2017**, *76*, 1188−1195.

(14) Li, B.; Kan, L.; Zhang, X.; Li, J.; Li, R.; Gui, Q.; Ma, F.; He, N.; Wang. Wei, H.; Wang, Y.; Wei, H. Biomimetic bone-like hydroxyapatite by mineralization on supramolecular porous fiber networks. *Langmuir* **2017**, *33* (34), 8493−8502.

(15) Nazeer, M. A.; Yilgör, E.; Yilgör, I. Intercalated chitosan/hydroxyapatite nanocomposites: Promising materials for bone tissue engineering applications. *Carbohyd. Polym* **2017**, *175*, 38−46.

(16) Szcześ, A.; Hołysz, L.; Chibowski, E. Synthesis of hydroxyapatite for biomedical applications. *Adv. Colloid Interface* **2017**, *249*, 321−330.
(17) Yao, Q.; Cosme, J. G.; Xu, T.; Miszuk, J. M.; Picciani, P. H.; Fong, H.; Sun, H. Three dimensional electrospun PCL/PLA blend nanofibrous scaffolds with significantly improved stem cells osteogenic differentiation and cranial bone formation. *Biomaterials* **2017**, *115*, 115−127.
(18) Kumar, A.; Mungali, M.; Dwivedi, A. K.; Meenakshi; Sakshi. Assessment of nano-hydroxyapatite (nHAP) adequacy in contemporary farming for soil health stipulation: A comprehensive review. *Int. J. Of Adv. Biochem Res.* **2024**, *8* (3), 142−148.
(19) Hafez, A.; Hafez, F.; Khedr, M.; Ibrahim, O.; Sabry, R.; Abdel-Wahhab, P. M. A. Safety evaluation of needle-like hydroxyapatite nanoparticles in female rats. *Egy Pharmaceut. J.* **2012**, *11* (2), 67−72.
(20) Bee, S.-L.; Bustami, Y.; Ul-Hamid, A.; Lim, K.; Hamid, Z. A. A. Synthesis of silver nanoparticle-decorated hydroxyapatite nanocomposite with combined bioactivity and antibacterial properties. *J. Mater. Sci.: mater. Med.* **2021**, *32* (9), 106.
(21) Bi, J.; Mo, C.; Li, S.; Huang, M.; Lin, Y.; Yuan, P.; Liu, Z.; Jia, B.; Xu, S. Immunotoxicity of metal and metal oxide nanoparticles: from toxic mechanisms to metabolism and outcomes. *Biomater. Sci.* **2023**, *11* (12), 4151−4183.
(22) (a) Remya, N. S.; Syama, S.; Gayathri, V.; Varma, H. K.; Mohanan, P. V. An in vitro study on the interaction of hydroxyapatite nanoparticles and bone marrow mesenchymal stem cells for assessing the toxicological behaviour. *Colloids Surf., B* **2014**, *117*, 389−397. (b) Remya, N. S.; Syama, S.; Gayathri, V.; Varma, H. K.; Mohanan, P. V. Corrigendum to "An in vitro study on the interaction of hydroxyapatite nanoparticles and bone marrow mesenchymal stem cells for assessing the toxicological behaviour" [Colloids Surf. B: Biointerfaces 117 (2014) 389−397]. *Erratum in: Colloids Surf. B Biointerfaces* **2021**, *207*, 112000.
(23) Nayak, A. K. Hydroxyapatite synthesis methodologies: An overview. *Int. J. ChemTech Res.* **2010**, *2* (2), 903−907.
(24) Gui, X.; Peng, W.; Xu, X.; Su, Z.; Liu, G.; Zhou, Z.; Kong, Q.; Li, Z.; Song, G.; Zhou, C.; Kong, Q. Synthesis and application of nanometer hydroxyapatite in biomedicine. *Nanotech Rev.* **2022**, *11* (1), 2154−2168.
(25) Alamri, S.; Nafady, N. A.; El-Sagheer, A. M.; El-Aal, M. A.; Mostafa, Y. S.; Hashem, M.; Hassan, E. A. Current Utility of Arbuscular Mycorrhizal Fungi and Hydroxyapatite Nanoparticles in Suppression of Tomato Root-Knot Nematode. *Agronomy* **2022**, *12*, 671.
(26) Howe, R. W. A parameter for expressing the suitability of an environment for insect development. *J. Stored Prod. Res.* **1971**, *7* (1), 63−65.
(27) Singh, D.; Boopathi, T. Callosobruchus chinensis (Coleoptera: Chrysomelidae): Biology, life table parameters, host preferences, and evaluation of green gram germplasm for resistance. *J. Stored Prod. Res.* **2022**, *95*, 101912.
(28) Pierantozzi, D.; Scalzone, A.; Jindal, S.; Stīpniece, L.; Šalma-Ancāne, K.; Dalgarno, K.; Gentile, P.; Mancuso, E. 3D Printed Sr-Containing Composite Scaffolds: Effect of Structural Design and Material Formulation towards New Strategies for Bone Tissue Engineering. *Compos. Sci. Technol.* **2020**, *191*, 108069.
(29) Said, A. E.-A.-A.; Abd El-Wahab, M. M.; El-Aal, M. A. The Catalytic Performance of Sulfated Zirconia in the Dehydration of Methanol to Dimethyl Ether. *J. Mol. Catal. A: chem.* **2014**, *394*, 40−47.
(30) Ali, H. M.; Ibrahim, S. M.; Abo Zeid, E. F.; Al-Hossainy, A. F.; El-Aal, M. A. A Comparative Study of Cu-Anchored 0D and 1D ZnO Nanostructures for the Reduction of Organic Pollutants in Water. *RSC Adv.* **2022**, *12*, 16496−16509.
(31) Bouchelos, K. T. *Insects of warehouses and food*; Embryo Publications: Athens, Greece, 2018; pp. 133.
(32) Stamopoulos, D. K. *Enemies of Stored Products Museums and Homes*; University of Thessaly Press: Volos, Greece, 2013; pp. 246.
(33) Du, M.; Chen, J.; Liu, K.; Xing, H.; Song, C. Recent advances in biomedical engineering of nano-hydroxyapatite including dentistry, cancer treatment and bone repair. *Composites, Part B* **2021**, *215*, 108790.
(34) Ibrahim, M.; Labaki, M.; Giraudon, J. M.; Lamonier, J. F. Hydroxyapatite, a multifunctional material for air, water and soil pollution control: A review. *J. Hazard Mater.* **2020**, *383*, 121139.
(35) Balasooriya, I. L.; Chen, J.; Korale Gedara, S. M.; Han, Y.; Wickramaratne, M. N. Applications of nano hydroxyapatite as adsorbents: A review. *Nanomaterials* **2022**, *12* (14), 2324.
(36) Oonishi, H.; Oonishi, H., Jr; Kim, S. C.; Hench, L. L.; Wilson, J.; Tsuji, E.; Oomamiuda, K. Clinical application of hydroxyapatite. In *Bioceramics and their clinical applications*; Woodhead Publishing, 2008, pp. 606−687. .
(37) Zhang, K.; Zhou, Y.; Xiao, C.; Zhao, W.; Wu, H.; Tang, J.; Zhang, X.; Yu, S.; Li, X.; Min, L.; Yu, Z. Application of hydroxyapatite nanoparticles in tumor-associated bone segmental defect. *Sci. Adv.* **2019**, *5* (8), No. eaax6946.
(38) Huang, A.; Dai, H.; Wu, X.; Zhao, Z.; Wu, Y. 2019."Synthesis and characterization of mesoporous hydroxyapatite powder by microemulsion technique". *J. Of Mater. Res. Technol.* **2019**, *8* (3), 3158−3166.
(39) Tran, T. Q.; Pham Minh, D.; Phan, T. S.; Pham, Q. N.; Nguyen Xuan, H. Dry reforming of methane over calcium-deficient hydroxyapatite supported cobalt and nickel catalysts. *Chem. Eng. Sci.* **2020**, *228*, 115975.
(40) Ibrahim, A. M.; Ali, A. M. Silver and zinc oxide nanoparticles induce developmental and physiological changes in the larval and pupal stages of Spodoptera littoralis (Lepidoptera: Noctuidae). *J. Asia-Pacific Entomol.* **2018**, *21* (4), 1373−1378.
(41) Ibrahim, A. M. A.; Thabet, M. A.; Ali, A. M. Physiological and developmental dysfunctions in the dengue vector Culex pipiens (Diptera: Culicidae) immature stages following treatment with zinc oxide nanoparticles. *Pestic. Biochem. Physiol.* **2023**, *192*, 105395.
(42) Ibrahim, A. M. A.; Alfuhaid, N. A.; Thabet, M. A.; Ali, A. M. Chronic effects induced by zinc oxide nanoparticles against larvae of the northern house mosquito Culex pipiens (Diptera: Culicidae). *Int. J. Trop Insect Sci.* **2023**, *43* (6), 1937−1945.
(43) Yasur, J.; Pathpati, U. R. Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology. *Chemosphere* **2015**, *124*, 92−102.
(44) Bacchetta, R.; Santo, N.; Valenti, I.; Maggioni, D.; Longhi, M.; Tremolada, P. Comparative toxicity of three differently shaped carbon nanomaterials on Daphnia magna: does a shape effect exist? *Nanotoxicology* **2018**, *12* (3), 201−223.
(45) Mohamed, D. S.; Al-Fuhaid, N. A.; Abeed, A. A. O.; Ibrahim, A. M. A novel pyrazole−pyridine derivative (PPD) targets specific biological pathways in the larval stages of the northern house mosquito Culex pipiens Linnaeus (Diptera: Culicidae). *J. Basic Appl. Zool.* **2023**, *84* (1), 29.
(46) Battiston, E.; Antonielli, L.; Di Marco, S.; Fontaine, F.; Mugnai, L. Innovative delivery of Cu (II) ions by a nanostructured hydroxyapatite: potential application in planta to enhance the sustainable control of Plasmopara viticola. *Phytopathology* **2019**, *109* (5), 748−759.
(47) Pittarate, S.; Rajula, J.; Rahman, A.; Vivekanandhan, P.; Thungrabeab, M.; Mekchay, S.; Krutmuang, P. Insecticidal effect of zinc oxide nanoparticles against Spodoptera frugiperda under laboratory conditions. *Insects* **2021**, *12* (11), 1017.
(48) Raj, A.; Shah, P.; Agrawal, N. Dose-dependent effect of silver nanoparticles (AgNPs) on fertility and survival of Drosophila: An in-vivo study. *PLoS One* **2017**, *12* (5), No. e0178051.
(49) Dziewięcka, M.; Witas, P.; Karpeta-Kaczmarek, J.; Kwaśniewska, J.; Flasz, B.; Balin, K.; Augustyniak, M. Reduced fecundity and cellular changes in Acheta domesticus after multi-generational exposure to graphene oxide nanoparticles in food. *Sci. Total Environ.* **2018**, *635*, 947−955.
(50) Taylot, T. A. *Crop Pests and Diseases Studies in the Development of African Resources*; Oxford University Press, 1977, Vol. 4, pp. 70.

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732

(51) Ebeling, W. Sorptive dusts for pest control. *Ann. Rev. Entomol.* **1971**, *16* (1), 123−158.
(52) Sugiura, M.; Horibe, Y.; Kawada, H.; Takagi, M. Insect spiracle as the main penetration route of pyrethroids. *Pest. Biochem. Physiol.* **2008**, *91*, 135−140.
(53) Debnath, N.; Das, S.; Seth, D.; Chandra, R.; Bhattacharya, S. C.; Goswami, A. Entomotoxic effect of silica nanoparticles against Sitophilus oryzae (L.). *J. Pest Sci.* **2011**, *84*, 99−105.
(54) Arumugam, G.; Velayutham, V.; Shanmugavel, S.; Sundaram, J. Efficacy of nanostructured silica as a stored pulse protector against the infestation of bruchid beetle, Callosobruchus maculatus (Coleoptera: Bruchidae). *Appl. Nanosci.* **2016**, *6*, 445−450.
(55) Fields, P.; Korunic, Z. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. *Jo Stored Prod Res.* **2000**, *36* (1), 1−13.
(56) Ayoub, H. A.; Khairy, M.; Elsaid, S.; Rashwan, F. A.; Abdel-Hafez, H. F. Pesticidal activity of nanostructured metal oxides for generation of alternative pesticide formulations. *J. Agric. Food Chem.* **2018**, *66* (22), 5491−5498.
(57) Ayoub, H. A.; Khairy, M.; Rashwan, F. A.; et al. Synthesis and characterization of silica nanostructures for cotton leaf worm control. *J. Nanostruct. Chem.* **2017**, *7*, 91−100.
(58) Debnath, N.; Das, S.; Seth, D.; Chandra, R.; Bhattacharya, S. C.; Goswami, A. Entomotoxic effect of silica nanoparticles against Sitophilus oryzae (L.). *J. Pest Sci.* **2011**, *84*, 99−105.
(59) Debnath, N.; Mitra, S.; Das, S.; Goswami, A. Synthesis of surface functionalized silica nanoparticles and their use as entomotoxic nanocides. *Powder Technol.* **2012**, *221*, 252−256.
(60) Adamski, Z. Exposure to carbaryl leads to ultrastructural changes and alters activity of antioxidant enzymes in Spodoptera exigua (Lepidoptera: Noctuidae). *Invert Biol.* **2007**, *126*, 191−201.

https://doi.org/10.1021/acsomega.5c00882
ACS Omega 2025, 10, 10724−10732