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RESEARCH PAPER

Evaluation of the Efficacy of Copper Oxide and Chromium Oxide as Nanopesticides in Controlling Sub-Adult Stages of the Great Wax Moth

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ABSTRACT

This study aim to evaluate the effectiveness of copper oxide nanoparticles (CuO-NPs) and chromium oxide nanoparticles (Cr_2O_3-NPs) as nanopesticides to control sub-adult stages of the insect pest known as the great waxworm (*Galleria mellonella*). This insect is a major pest that attacks honeybees and causes significant economic losses to hives and stores. The experiment was carried out in the laboratories of the Faculty of Education for Pure Sciences at Kirkuk University during the period from 1 January 2025 to 1 April 2025. The concentrations used for both nanomaterials included 250, 500, and 1000 ppm, in addition to a control group. The statistical significance of the results was determined at the $p \leq 0.05$ level. The results showed that both types of nanoparticles had significant negative effects on the greater waxworm, and these effects were directly proportional to the increase in concentration. In the egg stage, an enhancement in the average hatching time and a reduction in the hatching rate were observed. In the larval stage, the duration of larval development increased and mortality increased significantly. These adverse effects continued in the pupal stage, where the duration of the pupal stage was increased and mortality was also increased. These effects are attributed to the ability of nanoparticles to induce oxidative stress and cell damage in the developmental stages. This study confirms that copper oxide and chromium oxide nanoparticles are effective in inhibiting the growth and increasing the mortality of wax worm stages.

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INTRODUCTION

Insects are the most successful and diverse group of animals on earth and have shared complex relationships with humans. Although they are indispensable as pollinators of crops, they also act as major destroyers in stores and fields and transmit many diseases to humans, livestock and other animals [1]. *Galleria mellonella* of the order Pyralidae Lepidoptera is one of the most prominent insect pests that attack honeybees [2]. It is one of the most destructive economic pests of hives and wax foundations due to its rapid reproduction and voracious larvae, which attack wax discs and pollen both inside the hive and in the store [3]. Their larvae feed mainly on stored or old wax discs that are dark in color due to their protein content, as well as feeding in weakened honeybee cells on all wax and pollen contents

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of the frame, causing the disappearance of the wax coverings of the six eyes or what is known as the Bald brood and impeding the movement of the brood [4]. They also hinder the movement of workers between frames by producing dense silk threads between the frames, which causes hive weakness, bee death and the occurrence of Gallerias. The faecal waste of the larvae contains spores of the American brood rot bacterium Paenibacillus larvae [5]. The economic damage caused by insect pests is enormous [6]. So we must adopt advanced pest management strategies to reduce economic losses. Nanotechnology is a promising technology for control within integrated pest management (IPM) programs [7]. Insecticide formulations using nanomaterials as active ingredient carriers have shown promising results for the reduction of agricultural and stored pests and disease vectors [1]. A variety of phytochemical groups act as reducing and stabilizing agents during the biosynthesis of metal nanoparticles that can act as bio-nano pesticides. Moreover, green synthesis of metal nanoparticles or nano-conjugates has experimentally demonstrated success in controlling [8]. These particles possess new physical, chemical, and biological properties [9], which are considered as safe alternative within the integrated control framework and as feed inhibitors, repellents, and growth inhibitors against different species of stored insects [10]. CuO and Cr_2O_3 are among the most important compounds. These materials are effective nanopesticide against some storage insects [11]. Their effect is attributed to ability for production of reactive oxygen species (ROS) and oxidative stress, which leads to membrane damage and inactivation of insect proteins and enzymes. Our study aimed to evaluate the efficiency of some integrated control agents as compared with the sub-adult stages of the large waxworm. The effect of different concentrations of CuO and Cr_2O_3 nanomaterials on some sub-adult stages (egg, larva and pupa) were studied by measuring egg incubation rate, hatching rate, larval and pupal development rate as well as mortality rate in both stages.

MATERIALS AND METHODS

Chemicals

CuO and Cr_2O_3 nanoparticles were used as nanopesticides against greater wax moth (*Galleria mellonella*). The physical properties of CuO and Cr_2O_3 were analyzed using a Horiba SZ-100 on Sunday, 26 November 2023. The results showed that the particle size ranged from 20-50 nm. The Zeta potential for Cr_2O_3 was -22.33 mV and for CuO was 95.6 mV, indicating the purity and high stability of the samples.

Preparation of nanosuspensions (dilutions)

CuO and Cr_2O_3 nanosuspensions were prepared using deionized water as a solvent and polyvinyl alcohol (PVA) as a dispersant. 400 mL of each concentration (1000, 500 and 250 ppm) of materials was prepared using magnetic stirring. After preparation, the dilutions were stored in opaque glass bottles to protect from light. The nozzles were wrapped with PVC wrap to prevent contamination and evaporation, and kept at room temperature for the duration of the experiment. The dilutions were replenished weekly to ensure stable efficacy.

Breeding the great wax moth

A culture of the great wax moth (*Galleria mellonella* L.) was established using live specimens obtained from infected apiaries in some villages of Hawija district in Kirkuk Governorate, Iraq, after diagnosis from the Natural History Museum at the University of Baghdad. The rearing system was based on the use of old beeswax from abandoned hives sterilized by freezing method. Specimens were placed in sterilized plastic vials inside an incubator under controlled environmental conditions, where we maintained a constant temperature of 30±1°C and relative humidity of 70±5% to ensure optimal insect growth [12]. To propagate the insects in the laboratory, we prepared special plastic containers containing pieces of beeswax as food for the larvae, and added black cardboard strips with dimensions of 10 × 5 cm to facilitate egg laying. Five pairs of adult insects (five females and five males) were placed in each container. To ensure the success of the rearing process, the containers were covered with a muslin cloth and the lids were securely fastened with rubber bands. We monitored the life cycle on a daily basis and renewed the colony after each generation to maintain a healthy strain of insects. As the farm expanded, we moved the culture into larger incubators to ensure continuity of production. These large incubators were designed to provide optimal environmental conditions that ensure consistent reproduction and growth rates for the insects. The breeding system follows strict

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protocols to ensure the quality of the samples produced for research purposes and various scientific experiments [13].

Experiment design

The bioassay was carried out to evaluate the effect of CuO and Cr_2O_3 on the great wax moth (*Galleria mellonella*) using three different concentrations of each nanomaterial (1000, 500, and 250 ppm), in addition to a control group. The experiment included each of the different life stages of the insect (eggs, larvae, pupae) and individuals were evenly distributed among the different treatments. For each stage, groups were treated with one of the specific concentrations of each substance, as well as a parallel control group. All treatments were carried out in triplicate for each concentration, and individuals were monitored daily to record toxicological effects such as mortality and developmental duration. The environmental conditions (temperature, humidity and lighting) were kept constant throughout the experiment to ensure the accuracy of the results.

Egg treatment

For the purpose of obtaining eggs and treating them with Nanomaterials, a number of stinging insects of males and females were placed in glass bottles, and inside the bottle, black leaves were placed on the bottle. So that the stingingings insects contain the eggs on them, the external whole insects were fed with honey solution at a concentration of 10% by placing the solution in flat bottles, the fungi were placed on them, and the solution was replaced daily. Bottle nozzle was fed. The vial was placed in the incubator at a temperature of 30% +1 m and a relative humidity of 70±5%. Eggs were then taken one day old and treated with the substances under study in addition to the treatment of agaric (distilled water treatment) by surface treatment. In this experiment, a micro applicator syringe was used to cover the surface of the egg with 1 microtiter per egg, and three replicates were used for each treatment, with each replicate containing 25 eggs for each concentration. Three replicates were used for each treatment with 25 eggs per replicate for each concentration, in addition to the nodule treatment. The obtained eggs were placed in plastic dishes with a little wax to facilitate the feeding of larvae upon hatching under the mentioned conditions [14]. The experiments were carried out according to the methodology and then placed in the incubator under the previously mentioned rearing conditions with daily monitoring to record the results and the following was calculated:

- Average incubation time
- Hatching rate

The 10-day-old larvae of the great waxworm were treated with the nanomaterials used in this study using a micro applicator where 0.5 microlitre was applied to each larva and three replicates for each concentration and in each replicate 10 larvae, in addition to the control treatment with sterile distilled water. Then the treated larvae were placed in 80 cm^3 plastic bottles and thin cotton. The treatments were incubated in the incubator under the previously mentioned rearing conditions [15], and the calculation was made:

- Duration of larval and pupal stage.
- Killing percentage in the larval and pupal stage.

Treatment of pupae

To obtain one-day-old great waxworm pupae, the caterpillars were taken at the beginning of weaving and transferred to Petri dishes to facilitate observation of pupal formation, after that, one-day-old pupae were taken and treated by dipping them with nanomaterials in the same concentrations as before and three replicates with 5 pupae per replicate, in addition to the control sample treated with distilled water and then calculated:

- Duration of the pupal stage.
- Killing percentage in the metaphase

Statistical analysis

The experiment was analyzed using SPSS software (version 19). Data on mortality and the effect of different nanoparticle treatments on the life stages of *Galleria mellonella* were collected and processed using One-Way ANOVA to determine significant differences between the treatments at the 0.05 significance level, Duncan's Multiple Range Test was performed to compare means and identify the most effective treatments [16]. Homogeneity of variance and basic statistical assumptions were confirmed before applying the tests [16].

RESULTS AND DISCUSSION

Characterizations

The morphological properties of the CuO sample were analyzed using transmission electron

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microscopy (TEM). The microscopic images in Fig. 1 showed that the nanoparticles were formed as irregular aggregates. The TEM image, at a scale of 1 µm, shows large agglomerates consisting of closely packed nanoparticles, indicating random particle growth. Irregular surfaces indicate the presence of heterogeneous properties in the structure, which may affect the physical and chemical properties of the materials when used in practical applications.

To check the stability of the CuO particles in the aqueous medium, other measurements were made using a HORIBA SZ-100, like the Zeta Potential, which recorded a negative value of -22.3 mV. This value reflects that the particles have a relatively weak negative surface charge, indicating moderate stability in the suspension. This is due to the low electrostatic repulsive forces between the particles, which increases the likelihood of agglomeration over time. The value of the electrophoretic mobility was -0.000115 $cm^2/V\text{-}s$, which is consistent with the limited negative particle charge. Based on these results, the sample has acceptable properties in terms of size and morphology, but its stability in aqueous solutions may need to be improved by using stabilizing agents or modifying the synthesis conditions.

The TEM images of Cr_2O_3 samples in Fig. 2 showed irregularly shaped structures with

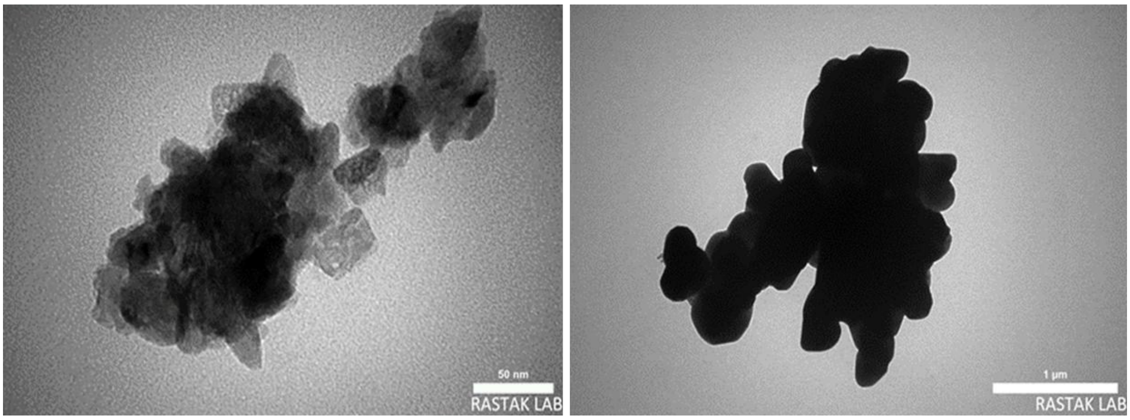

Fig. 1. TEM images of CuO nanoparticles.

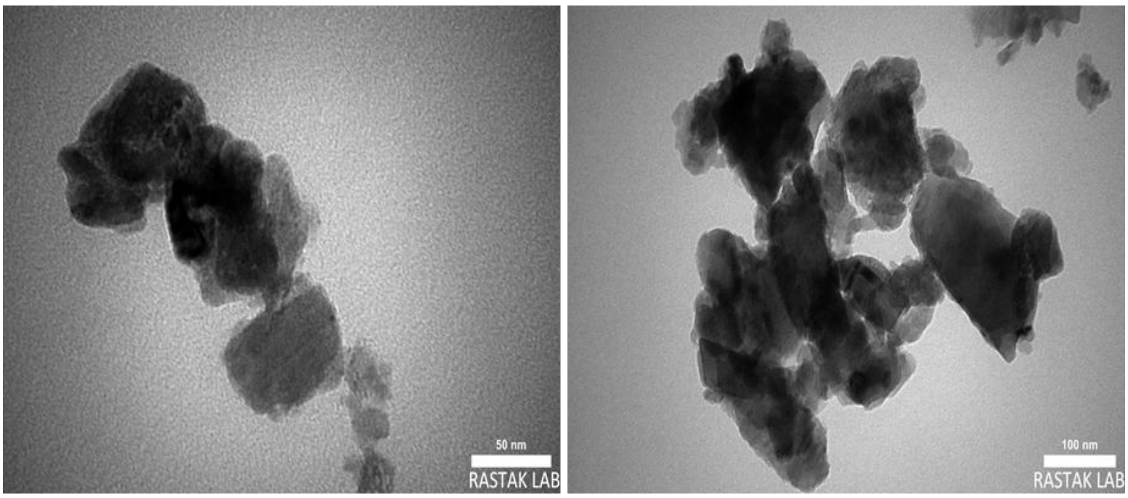

Fig. 2. TEM images of Cr_2O_3 nanoparticles.

partial agglomeration. The resulting particles are approximately 20-60 nm in size and have irregular surfaces, indicating a non-crystalline or semi-crystalline structure. Overall, the images show that the particles are clumped but remain within the acceptable nano scale range, reflecting the stable nanostructured particles.

A high positive Zeta Potential value of +95.5 mV was recorded, indicating a very high stability of the nanoparticles and preventing them from agglomerating, which is much greater than the minimum acceptable value (±30 mV) to ensure particle stability. The measurement also showed a value of 0.000494 cm²/V-s, indicating that the particles possess a strong positive surface charge that promotes electrostatic repulsion between them. These results show that the prepared nanomaterial has structural and electrochemical properties that make it an excellent candidate for diverse applications in biological, environmental and industrial fields, such as drug delivery systems, nano catalysts, water treatment, and microsensors.

Effect of the studied treatments on egg incubation time and hatching rate

The present study showed the effect of CuO-NPs and Cr_2O_3-NPs at different concentrations on the mean hatching time and hatching rate of great waxworm (*Galleria mellonella*). The analysis was performed at a probability level of 0.05. Statistical details and specific averages can be found in the Table 1.

It was observed that the treatment of waxworm with CuO-NPs resulted in a significant enhancement in the average hatching time and a reduction in the hatching rate with increasing concentration. Similarly, Cr_2O_3-NPs caused a significant enhancement in the average hatching time and a reduction in the hatching rate of the adult waxworm, especially at high concentrations. Although direct research on the insect toxicity of Cr_2O_3-NPs is less common compared to some metal nanoparticles, this effect is consistent with the results of previous studies showing the toxicity of metal oxide nanoparticles on insects, egg incubation period or hatching time. For example, Aziz and his group [17] found that Zn-NPs, Se-NPs and Ag-NPs could prolong different developmental stages in a direct proportion to the nanomaterial concentration when used to control grain beetle, especially egg incubation period if it increased from 7. 66 to 11. This effect is attributed to the ability of these particles to cause oxidative stress and cell damage, negatively affecting embryonic development and the ability to hatch. These common mechanisms could also explain the observed effects on hatching, where interference with physiological pathways leads to prolonged development and reduced survival

Table 1. Effect of different treatments on egg incubation rate and hatching rate.

Treatment	Conc. (ppm)	Mean hatch time (days)	Hatch rate (%)
Control	0	11.2 A	87.3 a
CuO-NPs	250	11.9 Ab	80.6 b
	500	13.2 C	73.2 c
	1000	14.8 D	60.4 d
Cr_2O_3-NPs	250	12.5 B	83.9 ab
	500	14.0 Cd	76.3 bc
	1000	1.5 1 D	65.1 e

Averages indicate significant differences at the probability level (p≤0.05)

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rates. Another study using chitosan on thaged grain beetle showed the same effect in prolonging egg incubation, which hinders the respiration process and prevents the access of oxygen to the embryo and thus reduces the hatching process [18, 19].

Effect of the studied treatments on larval development time and mortality rate

The present study showed the effect of CuO-NPs and Cr_2O_3-NPs at different concentrations on the duration of larval development and killing rate. Statistical analysis was performed at a probability level of 0.05. The statistical details and averages shown in the Table 2 showed an enhancement in the duration of larval development and the killing rate in the treated groups when compared to the control.

In general, it was observed that the larval development time of the great waxworm increased significantly with increasing concentration of both types of nanoparticles as compared to the control

Table 2. Effect of different treatments on larval development rate and killing rate.

Treatment	Conc. (ppm)	Larval duration (days)	Mortality rate (%)
Control	0	26.4 A	9.5 e
CuO-NPs	250	27.3 Ab	32.8 d
	500	28.5 Bc	52.7 bc
	1000	30.1 D	70.5 a
Cr_2O_3-NPs	250	27.9 B	38.2 cd
	500	29.2 C	61.4 ab
	1000	30.6 D	68.9 a

Different letters below the means indicate significant differences at the probability level ($p \leq 0.05$)

Table 3. Effect of the studied parameters on the duration of the virgin phase and the mortality rate.

Treatment	Conc. (ppm)	Pupal duration (days)	Pupal mortality rate (%)
Control	0	9.5 A	7 E
CuO-NPs	250	10.1 Ab	19.5 D
	500	10.8 Bc	35.2 C
	1000	11.6 D	54.7 A
Cr_2O_3-NPs	250	10.3 Ab	22.6 D
	500	11.0 C	41.3 B
	1000	11.7 D	52.9 A

Different letters below the means indicate significant differences at a probability level ($p \leq 0.05$).

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group. Larvae in the control group recorded the short developmental duration, while treatment with the highest concentrations (1000 ppm) of both CuO-NPs and Cr_2O_3-NPs resulted in the long developmental duration. In contrast, the mortality rate among waxworm larvae increased significantly with increasing nanoparticle concentration. The control group recorded the lowest mortality rate, while treatment with the highest concentrations of CuO-NPs and Cr_2O_3-NPs resulted in the highest mortality rates. The highest concentration of CuO-NPs (1000 ppm) caused the highest mortality, followed closely by Cr_2O_3-NPs at the same concentration. These results clearly indicate that both types of nanoparticles, especially at higher concentrations, have a negative and inhibitory effect on larval development and increase mortality. The findings of this study on the increased duration of larval development and higher mortality of the great waxworm exposed to CuO-NPs and Cr_2O_3-NPs are consistent with the ecotoxicity and insecticidal potential of metal nanoparticles. CuO-NPs have been shown to be effective as insecticides against great waxworm larvae in previous studies.

Effect of the studied treatments on pupal stage duration and kill rate

The results showed the effects of CuO-NPs and Cr_2O_3-NPs at different concentrations on the pupal stage of the great waxworm, in terms of the duration of this stage and its mortality rate (Table 3). Statistical analysis of these data was performed at a probability level of 0.05.

The results showed that nanoparticle treatment resulted in an enhancement in the duration of the pupal phase with increasing the concentration for both materials. While the short pupal duration was recorded in the control group, high concentrations of CuO-NPs and Cr_2O_3-NPs resulted in a significant extension of this duration, indicating interference in the natural metamorphosis process. In parallel, a significant enhancement in mortality during the pupal stage was observed in the nanoparticle-treated worms. The control group showed low efficiency in terms of mortality, while increasing concentrations of CuO-NPs and Cr_2O_3-NPs caused a significant enhancement in mortality rates. Results show that the highest mortality rates in this phase were associated with the maximum concentrations of both types of nanoparticles. These confirm that exposure to resulting nanomaterials has direct detrimental effects on the viability of the great waxworm during the pupal stage. The effects of CuO-NPs on insect developmental stages have been documented. For example, it has been found that CuO-NPs can cause developmental delays and high mortality rates in the pupal stage of other insects like Drosophila melanogaster [20]. This supports the hypothesis that CuO-NPs interfere with the underlying cellular and molecular mechanisms that control insect metamorphosis.

Studies suggest that Cr_2O_3 exposure can cause disruptions in growth and development at sensitive stages of insect life, leading to abnormalities or failure to metamorphose. For example, the effects of Cr_2O_3 compounds on pre-adult (including pupal) stages in some species of aquatic insects are indicative of their potential for developmental toxicity. This supports the interpretation that Cr_2O_3-NPs may affect complex metamorphic processes in the pupal stage.

CONCLUSION

This study showed that CuO-NPs and Cr_2O_3-NPs possess systemic toxicity across all sub-adult stages of *Galleria mellonella*, including eggs, larvae, and pupae. The negative effect of these nanoparticles, both on developmental duration and survival rate, was found to be directly dose-dependent. In particular, the treatment resulted in a prolongation of the mean hatching time, the duration of larval development, and the duration of the pupal stage. In parallel, a significant enhancement in mortality rates was observed across the egg, larval, and pupal stages. These results were attributed to the potential mechanisms of nanotoxicity, which include the ability of nanoparticles to induce oxidative stress and cell damage within insect tissues, disrupting vital biological processes of the insect and negatively affecting its growth and viability. It can be concluded that CuO-NPs and Cr_2O_3-NPs have the potential as effective biocontrol agents in IPM programs, due to their ability to negatively affect the life cycle of the great waxworm in its sub-adult stages.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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