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Article

Evaluation of the Effectiveness of Copper Oxide and Chromium Oxide Nanoparticles as Nano-Pesticides Against the Greater Wax Moth (Galleria mellonella)

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Abstract: This study was conducted in the laboratory of the College of Education for Pure Sciences at the University of Kirkuk from January 1, 2025, to April 1, 2025, with the aim of evaluating the effects of nanomaterials such as copper oxide (CuO) and chromium oxide (Cr_2O_3) as nano-pesticides against the greater wax moth (Galleria mellonella). Adult insects were treated with these nanomaterials, and their effects on the development of different life stages (eggs, larvae, pupae) were studied by measuring egg count, incubation period, hatching rate, and mortality rates. The results showed that the nanomaterials had a clear dose-dependent effect. At a concentration of 1000 ppm of copper oxide nanoparticles, the egg count significantly decreased to 47.8 eggs compared to 300 eggs in the control group. The incubation period also increased to 15.2 days at the same concentration, compared to 9.2 days in the control group. As for the hatching rate, it significantly decreased to 36.6% with copper oxide nanoparticles, reflecting the significant impact of high concentrations. Additionally, an increase in mortality rates in various stages (larvae and pupae) was recorded, with the mortality rate reaching 42% at 1000 ppm of copper oxide nanoparticles. Similar results were obtained with chromium oxide nanoparticles, which exhibited similar effects but with slight differences in efficacy between the two compounds. These findings suggest that copper oxide and chromium oxide nanoparticles can be effective insecticides in controlling the greater wax moth and enhance the potential use of nanotechnology in integrated pest management programs in an environmentally safe and effective manner.

Keywords: Copper Oxide Nanoparticles, Chromium Oxide Nanoparticles, Greater Wax Moth, Nano-Pesticides, Integrated Pest Management

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1. Introduction

Insects are among the most successful and diverse animal groups on Earth, and they have a complex relationship with humans. Despite their significant role as pollinators of crops, they also pose major threats to stores and fields and transmit numerous diseases to humans and other animals (Gupta et al., 2023) One of the prominent pests attacking honeybees is the greater wax moth, Galleria mellonella, belonging to the order Lepidoptera, family Pyralidae (Özgör, 2021). This insect is an economically destructive pest of hive cells and wax foundations, as it reproduces rapidly and its larvae feed on wax and pollen inside the cells or stores (Hood, 2010). The larvae depend on stored or old wax combs rich in proteins, and they also feed on all the contents of the honeybee frames, including wax and pollen, leading to the destruction of the brood's wax covers, a phenomenon known as bald brood (Ellis et al., 2013).

Furthermore, the larvae hinder the foraging of worker bees between frames by producing dense silk threads that prevent movement and feeding, weakening the hive and causing bee mortality, a condition called Galleriasis due to malnutrition (Ellis et al., 2013). The larvae's fecal waste contains spores of the American Foulbrood bacterium Paenibacillus larvae, which promotes the spread of diseases to honeybee colonies (Sulborska et al., 2019).

The economic damage caused by insect pests is immense (Oliveria et al., 2013), necessitating advanced pest management strategies to significantly reduce economic losses. Among these strategies is nanotechnology, which is a promising tool for pest control within Integrated Pest Management (IPM) programs (EL-Wakeli et al., 2017). This technology is highly effective, offering properties that any effective insecticide must possess, such as increased spread, ensuring better contact with insect pests, as well as providing a selective, targeted, long-lasting, and environmentally safe pesticide due to the small quantities used that minimize accumulation (Zannat et al., 2021). Studies have shown that insecticide formulations using nanomaterials as carriers for active ingredients have shown promising results in reducing agricultural and stored pests as well as disease vectors (Gupta et al., 2023). Various plant chemical groups act as reducing and stabilizing agents during the biosynthesis of secondary metallic nanoparticles, which can be used as bio-nano-pesticides. The green synthesis of these nanoparticles has been experimentally successful in combating stored food product insects (Baliyarsingh & Pradhan, 2023). It is noteworthy that these nanoparticles, ranging in size from 1 to 100 nanometers, possess new physical, chemical, and biological properties that make them effective in integrated pest management. They serve as a safe alternative to traditional chemical pesticides due to their high effectiveness and non-toxicity to living organisms (Firozjaee et al., 2018; Al-Atai, 2018). These nanoparticles are also known to be feed inhibitors, repellents, and growth inhibitors against many stored pests, such as the sawtoothed grain beetle Oryzaephilus surinamensis (Jamal et al., 2024a; Jamal et al., 2024b), the lesser grain borer Rhyzopertha dominica (Aziz et al., 2022), and the greater wax moth (Özgör, 2021). Among the most commonly used nanomaterials in pest control are metal oxides, such as copper and chromium oxides. Copper oxide is considered an effective nano-pesticide against some stored pests (Rai et al., 2018). Its effect is due to its ability to generate reactive oxygen species (ROS), which cause oxidative stress, leading to membrane damage and the disruption of insect proteins and enzymes (Aruna et al., 2022).

Based on the above, our study aims to evaluate the effect of treatment on adult insects only. We will focus on studying the effects of this treatment on egg count, hatching rate, and incubation period in these insects. Additionally, we will study the impact of treatment on the larval and pupal stages resulting from the growth of eggs from treated adult insects, in terms of the duration of larval and pupal development, as well as the mortality or death rate in these stages.

2. Materials and Methods

Chemicals Used : Copper oxide nanoparticles (CuO-NPs) and chromium oxide nanoparticles (Cr_2O_3-NPs) were used as nano-pesticides against the greater wax moth (Galleria mellonella). The physical properties of these nanoparticles were analyzed using the Horiba SZ-100 on Sunday, November 26, 2023. The results showed that the particle size ranged between 20–50 nanometers. The Zeta potential for chromium oxide was -22.33 mV, and for copper oxide, it was 95.6 mV, indicating high purity and stability of the samples.

Reparation of Nanoparticle Suspensions (Dilutions) \ Suspensions of copper oxide and chromium oxide nanoparticles were prepared using deionized water as the solvent, with the addition of polyvinyl alcohol (PVA) as a dispersant. A total of 400 ml of each concentration (1000, 500, and 250 ppm) for each material was prepared using magnetic stirring to ensure homogeneity. After preparation, the dilutions were stored in opaque glass bottles to protect them from light, with the bottle openings sealed with food-grade PVC wrap to prevent contamination and evaporation. The suspensions were stored at room temperature throughout the experiment. The dilutions were refreshed weekly to ensure consistency in their effectiveness.

Rearing of Greater Wax Moth : A colony of Galleria mellonella was established using live samples obtained from infected beehives in some villages of Hawija district, Kirkuk province, Iraq. The colony was confirmed by the Natural History Museum of Baghdad University. The rearing system used old sterilized honeycomb, which was frozen for disinfection. The samples were placed in sterile plastic containers inside an incubator under controlled environmental conditions, with a stable temperature of 30±1°C and relative humidity of 70±5% to ensure optimal insect growth (Taqi, 2007; Karim, 2011).

To propagate the insects in the laboratory, we prepared special plastic containers containing pieces of honeycomb as food for the larvae, with black cardboard strips measuring 10×5 cm added to facilitate egg-laying. Each container housed five pairs of adult insects (five females and five males). A sugar solution was provided in small containers with cotton pads to feed the adult insects. To ensure the success of the rearing process, the containers were covered with muslin fabric for proper ventilation, while preventing the escape of insects. The covers were securely fixed using elastic bands. The life cycle was monitored daily, and the colony was replenished after each generation to maintain a healthy strain of insects. As the colony expanded, the culture was transferred to larger incubators to ensure continuous production. These larger incubators were designed to provide ideal environmental conditions for stable reproduction and growth rates for the insects. The rearing system followed precise protocols to ensure the quality of the samples produced for research purposes (Singh, 2014).

Experimental Design: The bioassay was carried out to evaluate the effects of copper oxide and chromium oxide nanoparticles on Galleria mellonella using three different concentrations for each nanoparticle (1000, 500, and 250 ppm), in addition to a control group that was not exposed to any nanoparticles. The experiment included different life stages of the insect (eggs, larvae, pupae), with individuals distributed evenly across the different treatments. Separate groups were allocated to each concentration of each nanoparticle, alongside a parallel control group. Three replicates were performed for each concentration, and individuals were monitored daily to record toxic effects such as mortality rates and developmental durations. Environmental conditions (temperature, humidity, and lighting) were kept constant during the experiment to ensure accurate results.

The adult insects were treated with nanoparticles, as described by Ali (2022), by applying a specific concentration of the pesticide to each insect individually. The treatment was applied using a micro applicator for topical application, where 1 microliter of the pesticide solution was placed on the ventral side of the insect's thorax. Three different concentrations of the pesticide were used, with each concentration repeated three times to ensure precision. The control group followed the same treatment procedure but without the addition of the pesticide. After treatment, the insects were placed in 1-liter plastic bottles and provided with a 10% honey solution. The insects were kept under the same rearing conditions as the control group. Some indicators were recorded.

Statistical Analysis : The data obtained from the experiment were analyzed using SPSS (version 19). Data related to mortality rates and the effect of different nanoparticle treatments on the life stages of Galleria mellonella were analyzed using one-way ANOVA to determine significant differences between treatments at a significance level of 0.05. Duncan's Multiple Range Test was used for pairwise comparison of means to identify the most effective treatments. The homogeneity of variance and basic statistical assumptions were verified before applying the tests (Duncan, 1995).

3. Results and Discussion

Effect of Copper Oxide and Chromium Oxide Nanoparticles on Reproductive Output and Egg Development in Adult Insects

This study aimed to evaluate the effects of copper oxide nanoparticles (CuO-NPs) and chromium oxide nanoparticles (Cr_2O_3-NPs) at different concentrations on the reproductive output of adult insects and egg development. Table (1) below presents the key quantitative results obtained from this experiment, which form the basis for all subsequent conclusions and interpretations. Table (1) below illustrates the dose-dependent effects of copper oxide nanoparticles (CuO-NPs) and chromium oxide nanoparticles (Cr_2O_3-NPs) on the average egg count, incubation period, and hatching rate in adult insects. Different letters within each column indicate statistically significant differences between treatments, highlighting a clear concentration response in most of the measured parameters.

Table 1. Effect of Different Treatments on the Incubation Rate, Egg Count, and Hatching Percentage in the Adult Stage of the Insect

Treatment	Concentration (p.p.m)	Average Egg Count	Incubation Period (Days)	Percentage of Hatched Eggs
Control	0	300 A	9.2 c	86 A
CuO-NPs	250	285 A	9.8 c	83.5 a
	500	190 B	12.3 b	62.4 b
	1000	47.8 C	15.2 a	36.6 c
Cr_2O_3-NPs	250	270 A	9.8 c	85.2 a
	500	175 B	12.0 b	60.7 b
	1000	45.2 C	14.9 a	39.0 c

Note: Different letters within the same column indicate statistically significant differences at a significance level of $p < 0.05$.

The results showed that the nanoparticles had a clear dose-dependent effect on the average egg count. In the control group, the average egg count was 300 eggs. At a concentration of 250 ppm, both copper oxide nanoparticles (285 eggs) and chromium oxide nanoparticles (270 eggs) showed no statistically significant difference compared to the control group, as they all belonged to the statistical group "a." This indicates a clear threshold effect, where lower concentrations (250 ppm) did not produce statistically significant harmful effects on the biological parameters measured compared to the control group.

As the concentration increased, a significant decrease in egg count was observed. At 500 ppm, the average egg count dropped to 190 eggs with copper oxide nanoparticles, representing a decrease of about 36.7% compared to the control group, and to 175 eggs with chromium oxide nanoparticles, a decrease of about 41.7%. Both values belong to statistical group "b," indicating significant differences from the control group. At the highest concentration, 1000 ppm, the decline was more pronounced, with the average egg count reaching 47.8 eggs with copper oxide nanoparticles (a decrease of about 84.1%) and

45.2 eggs with chromium oxide nanoparticles (a decrease of about 84.9%). These values belong to statistical group "c," indicating highly significant differences from the control group and the lower concentrations. Overall, both nanoparticles showed similar dose-dependent reductions, with chromium oxide nanoparticles at 500 ppm resulting in a slightly lower egg count than copper oxide nanoparticles, although both were in statistical group "b." At 1000 ppm, the effects were nearly identical.

These results are consistent with several studies that have highlighted the role of nanomaterials in reducing egg production. For instance, Al-Fatlawy et al. (2023) demonstrated that direct intervention in reproductive processes is one of the key ways in which nanomaterials affect egg production in insects. Their comprehensive study on the red flour beetle Tribolium castaneum showed that silicon oxide nanoparticles had a profound effect on egg-laying, where the average egg count from females exposed to a 50 mg/kg dose dropped to zero eggs, compared to 98.1 eggs in the control group. This drastic reduction likely indicates deep intervention in the physiological mechanisms responsible for egg formation and gamete maturation. This effect was shown to be dose-dependent, further confirming a direct causal relationship between the concentration of the nanoparticle material and its impact on reproductive output.

The incubation period of the eggs was also affected by the nanoparticle treatment in a dose-dependent manner. In the control group, the incubation period was 9.2 days. At a concentration of 250 ppm, neither copper oxide nanoparticles (9.8 days) nor chromium oxide nanoparticles (9.8 days) showed any statistically significant difference compared to the control group, as all values belonged to statistical group "c."

As the concentration increased, the incubation period increased significantly. At 500 ppm, the incubation period increased to 12.3 days with copper oxide nanoparticles (an increase of about 33.7%) and 12.0 days with chromium oxide nanoparticles (an increase of about 30.4%). Both values belonged to statistical group "b," indicating significant differences from the control group. At the highest concentration, 1000 ppm, the incubation period reached 15.2 days with copper oxide nanoparticles (an increase of about 65.2%) and 14.9 days with chromium oxide nanoparticles (an increase of about 62.0%). These values belonged to statistical group "a," indicating the longest incubation periods and the largest significant differences. Both nanoparticles showed very similar and dose-dependent increases in the incubation period, with no notable differences between the two types.

These results align with Ali (2022), who used zinc oxide nanoparticles and titanium dioxide nanoparticles on Galleria mellonella, leading to an increase in the incubation period from 10.2 days to 16 days. These results were somewhat proportional to the concentration of the nanoparticle solution and could be attributed to what Paur et al. (2011) mentioned—that the size of nanoparticles is comparable to the size of cellular proteins, allowing nanoparticles to cross biological system barriers, including selective permeability cell membranes. This capacity to control the movement of large or small molecules into and out of cells likely contributed to the aforementioned results.

The concentrations of nanoparticles also had a significant negative effect on the hatching rate. In the control group, the hatching rate was 86%. At 250 ppm, neither copper oxide nanoparticles (83.5%) nor chromium oxide nanoparticles (85.2%) showed any statistically significant difference compared to the control group, as all values belonged to statistical group "a." This reinforces the observation of a threshold effect at low concentrations. As the concentration increased, the hatching rate decreased sharply. At 500 ppm, the rate dropped to 62.4% with copper oxide nanoparticles (a decrease of about 27.4%) and to 60.7% with chromium oxide nanoparticles (a decrease of about 29.4%). Both values belonged to statistical group "b," indicating significant differences from the control group. At the highest concentration, 1000 ppm, the decrease was even more pronounced, with the rate reaching 36.6% with copper oxide nanoparticles (a decrease of about 57.4%)

and 39.0% with chromium oxide nanoparticles (a decrease of about 54.7%). These values belonged to statistical group "c," indicating the lowest hatching rates and the largest significant differences.

Overall, both nanoparticles showed similar dose-dependent reductions in the hatching rate. At 500 ppm, chromium oxide nanoparticles resulted in a slightly lower hatching rate, while at 1000 ppm, copper oxide nanoparticles had a slightly lower hatching rate, but both were within the same statistical group. These results are consistent with Alfy et al. (2020), who demonstrated the effect of chitosan nanoparticles on Spodoptera littoralis, leading to inhibition of egg hatching and shortened larval and pupal lifespan.

Effect of Treating the Greater Wax Moth (Galleria mellonella) with Different Concentrations of Nanomaterials on Larval Duration and Mortality Rate

This study aims to evaluate the effect of treatment with varying concentrations of copper oxide nanoparticles (CuO-NPs) on Galleria mellonella, focusing on two main variables: larval duration and mortality rate. The experiment was designed with a control group (no treatment) and three concentrations of the nanoparticle (250, 500, and 1000 ppm), with statistical differences calculated at a significance level of 0.05% using Duncan's Multiple Range Test. Table (2) summarizes the key results of the experiment.

The following table details the effect of different concentrations of nanoparticles (CuO-NPs and Cr_2O_3-NPs) on larval duration and mortality rate in Galleria mellonella, compared to the control group and at a probability level ($p \leq 0.05$).

Table 2. Effect of Treating the Greater Wax Moth with Different Concentrations of Nanomaterials on Larval Duration and Mortality Rate

Treatment	Concentration (p.p.m)	Pupa Duration (Days)	Mortality Rate (%)
Control	0 ppm	9.0 D	7.5 e
CuO-NPs	250 ppm	9.7 Cd	33.5 cd
	500 ppm	10.5 B	35.4 bc
	1000 ppm	11.1 A	37.2 b
Cr_2O_3-NPs	250 ppm	9.5 Cd	31.2 d
	500 ppm	10.3 Bc	34.5 c
	1000 ppm	10.8 Ab	40.7 a

Note: Different letters within the same column indicate statistically significant differences at $p < 0.05$.

The results indicate that both types of nanoparticles, copper oxide nanoparticles (CuO-NPs) and chromium oxide nanoparticles (Cr_2O_3-NPs), had a clear effect on both pupal duration and mortality rate in Galleria mellonella compared to the control group. An increase in pupal duration was observed with increasing concentrations of nanomaterials, reaching its highest at 1000 ppm for both treatments. Additionally, all concentrations of the nanomaterials resulted in a noticeable increase in the mortality rate, indicating their effectiveness in controlling this pest.

Looking at the differences between the types of nanomaterials, it appears that chromium oxide nanoparticles (Cr_2O_3-NPs) were slightly more effective in increasing the mortality rate at the highest concentration (1000 ppm), with a mortality rate of 40.7% compared to 37.2% for copper oxide nanoparticles at the same concentration. However, the significant differences between concentrations and within each group confirm that the effect is directly dependent on concentration, and that both materials have promising potential in controlling the greater wax moth. These results align with the study by AbdulRahman & Mohammad (2022), which indicated that pupal stages resulting from insects treated with different concentrations of nanomaterials showed a clear response, with the average larval duration increasing from 8.5 days in the control group to 31.5 days at higher concentrations, and the mortality rate rising from 6.5% in the control group to 39.7% at higher nanomaterial concentrations. This response was positively correlated with the concentration of the nanomaterial.

This effect can be explained by the deep impact of nanomaterials at the molecular and cellular levels. Recent research in a comprehensive study on Drosophila melanogaster using multi-cationic star-shaped nanoparticles (SPc) showed fundamental changes in the gene expression of genes involved in metabolism, innate immunity, stress response, and hormone production in larvae. These genetic changes affect the insect's ability to adapt to the environment and complete its life cycle successfully, explaining the increase in mortality rates and prolonged developmental times (Yan et al., 2022). Additionally, genetic analysis shows that nanomaterials interfere with essential metabolic pathways such as pentose and glucuronate transformations, glycerolipid metabolism, folate synthesis, and amino sugar and nucleotide metabolism. This interference in basic metabolic processes leads to an imbalance in energy and raw materials required for growth and development, resulting in delayed metamorphosis and increased mortality rates, particularly during critical stages like the pupal stage (Sun et al., 2022).

4. Conclusion

The study demonstrated that copper oxide and chromium oxide nanoparticles proved effective as insecticides against the greater wax moth (Galleria mellonella). As concentrations increased (1000 ppm), egg count decreased, incubation period increased, leading to a significant reduction in hatching rate. The toxic effect was dose-dependent, with increased mortality rates observed in various stages with higher concentrations. Nanomaterials affected all life stages (eggs, larvae, pupae), causing a reduction in the development of immature stages and an increase in mortality rates. Regarding the comparison between copper oxide and chromium oxide nanoparticles, both exhibited similar effects, with chromium oxide nanoparticles showing a slight advantage in some cases. Nanomaterials can be considered a promising option for integrated pest management (IPM), given their high effectiveness and environmental safety, as small quantities are used, reducing pesticide accumulation in the environment.

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