Carbon-based nanocide against the red flour beetle *Tribolium castaneum*

Abbas Abdull Ammer[1], Abdulla N. Ali[2], and Ruqaya Ali Kadhim[2]

ABSTRACT

Tribolium castaneum (Herbst, 1797) is a pest of stored food that inflicts significant economic damage. Developing innovative methods for controlling this pest can benefit insect and integrated pest management system development. A laboratory study was conducted to assess the sub-lethal effects of carbon nanobiocide against the *T. castaneum* pest. This paper evaluated how carbon nanotubes influence several life characteristics of *the T. castaneum* insect. The larval mortality was reduced, yielding a mean rank mortality score of 33.3 at a 0.1 mg/mL concentration. The mean ranked adult mortality score for the concentration of 0.1mg/mL was 36.7. The fecundity of females, measured by the number of eggs laid, varied across different CNT treatments, with the 0.05 mg/mL concentration resulting in nearly no eggs laid per female compared to the other CNTs concentrations employed in this work. The germination rates of wheat seeds subjected to different CNTs concentrations across all studied materials were analyzed. To our knowledge, there is no published research examining the impact of CNTs on stored product insects. Thus, this study serves as a reference for future research by analyzing the merits and demerits of different CNT concentrations on stored pests such as the red flour beetle and forecasting their developmental trajectories.

Keywords: *Tribolium castaneum*, stored pest, wheat flour, Nanoparticles, carbon nanotubes CNTs.
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INTRODUCTION

Cereals and their derivatives constitute an essential food source, a fundamental staple for several populations. Wheat (*Triticum aestivum*) is a vital cereal crop of significant global economic importance. Improving grain harvests is crucial for increasing wheat production and food security amid continued population expansion (Ahmad *et al.,* 2024). Wheat is an essential cereal that provides roughly 20% of human calorie consumption and more than 25% of protein. It possesses a notable amount of dietary fiber (Khaleghdoust, 2025; Sharma and Sharma, 2025)

Tribolium castaneum, commonly known as the red flour beetle, is recognized as a secondary polyphagous pest found in 246 varieties of grain and processed storage products, primarily flour and starchy meals, around the world (Kang and Lee, 2018). This stored pest is a global pest of stored products, resulting in significant infestations in raw grain storage, cereal processing plants, warehouses, retail outlets, and domestic granaries. *T. castaneum* is a pest that affects wheat and rice flour, causing damage to stored grains and other products, which results in significant economic losses, particularly in warmer regions around the world. It invades grains that have already been compromised by other pests or during harvesting and storage (Chaubey, 2023). Wheat flour was predominantly favorable to the red flour beetle (Halliday *et al.,* 2019). Providing insects with appropriate nourishment may result in rapid development, elevated reproductive rates, and reduced mortality (Fardisi *et al.,* 2019). *T. castaneum* adult females lay their eggs on flour, complete their life cycle, and diminish the nutritional value of the grain. In severe infestations, flour smells unpleasant and loses nutritional and market value (Shamjana and Grace, 2021). Various insecticides have been used to control *T.*

castaneum by targeting its neurological areas, causing irreversible neurological damage and insect death. It has lowered infection rates, protected stored products for years, and is straightforward (Collins and Schlipalius, 2018). The constant use of chemicals in storage has increased *T. castaneum's* insecticide resistance and created resistant alleles in subsequent generations. Many nations reported insecticide-resistant *T. castaneum* in grains and cereals in transit and storage (Kumar and Morrison, 1965). Chemical pesticides present other issues, including safeguarding crops and human health and environmental pollution (Khandehroo *et al.,* 2024). Nanomaterials denote a category of substances that display exceptional physical, chemical, and biological qualities compared to conventional materials, due to their unique structures and features. Adsorbents, serving as the trapping medium in extraction technologies, are critical components that influence the precision and efficacy of sample preparation methods (Wu *et al.,* 2020). Compared to traditional adsorbents, nanomaterials demonstrate superior efficacy in extracting, enriching, and purifying organic contaminants in food, significantly enhancing instrumental analyses' precision, accuracy, and sensitivity (Qiu *et al.,* 2023).

Nanomaterials demonstrate superior efficacy in extracting, enriching, and purifying organic contaminants in food, significantly improving instrumental assessments' precision, accuracy, and sensitivity. Due to their extensive surface area and distinctive physicochemical characteristics, nanomaterials can selectively identify target pesticides, including organophosphates and organochlorines. This capability minimizes interference from sample matrices, thereby effectively adsorbing and concentrating pesticide residues, which enhances detection sensitivity and accuracy (Qiu *et al.,* 2023). Nanotechnology has emerged as the most promising innovative method for managing stored-product insects. Since both larvae and adults consume grain and grain products, this results in significant economic losses (Chaubey, 2023). This study employed laboratory tests to assess the impact of carbon nanotubes (CNTs) against the life characteristics of the grain pest *T. castaneum*, including adult and larval mortality and eggs laid per female. This study aimed to evaluate the effect of carbon nanobiocide (CNC) concentrations on the red flour beetle, providing research avenues and insights into the advancement and utilization of nanomaterials for identifying new pesticides to control stored pests.

MATERIALS AND METHODS

Cultivating insects

Wheat samples infested with over a thousand insects were retrieved from the local grain market and brought to the Plant Protection Department's insect laboratory. The samples were collected in plastic receptacles covered with muslin cloth to facilitate aeration. The adults of *T. castaneum* were segregated using a camel hairbrush. Red flour beetles were bred for multiple generations in plastic containers filled with wheat flour, covered with muslin to ensure adequate ventilation and prevent insect escape.

Preparing the Carbon Nanotube nanoparticles

Carbon nanotube nanoparticles were synthesized and produced in the chemical laboratory, Faculty of Science, University of Kufa, as previously mentioned in detail, see (Ali *et al.,* 2023).

Treating *T. castaneum* adults and larval stages

Carbon nanobiocide (CNCs) were applied to adult and larval instars at different concentrations (0.05mg/mL, 0.03mg/mL, and 0.1% mg/mL), respectively. This was done because the organisms are actively consuming food during these stages. In contrast to carbon nanotubes, the control treatment consisted of distilled water at the facility. In addition to those lying on their backs with their legs up or remaining motionless, the red flour beetle exhibiting ataxia (active movement that seems to be disorganized) was assessed to have passed away at one, two, and three days after exposure (Heinz-Castro *et al.,* 2021). During a research study, using three replicates, each consisting of ten subjects at each treatment, was customary.

Red flour beetle female fecundity: laid eggs

Fecundity was measured after exposing female red flour beetles to varying quantities of carbon nanotubes (CNTs). A Petri dish was used to place ten selected females. Under normal circumstances, all the females were placed in Petri dishes. One replication is provided by each wheat flour-containing Petri dish. Until the end of their lives, the egg output of the females was meticulously documented. Only disinfected water was administered to the control group.

Statistical analysis

The JMP16 Pro® program was used to perform ANOVA. The effects of nanomaterials on red flour beetles were assessed using Wilcoxon/Kruskal-Wallis H and Tukey's HSD tests. Abbott's method adjusted the control group's cumulative mortality for natural deaths (Abbott, 1925) .Kruskal-Wallis statistics test the effect of CNTs on the number of eggs laid per female. All data analyses used a 0.05 level of significance.

RESULTS

Red flour beetle adult mortality

A Kruskal-Wallis H test showed a statistically significant difference in the red flour beetle adult mortality between the different CNT treatments, $\chi2 = 27.8410$, $p = 0.0001$, with a mean rank adult mortality score of 36.7 for the concentration 0.1mg/mL, 12.13 for the concentration 0.05 mg/mL, and 20.16 for the concentration 0.03 mg/mL, Figure (1).

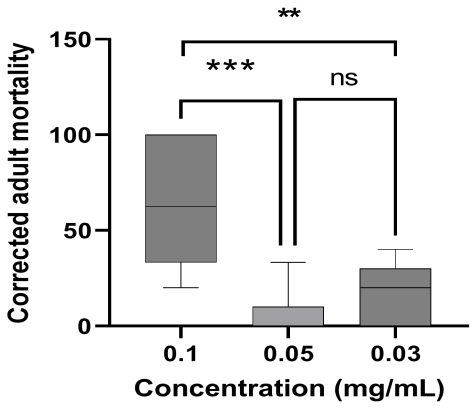

Figure 1. Corrected mortality rate of adult red flour beetle. P<0.0001, **= significant, NS= non-significant.

Red flour beetle larval mortality

There was a statistically significant difference in the death rate of the larval stages of the red flour beetle, *T. castaneum*, between the different CNTs treatments: $\chi2 = 15.92$, $p = 0.0003$, with a mean rank larval mortality score of 33.3667 for the concentration 0.1mg/mL, 15.0 for the concentration 0.05 mg/mL, and 20.6333 for the concentration 0.03 mg/mL, Figure (2).

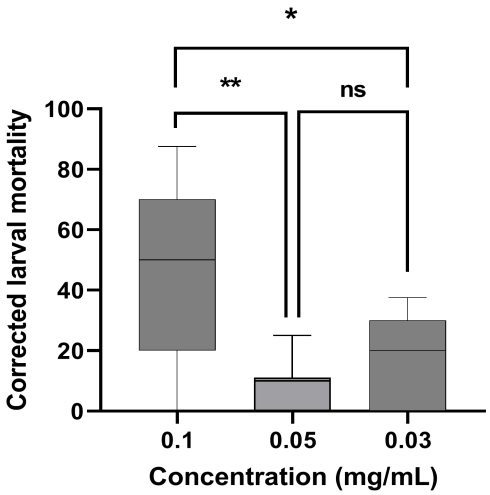

Figure 2. The corrected mortality rate of red beetle larvae. ** indicates significantly significant ($P < 0.0001$). Unconnected bars were similar. *= 0.02, **= 0.0003, ns= non- significant at p. value = 0.05.

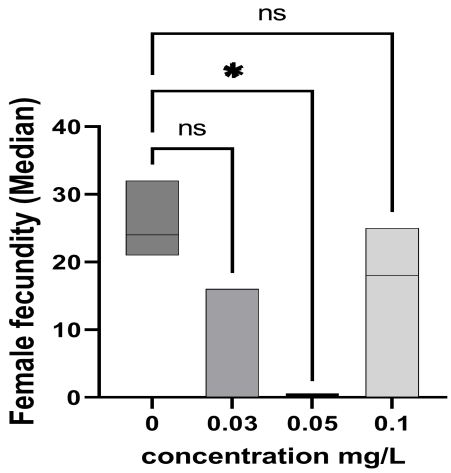

Figure 3. Number of eggs oviposited by female red beetles following carbon nanobiocide exposure. $P > 0.05$, ns = not significant. *= 0.0397, ns= non-significant at p. value = 0.05.

Laid eggs per female (female fecundity)

The number of eggs laid by each female, generally known as female fecundity, varies according to the CNTs treatment. The 0.05 mg/mL concentration indicated essentially no eggs laid by each female compared to the other CNTs concentrations used in this study. Kruskal-Wallis statistics = 0.0338, p= 0.0338, (Figure 3).

Wheat seed germination

The results of the wheat seed germination experiment, which involved exposure to carbon nanobiocide, indicate a significant difference in the treatments applied to promote seed germination ($F_{3,8} = 4.250$, $P = 0.0452$). The 0.1 mg/mL concentration exhibited a much higher germination rate of approximately 93.3% compared to the concentration of 0.05, which yielded a germination rate of 76.6%, as shown in Figure 4.

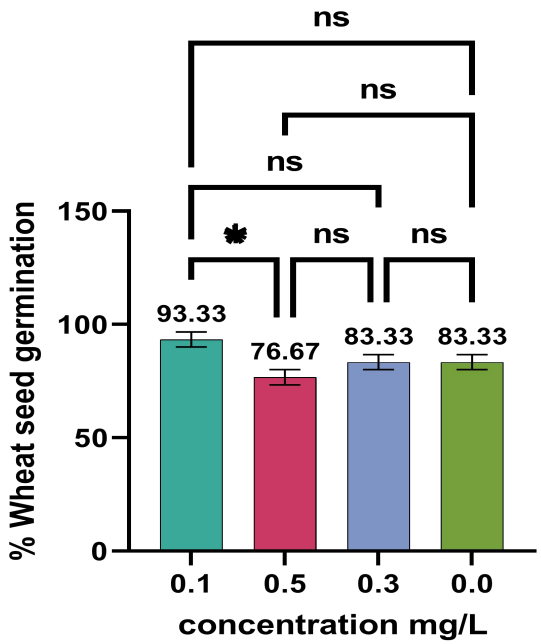

Figure 4. Wheat seed germination percentage. *= significant, NS= non- significant at p. value = 0.05.

DISCUSSION

The rising expenses of pesticides, perilous handling, lingering impacts, and adverse effects on humans and the environment are significant considerations (Islam *et al.,* 2016). Considering the insecticide resistance in *T. castaneum* and the detrimental effects of chemicals, environmentally sustainable methods should be employed to control the target pest. These methodologies may encompass biological, physical, and ecological strategies (Talukder and Miyata, 2002). This study assessed the impact of CNTs on several life cycle parameters, including adult and larval mortality rates and the fecundity of *T. castaneum*, measured based on the number of eggs produced by each female. The results of our study revealed that egg hatching was minimal at a concentration of 0.05 mg/L compared to other assessed CNT concentrations. Moreover, adult and larval mortality rates differed between various CNT treatments. The wheat seed germination treatment results revealed that the germination rate 0.1 was 93.3%, compared to 76.6% at the significance level (0.05). The current findings demonstrated a harmful effect of CNTs on both adult and larval stages of *T. castaneum*. The exoskeleton of insects is susceptible to penetration by nanoparticles and engage with thiol groups of the amino acid cysteine that may harm DNA, RNA, and proteins, potentially resulting in cell death (Al-Esawy *et al.,* 2021; Banerjee *et al.,* 2010). Research has shown that adult *Drosophila spp.* Subjected to dry CNTs exhibited reduced mobility and heightened mortality rates (Liu *et al.,* 2009). The efficacy of carbon nanotubes (CNTs) against *T. granarium* indicates significant promise for their use in pest management. Nevertheless, these outcomes must be considered in light of possible environmental and health repercussions (Al-Esawy *et al.,* 2021). *T. castaneum* exhibited susceptibility to environmentally friendly synthetic copper nanoparticles (CuNPs) (Abdel-Megeed *et al.,* 2024) generated from bacterial extracts of *Acacia cornigera* and *Annona purpurea* (El-Saadony *et al.,* 2020). These findings should be considered in relation to environmental and health risks. To safely and effectively apply diverse NPs in the real world, more research on long-term effects, economic feasibility, and regulatory permission is needed (Kadir *et al.,* 2025). Carbon nanotubes may be a new storage pest control method that improves seed germination, reduces toxicity, and degrades quickly (Kadir *et al.,* 2025).

Most pests in stored commodities exhibit resistance to insecticides. The pest *T. castaneum* destroys stored grains, flour, and rice bran. This insecticide-resistant, omnivorous beetle endures all challenges. The extensive application of insecticides, management practices, fitness levels, and the genetic background of *T. castaneum* influence resistance. Finding a new, environmentally friendly method to control stored pests like the red flour beetle *T. castaneum* without triggering or postponing pesticide resistance is of the utmost importance. Carbon nanotubes demonstrated a promising insecticide to control the red flour beetle. The germination of wheat seeds remained unaffected primarily following exposure to CNTs, suggesting the potential application of these nanomaterials in pest management.

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Declaration of authorship contribution

Abbas Abdul Ameer: Conceptualization, collecting Data, running laboratory treatment, and visualizing data, Investigation, Methodology, Resources, Software development, Writing – original draft. Abdulla N. Ali: Formal analysis, oversight, validation, visualization. Ruqaya Alghazali: A formal analysis. Composition – initial draft, Composition – evaluation and revision. All authors assert the absence of any conflict of interest.

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