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Research Article

Eco-Friendly Management of the Khapra Beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), in Stored Wheat Using *Carica papaya* Leaf-Mediated Copper Oxide Nanoparticles

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ABSTRACT

The khapra beetle, *Trogoderma granarium*, is a destructive stored-grain pest that threatens food security and requires effective, environmentally compatible management strategies. This study evaluated the toxicity and repellency of aqueous *Carica papaya* leaf extracts and green-synthesized copper oxide nanoparticles (CuONPs) against *T. granarium* larvae and characterized the synthesized nanomaterial using UV–visible and Fourier-transform infrared (FTIR) spectroscopy. Aqueous leaf extracts exhibited significant concentration- and time-dependent larvicidal activity ($P < 0.001$), with maximum mortality of 62% at 60% concentration after 8 days. Their repellency ranged from 20.5% to 67.17%. CuONPs demonstrated significantly greater toxicity against third-instar than fifth-instar larvae ($P < 0.001$), with maximum mortality of 85.51% and 77.17%, respectively, at 1,500 µg/mL after eight days. Maximum repellency reached 80.50% at the same concentration within 24 h. The estimated LC_{50} values declined with increasing exposure duration, reaching 327.14 µg/mL for third-instar and 505.45 µg/mL for fifth-instar larvae after 8 days. UV–visible spectroscopy revealed prominent absorption peaks at approximately 230 and 310 nm, while FTIR analysis indicated functional groups potentially involved in nanoparticle formation and stabilization. Overall, *C. papaya*-mediated CuONPs exhibited promising insecticidal and repellent activities against *T. granarium*, supporting further investigation of their potential for stored-grain pest management. Further physicochemical characterization, toxicity assessment, and validation under practical storage conditions are required before their application can be recommended.

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Introduction

Agriculture is a cornerstone of Pakistan's economy and plays a pivotal role in the economic development, food security, and livelihoods of populations in developing countries. Globally, it remains a fundamental economic sector, particularly in agrarian economies (Priyanka, 2019; Kamal et al., 2022; Jaffar, 2024). Cereal crops, including maize, oats, wheat, rye, millet, sorghum, and rice, constitute major dietary staples across Asia. A substantial proportion of cereal production is stored after harvest to ensure year-round availability and market stability. In South Asia, approximately 50–80%

of harvested grain is stored, while an estimated 65–75% of wheat, Pakistan's principal staple food, is stored at the farm level or in warehouses, commonly in gunny sacks (Gatto et al., 2021; Qian et al., 2022; Ahsan et al., 2023). However, post-harvest losses remain a major constraint on food security and agricultural sustainability. In Pakistan, reported post-harvest losses of rice, wheat, and maize are approximately 17.1%, 15.3%, and 12.6%, respectively, with insect pests, molds, birds, and rodents contributing substantially to deterioration during storage (Wike et al., 2023).

Among stored-grain insect pests, the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is particularly destructive because of its ability to survive under adverse environmental conditions and infest a wide range of stored commodities. Wheat damage attributed to this pest has been reported to range from 6% to 33%, with losses reaching as high as 73% under severe infestation, whereas estimates of wheat damage in Pakistan range from 3% to 25% (Saeed et al., 2020; Usman et al., 2022). The khapra beetle is recognized internationally as a major quarantine pest and is considered among the most economically important insect pests of stored products. Its occurrence in numerous countries, including the United States, highlights its significance for international grain trade and biosecurity (Usman et al., 2022; Wu et al., 2023). Infestation can also result in substantial quantitative and economic losses. For example, wheat weight losses of approximately 32.7 kg per ton after 120 days of exposure, equivalent to 3.27% of the initial grain weight, have been reported, together with associated reductions in market value (Zulfikar et al., 2020; Karso, 2023). These losses highlight the need for effective, economical, and environmentally sustainable strategies to protect stored wheat against this persistent pest.

Several management strategies have been employed against *T. granarium*, including physical, mechanical, botanical, and chemical approaches. Physical methods include heat treatment, solar drying, and the application of inert materials, such as amorphous silica and fine sand, to reduce insect survival. Botanical products derived from plants such as neem, garlic, castor, mint, and other species have also been investigated for their insecticidal or repellent properties. However, chemical control, particularly fumigation with phosphine, has remained an important component of stored-grain pest management. Other fumigants and contact insecticides have also been used, although their suitability depends on regulatory restrictions, commodity safety, and application conditions (Ahmedani et al., 2007). The repeated and intensive use of conventional insecticides and fumigants has raised concerns about insecticide resistance, chemical residues, environmental contamination, and risks to human health. Resistance to phosphine has been documented in multiple stored-product insect species, compromising the long-term effectiveness of fumigation-based control programs (Bell and Wilson, 1995; Sunil, 2017). These limitations emphasize the importance of developing alternative pest-management strategies that are effective, safer, and compatible with integrated pest management (IPM).

Plant-derived materials offer a promising alternative because they contain diverse bioactive compounds that may exhibit insecticidal, repellent, antifeedant, or growth-regulating effects. Various botanical preparations have been investigated against stored-product insect pests, including extracts of *Solanum nigrum*, *Citrus reticulata*, *Datura stramonium*, and *Azadirachta indica*, as well as powders or extracts derived from ginger, turmeric, garlic, *Eucalyptus* spp., rosemary, *Nigella sativa*, and *Peganum harmala* (Ali et al., 2022; Abbas et al., 2024). Despite their potential, the practical application of botanical products may be constrained by variability in their chemical composition, limited stability, rapid degradation, and inconsistent efficacy under different storage conditions. Green nanotechnology may help address some of these limitations by using plant extracts as reducing, capping, or stabilizing agents during nanoparticle synthesis. This approach can reduce reliance on certain conventional chemical reagents and may produce nanomaterials with physicochemical properties that enhance their interaction with target pests. However, the environmental safety and practical suitability of each formulation must be evaluated rather than assumed.

Green-synthesized metal and metal oxide nanoparticles, including silver (AgNPs), silica (SiO_2), aluminium oxide (Al_2O_3), zinc oxide (ZnO), titanium dioxide (TiO_2), and copper oxide (CuO), have attracted increasing interest for the management of stored-product insect pests. Their biological activity may be influenced by particle size, morphology, surface chemistry, concentration, and formulation, as well as by the insect species and developmental stage targeted. Characterization techniques such as ultraviolet–visible (UV–Vis)

spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) are commonly employed to assess their optical properties, functional groups, crystalline structure, and surface morphology, respectively (Shahid et al., 2021; Zannat et al., 2021; Kannan et al., 2022). Previous studies have investigated nanoparticle-based formulations against important stored-product pests, including *Sitophilus oryzae*, *Tribolium castaneum*, *Rhyzopertha dominica*, and *T. granarium*. For example, green-synthesized zinc oxide nanoparticles reportedly caused 46.12% mortality and 79.17% repellency against the khapra beetle (Haider et al., 2020). Such findings support further investigation of plant-mediated nanomaterials as potential components of sustainable stored-grain pest management. Nevertheless, their efficacy, dose-dependent effects, and potential impacts on grain quality and non-target organisms require systematic evaluation.

Carica papaya is a widely available plant whose leaves contain diverse phytochemicals that may contribute to the reduction, stabilization, and biological activity of green-synthesized nanoparticles. However, the potential of *C. papaya* leaf-mediated copper oxide nanoparticles (CuONPs) against *T. granarium* requires further investigation, particularly in relation to larval mortality and their suitability for protecting stored wheat. Accordingly, the present study aimed to synthesize and characterize CuONPs using aqueous *C. papaya* leaf extract and evaluate their insecticidal efficacy against *T. granarium* larvae. The study also sought to investigate the potential of this plant-mediated nanomaterial as an alternative approach to managing khapra beetle infestations in stored wheat. The findings may contribute to the development of plant-mediated nanotechnology-based strategies for stored-grain protection, subject to further assessment of grain quality, environmental safety, and practical applicability.

Materials and Methods

Collection and rearing of *T. granarium*

The experimental population of *T, granarium* was obtained from naturally infested stored wheat and maintained as a laboratory culture on wheat kernels under controlled conditions. Larvae were reared until the required developmental stages were attained. Healthy, active third- and fifth-instar larvae were selected for the bioassays. Fifth-instar larvae were used to assess the insecticidal and repellent activities of the aqueous *C. papaya* leaf extract, whereas third- and fifth-instar larvae were used in the bioassays evaluating the activity of green-synthesized copper oxide nanoparticles (CuONPs).

Preparation of wheat and experimental setup

Wheat kernels were selected as the substrate for the bioassays because wheat is a suitable host of *T. granarium*. Before use, the kernels were cleaned to remove damaged grains, foreign matter, and other impurities. For the mortality bioassays, 10 g of wheat kernels was placed in each Petri dish (60 mm diameter × 20 mm height). Larger Petri dishes (100 mm diameter × 20 mm height) were used for the repellency bioassays.

Unless otherwise specified, the experimental units were maintained under laboratory conditions at 30 ± 2°C and 65 ± 5% relative humidity (RH). The required number of larvae was introduced into each experimental unit according to the respective bioassay design. Experimental units and stored wheat kernels were inspected visually throughout the experimental period.

Preparation of Aqueous *C. papaya* leaf extract

Fresh, healthy, mature leaves of *C. papaya* were collected from the fields of the Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The leaves were thoroughly washed under running tap water to remove adhering dust and surface contaminants, rinsed with distilled water, and shade-dried under ambient laboratory conditions in the Integrated Pest Management (IPM) Laboratory. The dried leaves were ground into a fine powder using an electric grinder and stored in airtight containers until extraction.

For aqueous extraction, 10 g of leaf powder was weighed using a digital balance and mixed with 150 mL of distilled water to obtain a 6.67% (w/v) preparation. The mixture was heated on a hot plate at 100°C for 20–25 min, with occasional stirring, to facilitate the extraction of water-soluble phytochemicals. After cooling to room temperature, the mixture was filtered through Whatman filter paper to remove plant residues. The resulting aqueous filtrate was collected and used as the stock extract for subsequent bioassays.

Insecticidal activity of aqueous *C. papaya* leaf extract

The insecticidal activity of the aqueous *C. papaya* leaf extract was evaluated against healthy fifth-instar larvae of *T. granarium*. Four extract concentrations (15, 30, 45, and 60%) were tested, with distilled water serving as the control. Each treatment was replicated three times, with 20 larvae per replicate.

A surface-treatment bioassay was employed. Each Petri dish was treated with the corresponding extract concentration using a cotton pad and allowed to dry under laboratory conditions. Subsequently, 10 g of wheat kernels and 20 fifth-instar larvae were introduced into each dish. Control dishes were treated with distilled water following the same procedure. Larval mortality was assessed at 2, 4, and 8 days after treatment (DAT). Larvae were considered dead when they failed to exhibit movement in response to gentle stimulation with a camel-hair brush.

Repellency of aqueous *C. papaya* leaf extract

The repellent activity of the aqueous *C. papaya* leaf extract against *T. granarium* larvae was assessed using a two-choice filter-paper bioassay. Petri dishes measuring 100 mm in diameter × 20 mm in height were used. Filter paper was cut to fit each dish and divided into two equal halves. One half was treated with the respective concentration of the aqueous leaf extract, while the other was treated with distilled water. Both halves were allowed to dry before being placed together in the Petri dish.

Each concentration was tested in three replicates, with 10 larvae released at the center of each dish. The larvae were allowed to move freely for 24 h under laboratory conditions of approximately 30°C and 65% RH. At the end of the exposure period, the numbers of larvae present on the treated and untreated filter-paper halves were recorded.

Percentage repellency (PR) was calculated using the following equation:

$$\text{Percentage repellency (PR)} = \frac{(\text{Nc} - \text{Nt})}{(\text{Nc} + \text{Nt})} \times 100$$

Where: PR = percentage repellency; Nc = number of larvae on the untreated area; and Nt = number of larvae on the treated area. Positive values indicated repellency, whereas negative values indicated attraction.

Green synthesis of CuONPs

CuONPs were synthesized using *C. papaya* leaf extract as a biological source of phytochemicals potentially involved in nanoparticle formation and stabilization. The synthesis procedure was based on the general methodology described by Bharathi and Jothiramalingam (2018), with modifications where applicable.

Briefly, 50 mL of filtered aqueous leaf extract was mixed with 10 mL of 5 M sodium hydroxide (NaOH) and 50 mL of 1 M copper (II) chloride ($CuCl_2$) solution. The reaction mixture was heated on a hot plate at 90°C for 90 min under stirring at 12 rpm. The beaker was covered with aluminium foil to minimize contamination and evaporation. Following the reaction, the mixture was allowed to cool and stand for 24 h.

The resulting suspension was transferred into centrifuge tubes and centrifuged at 5,000 rpm for 10 min to separate the precipitate from the supernatant. The precipitate was collected and transferred to a clean porcelain dish, and then oven-dried at 100°C for approximately 3 h or until the moisture had evaporated. The dried material was carefully scraped from the dish and stored in clean vials for subsequent characterization and bioassays.

Preparation of CuONP stock suspension and test concentrations

The dried CuONP material was dispersed in distilled water and subjected to ultrasonication to improve dispersion and reduce particle agglomeration (Figure 1). A stock suspension with a nominal concentration of 2,000 µg/mL was prepared. The required mass of CuONPs was calculated using the following equation:

$$\text{Mass of solute required (mg)} = \frac{\text{Concentration required (µg/mL)} \times \text{Volume required (ml)}}{1000}$$

The required test concentrations were prepared from the stock solution using the dilution equation:

$$C_1V_1 = C_2V_2$$

Where C_1 is the stock concentration, V_1 is the volume of stock suspension required, C_2 is the desired final concentration, and V_2 is the final volume of the working suspension.

Five CuONP concentrations were prepared: 500, 750, 1,000, 1,250, and 1,500 µg/mL.

Mortality bioassay of green-synthesized CuONPs

The insecticidal activity of green-synthesized CuONPs was evaluated against third- and fifth-instar larvae of *T. granarium* using surface-treatment and food-treatment bioassays.

For the surface-treatment bioassay, Petri dishes (60 × 20 mm) were treated with the respective CuONP concentrations using cotton pads and allowed to air-dry under laboratory conditions. For the food-treatment bioassay, 10 g of wheat kernels per experimental unit were treated with the corresponding nanoparticle concentrations and air-dried until the treated kernels were dry.

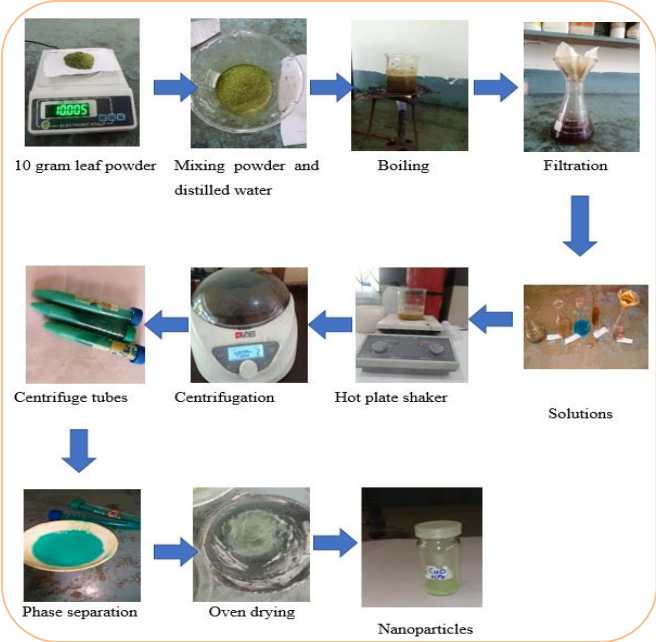


Figure 1. Preparation of CuONP stock suspension and test concentrations.

Each treatment consisted of three replicates, with 20 larvae per replicate. The treated Petri dishes or wheat kernels, as appropriate, were placed in the respective experimental units. Larval mortality was recorded at 2, 4, 6, and 8 DAT. Larvae that failed to move following gentle stimulation with a camel-hair brush were considered dead. Percentage mortality was calculated as follows:

$$PM = \frac{Mt}{T} \times 100$$

Where: PM = percentage mortality; Mt = the number of dead insects in the treatment; and T = the total number of insects initially introduced into the experimental unit. To account for natural mortality in the control, corrected mortality was calculated using Abbott's formula:

$$CPM = 1 - \left(\frac{Xt}{Xc}\right) \times 100$$

Where: CPM = corrected percentage mortality; Xt = number of live insects in the treatment; and Xc = number of live insects in the control.

Repellency bioassay of green-synthesized CuO NPs

The repellency of green-synthesized CuONPs against *T. granarium* larvae was evaluated using a two-choice filter-paper bioassay. Filter paper was cut to fit Petri dishes (100 × 20 mm) and divided into two equal halves. One half was treated with the respective CuONP concentration, whereas the other was treated with distilled water as the untreated control. The treated and untreated halves were air-dried for approximately 30 min and then reassembled in the Petri dish.

Each treatment was replicated three times, with 10 larvae released at the center of each experimental unit. The larvae were allowed to move freely for 24 h under dark conditions at approximately 30°C and 65% RH. At the end of the exposure period, the numbers of larvae present on the treated and untreated halves were recorded. Percentage repellency was calculated as follows:

$$\text{Percentage repellency (PR)} = \frac{(Nc - Nt)}{(Nc + Nt)} \times 100$$

Where: PR = percentage repellency; Nc = the numbers of insects recorded on the untreated filter-paper surfaces; and Nt = the numbers of insects recorded on the treated filter-paper surfaces. Positive values indicated repellency, whereas negative values indicated attraction.

Characterization of Green-Synthesized CuONPs

The synthesized CuONPs were characterized using ultraviolet–visible (UV–Vis) and Fourier-transform infrared (FTIR) spectroscopy at the Analytical Laboratory, Department of Chemistry, University of the Punjab, Lahore, Pakistan.

UV–visible spectroscopy

UV–Vis spectroscopy was used to examine the optical properties of the synthesized nanoparticles and provide preliminary evidence of nanoparticle formation. Before analysis, the sample was diluted with deionized water. Absorbance spectra were recorded over the wavelength range of 190–790 nm.

Fourier-transform infrared spectroscopy

FTIR spectroscopy was performed to identify the functional groups associated with phytochemicals potentially involved in the formation and stabilization of the green-synthesized CuONPs. Spectral measurements were obtained over the range of 500–4000 cm^{-1}.

Assessment of physical grain quality and insect population dynamics

During the storage period, treated wheat kernels and their corresponding experimental units were examined visually at the designated observation intervals. Changes in physical grain quality, including visible insect damage, discoloration, and other observable treatment-related alterations, were documented. The numbers of live and dead insects were also recorded at each assessment to monitor insect population dynamics and evaluate treatment-associated effects during storage.

Statistical analysis

Experimental data were analyzed using Statistix version 8.1. Analysis of variance (ANOVA) was performed to evaluate the effects of treatment, nanoparticle concentration, exposure period, and their interactions on insect mortality and repellency, as applicable to the experimental design. Treatment means were compared using the least significant difference (LSD) test at the 5% significance level. Probit analysis was conducted using Minitab to estimate the median and 90% lethal concentrations (LC_{50} and LC_{90}, respectively) of the tested treatments.

Results

Toxicity and repellency of aqueous *C. papaya* leaf extracts against *T. granarium* larvae

All tested concentrations of aqueous *C. papaya* leaf extracts exhibited significant larvicidal activity against *T. granarium* ($P < 0.001$), with mortality increasing progressively with both extract concentration and exposure duration. The highest larval mortality (62%) was recorded at the highest extract concentration (60%) after 8 days of exposure, followed by 45% mortality at the same concentration after 6 days (Figure 2). These findings indicate a concentration- and time-dependent toxic effect of the aqueous leaf extracts against *T. granarium* larvae.

The extracts also exhibited significant repellent activity against *T. granarium* larvae ($P < 0.001$). Mean repellency ranged from 20.5 ± 0.21% to 67.17 ± 0.81% across the tested concentrations (15, 30, 45, and 60%) (Figure 3).

Toxicity of CuONPs against third- and fifth-instar larvae of *T. granarium*

Green-synthesized CuONPs caused significantly greater mortality in third-instar larvae than in fifth-instar larvae across all assessment periods ($P < 0.001$). Larval mortality increased with increasing CuONP concentration and exposure duration in both instars. The highest mean mortality was recorded at 1,500 µg/mL after 8 days of treatment, reaching 85.51 ± 1.39% in third-instar larvae and 77.17 ± 0.87% in fifth-instar larvae. In contrast, the control treatments exhibited the lowest mortality, with 3.84 ± 0.21% and 3.84 ± 0.10% mortality in third- and fifth-instar larvae, respectively, after 8 days. Across the tested concentrations, mortality in third-instar larvae ranged from 25.51 ± 0.41% to 85.51 ± 1.39%, whereas that in fifth-instar larvae ranged from 22.17 ± 0.41% to 77.17 ± 0.87% over the 8-day assessment period (Figure 4).

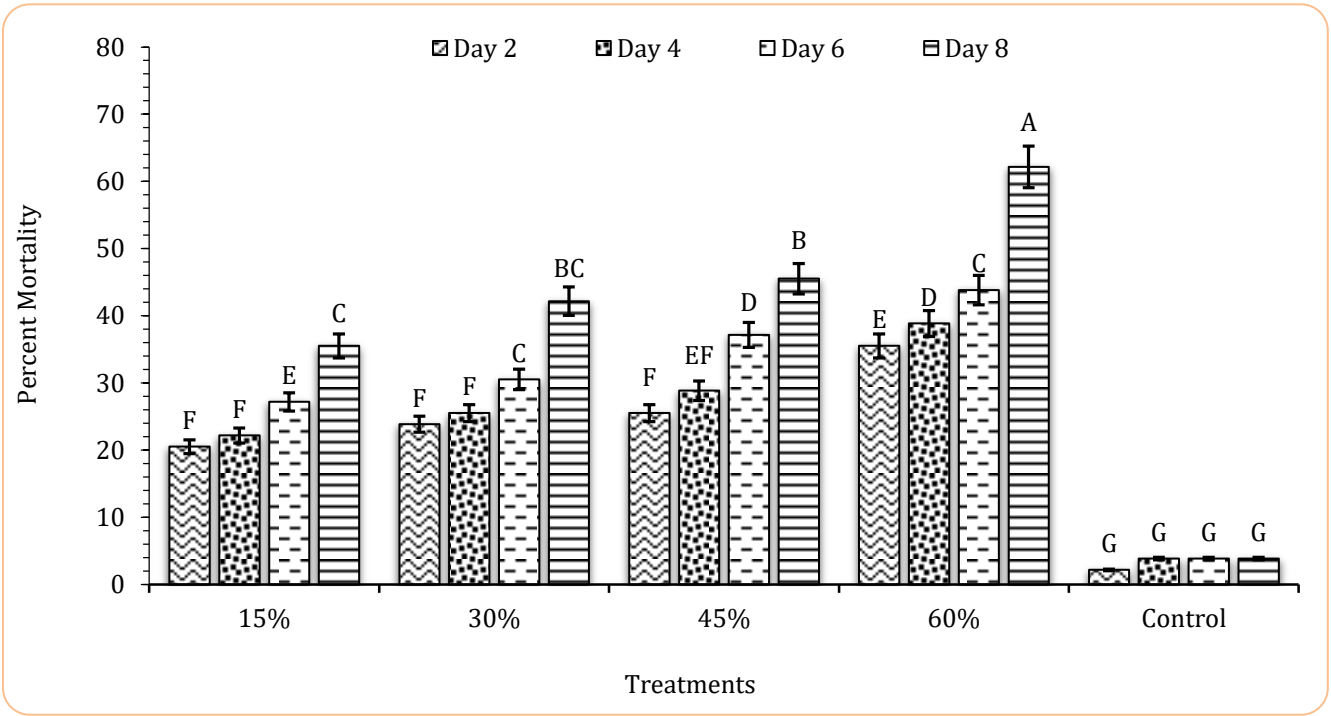


Figure 2. Percentage means and standard errors (SE) of *T. granarium* larval mortality following 2, 4, 6 and 8 days of exposure to aqueous extracts of *C. papaya* leaves. Means sharing the same letters do not differ significantly ($t > 0.05$) according to the LSD test.

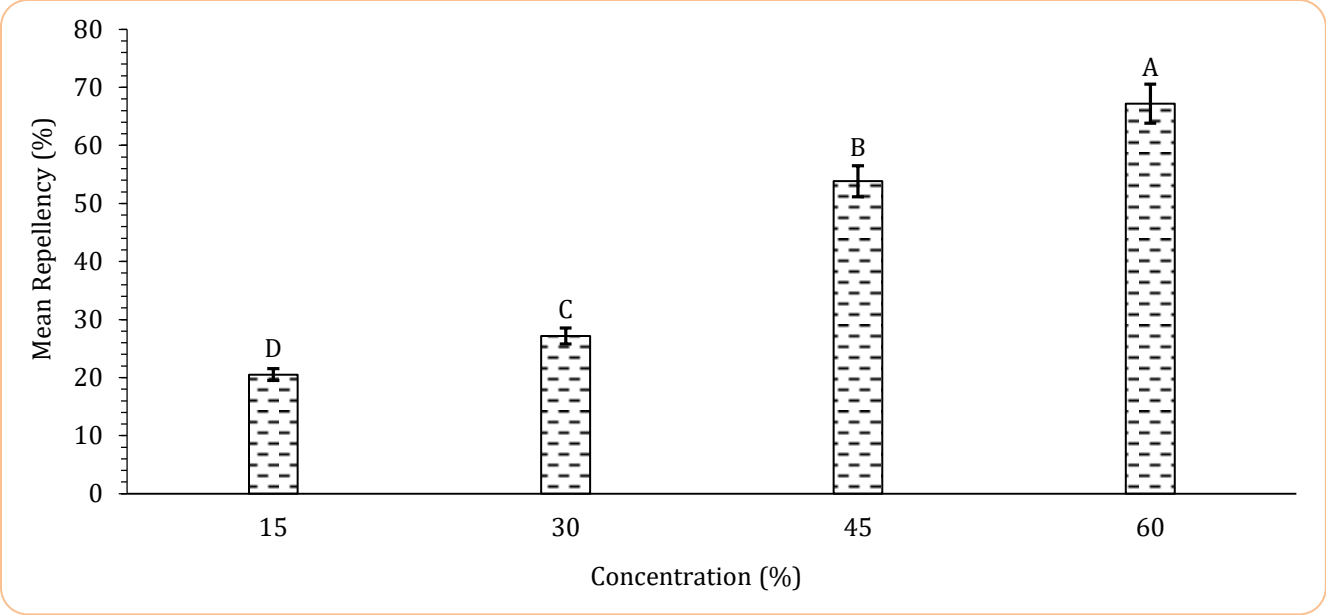


Figure 3. Percent repellency of various concentrations of *C. papaya* aqueous leaf extracts against larvae of *T. granarium.*

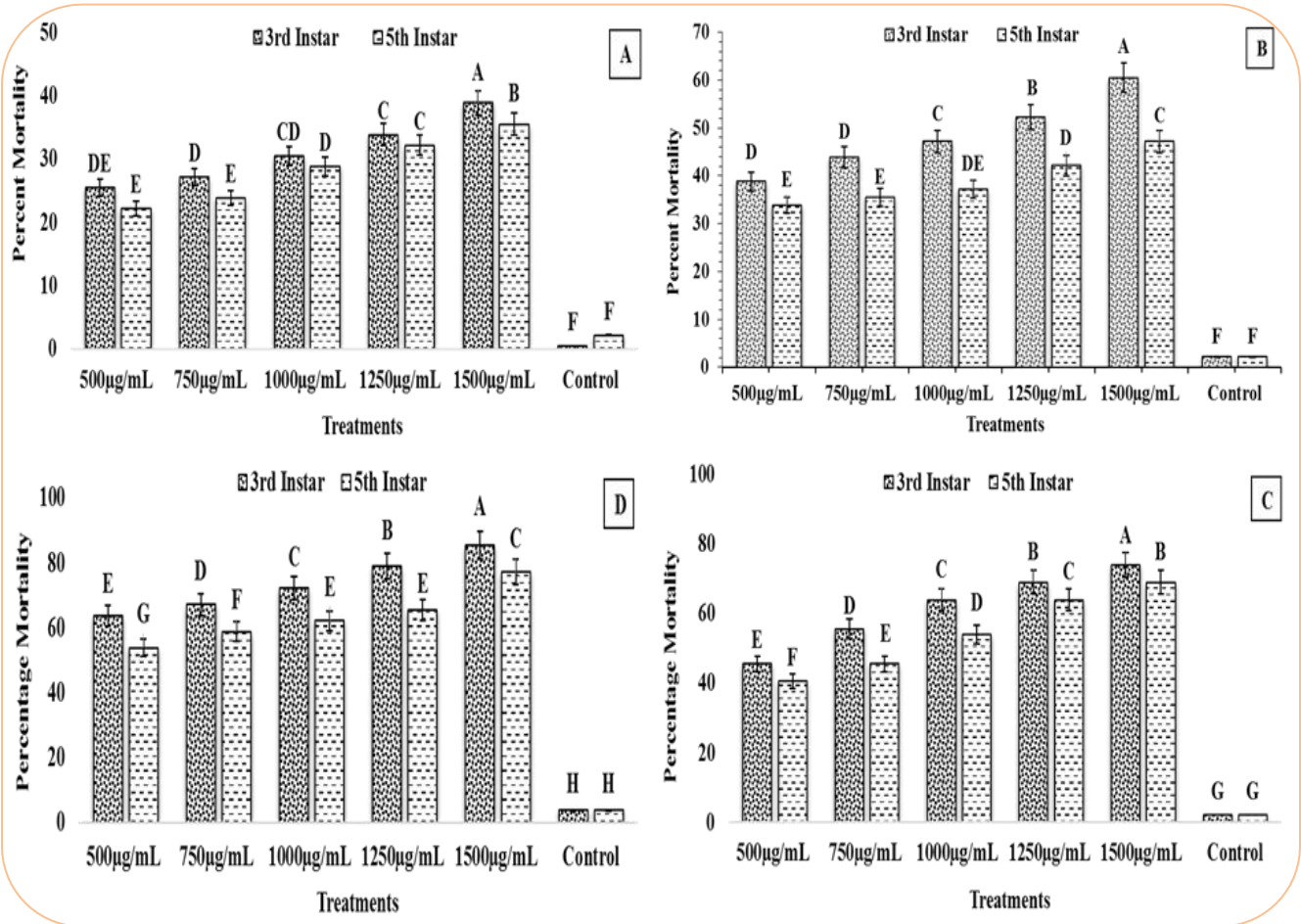


Figure 4. Mean percentage mortalities caused by various concentrations of CuONPs in 3rd and 5th Instar larvae of *T. granarium* A = 2 day, B = 4 day, C = 6 day, and D = 8 day.

Repellent activity of CuONPs against *T. granarium* larvae within 24 h

CuONPs exhibited a highly significant concentration-dependent repellent effect against *T. granarium* larvae within 24 h ($P < 0.001$). The highest mean repellency (80.50 ± 1.40%) was recorded at 1,500 µg/mL, followed by 73.83 ± 1.40% at both 1,200 and 1,000 µg/mL. Repellency decreased to 67.17 ± 1.33% and 60.50 ± 0.99% at 750 and 500 µg/mL, respectively (Figure 5).

Characterization of Green-Synthesized CuONPs using *C. papaya* leaf extract

UV–visible spectroscopy

UV–visible spectroscopy was employed to investigate the optical properties of the green-synthesized CuONPs produced using *C. papaya* leaf extract. The sample was diluted with deionized water and scanned over a wavelength range of 190–790 nm. Absorbance was recorded over the instrument's specified range of −1.000 to 6.000 absorbance units (a.u.). The resulting spectrum illustrates the wavelength-dependent absorbance of the sample (Figure 6).

Two prominent absorption peaks were observed at approximately 230 and 310 nm, with absorbance values of about 4.0 a.u. These absorption features may be associated with the optical characteristics of the synthesized copper-based nanomaterial and are consistent with wavelength ranges reported in previous studies on plant-mediated copper and CuONPs (Sankar et al., 2014; Bharathi and Jothiramalingam, 2018; Turakhia et al., 2020; Phang et al., 2021; Gayathri et al., 2023).

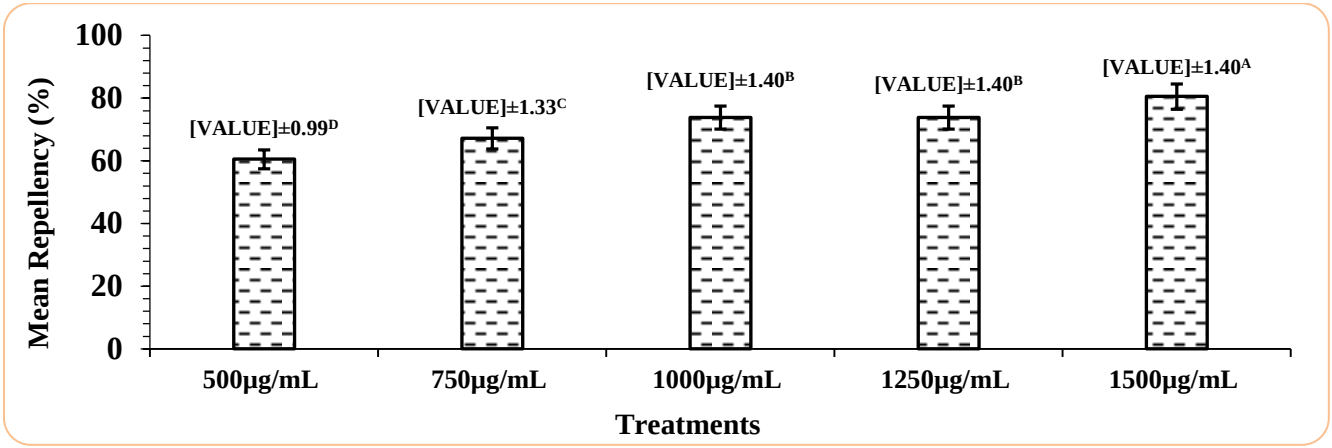


Figure 5. Percent repellency of CuONPs on larvae of *T. granarium* within 24 h.

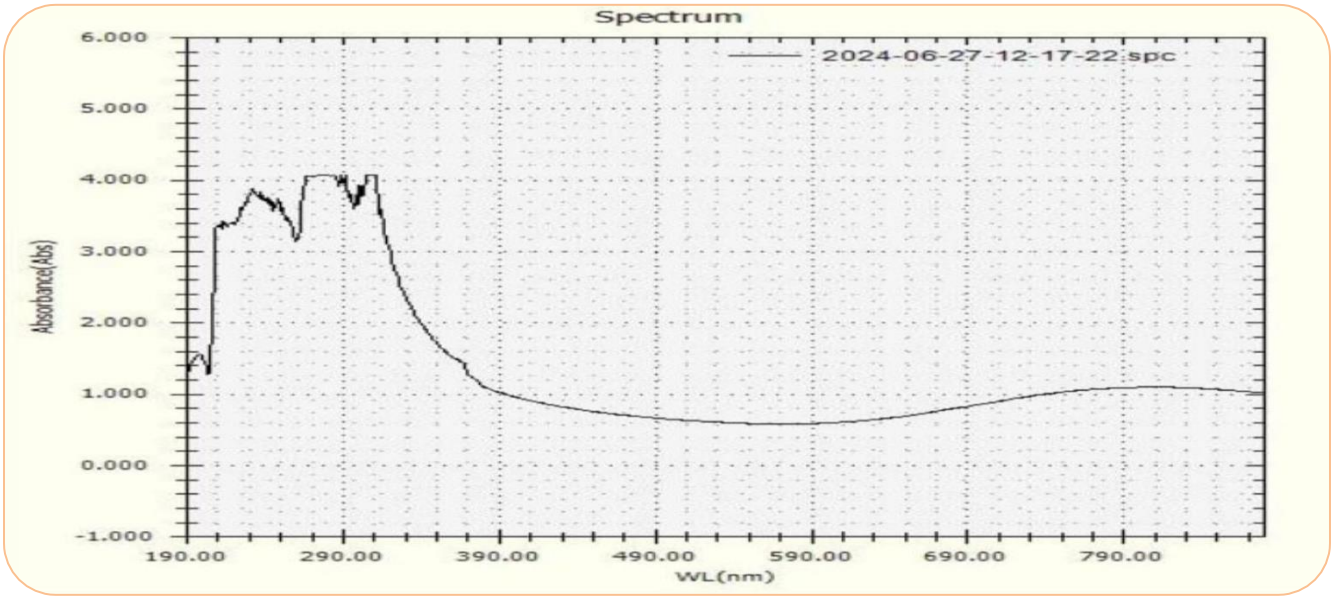


Figure 6. UV-Visible spectroscopy report of green synthesized CuONPs from *C. papaya* leaves.

An increase in peak intensity may indicate a higher concentration of light-absorbing species in the sample, whereas the positions of absorption bands are influenced by factors such as solvent composition, pH, stabilizing agents, and nanoparticle size. The sharp absorption peaks observed at 230 and 310 nm may be associated with the presence of small nanoparticles and their surface-related optical properties (Turakhia et al., 2020). Absorption in the 230–290 nm range has been associated with phenolic compounds, including tannins, and flavonoids, whereas absorption in the 330–420 nm range may indicate the presence of phenolic acids and their derivatives (Figure 7) (Bharathi and Jothiramalingam, 2018). These spectral features suggest the presence of flavonoids, tannins, and other phenolic compounds in the sample.

FTIR spectroscopy

FTIR spectroscopy was employed to identify the functional groups associated with the *C. papaya* leaf extract and to investigate their potential roles in the bioreduction, formation, and stabilization of CuONPs. The FTIR spectrum was recorded over the wavenumber range of 500–4000 cm^{-1}, with transmittance values ranging from 35% to 100%. Seven absorption bands were observed, spanning 816.3–3410.0 cm^{-1} (Figure 8). The band at 3410.0 cm^{-1} was tentatively attributed to N–H stretching vibrations, potentially indicating the presence of amide-containing compounds (Bharathi and Jothiramalingam, 2018). The band at 3320.1 cm^{-1} was assigned to O–H stretching vibrations associated with hydrogen-bonded hydroxyl groups in alcohols, phenolic compounds, and water (Sankar et al., 2014). The band at 1636.8 cm^{-1} was attributed to C=O stretching and/or other overlapping vibrations, potentially associated with carbonyl-containing compounds, including ketones, aldehydes, carboxylic acids, esters, and amides. The band at 980.8 cm^{-1} was tentatively assigned to C–O-related vibrations, which may be associated with alcohols or ethers. The band at 913.2 cm^{-1} was attributed to vibrations potentially associated with aromatic or other organic structures; however, this assignment requires further verification. The band at 816.3 cm^{-1} may correspond to out-of-plane bending vibrations of substituted aromatic groups or other fingerprint-region vibrations. The presence of copper–oxygen bonds should be confirmed by examining characteristic Cu–O vibrational bands, particularly in the lower-wavenumber region, using the complete spectrum.

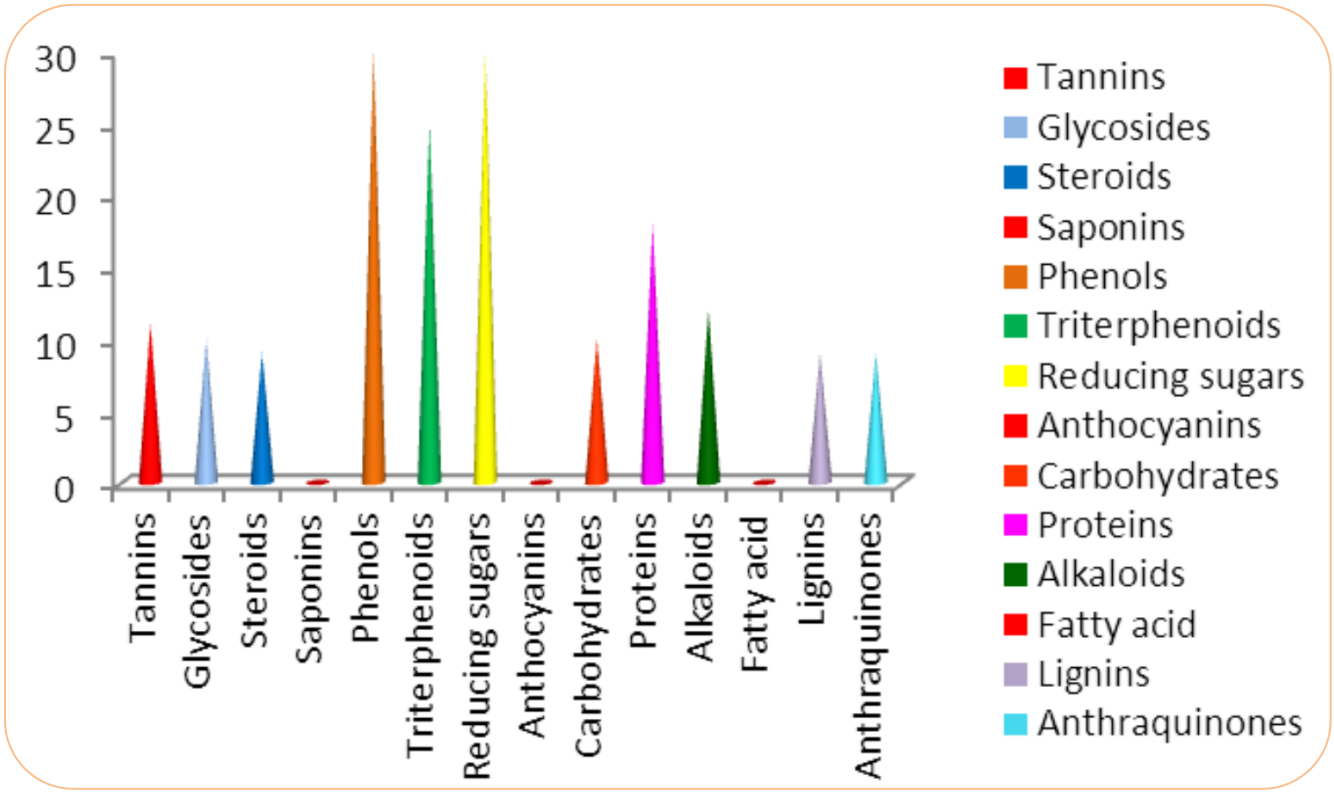


Figure 7. Graphical representation of phytochemical analysis of green synthesized CuONPs from *C. papaya* leaves.

Collectively, the observed absorption bands suggest that oxygen- and nitrogen-containing functional groups in the *C. papaya* leaf extract may participate in the formation and stabilization of CuONPs. Phytochemicals such as flavonoids, phenolic compounds, alkaloids, and other organic constituents may contribute to these processes through their hydroxyl, carbonyl, and nitrogen-containing functional groups. However, the specific identities of these compounds cannot be established from FTIR data alone and require complementary phytochemical or analytical characterization.

Generally, the FTIR results provide preliminary evidence for the involvement of plant-derived functional groups in the synthesis and surface stabilization of CuONPs.

Lethal concentrations (LC50 and LC90) of CuONPs against different larval instars of *T. granarium*

Third-instar larvae

Table 1 presents the median LC_{50} and 90% LC_{90} of CuONPs against third-instar larvae of *T. granarium* following 2, 4, 6, and 8 days of exposure. The estimated LC_{50} values decreased progressively from 3,843.64 µg/mL after 2 days to 1,085.93, 639.12, and 327.14 µg/mL after 4, 6, and 8 days, respectively. Similarly, the LC_{90} values decreased from 52,526.2 µg/mL at 2 days to 8,027.84, 3,215.94, and 2,433.60 µg/mL at 4, 6, and 8 days, respectively. These results indicate an increase in the toxicity of CuONPs with prolonged exposure.

The corresponding fiducial limits for LC_{50} estimates were 1,899.63 to 4.75188×10^{11}, 862.31–1,513.50, 436.72–772.13, and 110.32–481.29 µg/mL after 2, 4, 6, and 8 days, respectively. For LC_{90}, the fiducial limits were 7,587.05 to 1.92211×10^{28}, 3,627.57–160,392, 2,200.17–7,986.09, and 1,717.42–6,401.76 µg/mL, respectively. The estimated probit regression slopes were 0.459 ± 0.21, 0.60 ± 0.17, 0.74 ± 0.61, and 0.59 ± 0.51 after 2, 4, 6, and 8 days, respectively.

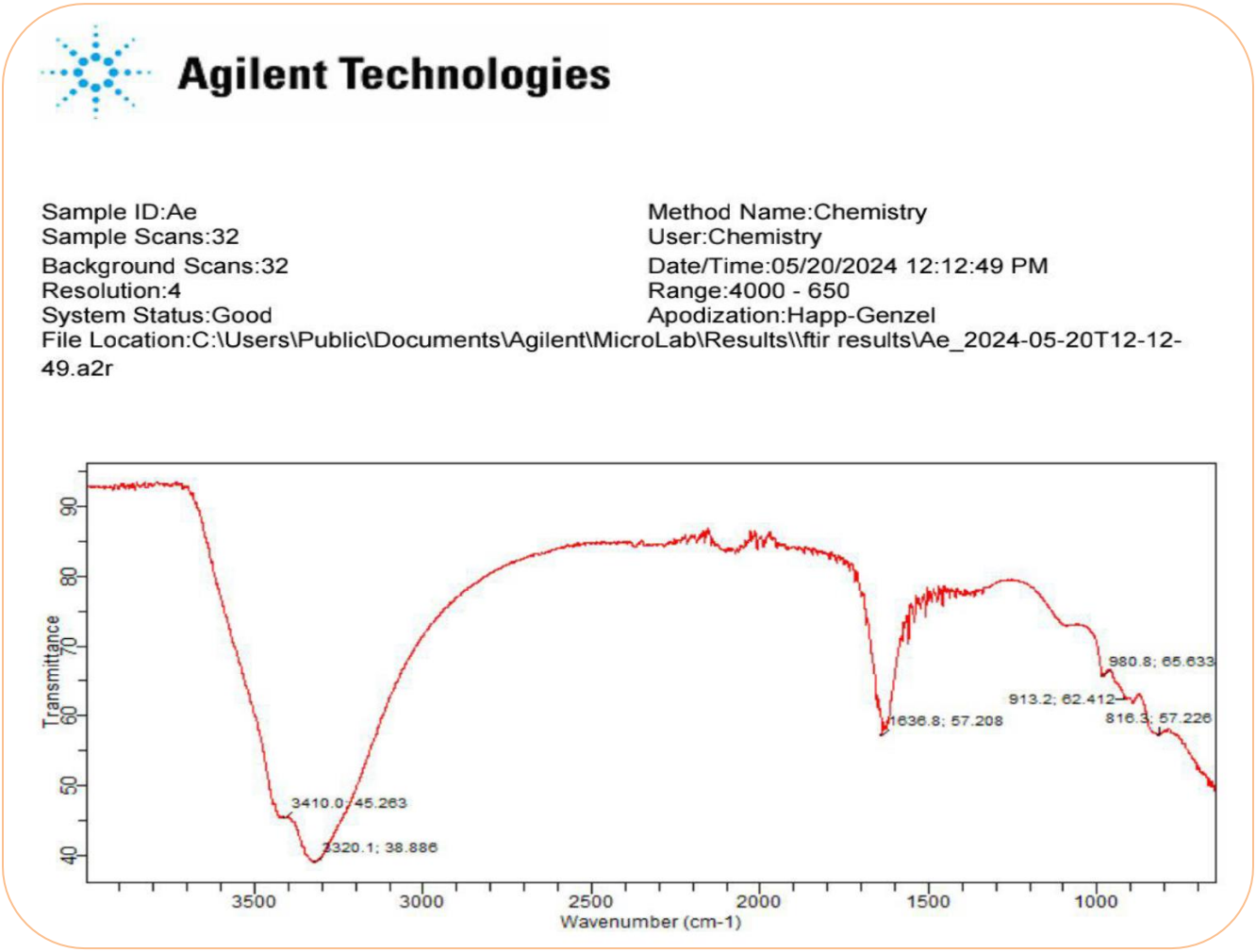


Figure 8. FTIR- spectroscopy report of green synthesized CuONPs from C. papaya leaves.

Table 1. Lethal concentration (LC_{50} and LC_{90}) of leaf extracts of *C. papaya* against 3rd Instar larvae of *T. granarium.*

Days	LC_{50} (µg/mL)	Fiducial Limit	LC_{90} (µg/mL)	Fiducial Limit	Slope ± S.E.	Goodness of fit test		
						χ^2	D.F.	P
Day-2	3843.64	1899.63-	52526.2	7587.05-	0.459 ± 0.21	0.355803	3	0.949
Day-4	1085.93	862.308-	8027.84	3627.57-	0.60 ± 00.17	0.903673	3	0.825
Day-6	639.121	436.723-	3215.94	2200.17-	0.74 ± 0.61	0.0025307	3	1.000
Day-8	327.137	110.315-	2433.60	1717.42-	0.59 ± 0.51	1.62555	3	0.654

Fifth-instar larvae

Table 2 summarizes the LC_{50} and LC_{90} estimates for CuONPs against fifth-instar larvae of *T. granarium* following 2, 4, 6, and 8 days of exposure. The LC_{50} values decreased from 3,888.35 µg/mL after 2 days to 2,305.00, 846.40, and 505.45 µg/mL after 4, 6, and 8 days, respectively. The corresponding LC_{90} values were 32,668.8, 38,696.8, 3,689.54, and 4,601.59 µg/mL, respectively. Overall, the decline in LC_{50} estimates suggests increased toxicity with prolonged exposure, although the LC_{90} estimates did not decrease consistently across all exposure periods.

The fiducial limits for LC_{50} were 2,026.82–1,642,226, 1,424.70–8,325,608, 678.73–985.33, and 199.91–678.37 µg/mL after 2, 4, 6, and 8 days, respectively. For LC_{90}, the corresponding limits were 6,709.77 to 1.61842×10^{11}, 6,626.20 to 4.95063×10^{19}, 2,458.39–9,606.03, and 2,539.15–38,516.2 µg/mL, respectively. The estimated probit regression slopes were 0.564 ± 0.235, 0.456 ± 0.196, 0.815 ± 0.173, and 0.544 ± 0.159 after 2, 4, 6, and 8 days, respectively.

Discussion

T. granarium, commonly known as the khapra beetle, is a destructive insect pest of stored grains that can cause substantial postharvest losses worldwide. Conventional management relies heavily on synthetic insecticides and fumigants, raising concerns regarding environmental contamination, human health risks, grain quality, and the development of insecticide resistance. The present study investigated the insecticidal and repellent potential of aqueous *C. papaya* leaf extract and green-synthesized CuONPs against khapra beetle larvae.

Previous studies have demonstrated the insecticidal potential of *C. papaya* leaf-derived products against several stored-product pests, including the red flour beetle, *T. castaneum* (Mangang et al., 2020); maize weevil, *S. zeamais* (Oboho et al., 2022); cowpea weevil, *Callosobruchus maculatus* (Arinola et al., 2021; Nyamador et al., 2022); rice weevil, *S. oryzae* (Okore et al., 2022); and lesser grain borer, *R. dominica* (Shukla et al., 2020). To the best of our knowledge, no published study identified during the preparation of this manuscript has specifically evaluated *C. papaya*-mediated CuONPs against *T. granarium*. This study therefore provides preliminary evidence of the potential application of this plant-based nanomaterial for managing this important stored-grain pest. The observed insecticidal activity is consistent with the findings of Shah et al. (2022), although direct comparisons should account for differences in experimental conditions, formulations, and exposure periods.

Treatment-associated changes in larval appearance and development were also observed. Larvae exposed to the CuONPs developed a greyish-black coloration after 8 days, suggesting possible treatment-related physiological or structural changes. However, this observation alone does not establish the underlying mechanism of toxicity. Adults emerging from nanoparticle-treated Petri dishes appeared earlier, while the number of larvae in the F_1 generation was lower than that in the untreated control. These findings suggest that exposure may influence developmental timing and subsequent population establishment. However, further experiments are required to determine whether these effects result from direct toxicity, sublethal physiological stress, altered reproductive performance, or other treatment-related factors.

The insecticidal activity of *C. papaya* leaf extract may be associated with its diverse phytochemical constituents, including alkaloids, flavonoids, tannins, and other phenolic compounds. Rahayu et al. (2024) discussed the potential biological activities of papaya-leaf phytochemicals, including alkaloids such as carpaine and related compounds, and the possible involvement of midgut damage and oxidative stress in insect mortality. Phenolic compounds and flavonoids may also influence insect physiology through oxidative processes and

disruption of cellular functions. However, these mechanisms were not directly investigated in the present study and should therefore be regarded as reasonable explanations rather than confirmed modes of action against *T. granarium*. Histological examination, biochemical assays, and targeted analyses of oxidative stress markers would help establish the mechanisms responsible for the observed larval mortality.

Table 2. Lethal concentration (LC_{50} and LC_{90}) of leaf extracts of *C. papaya* against 5th Instar larvae of *T. granarium.*

Days	LC_{50} (µg/mL)	Fiducial Limit	LC_{90} (µg/mL)	Fiducial Limit	Slope ± S.E.	Goodness of fit test		
						χ^2	D.F.	P
Day-	3888.35	2026.82-	32668.8	6709.77-	0.56 ± 0.23	0.38	3	0.944
Day-	2305.00	1424.70-	38696.8	6626.20-	0.45 ± 0.19	0.61	3	0.892
Day-	846.400	678.731-	3689.54	2458.39-	0.81 ± 0.17	1.06	3	0.785
Day-	505.452	199.909-	4601.59	2539.15-	0.54 ± 0.15	2.15	3	0.536

Green synthesis and physicochemical characterization are important for assessing the identity and surface chemistry of plant-mediated nanomaterials. UV–visible spectroscopy revealed absorption peaks at 230 and 310 nm, with reported absorbance values of up to 4.000 absorbance units. These spectral features may indicate the presence of plant-derived chromophores and changes associated with nanoparticle synthesis, as reported in previous studies (Bharathi and Jothiramalingam, 2018; Gayathri et al., 2023). Nevertheless, UV–visible spectroscopy alone is insufficient to conclusively establish the formation and phase purity of CuONPs. Additional techniques, particularly X-ray diffraction (XRD), transmission or scanning electron microscopy (TEM/SEM), and energy-dispersive X-ray spectroscopy (EDS), would provide stronger evidence of crystalline phase, particle morphology, size distribution, and elemental composition. The FTIR spectrum of the green-synthesized CuONPs exhibited absorption bands at 3410.0, 3320.1, 1636.8, 980.8, 913.2, and 816.3 cm^{-1}. The bands in the higher-wavenumber region may be associated with O–H and N–H stretching vibrations, suggesting the presence of hydroxyl-containing phytochemicals and potentially nitrogen-containing functional groups. The band near 1636.8 cm^{-1} may correspond to carbonyl-related vibrations, amide groups, or other overlapping vibrations, while the bands at lower wavenumbers may reflect C–O and other fingerprint-region vibrations. These assignments should be interpreted cautiously because individual absorption bands may have multiple origins, and the precise assignment of Cu–O vibrations requires confirmation from the complete spectrum and supporting characterization data. Collectively, the FTIR results suggest that *C. papaya*-derived phytochemicals may contribute to the surface functionalization and stabilization of the synthesized material. However, confirmation of nanoparticle formation and the specific role of individual phytochemicals require complementary analytical evidence. These observations are broadly consistent with previous reports on plant-mediated nanoparticle synthesis and characterization (Bharathi and Jothiramalingam, 2018; Phang et al., 2021; Gayathri et al., 2023).

Conclusions

This study demonstrated the insecticidal and repellent potential of aqueous *C. papaya* leaf extract and green-synthesized CuONPs against *T. granarium* under laboratory conditions. The aqueous leaf extract produced a maximum larval mortality of 62.17 ± 0.91% and repellency of 67.17 ± 0.81% at a concentration of 60%. In comparison, CuONPs achieved mortality rates of 85.51 ± 1.39% in third-instar larvae and 77.17 ± 0.87% in fifth-instar larvae at 1500 µg/mL after 8 days of exposure. The estimated LC_{50} values were 327.14 µg/mL for third-instar larvae and 505.45 µg/mL for fifth-instar larvae, indicating greater susceptibility of the younger larval stage under the experimental conditions. The nanoparticle treatment also achieved a reported maximum repellency of 80.50%.

These findings suggest that green-synthesized CuONPs may offer greater insecticidal efficacy than aqueous *C. papaya* leaf extract against *T. granarium* and warrant further investigation as a potential component of integrated stored-grain pest management. However, their practical application requires validation through grain-storage trials, assessment of residues and grain quality, and comprehensive evaluation of human and environmental safety. Further research should also

optimize formulation and application rates and establish the mechanisms underlying insect mortality and repellency. Until these aspects are addressed, the findings should be considered promising laboratory evidence rather than confirmation of a commercially viable or environmentally safe grain-protection technology.

Author Contributions

MI and SA conceptualized and designed the experiment. AR conducted the field investigations and carried out the experimental work. MI and SA provided overall supervision and ensured methodological rigour and analytical consistency throughout the study. AI, AS, EA, and SNBR contributed as co-supervisors, providing support in visualization, validation, and manuscript review and editing. All authors critically reviewed the manuscript, contributed to its intellectual refinement, cross-checked the data and analyses, and approved the final version for submission.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 9: Industry, Innovation and Infrastructure
SDG 12: Responsible Consumption and Production

Policy Addressed

This study addresses policies related to food security and postharvest loss reduction, sustainable stored-grain pest management, green nanotechnology, pesticide risk reduction, and environmental safety. It also supports agricultural innovation and stored-grain biosecurity.

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