Transforming micronutrients into Nano-pesticides efficacy of Copper oxide nanoparticles as innovative pesticides against Diaphania pulverulentalis

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ABSTRACT

Sericulturists are facing problem with significant losses in mulberry leaf yield due to pests infestation throughout the year. In order to control the pests infestation in mulberry, a research was carried to explore the efficacy of copper oxide nanoparticles (CuO-NP) as nanopesticides in pest management and ecofriendly alternatives to traditional pesticides. Synthesis of CuO-NPs was achieved by Hydrothermal technique and confirmed through XRD, FTIR, DRS characterization. CuO-NPs showed effective toxicity against *Diaphania pulverulentalis* with 100% larval mortality at concentrations of 400 and 500mg/l in ultrapure water. While concentration of 300, 200 and 200mg/l observed 93.33%, 71.66% and 55% of larval mortality. Pupal mortality observed in 100-500mg/l concentration of CuO-NPs as nanopesticides. The LC_{30}, LC_{50}, and LC_{90} values post-treatment are 0.000, 0.002 and 0.0136 respectively. Statistical significance between pest mortality and CuO-NPs concentration was observed at both the 16-hur and 24-hur time points. The study revealed that, CuO-NPs act as pesticides against leaf roller and need further in-depth research on its toxicity to other beneficial insects or non-targeted species.

Keywords CuO NPs; Leaf roller; XRD; FTIR; DRS; Toxicity
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INTRODUCTION

Nanomaterials have gained widespread use in agriculture field as nano fertilisers, nanoemulsion, nanosuspension and nano pesticides for crop management. These nanomaterial-based formulations offer improved action and reduced harm to non-target species compared to old methods like aluminum phosphide (Poonam Jasrotia *et al*., 2022). Eco-friendly and cost effective approaches through green synthesis of nanomaterials using plant extracts to produce essential metal oxides like iron, zinc, and copper, further focus need on minimizing the toxicity while increase the biological efficacy of CuO-NPs (Sunday Adewale Akintelu *et al.,* 2020;Haifaa Abass Hussein *et al.,* 2021). Copper based nanomaterials such as copper hydroxide (Cu $(OH)_2$ nanowires, Cu and CuO-NPs, emerged as promising alternatives to traditional copper-based agro-chemicals by their potential as nano pesticides and nano fertilisers (Jose Luis Lasso-Robledo *et al.,* 2022). CuO nanoparticles minimizing environmental impact for targeted delivery of pesticides and play a role in eco-friendly photo degradation of specific pesticides and it act as potent pesticides with their unique properties in agriculture field (Mohamed Amine Gacem *et al.,* 2022). Green synthesis of highly pure and crystalline CuO nanoparticles using Calotropis procera leaf extract with monoclinic-shaped particles with band gap energy of 2.35 eV (Rayapa Reddy, 2017) and *Enicostemma axillare* and *Azadirachta indica* leaf extract method's efficiency in producing CuO-NPs with a particle size of 30nm and 40-100nm by reducing toxic level with its significant biological activities of antimicrobial and antioxidant (Suresh Chand Mali *et al.,* 2019; Muhammad Aamir Manzoor *et al.,* 2023). Biologically synthesised *Pseudomonas fluorescence* (Gram-negative bacteria) successfully bio-transformed copper nanoparticles (CuNPs) effective against the stored grain pest *Tribolium castaneum* (Mohamed *et al.,* 2020). CuO and ZnO nanoparticles are synthesized by using *Lantana camara* leaf extracts is more effective in protecting rubber wood against *Lyctus africanus*, a major dry wood pest and demonstrating promise as an environmentally friendly wood preservative. (Shiny *et al.,* 2021). Abnormalities in reproductive and disturbance in population by increase apoptosis and DNA damage through disregulation of antioxidant system in Blaps sulcate observed by application of CuO NPs (LD_{50}), emphasize the lasting effects of CuO-NPs on insect physiology (Lamia *et al.,* 2022). Nano CuO demonstrated effective control of the cotton leaf worm, inducing 100% mortality and exhibited promising photo degradation properties against the pesticide methomyl (Shaker *et al.,* 2016). Copper sulphide nanoparticles (CuSNPs) demonstrated potent larvicidal effects against *Aedes aegypti*, a vector for dengue and chikungunya with 100% mortality (Sandhu *et al.,* 2022).

Copper oxide and copper oxide doped with silver nano sheets significantly effect on *Spodoptera littoralis* larval mortality with CuO showing a higher impact than CuO:Ag. Pupal mortality and deformities increased in larvae treated with CuO-Ag and incorporation of nanosheets into the diet also influences the timing of larval development. Potential of nano sheets, particularly CuO more effective and safer insecticides with implications for pest population fluctuation. (Atwa *et al.,* 2017).

In order to control the leaf roller infestation in mulberry, a research was carried out on copper oxide nanoparticles as nanopesticides and its effect on pest control and eco-friendly alternatives to traditional pesticides.

MATERIALS AND METHODS

Materials used for our study include CuSO4 and CuCl3 (Sigma aldrich), NaOH (99%, Sigma-Aldrich, India), Ethanol (99.9% Alfa Aesar, India), all chemicals used were analytical grade.

Synthesis of Copper oxide nanoparticles:

Copper oxide nanoparticles were synthesised by Hydrothermal method with minor modification (Mohammed Ajmi Abd *et al.,* 2019). Dissolving 0.2M of Copper sulfate pentahydrate (CuSO4.5H2O) in 50ml distilled water to obtain blue solution stirred for 30 minute (A), subsequently 0.5 g of jeera extract was dissolved in 10ml ultrapure water and stirred at 680rpm until complete dissolution (B). Mix both solution A and B then 1M of sodiuom hydroxide (NaOH) solution was added by drop-wise until pH reached 8 and kept in magnetic stirring (Remi-2MLH model) for 2 h. After the solution was transferred into the Teflon liner (autoclave) and placed in hydrothermal reactor (SS316), kept in hot air oven at 120° C for 20 hours., synthesis was carried out for 20h at 120°C. The obtained precipitate was centrifuged at 10000rpm (REMI-124), washed five times with ultra-pure water, followed by final wash with 5ml of ethanol. The pellets was dried in hot air oven at 60° C for 1 hur, the nanoparticles obtained were further processed by crushing with an agate mortar and pestle to get nano sized particles.

Characterization

UV-Vis Spectrum: UV-visible spectrum was recorded on using UV Shimadzu (26001) spectrophotometer.

FT-IR Spectroscopy: FT-IR spectroscopy was measured using FT-IR, model: Shimadzu-01163.The samples were mixed uniformly with potassium bromide at 1:10 (sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powders (mixture of sample and

KBr) at pressure of 5 tons for 5 min in a hydraulic press. The discs were scanned in the range of 400–4000 cm^{-1} to obtain FT-IR spectra.

X-Ray Diffraction (XRD): XRD patterns recorded on a Rigaku-Miniflex model. The diffractometer was controlled and operated by a PC computer with the programs Smart Lab studios-2 at 2θ with 5° per minute scan speed (25 minutes/sample).

Dynamic Light Scattering (DLS): It allows to measure the size distribution of particles in the sub-micron range. The CZnO nanoparticles size was measured using instrument type Light sizer-500 (Anton Paar).

CuO NPs toxicity against leaf roller: Toxicity activity towards *Diaphania pulverulentalis* (Lepidoptera : Pyralidae), larvae were collected from Sericulturist mulberry garden, Doddamudugere, Tamilnadu-638461. Assay was evaluated through spray CuO-NPs on mulberry leaf then fed to leaf roller with concentration 100-500 microgram per litre of ultrapure water.

Insecticidal toxicity assay: To quantify the efficiency of CuO NPs insecticides, when accurate counts of live and dead insects in treated groups are available, it's important to account for mortality due to natural causes. Total 60 larvae per dose with tree replication were selected and treated with five doses of CuO NPs (100, 200, 300, 400 and 500 mg/l) against leaf roller and mortality recorded for 8, 16 and 24 hours of post treatment. The insect population data is used to calculate the corrected efficacy percentage (Abbott, 1925).

Analysis of statistics

The mortality data was used to calculate the corrected mortality using Abbott's correction technique. The slopes and regression equations for LC_{30}, LC_{50}, and LC_{90} were determined using probit analysis with the corrected mortality values (Finney, 1971). Mortality was evaluated using one-way ANOVA and Tukey's HSD test ($p < 0.05$). Analyses were conducted using SPSS and Origin Pro statistical software.

RESULT AND DISCUSSION

Characterization of CuO-NPs

Powder XRD analysis

A monoclinic structure of copper oxide nanoparticles was confirmed through a Powder X-ray diffraction study, showed peaks at 4.685 Å, 3.423 Å, 5.132 Å, 99.52° and a volume of 81.17 Å. The CuO nanoparticles confirmed by comparing diffraction peaks with the Joint committee on powder diffraction standards (JCPDS) Card No: 01-089-2529 showing a close match with reported values. Crystal sizes for various Full Width Half Maximum (FWHM) values calculated by Debye–Scherrer equation, D = K/cos θ. CuO phase was identified with diffraction peaks at 35.09° and 38.7° corresponding to the (022) and (111) atomic planes, respectively. The mean of average crystallite size ranged from 15 nm to 23 nm (Figure 1).

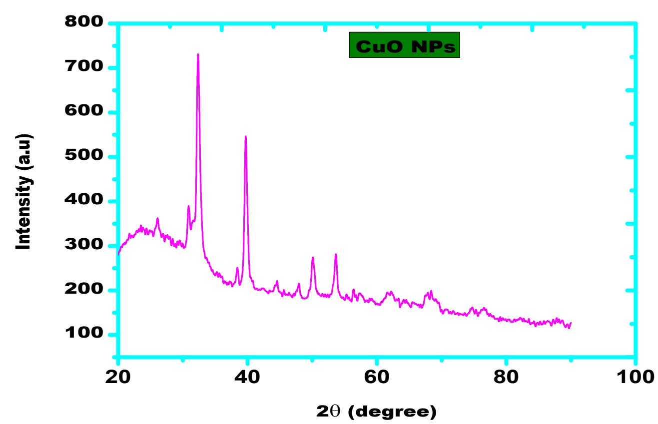


Figure 1. XRD pattern of Copper oxide nanoparticles.

FT-IR Analysis

Fourier Transform Infrared spectroscopy (FT-IR) is a technique used to determine functional groups of compound. The FT-IR spectra of CuO NPs shown in Figure 2. The strong intensity peak at 3473 cm-1 assigned to amine (NH) group, peak at 1634 cm-1 assigned to amide (COO-) while the very intense peak positioned at 1114 cm-1 revealed the presence of (O-H) stretching for alkyl (Figure 2).

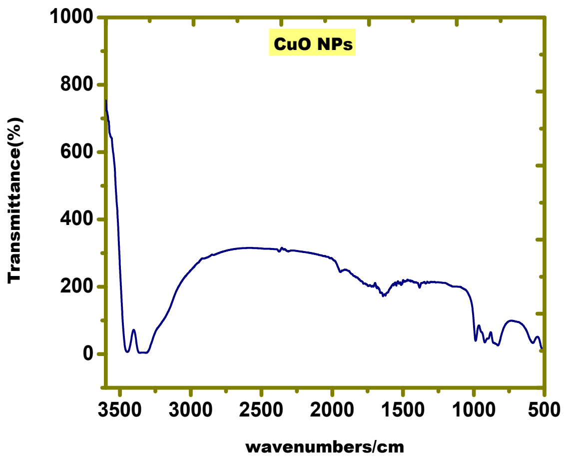


Figure 2. FT-IR pattern of Copper oxide nanoparticles.

UV-Visible analysis

Ultraviolet-visible Diffusion Reflectance Spectroscopy (UV-Vis DRS) assay were done for optical characterization, in order to calculate the Band gap energy of copper oxide nanoparticles. UV-Vis – Kubelka Munk function bandgap technique was used to calculate the direct band gap energy of CuO. Eg value of CuO-NPs according to the following equation Eg = h freq, The band gap energy was calculated in terms of eV. Eg is the band gap energy, h is plank's constant and freq is the frequency of the emitted radiation. The direct band gap of CuO is calculated value with 2.67 eV (Figure 3).

Dynamic Light Scattering (DLS) analysis

Dynamic Light Scattering technique were done for Copper oxide nanoparticles, in order to know the crystal size (nm). One milligram of CuO NPs dispersed in 8ml of ultrapure water then sonicated for 40 minutes, subsequently pour the suspension into cuvette, immediately run DLS (Lite sizer-500, Anton Paar GmbH). The nanometre of CuO NPs was recorded with the range of 12-15nm (Figure 4).

CuO-NPs against *Diaphania pulverulentalis*

In a toxicity bioassay, leaf roller larvae were collected from a farmer's field to assess the effects of copper oxide nanoparticles (CuO NPs). The experiment utilized 20 larvae per concentration, with three replications for each treatment. CuO NPs were prepared at concentrations of 100, 200, 300, 400, and 500 mg/L in ultrapure water, dispersed using a medical ultrasonic cleaner for 40 minutes at room temperature.

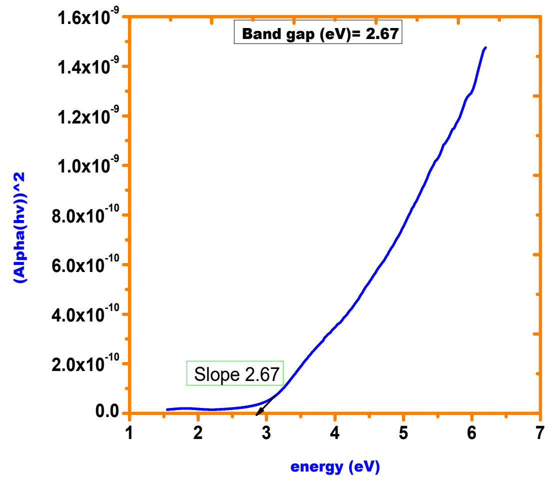


Figure 3. Bandgap energy of CuO NPs calculated by UV-Vis DRS

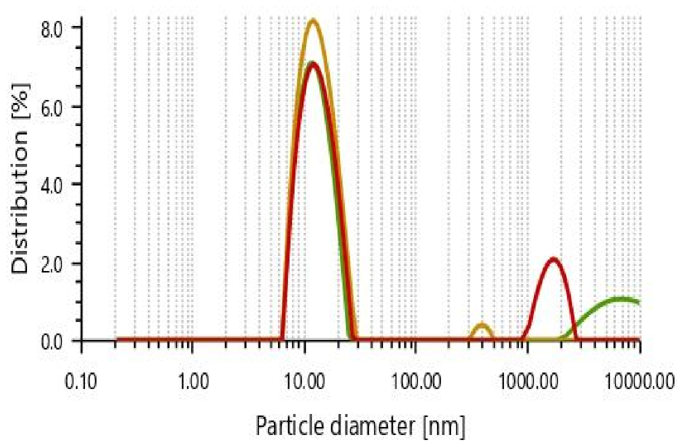


Figure 4. Bandgap energy of CuO NPs calculated by UV-Vis DRS.

This nanoparticle solution was sprayed onto two mulberry leaves, which were subsequently dried in the shade for 2 hours before being fed to the larvae (Photo 1). Larval abnormalities and mortality rates were recorded from 6hr to 24 hours post-treatment. All treated larvae exhibited signs of abnormality, such as reduced movement, decreased feeding capacity and diminished activity after 24 hurs of exposure. Mortality was observed starting at 16 hours post-treatment. The highest larvicidal effect was observed at CuO NP concentrations of 400 and 500 mg/L, both resulting in 100% larval mortality. Mortality rates for the other concentrations were as follows: 300, 200 and 100 mg/L with 93.33%, 71.66% and 55% larval mortality (Table 1). These findings indicate that CuO-NPs have a significant dose-dependent toxic effect on leaf roller larvae, with higher concentrations leading to greater mortality and more pronounced larval abnormalities. At concentrations of 100-200 mg/L, CuO nanoparticles (CuO-NPs) exhibited lower larval mortality compared to concentrations of 300-500 mg/L.

Probit Analysis

The mortality rate of *D. pulverulentalis* in response to a contact bioassay (Fig. 5) using CuO NPs solution is highly statistically significant. The 8hr LC_{50} value is 0.077 ($Y = 0.035x - 5.1$; $R^2 = 0.9061$; $p < 0.05$), and the 24 hrs LC_{50} value is 0.002 ($Y = 0.038x + 35.6$; $R^2 = 0.1462$; $p < 0.05$), as shown in Table 2. The chi-square values of LC_{30}, LC_{50} and LC_{90} values of post treatment is 2.795, 15.286 and 26.785. Statistical significance between the pest mortality and CuO-NPs concentration was observed at both the 16hr and 24hr time points.

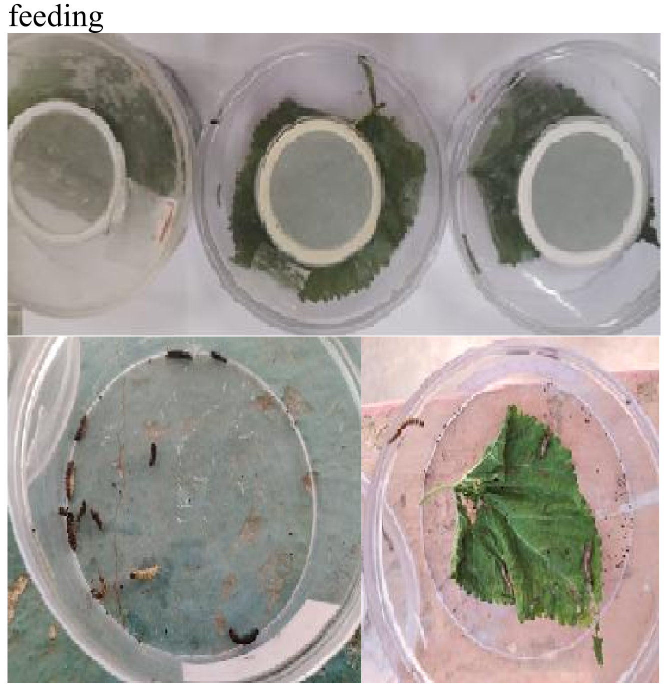
Cytotoxicity of CuO NPs

In the cytotoxicity study, L6 cells were selected to evaluate the toxicity of CuO nanoparticles (CuO NPs) using the MTT assay. The concentrations tested were 0, 50, 100, 150, and 200 µg/mL of CuO NPs. The results demonstrated a dose-dependent cytotoxic effect of CuO NPs on L6 cells. The highest toxicity was observed at the Concentration of 200 µg/mL, which resulted in 84% inhibition of cell viability. This was followed by a concentration of 150 µg/mL, which exhibited 77% inhibition of cell growth (Figure 5).

Table 1. Overview of CuO NP Treatmented Leaf Roller Larval Mortality and Survivability

Concentration of CuO-NPs	No. of larvae treated	Larval mortality (%)	Larval survivability (%)
100mg/l	60	55	45
200mg/l	60	71.66	28.34
300mg/l	60	93.33	6.67
400mg/l	60	100	0
500mg/l	60	100	0

Fig. 5. Toxicity bioassay protocol of CuO NPs against *Diaphania pulverulentalis* by per-oral feeding



The study revealed that, synthesized copper oxide nanoparticles by hydrothermal technique with plant extract and compound was confirmed by their characterization through XRD, FTIR and UV-Vis DRS analysis.

Table 2. Probit analysis of CuO NPs against leaf roller

Hours	LC_{30}	LC_{50}	Lc_{90}	Slope	Chi-square	Regression	R^2	Sig.
8	0.056	0.077	0.169	3.923	2.795	Y=0.035x - 5.1	0.9061	0.000
16	0.025	0.031	0.055	9.071	15.286	Y=0.134x – 13.8	0.878	0.000
24	0.000	0.002	0.136	1.985	97.157	Y= 0.038x + 35.6	0.1462	0.047

LC: lethal concentration; significant at $p < 0.05$ level

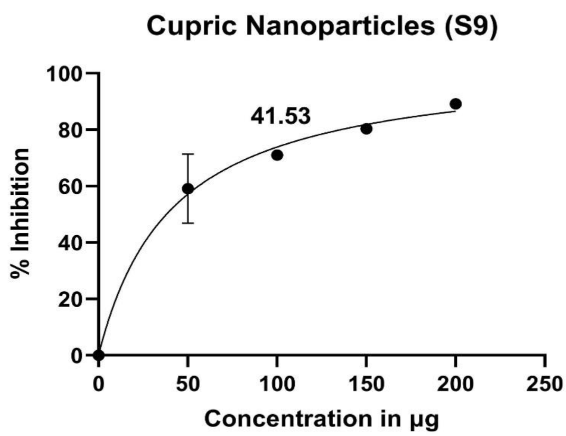


Figure 5. Cytotoxicity of CuO NPs on L6 cells

Copper oxide nanoparticles showed 100% toxicity in leaf roller larval and pupal mortality. Future study should focus on CuO NPs toxicity bioassay of silkworm. The data clearly indicate that CuO NPs have a substantial cytotoxic impact on L6 cells, with higher concentrations leading to greater inhibition of cell proliferation.

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AUTHOR'S CONTRIBUTION

All authors equally contribute the work and knowledge to complete the research work.

AUTHOR'S DECLARATION

We, the authors of this research paper, declare that we have no conflicts of interest to disclose regarding the research, its findings and integrity of the work.

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