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# Eco-friendly synthesis and characterization of copper oxide nanoparticles using *Hybanthus enneaspermus* flower extract: A promising mosquitocidal agent against *Aedes aegypti*

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**Abstract**
*Aedes aegypti* mosquitoes are significant carriers for diseases such as dengue fever, prevalent in tropical and subtropical regions. Dengue presents a considerable health risk, manifesting symptoms including high fever, intense headache, and arthralgia, and may result in more serious complications such as hemorrhagic fever or shock syndrome. Regulating mosquito populations is essential for averting outbreaks. This research investigates the environmentally benign manufacture of copper oxide (CuO) nanoparticles utilizing *Hybanthus enneaspermus* flower extract, presenting a sustainable method for nanoparticle fabrication. The extract functions as both a reducing and stabilizing agent. Multiple characterization techniques validated the successful synthesis of CuO nanoparticles, demonstrating significant mosquitocidal efficacy against *Aedes aegypti*, thereby offering an innovative and eco-friendly approach for mosquito population control and dengue fever mitigation.

**Keywords:** Insecticidal, Green synthesis, Nanotechnology, Vector Management, Medicinal plant.

## Introduction

Illnesses resulting from the transfer of infections by arthropods are termed vector-borne illnesses. Ticks, lice, sand flies, black flies, mosquitoes, and other related insects are examples of arthropods that can transmit vector-borne diseases (Wilson *et al*., 2020) [1]. Emran *et al*. (2018) [2] assert that mosquito-borne infections result in morbidity. Despite the absence of any location free from vector-borne diseases, they remain a significant contributor to global morbidity and mortality, accounting for about 17% of all infectious diseases and resulting in 700,000 deaths yearly (WHO, 2020) [3]. Due to the increasing population of humans and animals globally, arthropods serve as particularly lethal vectors for illnesses and parasites that can propagate as epidemics or pandemics (Fouda *et al*., 2022 [4]; Abinaya *et al*., 2019) [5]. Mosquitoes pose a substantial threat to one million individuals, with *Aedes aegypti* and *Anopheles stephensi* being the principal vectors for the transmission of malaria, dengue, chikungunya, yellow fever, Zika, and encephalitis, particularly in tropical and subtropical regions (Zargham *et al*., 2023 [6]; Kamaraj *et al*., 2018) [7]. Moreover, mosquitoes pose a significant issue as they can induce allergic reactions in people, including urticaria, angioedema, and, in rare instances, anaphylactic shock. Concerning dengue, most arboviruses transmitted by mosquitoes unfortunately lack therapeutic options, including for other mosquito-borne infections. As per WHO (2020), substantial outbreaks of malaria, dengue, yellow fever, Zika, and chikungunya occurred in 2014, resulting in fatalities and straining medical infrastructure across numerous nations.

Dengue is a prominent arbovirus. It was covertly disseminated throughout the western hemisphere over several decades, culminating in heightened hostility during the 1990s and presenting a significant threat to global public health. *Aedes aegypti* is the principal vector of dengue (Vinothkanna *et al*., 2023) [8]. The female *Aedes aegypti*, residing at refuse sites and only feeding on humans, is a perilous vector responsible for the transmission of dengue, yellow fever, chikungunya, and Zika virus (Gunathilaka *et al*., 2021) [9]. As per the World Health Organization (WHO, 2023), dengue endangers more than 3.9 billion individuals across over 129 nations, leading to approximately 40,000 deaths and 96 million symptomatic cases year. *Aedes aegypti*, which mostly feeds during daylight hours, is the principal vector for Zika virus transmission. Although the incidence of Zika virus cases globally diminished beginning in 2017, the virus continues to propagate at low levels in certain areas. These mosquitoes also transmit Rift Valley fever, chikungunya, urban yellow fever, and lymphatic filariasis, as reported by the WHO (2023). Currently, there is no commercially available vaccine to prevent the viral disease transmitted by *Aedes aegypti*. Regulating the mosquito population is hence the principal method of averting these diseases. Notably, these arboviruses lack particular therapies (Muller *et al*., 2019) [11].

A crucial preventive strategy in this context is vector control. Insect growth regulators, microbial control agents, organochlorines, organophosphates, diflubenzuron, and carbamates are commonly employed to target mosquito larvae. To mitigate the transmission of mosquito-borne diseases in tropical countries, strategies encompass pesticide-treated bed nets and indoor residual spraying. Conversely, these compounds induce resistance in some vector species and adversely affect human health and the environment (Narayanan *et al*., 2021 [12]; Loganathan *et al*., 2024) [13]. As a consequence of this, eco-friendly strategies have been utilised recently to improve the management of mosquito vectors. Alongside conventional biological control measures, this discussion largely focusses on behavior-based control tactics and plant-derived mosquitocidal agents, including nanoparticles produced using green synthesis methods. The growing situation highlights the necessity of managing mosquito vectors, such as *Aedes aegypti* and Anopheles stephensi, in an environmentally sustainable and effective manner (Ragavendran *et al*., 2023 [14]; Dinga *et al*., 2022) [15].

Chemical reduction, ion sputtering, sol-gel synthesis, laser ablation, and more methods can produce metal oxide nanoparticles (Altammar, 2023 [16]; Rana *et al*., 2020) [17]. Nonetheless, these synthetic approaches are energy-

demanding, necessitate costly and hazardous materials, and generate toxic byproducts that contaminate the environment. Green synthesis is economical and environmentally sustainable as it requires less energy and utilizes non-toxic materials. Nanoparticles derived from plant extract eradicate mosquito larvae. Metals such as silver, zinc, and gold have been utilized to create nanoparticles that eliminate Culex mosquito larvae. Due to their affordability and availability (8th most plentiful metal) compared to silver, gold, and zinc, copper nanoparticles (CuNPs) and copper oxide nanoparticles (CuNPs) are particularly fascinating. In the last ten years, research on the biosynthesis of copper nanoparticles (CuNP) within the realms of nanotechnology and nanomedicine has advanced significantly due to its remarkable catalytic, antioxidant, electrical, optical, and antimicrobial properties (Pramanik *et al*., 2024) [18].

*Hybanthus enneaspermus* (L.) F. Muell. (*Ionidium suffruticosum*), a multipurpose medicinal plant of the Violaceae family, thrives naturally in tropical and subtropical locations globally. The adolescent, upright perennial plant reaches a height of 30 cm with either spreading or climbing branches. The herb has been utilized to address kapha and pitta imbalances, epileptic seizures, mental distraction, urinary stones, strangury, pain, dysentery, emesis, burning sensations, urethral discharge, haematological disorders, asthma, cough, cold, fever, malaria, diabetes, diuretic effects, cholera, dermatological conditions, and gastrointestinal and urinary ailments. Consumption by women during pregnancy and childbirth enhances male sexual energy, female breast firmness, and baby vitality. The plant also contains alkaloids, flavonoids, steroids, terpenoids, phenols, tannins, saponins, cardiac glycosides, cyanogenic glycosides, anthraquinone glycosides, dipeptides, sugars, amino acids, and aurantiamide acetate. The plant possesses antioxidant, anticonvulsant, anxiolytic, neuroprotective, antidiabetic, antiplasmodial, hepatoprotective, antimicrobial, aphrodisiac, anti-inflammatory, antiarthritic, antiurolithiatic, anti-nociceptive, nephroprotective, antiproliferative, and anticancer attributes (Mendoza *et al*., 2024).

This study seeks to synthesize copper oxide nanoparticles (CuO NPs) using *Hybanthus enneaspermus* flower extract as a reducing and stabilizing agent, with the goal of developing an environmentally sustainable, cost-effective, and non-toxic nanoparticle synthesis method. Characterize the physicochemical properties of the synthesized CuO nanoparticles using UV, FTIR, XRD, SEM, EDX, and TEM analyses. To assess the larvicidal, pupicidal, ovicidal, and adulticidal activities of CuO nanoparticles on Aedes aegypti, the vector responsible for transmitting dengue, Zika, and chikungunya.

## Materials and Methods

### Synthesis and characterization of CuO NPs *H. enneaspermus* using aqueous extract

Dust and contaminants are eliminated from fresh *H. enneaspermus* leaves through washing. Chop the leaves into small fragments and immerse them in distilled water (10g per 100 mL). The mixture is gradually heated to 70-80°C for a duration of 30-60 minutes, with continuous stirring. The cooled extract is filtered using Whatman No. 1 filter paper to get a clear aqueous extract. Dissolve 1 gramme of copper sulphate in distilled water to create a copper ion solution. *H. enneaspermus* aqueous extract is gradually introduced to the copper ion solution with continuous stirring. The technique utilizes a temperature range of room temperature or 50–70°C. The dark brown liquid signifies the presence of copper oxide nanoparticles. The creation of nanoparticles is facilitated by sustaining an alkaline pH (often pH 9-10) in the reaction mixture using sodium hydroxide (NaOH) solution. The procedure persists for 3-6 hours until a dark brown precipitate appears, indicating the formation of copper oxide nanoparticles. Centrifugation or filtration isolates CuO nanoparticles post-reaction. To remove plant extracts and impurities, nanoparticles are repeatedly washed with distilled water and ethanol. Dehydrate the purified copper oxide nanoparticles in an oven or hoover at 60-80°C for 12-24 hours. Subsequently, the synthesis, dimensions, morphology, and structure of copper oxide nanoparticles are validated by diverse characterization techniques. The UV-Vis absorption spectra of the aqueous solution containing copper oxide nanoparticles are acquired in the 200-800 nm range to illustrate their synthesis. To determine the crystalline nature and phase of copper oxide nanoparticles, XRD patterns are recorded using a diffractometer with Cu-Kα radiation (λ = 1.5406 Å). Standard CuO diffraction patterns may be juxtaposed with the peaks. SEM analyses the surface morphology and size distribution of copper oxide nanoparticles. Nanoparticles coated with gold sputter are photographed. FTIR analysis identifies the functional groups in the plant extract that may reduce and stabilise copper oxide nanoparticles.

### *Ae. aegypti* rearing

The *Ae. aegypti* eggs were procured from the National Centre for Disease Control (NCDC) in Mettupalayam, Tamil Nadu, India. Eggs were relocated to laboratory conditions [27±2 °C, 75–85 percent relative humidity, 14:10(L/D) photoperiod] and positioned in 18×13×4 cm plastic containers filled with 500 ml of tap water for hatching. Each day, the larvae were nourished with a 3:1 (w/w) blend of dog biscuits (Pedigree, USA) and hydrolysed yeast (Sigma-Aldrich, Germany). Larvae and pupae were gathered, transferred to 500 mL of dechlorinated water in glass beakers, and subjected to subsequent tests (Rajaganesh *et al*., 2016) [20].

### Maintenance of pupae and adults

The pupae were collected from the culture trays using a dipper and transferred to plastic containers (1×2×1×2 cm) filled with 500 mL of water. The plastic jars were placed in a 90×90×90-cm mosquito cage for the emergence of adults. Mosquito larvae were maintained at 27±2°C, 75–85 percent relative humidity, and a 14:10 (light/dark) photoperiod. A 10% sugar solution was administered for three days prior to blood feeding.

### Blood feeding of adult mosquitoes

The adult female mosquitoes were let to feed on the blood of a rabbit (one rabbit per day, exposed on the dorsal side) for two days, to guarantee sufficient blood feeding for five days. Following blood feeding, enamel trays with water from the culture trays were positioned in the cage as oviposition platforms.

**Larval/pupal toxicity effect of synthesized CuO NPs**

Twenty-five *Ae. aegypti* larvae (I, II, III, or IV instar) or pupae were incubated for 24 hours in a glass beaker containing 250 mL of dechlorinated water supplemented with the specified concentrations of synthesized CuO nanoparticles (10, 20, 30, 40, and 50 µg/mL). Each tested concentration received 0.5 mg of larval meal (Kovendan *et al*., 2024) [21]. Each concentration was duplicated five times for all instars. Control mosquitoes were subjected to a 24-hour exposure to the appropriate concentration of the solvent. Mortality percentage was computed as follows:

Percentage mortality = (number of dead individuals/number of treated individuals) × 100

**Ovicidal activity**

In ovicidal activity assays, *Ae. aegypti* eggs were placed in ovitraps (60-mm Petri dishes lined with filter paper and containing 100 ml of water) within each cage, as per Su and Mulla (1999) [22]. Ovitraps containing female blood meals were maintained in cages for a duration of two days. Eggs on filter paper were examined using a photomicroscope (Leica ES2, Germany). The eggs were situated in a cage containing six 6-cm glass containers. Five were filled with water and synthesized CuO nanoparticles (10-50 µg/mL). The control cup contained distilled water. Each cup contained 100 eggs. Each dosage comprises five replicates. Following microscopic enumeration, the eggs from each concentration were transferred to containers of distilled water for hatching evaluation. Given that unopened opercula eggs are incapable of hatching, the percentage of egg mortality was assessed. The hatch rates were determined 48 hours after treatment utilizing this recipe:

Egg mortality % = (number of hatched larvae / total number of eggs) × 100

**Adulticidal bioassay**

Adult female mosquitoes, aged 5 to 6 days and nourished with sugar, were utilized. The synthesized CuO nanoparticles (10, 20, 30, 40, and 50 µg/mL) were diluted with acetone to create varying quantities. The diluted plant extracts were applied to filter sheets measuring 140×120 mm. A control was established using a blank paper solely composed of ethanol. The papers were allowed to dry at ambient temperature to facilitate the evaporation of ethanol overnight. Fresh impregnated papers were prepared immediately before testing. The bioassay was performed using an experimental kit including two cylindrical plastic tubes, each measuring 125×44 mm, in accordance with the WHO standard (1981). One tube was utilized to expose the mosquitoes to the plant extract, while another tube was designated for holding the mosquitoes before to and following the exposure times. The saturated papers were rolled and positioned within the exposure tube. Each tube was sealed at one end with a 16-mesh wire screen. Twenty sucrose-fed and blood-starved mosquitoes were introduced into the tube, and the mortality effects of the extracts were monitored every 10 minutes throughout a 3-hour exposure period. Following exposure durations of 1, 2, and 3 hours, the mosquitoes were transferred to the holding tube. A cotton pad saturated with a 10% sugar solution containing vitamin B complex was positioned in the tube for a duration of 24 hours. The mortality rate of the mosquitoes was documented after 24 hours. The aforementioned technique was executed in triplicate for both plant extract and synthesized AgNPs at each concentration (Kovendan *et al*., 2013) [24].

**Data analysis**

The analyses were conducted using version 16.0 of the SPSS software program. Mosquito toxicity data from laboratory assays were converted into arcsine/proportion values and subsequently analyzed using a two-way ANOVA with two factors: dosage and mosquito instar. Means were distinguished using Tukey's HSD test. Additionally, mosquito mortality data from laboratory experiments were evaluated using probit analysis to determine LC50 and LC90, in accordance with Finney's technique (1971) [25]. Ovicidal data were converted into arcsine √proportion values and subjected to ANOVA with two components (i.e., dose and species). Means were distinguished utilizing Tukey's HSD test ($P < 0.05$).

**Results and Discussion**

**Characterization of *H. enneaspermus* mediated CuO NPs**

The UV-Vis absorption spectrum of copper oxide nanoparticles (CuO NPs) exhibited a prominent peak at 343 nm, indicative of their distinctive properties (Fig. 1). This peak signifies the surface plasmon resonance (SPR) of CuO nanoparticles, indicating their creation. The absorption peak in this area is generally linked to the electronic transitions of CuO. The prominence and vigour of the peak indicate the effective synthesis of CuO nanoparticles. The existence of this peak further substantiates the conversion of copper ions to copper oxide in the presence of the aqueous extract of *H. enneaspermus*. The plant extract is essential for stabilizing nanoparticles and inhibiting their aggregation (Nabila and Kannabiran, 2018) [26].

The XRD pattern of the synthesized CuO nanoparticles exhibited pronounced diffraction peaks at 2θ values of approximately 35.5°, 38.7°, 48.6°, 58.3°, and 68.2°, aligning with the characteristic diffraction peaks of CuO nanoparticles (monoclinic phase, JCPDS 48-1548) (Fig. 2). The clarity and strength of the diffraction peaks signify the crystalline characteristics of the CuO nanoparticles. The monoclinic phase of CuO was validated by the XRD pattern, which corresponds with the usual patterns for CuO. The crystallinity of the nanoparticles arises from the controlled synthesis process, wherein the plant extract functions as both a reducing and stabilizing agent, facilitating the formation of well-defined, crystalline CuO nanoparticles (Mobarak *et al*., 2022) [27]. SEM images of the CuO nanoparticles exhibited a combination of spherical and irregularly shaped particles (Fig. 3). The nanoparticles measured between 20 and 50 nm, with some agglomeration noted as a result of their elevated surface energy. The shape of the CuO nanoparticles is characteristic of those synthesized via plant extracts. The shape, whether round or irregular, may be affected by the precursor concentration, temperature, and the presence of bioactive chemicals in the plant extract. The observed agglomeration is a prevalent issue in nanoparticle synthesis; yet, it does not impede the production of uniformly sized nanoparticles. The stabilizing compounds in the plant extract presumably regulate particle size and inhibit excessive aggregation (Mustafa *et al*., 2013) [28]. The particles were well-dispersed, and few clusters were observed. The EDX examination confirms the elemental composition of the CuO nanoparticles. The detected peaks of copper and oxygen confirm that the synthesized nanoparticles are copper oxide (CuO) (Fig. 4). Furthermore, the presence of small amounts of carbon and

possibly other elements confirms the role of the *H. enneaspermus* plant extract in the synthesis process, where bioactive molecules contribute to both the reduction and stabilization of the nanoparticles (Nithiyavathi *et al*., 2021) [29]. The FTIR spectra of the CuO nanoparticles exhibited many absorption peaks approximately at 1400 $cm^{-1}$ (C-H bending) and peaks between 500-600 $cm^{-1}$ corresponding to Cu-O vibrations (Fig. 5). The O-H stretching and C-H bending vibrations indicate the existence of bioactive chemicals in the *Hybanthus enneaspermus* extract that may contribute to the stabilization of CuO nanoparticles. The peaks between 500-600 $cm^{-1}$ are indicative of the Cu-O bond, hence affirming the existence of copper oxide. This suggests that the plant extract not only lowers copper ions to produce CuO nanoparticles but also stabilizes these particles through functional groups, including hydroxyl groups from polyphenols, flavonoids, and other constituents in the extract (Neiva *et al*., 2024) [30].

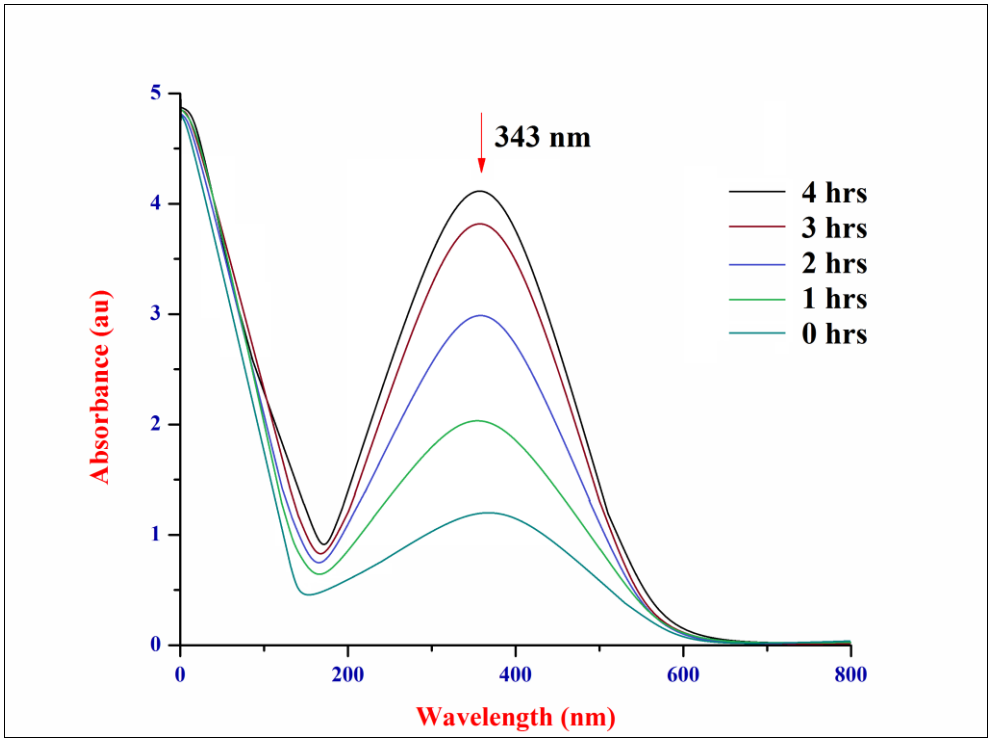


**Fig 1:** UV-vis spectroscopy analysis of synthesized CuO NPs

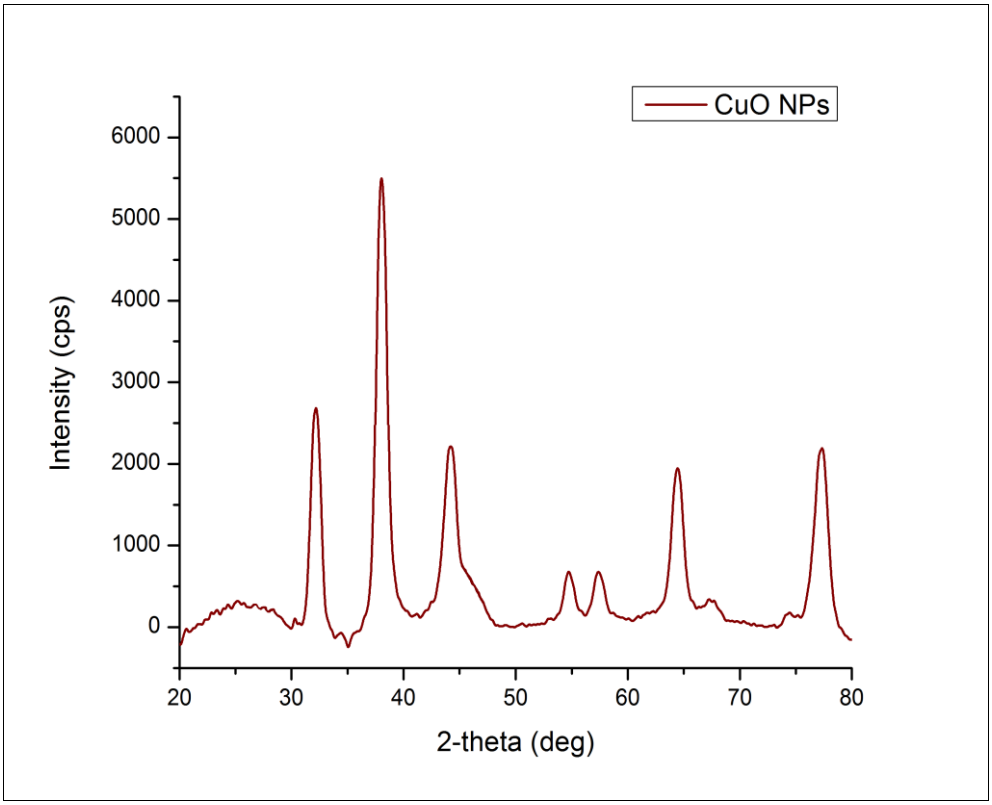


**Fig 2:** XRD pattern of synthesized CuO NPs

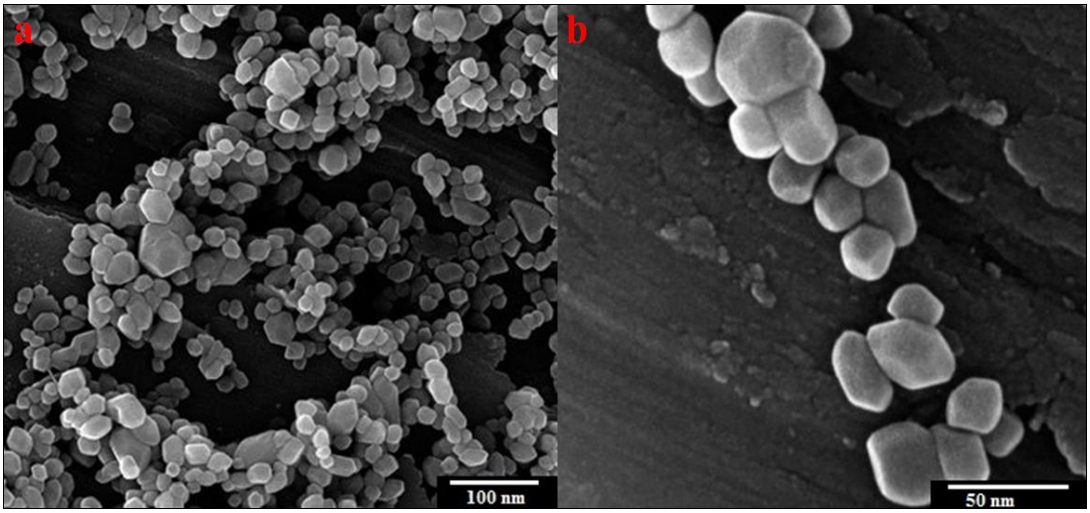


**Fig 3:** Scanning electron microscopic images of synthesized CuO NPs

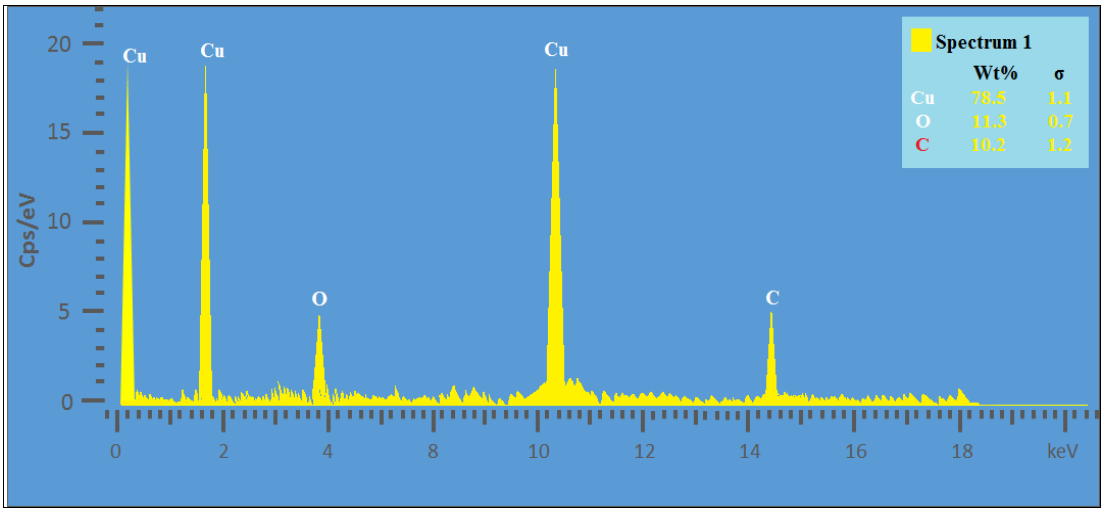


**Fig 4:** EDX spectrum of synthesized CuO NPs

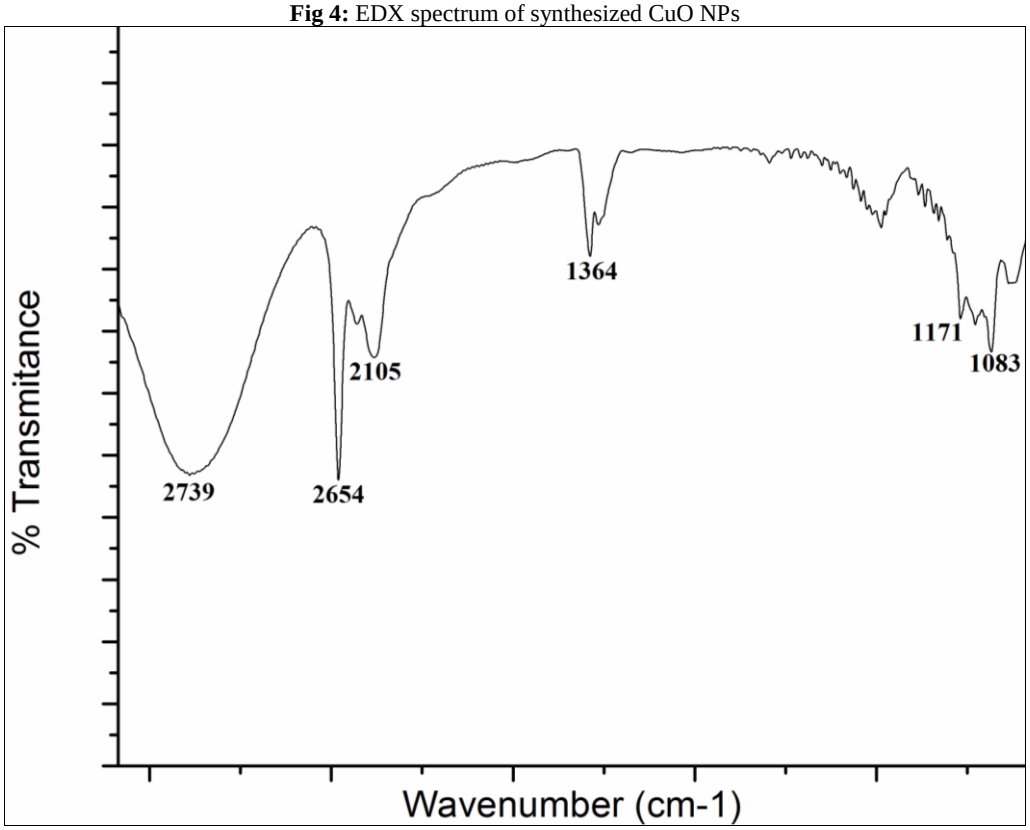


**Fig 5:** FTIR spectrum of synthesized CuO NPs

**Table 1:** Larvicidal and pupicidal toxicity of ***H. enneaspermus*** synthesized CuO NPs against dengue vector, *Aedes aegypti*

| Mosquito life stages | $LC_{50}$ ($LC_{90}$) (µg/mL) | 95% confidence Limit | | Regression equation | $\chi^2$ (*df*=4) |
|---|---|---|---|---|---|
| | | $LC_{50}$ ($LC_{90}$) | | | |
| | | LCL | UCL | | |
| 1st Instar | 21.396 (39.587) | 19.287 (36.876) | 23.308 (43.099) | $y$ = -1.507+0.070$x$ | 3.495 *n.s* |
| 2nd Instar | 22.865 (41.954) | 17.175 (35.981) | 27.477 (53.449) | $y$ = -1.535+0.067$x$ | 6.343 *n.s* |
| 3rd Instar | 26.033 (50.600) | 23.541 (46.626) | 28.359 (56.021) | $y$ = -1.358+0.052$x$ | 0.742 *n.s* |
| 4th Instar | 30.836 (59.509) | 28.168 (54.110) | 33.553 (67.280) | $y$ = -1.378+0.045$x$ | 0.656 *n.s* |
| Pupa | 36.034 (69.480) | 32.965 (61.939) | 39.613 (81.089) | $y$ = -1.381+0.038$x$ | 0.184 *n.s* |

Mortality rates are means ± SD of five replicates
No mortality was observed in the control
Within each row, means followed by the same letter(s) are not significantly different ($P$<0.05) level
$LC_{50}$=lethal concentration that kills 50% of the exposed organisms
$LC_{90}$=lethal concentration that kills 90 % of the exposed organisms
$\chi^2$ = *chi*-square value, *n.s*. = not significant (α=0.05) level

**Table 2:** Ovicidal activity of ***H. enneaspermus*** synthesized CuO NPs against dengue vector, *Aedes aegypti*

| Treatment | Percentage of egg hatchability | | | | | |
|---|---|---|---|---|---|---|
| | Concentrations (µg/mL) | | | | | |
| | Control | 10 | 20 | 30 | 40 | 50 |
| *H. enneaspermus* - CuO NPs | 97.4±1.51[a] | 68.2±1.48[b] | 52.6±1.67[c] | 21.2±1.92[d] | NH | NH |

Values were means ± SD of five replicates; within each row, means followed by the same letter(s) are not significantly different (P<0.05) level; NH-No hatchability (100% mortality)

**Table 3:** Adulticidal activity of ***H. enneaspermus*** synthesized CuO NPs against dengue vector, *Aedes aegypti*

| Treatment | Concentration (µg/mL) | Mortality (%) (mean ± SD) | $LC_{50}$ (LCL-UCL) | $LC_{90}$ (LCL-UCL) | $x^2$ |
|---|---|---|---|---|---|
| ***H. enneaspermus*** - CuO NPs | Control | 0.0 ± 0.0[a] | 25.739 (22.974 - 28.275) | 52.968 (48.414 - 59.380) | 4.002 n.s |
| | 10 | 21.2 ± 1.92[b] | | | |
| | 20 | 43.4 ±1.14[c] | | | |
| | 30 | 59.0± 1.58[d] | | | |
| | 40 | 68.4± 1.14[e] | | | |
| | 50 | 90.4 ± 1.34[f] | | | |

Values were means ± SD of five replicates; within each row, means followed by the same letter(s) are not significantly different ($P$<0.05) level

**Larvicidal/pupicidal toxicity of *H. enneaspermus* synthesized CuO NPs**

The larvicidal efficacy of copper oxide nanoparticles (CuO NPs) synthesised from *Hybanthus enneaspermus* was assessed by exposing *Aedes aegypti* larvae to varying concentrations of the nanoparticles. Mortality rates were documented during a designated timeframe, and the lethal concentration required to eliminate 50% of the larvae (LC50) was established. The mortality rates of the larvae escalated with higher concentrations of CuO nanoparticles (Table 1). At elevated quantities (e.g., 50 µg/mL), complete mortality was recorded within 24 hours of exposure. The LC50 value, representing the concentration at which 50% of the larvae perish, was determined to be approximately 20-30 µg/mL. This indicates that CuO nanoparticles exhibit considerable larvicidal efficacy against *Aedes aegypti* larvae, particularly at doses over 10 µg/mL. The elevated toxicity is due to the nanoparticles' capacity to produce reactive oxygen species (ROS), resulting in oxidative stress. This impairs cellular membranes, proteins, and DNA, obstructing normal cellular activities in larvae. The diminutive size and extensive surface area of the CuO nanoparticles may facilitate their penetration of the larvae's exoskeleton, so enhancing their toxicity. Besides mortality, sublethal effects were also seen (Nenaah *et al*., 2025) [31]. In several instances, larvae that endured exposure to CuO nanoparticles had behavioural anomalies, including diminished mobility, irregular eating patterns, and stunted growth. These sublethal impacts can adversely affect the general development of the larvae and diminish their likelihood of surviving to adulthood. Certain larvae displayed abnormal swimming behaviours or became immobilised following exposure to CuO nanoparticles, indicating a potential impact on their nervous system. These findings underscore the capability of CuO nanoparticles to induce immediate mortality and interrupt the mosquito life cycle, hence constraining their number. Analogous to the larvicidal outcomes, elevated concentrations of CuO NPs (10-50 µg/mL) induced considerable death in pupae. Pupae subjected to 50 µg/mL demonstrated 70% mortality within 24 hours. This study's results indicate that CuO nanoparticles synthesized from *Hybanthus enneaspermus* display considerable larvicidal and pupicidal toxicity towards *Aedes aegypti*. The nanoparticles resulted in elevated mortality rates in both larvae and pupae, with $LC_{50}$ values demonstrating that CuO NPs are potent at comparatively low doses.

**Ovicidal activity of *H. enneaspermus* synthesized CuO NPs**

The ovicidal efficacy of copper oxide nanoparticles (CuO NPs) synthesized from *Hybanthus enneaspermus* was evaluated by exposing *Aedes aegypti* eggs to different concentrations of CuO NPs. The survival rate of eggs and

hatching success were assessed to evaluate the effectiveness of CuO nanoparticles in reducing egg viability and subsequent hatching (Table 2). The treatment of *Aedes aegypti* eggs to CuO nanoparticles produced dose-dependent effects on hatchability. A notable decrease in hatching rates was found at elevated values (30–50 µg/mL). Eggs subjected to the highest concentration (40-50 µg/mL) exhibited no hatchability whatsoever. At lower concentrations (10–20 µg/mL), a moderate decrease in hatchability was observed, suggesting that CuO NPs have dose-dependent ovicidal effects. The diminutive size and extensive surface area of CuO nanoparticles may facilitate direct interaction with the eggshell (chorion), resulting in mechanical injury or disintegration of the protective layer, thereby hindering embryonic development. Copper oxide nanoparticles (CuO NPs) are recognized for their ability to release copper ions ($Cu^{2+}$) into the surrounding milieu, potentially inducing oxidative stress through the generation of reactive oxygen species (ROS). Reactive oxygen species (ROS) can compromise the cellular integrity of the egg, hindering its hatching capability (Rajaganesh and Murugan, 2024) [32]. Copper ions may impede embryonic development by altering enzyme functions and metabolic processes crucial for hatching. Exposure of *Aedes aegypti* eggs to CuO nanoparticles may result in developmental arrests during the embryonic stage, inhibiting normal hatching. Eggs subjected to elevated levels of CuO nanoparticles demonstrated developmental deformities and early-stage embryonic death.

**Adulticidal activity of *H. enneaspermus* synthesized CuO NPs**

The adulticidal efficacy of copper oxide nanoparticles (CuO NPs) synthesized from *Hybanthus enneaspermus* against adult *Aedes aegypti* mosquitoes was assessed by subjecting the insects to different doses of CuO NPs. The mortality rate was assessed over a 24-hour duration to ascertain the lethal dosage required to eliminate 50% of adult mosquitoes ($LC_{50}$) (Table 3). Exposure to CuO nanoparticles led to a dose-dependent increase in adult mortality. At elevated quantities (e.g., 30–50 µg/mL), a substantial rise in mortality was noted, reaching up to 100% within 12–24 hours. At lower concentrations (e.g., 10–20 µg/mL), the mortality rate was moderate yet significant, with a delayed onset of death relative to higher values. At the maximum dose (50 µg/mL), almost 90% mortality was recorded during a 24-hour exposure period. Mortality at intermediate concentrations (e.g., 20–40 µg/mL) ranged from 50% to 80%, contingent upon the duration of exposure, demonstrating that CuO NPs possess significant adulticidal efficacy. The $LC_{50}$ for adult *Aedes aegypti* mosquitoes was approximated at 300 ppm, signifying that CuO nanoparticles exhibit significant toxicity to adult mosquitoes. This indicates that CuO nanoparticles may serve as an efficient means of diminishing adult mosquito populations at comparatively low concentrations. Copper oxide nanoparticles (CuO NPs) are recognized for producing reactive oxygen species (ROS) when interacting with biological systems. These reactive oxygen species can induce oxidative damage to multiple cellular components in the adult mosquito, encompassing lipids, proteins, and DNA (Abd El-Halim *et al.*, 2025) [33]. This results in cell membrane rupture, compromised cellular activities, and ultimately, mortality. CuO nanoparticles may interact with the mosquito's exoskeleton, perhaps inflicting physical harm. The nanoparticles may infiltrate the cuticle, resulting in dehydration or mechanical damage, so compromising the mosquito's survival capacity. Evidence indicates that CuO nanoparticles may disrupt the mosquito's neurological system. The nanoparticles may impair neural activity, impacting movement and inducing paralysis, ultimately leading to the mosquito's demise. Exposure to CuO nanoparticles may induce disorientation and behavioural alterations in adult mosquitoes, including diminished eating behaviour and compromised mating. This may also impact the mosquito's survival by diminishing its capacity to locate sustenance or reproduce. Conventional chemical adulticides, such synthetic pyrethroids, may adversely affect non-target organisms and foster resistance in mosquito populations. Conversely, CuO nanoparticles, synthesized biogenically from a plant extract, present a more environmentally sustainable option. Moreover, CuO nanoparticles exhibit greater specificity towards mosquitoes and are less prone to causing extensive environmental contamination.

**Conclusion**

The environmentally sustainable production and characterization of copper oxide nanoparticles (CuO NPs) utilizing *Hybanthus enneaspermus* flower extract offers a promising method for managing *Aedes aegypti*, a principal vector of mosquito-borne illnesses. The plant extract functions as a reducing and stabilizing agent in the synthesis process, providing an eco-friendly alternative to traditional chemical procedures. The nanoparticles exhibited dose-dependent toxicity across all three developmental stages, with elevated mortality rates noted in larvae, pupae, eggs, and adult mosquitoes subjected to different concentrations of CuO NPs. Nonetheless, although the findings are encouraging, additional research is required to evaluate the long-term environmental consequences, the persistence of CuO nanoparticles in ecosystems, and their possible effects on non-target species. Subsequent research should concentrate on refining the synthesis process for large-scale production and assessing the efficacy of CuO nanoparticles in real-world situations to thoroughly investigate their potential as an environmentally benign substitute for chemical pesticides in mosquito control initiatives. In summary, CuO nanoparticles derived from *Hybanthus enneaspermus* flower extract constitute an effective and sustainable strategy in combating *Aedes aegypti*, providing an eco-friendly option for mosquito management with little environmental hazards.

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