



Plant-mediated copper oxide nanoparticles as eco-friendly mosquito larvicides: Larval bioassays and computational docking insights

Dr. K Vijayalakshmi*, Dr. N Vidhyulatha*

Department of Chemistry, Rajah Serfoji Government College, Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Abstract

The present study principally concentrated on developing environmentally responsive, plant-mediated, less dangerous copper oxide nanoparticles (CuO NPs) using *Clausena dentata* leaf extract and exploring the mosquito larvicidal action. The biosynthesized CuO NPs were characterized by UV-Visible, FT-IR, XRD, SEM, and EDX spectroscopy, which explain the development of CuO NPs. A mosquito larvicidal study of the green-synthesized CuO NPs was assessed, and the LC₅₀ value of 13.45 µg/mL shows the strong larvicidal potential of the synthesized NPs. This was further confirmed by the molecular docking study with mosquito larval protein (PDB Code: 6XYU), and the binding energy of -5.8 kcal/mol indicates the favorable contact with the chief amino acid residues. Hence, these findings reveal that the plant-mediated CuO NPs serve as a promising, ecological agent for mosquito larvae control.

Keywords: Copper oxide nanoparticles, *Clausena dentata*, green synthesis, larvicidal action, molecular docking, eco-friendly vector control

Introduction

Nowadays, Nanotechnology is a remarkable and rapidly advancing research domain. Its infinitesimal particles are extensively exploited through numerous sectors due to their effectiveness and cost-efficiency. Nanoparticles are currently essential in fields like medicine, chemistry, physics, engineering, and agriculture. In these regions, they behave as catalysts, sensors, and nano-adsorbents in the chemical industries and pitch in to nano-coating, energy storage, and nano-photonics [1].

In this context, metal oxide nanoparticles are noteworthy materials for their tremendous applications in biological and environmental applications such as drug delivery, bioimaging, vaccines, diagnostics, engineering, photocatalytic, dye removal, and many ecological remedies. These materials were fabricated through several methodologies, including sol-gel, co-precipitation, hydrothermal, micro-emulsion, and microwave-assisted synthesis [2]. They are very toxic to the environment, and the remedy to reduce the toxic nature is the bio fabrication, which is effective than the chemical approach. To reduce the ecological risk, green-synthesized nanoparticles are a promising factor, which is achieved by various phytochemicals present in the plants as reducing, capping, and stabilizing agents [3].

In this background, we employed the generation of green-synthesized CuO NPs using the extract of *Clausena dentata* leaf, which holds various phytochemicals like flavonoids, alkaloids, and polyphenols. Biosynthesized nanoparticles offer numerous benefits, including the reduction of hazardous solvents and toxic reagents and the mitigation of environmental concerns. Among these, mosquito propagation is one of the important problems to control, which was suppressed by the biofabricated CuO NPs due to their inherent capacity to reduce the propagation. In addition, the biomolecules present in the plant contribute to this mitigation in an environmentally friendly manner [4]. The efficacy of the larvicidal capacity of the green-

synthesized CuO NPs was explored with an IC₅₀ value of 13.45 mg/mL, revealing noteworthy toxicity, which was confirmed by a molecular docking study. Conclusively, the bio-fabricated CuO NPs showed their potential as an effective and eco-safe mosquito larvae vector control agent [5].

Materials and Methods

Materials

Copper acetate monohydrate [(CH₃COO)₂Cu·H₂O], sodium hydroxide (NaOH), and ethanol (C₂H₅OH) were obtained from Techno Scientific, Thanjavur. *Clausena dentata* leaves were collected from the green background of the Thanjavur district, Tamil Nadu, India, for the larvicidal bioassay. Late third- to early fourth-instar larvae of *Aedes aegypti* mosquitoes were collected from stagnant water sources in the local environment of Thanjavur.

Plant extract preparation

Freshly collected *Clausena dentata* leaves were cleaned with running tap water, followed by double-distilled water. About 20 g of the washed leaves were mixed with 200 mL of distilled water and boiled at 80°C for 30 min. Subsequently, the extract was filtered using Whatman No. 1 filter paper to eliminate impurities. The cultured extract was set aside for the following use at 4°C [6].

Biofabrication of CuO NPs

20 mL of 0.2 M (CH₃COO)₂Cu · H₂O solution were prepared and heated to 60°C using a magnetic stirrer. After that, 20 ml of the prepared leaf extract solution was added drop-wise at a constant temperature. The pH was adjusted to 10 by introducing 1 M NaOH solution, and the reaction mixture underwent continuous stirring for 3 hours to terminate the process of synthesis. Subsequently, centrifugation at 14,000 revolutions per minute for a period of 10 minutes was utilized to separate the synthesized nanoparticles, and the residue was thoroughly rinsed with

deionized water and ethanol to improve the level of purity. The biogenic CuO NPs were kept in a hot air oven at 140°C and then dried in a muffle furnace for 3 hours at 400°C [7].

Larvicidal Bioassay

The larvicidal activity was executed under the guidelines of the World Health Organization (WHO) with slight modifications. A 1000 ppm stock solution was prepared by dissolving bio-fabricated CuO NPs using deionized water, and the suspensions were diluted to concentrations of 10, 20, 30, 40, and 50 µg/mL. For the larval mortality assay, 10 mosquito larvae were shifted to plastic cups containing 100 mL of each trial solution [8]. Triplicates were carried out to ensure reproducibility, along with bare deionized water as a control. The test solutions were maintained under identical conditions, and the larval mortality was recorded after 24 hours [9]. When larvae did not react to light poking, they were deemed dead. The mortality rate was determined using the formula:

$$\% \text{ of mortality} = \frac{\text{number of dead larvae}}{\text{total larvae introduced}} \times 100 \text{ --- (1)}$$

Data Analysis

The mean percentage and the ± standard deviation were calculated from the triplicate mortality data. The median lethal concentration (LC₅₀) was estimated between doses that produced mortalities closest to 50% with a significance level of p < 0.05.

Results and discussions

UV analysis

Fig. 1 shows the UV-visible absorption spectrum of CuO NPs synthesized from *Clausena dentata* leaf extract, reported in the wavelength range between 200 and 1000 nm [10]. Two distinguished absorption peaks were observed at 214.3 nm and 375.3 nm, attributed to the surface plasmon response (SPR) absorption band, which confirms the formation of CuO NPs. As an attuned result, the UV absorption spectrum exhibits the formation of CuO NPs [11].

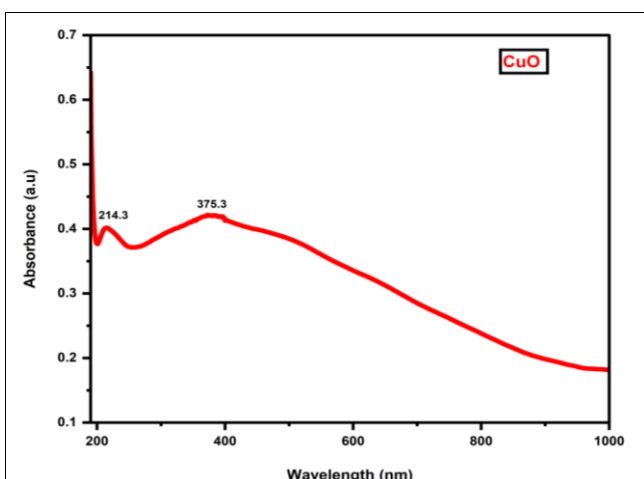


Fig 1: UV spectrum of the biosynthesized CuO NPs using *Clausena dentata* leaf extract

FT-IR analysis

Fig. 2 shows the FTIR spectrum of CuO NPs synthesized from *Clausena dentata* leaf extract [12]. A distinctive absorption band at 3432.78 cm⁻¹ indicates the presence of phenolic -OH, and the absorption bands observed at 1630.44

cm⁻¹ and 1470.06 cm⁻¹ are correlated with C=O stretching and C-H bending absorptions, respectively. Moreover, the absorption bands at 569.78 cm⁻¹ and 510.05 cm⁻¹ represent the Cu-O bond. The aforementioned information confirms the presence of photochemicals in the *Clausena dentata* leaf extract that acted as the reducing, capping, and stabilizing agents for the synthesis of CuO NPs process [13].

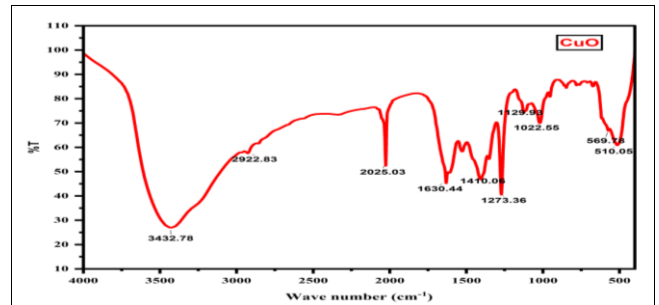


Fig 2: FT-IR spectrum of biosynthesized CuO NPs using *Clausena dentata* leaf extract

XRD analysis

Clausena dentata leaf extract was utilized to prepare CuO NPs, which were analyzed by x-ray diffraction pattern as shown in Fig. 3. Miller indices (110), (-111), (111), (202), (202), (-113), (311), (113), (221), and (004) are consistent with the monoclinic phase with COD number 1011148. This XRD analysis confirms the stability, crystalline nature, and phase purity and the successful formation of CuO NPs [14]. Moreover, the average size of the synthesized CuO NPs was determined by the following Scherrer equation (2).

$$D = \frac{k\lambda}{\beta \cos\theta} \text{ ----- (2)}$$

Where k is 0.94, β is the full width at half maximum of the peak, θ is the diffraction angle, and λ is the wavelength. The average particle size of the biosynthesized CuO NPs is 28 nm, which validates the nanoscale growth [15].

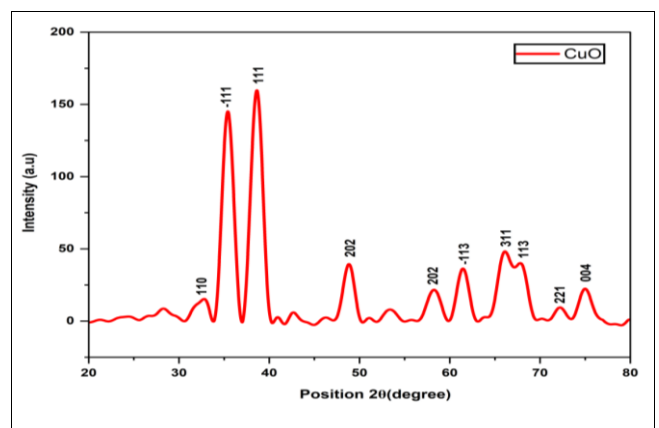


Fig 3: XRD patterns of CuO NPs synthesized using *Clausena dentata* leaf extract

SEM and EDS Analyses

Fig. 4a represents the surface structure of the biosynthesized CuO NPs, which visibly establishes the nanostructure, form of all the particles [16]. From this result, the SEM micrograph clearly reveals that CuO NPs are in cubic and spherical shapes, with less evidence of agglomerations, and the average particle diameter is 46.2 nm, confirming the

formation of biosynthesized CuO NPs. This is because bio-reduction frequently produces nanoparticles with different sizes and shapes of CuO NPs [17].

The surface elemental investigation of the biosynthesized CuO NPs and the corresponding weight percentages are shown in Fig. 4b, which reveals the presence of copper, oxygen, and carbon with weight percentages of 77.41%, 19.85%, and 2.74%, respectively [18]. The presence of carbon present in the EDS spectrum confirms the fabrication of CuO NPs using *Clausena dentata* leaf extract. Conclusively, the aforementioned SEM and EDS analyses corroborate the biofabrication and potential uses of CuO NPs [19].

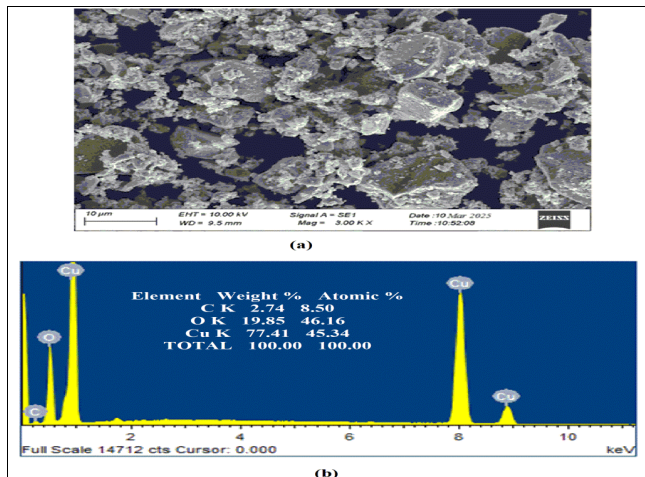


Fig 4: (a) SEM micrograph (b) EDS spectrum of CuO NPs synthesized using *Clausena dentata* leaf extract

Larvicidal activity

Fig. 5 shows the larvicidal bioassay of the different concentrations of the biosynthesized CuO NPs, indicating the effective control of larval endurance.

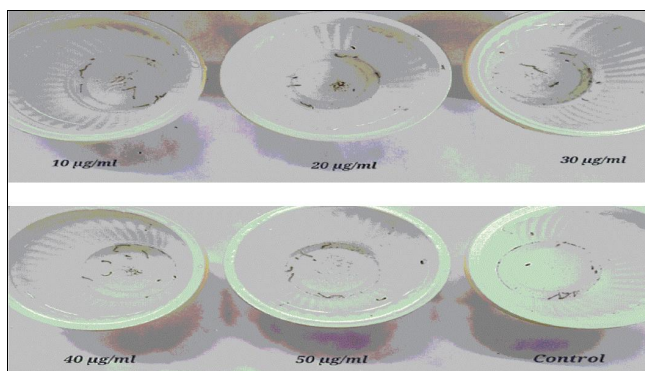


Fig 5: Larvicidal bioassay for different concentrations of CuO NPs synthesized using *Clausena dentata* leaf extract

Subsequently, Fig. 6 shows that the larval mortality increases in a dose-dependent manner even at 10 µg/mL, which confirms the strong deadly efficacy of synthesized CuO NPs [20]. As we see in Fig. 6, 44.73% of mortality was achieved at 10 µg/mL, and the utmost mortality was observed at 90.49% for 50 µg/mL, suggesting the effective penetration of the larval biological system by the biosynthesized CuO NPs [21]. The experimental results of the triplicate assay showed that the mortality rate is consistent. Half of the larval population mortality was obtained by the LC₅₀ value, which is 13.45 µg/mL, indicating the potential

larvicidal activity of the biosynthesized CuO NPs. It is making them promising and eco-friendly members for mosquito vector control [22].

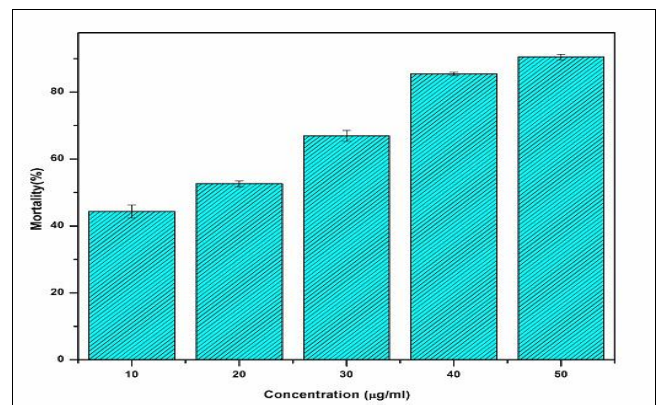


Fig 6: mortality rate for different concentrations of CuO NPs synthesized using *Clausena dentata* leaf extract

Molecular docking study

Fig. 7a shows the deep positioning of the CuO ligand with the active site of the 6XYU protein, suggesting a favorable interaction with a binding energy of -5.8 kcal/mol. The orientation is also displayed in Fig. 7a, indicating the mortality ability of the CuO ligand [23]. Further, Fig. 7b reveals the binding chief residues, such as Ser329, Thr275, and Lys403, of the protein with the ligand, confirming the stability of the protein-ligand complex of the larvae death. Consequently, Fig. 7c shows the hydrogen bonding interaction ranging from 2.08 to 3.14 Å corroborates the biological significance [24]. Fig. 7d further confirms the positioning of the ligand in the active site and the disruption of the structural integrity of the protein in the larvae. Conclusively, these theoretical evaluations suggest that the synthesized CuO NPs inhibit the biological process of the mosquito larvae [25].

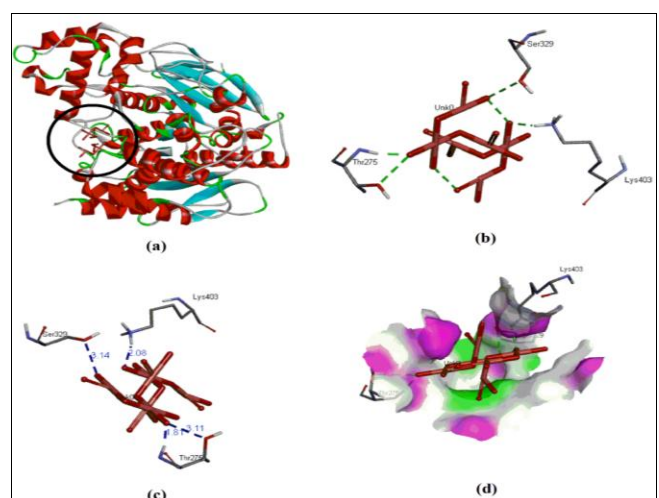


Fig 7: (a) 6XYU protein-CuO ligand binding (b) key amino acid residues (c) bond distance (d) hydrogen bonding

Conclusion

The CuO NPs were synthesized using *Clausena dentata* leaf extract as a sustainable and environmentally friendly approach. The structural, morphological, and elemental characterizations of the biosynthesized CuO NPs were illustrated by UV-Vis, FTIR, XRD, SEM, and EDS

analyses. The larvicidal activity of the synthesized CuO NPs revealed potential larvicidal activity with the LC50 value of 13.45 µg/mL, suggesting it as an effective alternative to conventional chemical larvicides. The theoretical evidence for the experimental outcomes was achieved by the molecular docking analysis with the mosquito larval protein 6XYU, and the binding energy of -5.8 kcal/mol indicates a strong affinity of CuO NPs against the target protein. Finally, all outcomes strongly suggest that the phytomediated CuO NPs reveal an eco-friendly, strong, and cost-effective candidate for mosquito larval control.

References

- Tadjarodi A, Roshani R. A green synthesis of copper oxide nanoparticles by the mechanochemical method. *Current Chemical Letters*,2014;3(4):215–220. doi: 10.5267/j.ccl.2014.7.001
- Punitha N, Mangalam M, Vijayalakshmi K, Selvan CSA. Microwave promoted synthesis of Schiff base ligand using natural acid catalyst and its nickel (II) and copper (II) complexes. Published online, 2015.
- Muhammed Shajin JS, Sankar S, Hasbiya NS, Jose L, Babu PS. Green synthesis of copper oxide (CuO) nanoparticles using *Phyllanthus emblica* extract and characterization studies. *Journal of Physics Conference Series*,2025;3038(1):012007. doi:10.1088/1742-6596/3038/1/012007
- Ali K, Sajid M, Abu Bakar S, Younus A, Ali H, Zahid Rashid M, *et al.* Synthesis of copper oxide (CuO) via coprecipitation method: tailoring structural and optical properties of CuO nanoparticles for optoelectronic device applications. *Hybrid Advances*,2024;6:100250. doi: 10.1016/j.hybadv.2024.100250
- Phiwdang K, Suphankij S, Mekprasart W, Pecharapa W. Synthesis of CuO nanoparticles by precipitation method using different precursors. *Energy Procedia*,2013;34:740–745. doi: 10.1016/j.egypro.2013.06.808
- Ikram A, Jamil S, Fasehullah M. Green synthesis of copper oxide nanoparticles from papaya/lemon tea extract and its application in degradation of methyl orange. *Materials Innovation*,2022;02(04):115–122. doi:10.54738/MI.2022.2401
- Vijayalakshmi K, Vidhyulatha N, Aldawood S, Alqahtani MS, Alshahrani MY, Alqahtani AM, *et al.* Computational and experimental investigation of biogenic zinc oxide nanoparticles synthesized from *Clausena dentata* leaf extract for α -amylase inhibition and K562 leukemia cell therapy. *Food Bioscience*,2025;68:106371. doi: 10.1016/j.fbio.2025.106371
- Divya Bharathi J, Suseem SR. Larvicidal activity of CuO and ZnO nanoparticles against *Aedes aegypti* and *Anopheles stephensi* mosquito vectors-a greener approach by *Phaseolus vulgaris* L. aqueous extract as bio-reductant. *Results in Chemistry*,2024;7:101408. doi: 10.1016/j.rechem.2024.101408
- Abd El-Halim MD, Almehezia AA, Elasley EAA, Alhaj Zen A, Kamel MHM, El-Sayed AA, *et al.* Larvicidal and adulticidal effect of green synthesis copper oxide nanoparticles using *Achillea fragrantissima* and its biological and ultra-structural impact on *Culex pipiens*. *Bulletin of the Chemical Society of Ethiopia*,2024;39(3):535–546. doi:10.4314/bcse.v39i3.11
- Sukumar S, Rudrasenan A, Padmanabhan Nambiar D. Green-synthesized rice-shaped copper oxide nanoparticles using *Caesalpinia bonducella* seed extract and their applications. *ACS Omega*,2020;5(2):1040–1051. doi:10.1021/acsomega.9b02857
- Nzilu DM, Madivoli ES, Makhanu DS, Wanakai SI, Kiprono GK, Kareru PG, *et al.* Green synthesis of copper oxide nanoparticles and its efficiency in degradation of rifampicin antibiotic. *Scientific Reports*,2023;13(1):14030. doi:10.1038/s41598-023-41119-z
- Gebrie HT, Assege MA, Meshesha DS, Abebe AT, Berhe AH, Desta MT, *et al.* Biosynthesis and characterization of copper oxide nanoparticles from *Plumbago zeylanica* leaf extract for antibacterial and antioxidant activities. *Scientific Reports*,2025;15(1):30656. doi:10.1038/s41598-025-10700-z
- Priya M, Venkatesan R, Deepa S, Krishnan M, Subramanian K, Rajendran R, *et al.* Green synthesis, characterization, antibacterial, and antifungal activity of copper oxide nanoparticles derived from *Morinda citrifolia* leaf extract. *Scientific Reports*,2023;13(1):18838. doi:10.1038/s41598-023-46002-5
- John AS, Gurumurthy K. Synthesis and characterization of CuO nanoparticles from bioleached copper through modified and optimized double precipitation method. *ACS Omega*,2025;10(10):10193–10198. doi:10.1021/acsomega.4c09064
- Sharma BK, Shah DV, Roy DR. Green synthesis of CuO nanoparticles using *Azadirachta indica* and its antibacterial activity for medicinal applications. *Materials Research Express*,2018;5(9):095033. doi:10.1088/2053-1591/aad91d
- Neiva J, Benzarti Z, Carvalho S, Devesa S. Green synthesis of CuO nanoparticles-structural, morphological, and dielectric characterization. *Materials*,2024;17(23):5709. doi:10.3390/ma17235709
- Černík M, Thekkae Padil VV. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. *International Journal of Nanomedicine*,2013;8:889–898. doi:10.2147/IJN.S40599
- Andualem WW, Sabir FK, Mohammed ET, Belay HH, Gonfa BA. Synthesis of copper oxide nanoparticles using plant leaf extract of *Catha edulis* and its antibacterial activity. *Journal of Nanotechnology*,2020;2020:1–10. doi:10.1155/2020/2932434
- Shafiq A, Jeong U, Han Y, Kim Y, Lee J, Kim BS, *et al.* Green synthesis of copper oxide nanoparticles from waste solar panels using *Piper nigrum* fruit extract and their antibacterial activity. *Catalysts*,2024;14(8):472. doi:10.3390/catal14080472
- Rajagopal G, Nivetha A, Sundar M, Panneerselvam T, Elumalai K, Ramesh M, *et al.* Mixed phytochemicals mediated synthesis of copper nanoparticles for anticancer and larvicidal applications. *Heliyon*,2021;7(6):07360. doi: 10.1016/j.heliyon.2021.e07360
- Velsankar K, R M AK, R P, V M, Sudhahar S. Green synthesis of CuO nanoparticles via *Allium sativum*

- extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities. Journal of Environmental Chemical Engineering,2020:8(5):104123. doi: 10.1016/j.jece.2020.104123
22. Prathap N, Dravid N, Kaarmukhilnilavan SR, Suresh S, Arivazhagan G, Ganesan P, *et al.* Copper oxide nanoparticles synthesized from *Indigofera linnaei* Ali and this plant's biological applications. Inorganics,2023:11(12):462. doi:10.3390/inorganics11120462
23. Ononamadu CJ, Abdalla M, Ihegboro GO, Abah J, Onoja SO, Eze PM, *et al.* In silico identification and study of potential anti-mosquito juvenile hormone binding protein (MJHBP) compounds as candidates for dengue virus-vector insecticides. Biochemical and Biophysical Reports,2021:28:101178. doi: 10.1016/j.bbrep.2021.101178
24. Murugesan R, Roshini S, Sasindran R, Santhoshkumar S, Sureshkumar J, Prabhu S *et al.* Insecticidal properties of *Ipomoea carnea* Jacq. flower against disease transmitting vector (*Aedes aegypti*): chemical profiling, bioassay, molecular docking, dynamic simulations and ecotoxicity validation. Pharmacological Research Natural Products,2025:9:100412. doi: 10.1016/j.prenap.2025.100412
25. Anita A, D S. In silico molecular docking study of plant-based compounds from medicinal plant *Lantana camara* L. against *Aedes aegypti* L. protein. International Journal of Mosquito Research,2022:9(6):97–106. doi: 10.22271/23487941.2022.v9.i6b.645