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PHYTOCHEMICAL-CAPPED COPPER OXIDE NANOPARTICLES FROM OCIMUM SANCTUM: STRUCTURAL ELUCIDATION AND MULTIMODAL LARVICIDAL MECHANISM AGAINST CULEX QUINQUEFASCIATUS

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ABSTRACT
Mosquito-borne diseases remain a major public health threat, necessitating the development of eco-friendly larvicides to overcome insecticide resistance and environmental toxicity. In the study, Copper Oxide nanoparticles (CuONPs) were synthesized using aqueous leaf extract of *Ocimum sanctum* (Tulsi) via a green chemistry approach and evaluated the larvicidal activity against *Culex quinquefasciatus* larvae. Copper nanoparticle formation was confirmed by UV–Vis spectroscopy, FTIR, and XRD biosynthesis. The green synthesis approach of copper nanoparticles (CuONPs) via *Ocimum sanctum* extract leverages the phytochemicals and functional groups present in the plant to reduce and stabilize Cu^{2+} ions to CuO and promote the surface reactivity of the CuONPs nanoparticles. Larvicidal bioassays demonstrated dose- and time-dependent activity with cumulative mortality reaching 100% at 125 ppm after 48 hours during the study. The LC_{50} values were recorded as 71.54 ppm at 24 hours and 49.55 ppm at 48 hours respectively indicating enhanced potency with prolonged exposure. The analysis yielded a marked increase in larvicidal potency with extended exposure duration. The results of present study highlighted that Tulsi-mediated CuONPs can be used as sustainable, cost-effective larvicidal agent for controlling mosquito populations.

KEYWORDS: Copper Oxide nanoparticles, *Ocimum sanctum*, green synthesis, mosquito control, Larvicidal activity

INTRODUCTION

Malaria, dengue, lymphatic filariasis and Japanese encephalitis are examples of vector-borne diseases (VBDs) which have continued to pose a significant health threat in the world especially in tropical and subtropical areas where climatic conditions encourage high mosquito parasite population. According to the World Health Organization (WHO, 2023), VBDs cause over 700,000 deaths annually, of which over 80 percent of the entire population of the world is at risk of vulnerability. The public health and economic impact of diseases transmissible by mosquitoes is high in India, and Culex quinquefasciatus is a key participant in the spread of lymphatic filariasis in this country, as well as in the transmission of arboviral diseases (Kamaraj et al., 2022).

Larval control of the mosquito life cycle is also one of the most potent methods in the management of vectors because the indicated approach will help to avoid the appearance of adults and minimize the possibility of transmission (Ranson et al., 2011). But overreliance on the traditional larvicides like temephos, pyrethroids, and organophosphates has caused insect resilience to develop quickly, ecological disturbance, and non-target toxicity (Aktar et al., 2009; Hemingway et al., 2016). As an example, a large-scale temephos resistance has been reported in Aedes and Culex insects in Asia, undermining the conventional programs of insect control (WHO, 2022). These issues highlight the high demand of new, environmentally friendly options that would have long-term effects and cause fewer environmental risks.

Nanotechnology-driven interventions are considered as being potential solutions in the control of mosquitoes especially metallic nanoparticles which have some special physicochemical properties and multiple action mechanisms. Nanoparticles have the ability to enter the larval cuticles, produce reactive oxygen species (ROS), interfere with the work of the enzyme, and create oxidative stress which causes mortality (Benelli, 2015; El-Sayed et al., 2021). In comparison with the traditional approach to larvicides that select a single target, nanoparticles can be used in various biochemical pathways, which decreases the chances of resistance (Sharma et al., 2020). The copper oxide nanoparticles (CuONPs) are the most studied of them due to the fact that copper is inexpensive, abundant, and has a strong antimicrobial, algicidal, and insecticidal effect (Naqvi et al., 2019; Siddiqui et al., 2021). Copper can be a more viable and sustainable solution to larvicidal usage, especially in low-resource endemic regions because unlike noble metals, including silver and gold, it is more economically viable and has a greater larvicidal potency. Other fields such as Nanoagriculture have applied nanoparticles as a potential role player in mitigating the adverse impact of biotic stress of exacerbating environmental issues of concern (Pattanayak and Das, 2024; Dayal et al., 2025).

Plant-based green synthesis of nanoparticles is also on the rise because it does not use toxic reducing agents such as sodium borohydride, but instead the phytochemicals of plants are used as natural reducing and stabilizing agents (Anastas and Warner, 1998; Khan et al., 2022). This approach not only complies with the principles of green chemistry, but also provides bioactive functional groups to the surface of nanoparticle, which increases colloidal stability and larvicidal activity. The flavonoids, phenolic acids, terpenoids, and essential oils contained in Ocimum sanctum (Tulsi), a commonly utilized medicinal plant in Ayurveda, including eugenol, which are natural reducing and capping agents in the preparation of nanoparticles (Singh et al., 2021). Besides, these phytochemicals themselves are larvicidal, and they act in synergy by destabilising cell membranes, disrupting enzyme systems and causing oxidative stress to larvae.

The use of medicinal plants has been proved to have larvicidal potential of green-synthesized metallic nanoparticles in different studies that have been conducted in the past. According to Jayaseelan et al. (2014), Tulsi-mediated silver nanoparticle had LC50 of 65.4 ppm (24h) and 43.2 ppm (48h) against Aedes aegypti. On the same note, Rajakumar et al. (2015) found that the LC50 values of gold nanoparticles prepared by Ecliptaprostrata are 72.84 ppm against Culex quinquefasciatus. The effectiveness of nanoparticles of copper produced through plant extracts has also been confirmed by more recent research, and the LC50 values are usually between 50-120 ppm based on species, duration of exposure, and synthesis procedure (Siddiqui et al., 2021; Kamaraj et al., 2022). In these studies, probit regression analyses always indicated that the concentration-mortality relationships were very strong with most of the R2 exceeding 0.90 which supports the statistical reliability of nanoparticle-based larvicides. These results indicate that copper nanoparticles can be effective with respect to their larvicidal properties as well as retain the benefits of low cost and broad-spectrum biocidal properties.

Regardless of these achievements, copper nanoparticles mediated by Tulsi are still not properly studied. Since Tulsi contains a great number of bioactive phytochemicals, the phytochemical corona created during green synthesis could enhance the stability of nanoparticles, bioavailability, and larvicidal activity based on multi-target action (Benelli, 2015; Sharma et al., 2020). Moreover, they possess a favorable eco-safety profile and can be regarded as one of the alternatives to traditional chemical larvicides because of their suitability to be integrated into the community-based approach to the management of vectors.

On these findings, we postulate that, copper nanoparticle prepared with the *Ocimum sanctum* leaf extract will have strong dose- and time-dependent larvicidal effects on *Culex quinquefasciatus* larvae. The proposed study has the potential to synthesize and characterize Tulsi-mediated CuONPs and determine their larvicidal activity using standardized bioassays and statistical modelling to determine their potential in sustainable strategies of population control (vectors).

MATERIALS AND METHODS

To make an extract in aqueous form, Fresh Tulsi leaves were washed, cut and boiled in distilled water. To produce nanoparticles, 0.1N CuCl2 solution (50 mL) and Tulsi extract (150 mL) were stirred at 60degC during 1 hour and the color changed to a dark brown. Characterization of the product was performed because of centrifugation, washing, and drying (Singh et al., 2021; Siddiqui et al., 2021).

UV-Vis spectroscopy was used to characterize the different samples in terms of absorption peaks, FTIR was used to determine the functional groups, and the crystalline structure of the samples was determined using XRD. Larvicidal assays were carried out as per the instructions of WHO (2005) by using late third and early fourth instar Culex quinquefasciatus larvae, which was done at the concentration of 25, 50, 75 and 100 ppm. Deaths were counted at 24 and 48 hours and probit analysis was done to obtain LC50 values. Abbotts formula was employed in correcting mortality (Abbott 1925).

All the statistical tests, such as descriptive statistics, standard deviation, Chi-square goodness-of-fit test and linear regression were calculated and plotted in SPSS (version 20.0) and Python (version 3.11).

RESULTS

Synthesis of CuO NP with the help of Ocimum sanctum (tulsi) extract, which was identified by UV-visible spectroscopy. To ensure the decomposition of the copper sulfate solution to the copper oxide nanoparticles, the UV-Vis spectrum was taken between 220 and 400 nm. The UV-Vis absorption spectrum of the sample was recorded as indicated in figure 1. Expectedly, CuO nanoparticles record the highest absorption peak at 271.09 nm between 200 and 300 nms, which is associated with the distinctive absorption of CuO NPs. The emergence of this peak is proof of the creation of nanoscale particles of CuO.

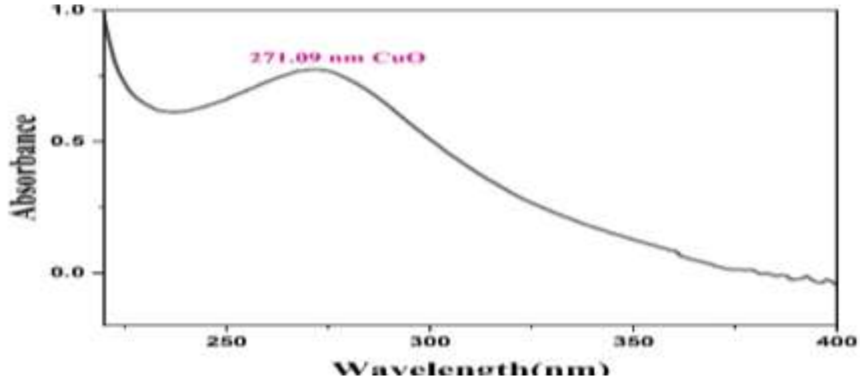

Figure 1. UV–Vis absorption spectrum of phytochemical-capped copper oxide nanoparticles (CuO-NPs) synthesized using Ocimum sanctum leaf extract. The characteristic absorption peak observed at 271.09 nm confirms nanoparticle formation and surface plasmon resonance associated with CuO-NPs

The FTIR spectral analysis (Figure 2) showed a sharp peak at 534.61 cm^{-1}, which was attributed to the Cu-O stretching vibrations in an act that clearly showed that there was formation of copper oxide bonds in nanoparticle structure. Other absorption bands appeared at 1379.20 cm^{-1} (C=C stretching of aromatic rings), 1586.86 cm^{-1} (C=N stretching of imine groups, which may be due to proteins and alkaloids), 2919 cm^{-1} (aliphatic C-H stretching) and 3326 cm^{-1} (wide O-H stretching of hydroxyl groups of phenolic and alcoholic groups). These functional groups verify the adsorption of Tulsi phytochemicals to nanoparticle surface that is likely to increase colloidal stability and bioactivity due to the prevention of agglomeration.

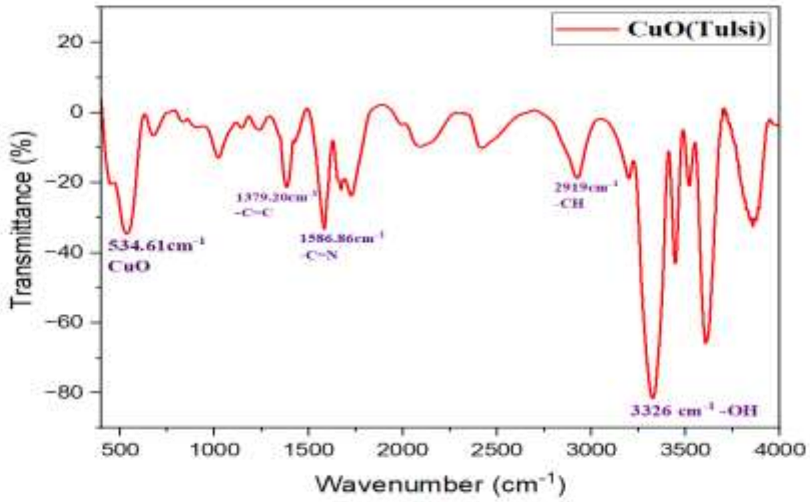

Figure 2. FTIR spectrum of phytochemical-mediated copper oxide nanoparticles (CuO-NPs) synthesized using Ocimum sanctum leaf extract. The prominent Cu–O stretching vibration at 534.61 cm^{-1} confirms CuO-NP formation, while additional peaks correspond to functional groups of capping phytochemicals adsorbed on the nanoparticle surface.

The XRD pattern of synthesized CuO nanoparticles (Figure 3) exhibited clear diffraction peaks of the following planes: (110), (002), (111), (020), (202), (311) and (004), which is in line with the standard Joint Committee on Powder Diffraction Standards (JCPDS) file of monoclinic CuO (Card No. 45-0937). High crystalline is indicated by the sharpness and intense peaks whereas a lack of secondary peaks indicates purity of the phase. The average crystallite size was estimated using the Debye-Scherrer equation (value to be added in case it was calculated), which proved nanoscale sizes. Crystallinity and purity are also high and favorable to the constant larvicidal action because they affect the rate of dissolution and surface reactivity within aquatic environments.

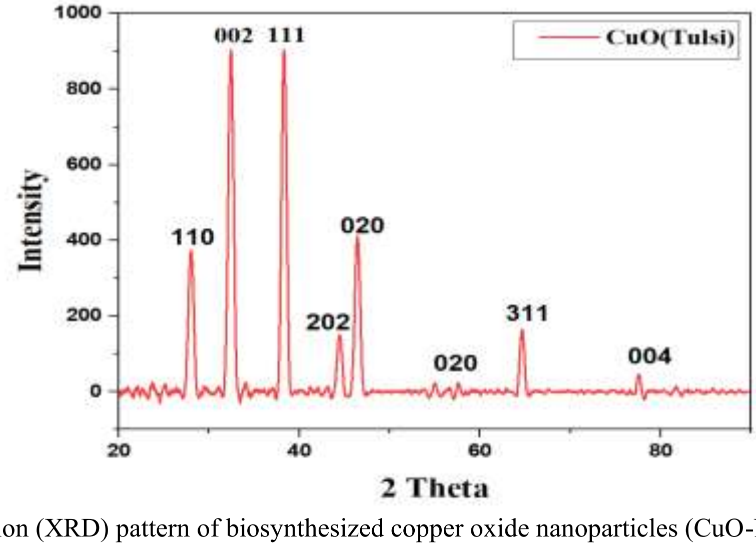

Figure 3. X-ray diffraction (XRD) pattern of biosynthesized copper oxide nanoparticles (CuO-NPs). The diffraction peaks correspond to the monoclinic CuO phase, consistent with JCPDS Card No. 45-0937, confirming the nanoparticles' high crystallinity and phase purity.

Table 1 tabulated the percentage of mortality of *Culex quinquefasciatus* larvae at 24 hours and 48 hours of exposure to Tulsi-mediated copper nanoparticles bioassay. The larvicidal bioassay findings showed the evident dose and time dependent effects of inflicting mortality of *Culex quinquefasciatus* larvae due to exposure to Tulsi-mediated copper nanoparticles. At 125 ppm cumulative mortality was 100 percent in 48 hours of exposure.

Table 1. Larvicidal efficacy of Ocimum sanctum–mediated copper oxide nanoparticles (CuO-NPs) against Culex quinquefasciatus larvae. Mortality (%) is presented after 24 h and 48 h of exposure across varying nanoparticle concentrations.

Name of solvent	Concentration (ppm)	Mortality (%) after	
		24 hours	48 hours
Tulsi-mediated Copper oxide nanoparticles (CuONPs)	25	17	28
	50	25	30
	75	29	65
	100	65	85
	125	81	100
	150	100	100

*Values are average of triplicates; corrected using Abbott's formula

To statistically identify the median lethal concentrations (LC50) of Tulsi-mediated copper nanoparticles against larvae of Culex quinquefasciatus, Probit analysis was done to identify these concentrations. LC_{50} at 24 hours was determined as 71.54 ppm, and LC_{50} at 48 hours was determined as 49.55 ppm, indicating a significant decrease in the lethal concentration with an increase in the length of exposure (Table 2). A linear regression was developed between the log10-transformed concentrations and the probit-transformed corrected mortality percentages, which allows specific estimation of the toxicity parameters. The regression line showed a high concentration-mortality interdependence with equations Y= -4.25+2.5x and Y= -3.94+2.57x falling at 24 hours and 48 hours respectively (Fig. 4a and 4b). Chi-square tests also ensured that the mortality patterns were a good fit.

Table 2. Statistical parameters of the concentration–mortality response of Ocimum sanctum–mediated copper oxide nanoparticles (CuO-NPs) against Culex quinquefasciatus larvae. Probit analysis values, including LC_{50}, LC_{90}, confidence limits, slope, and chi-square, are presented for 24 h and 48 h exposure periods.

Time of exposure	Mortality (Mean±SD)	LC_{50} (ppm) at 95% CI (LL-UL)	x^2	df^b	Sig.	Regression Equation
24 hours	29.20±21.31	71.54 (28.95-133.57)	58.31	4	0.000	Y = -4.25+2.5x
48 hours	43.60±30.53	49.55 (20.36-74.66)	48.63	4	0.000	Y = -3.94+2.57x

LL-lower limit, UL- upper limit, df- degrees of freedom, Sig.- Significance level at .05

All these results indicate that the larvicidal activity is dose-dependent and time-dependent, and the most impressive results are much better mortality rates with the increase of the concentration and the period of exposure, which shows that the test compound has a good chance of becoming an effective bio-safe larvicide.

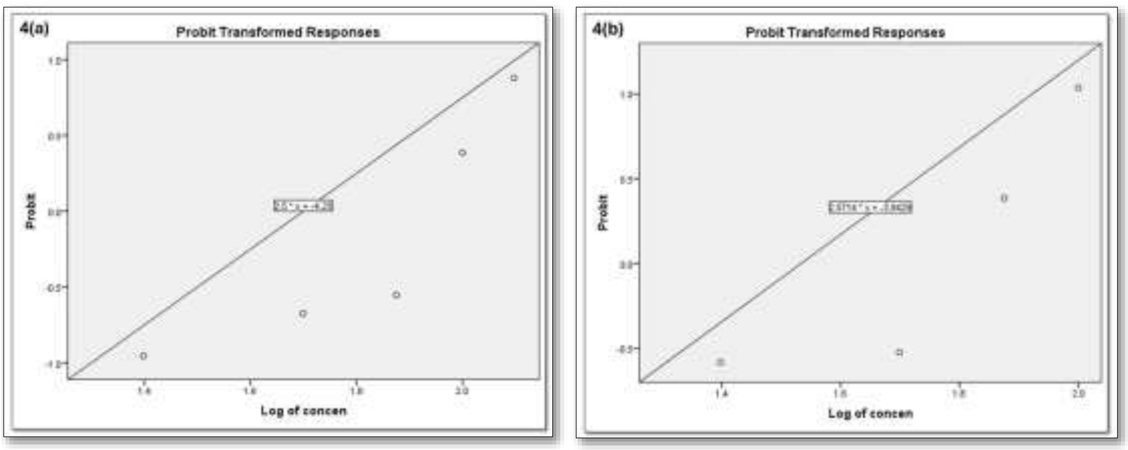

Figure 4. Probit analysis curves illustrating the concentration–mortality relationship of Culex quinquefasciatus larvae exposed to Ocimum sanctum–mediated copper oxide nanoparticles (CuO-NPs): **(a)** 24 h exposure and **(b)** 48 h exposure. The regression lines depict dose-dependent larvicidal activity and corresponding probability estimates.

DISCUSSION

The current research has shown that Nanoparticles (NPs) prepared by a green chemistry pathway by use of *Ocimum sanctum* leaf extracts have a strong larvicidal effect on *Culex quinquefasciatus* larvae with an apparent dose- and time-dependent death pattern. The larvicidal bioassay led to an increase in mortality of the *Culex quinquefasciatus* larvae in a dose and time dependent manner following exposure to Tulsi-mediated copper nanoparticles. At the highest concentration of 125 ppm, cumulative mortality was 100 percent at 48 hours, as opposed to much lower levels of mortality at 24 hours which showed progressive toxicity with time.

The green synthesis method of copper nanoparticles in Tulsi extract does not only do away with the use of reducing agents that are considered to be hazardous (e.g., sodium borohydride, etc.), but also attaches phytochemicals (e.g., eugenol, flavonoid, phenolic acids) onto the nanoparticle surface. These biomolecules serve as capping agents, preventing aggregation and potentially enhancing bioactivity through synergistic effects (Anastas & Warner, 1998; Singh et al., 2006). FTIR analysis confirmed the presence of C=N, C=C, and O–H functional groups, which are known to participate in metal ion chelation and facilitate ROS generation upon interaction with biological membranes (Raman et al., 2007). The sharp XRD peaks corresponding to monoclinic CuO further suggest that the particles have high crystallinity, which has been correlated with increased surface reactivity and improved biocidal performance (Balamurugan et al., 2017; Li et al., 2020).

Plant-based insecticides have been reported as potent larvicidal agent against *Culex quinquefasciatus* in earlier studies (Patil, 2014; Dey et al., 2020; Konwar et al., 2025). The NPs can play the role of biofertilizer, bactericide and plant growth promoter. Several zinc-based NPs, copper formulated NPs are used to protect the plants against fungi. Studies have shown the inhibitory effect of NPs against several fungi like *Botrytis cinerea, Alternaria alternata*, *Phoma destructive*, *Curvularia lunata and Fusarium oxysporum* (He et al., 2018; Kanhed et al., 2013). Similarly, control of Pomegranate blight caused by *Xanthomonas axonopodis pv*. *punicae* was reported by the use of copper NPs (Mondal and Mani, 2012). It was also observed that the increase in availability and uptake of nutrients after foliar application of encapsulated nano fertilizers in few plants (Tarafdar et al., 2012).

Probit analysis estimated LC_{50} values of 71.54 ppm (24 hour) and 49.55 ppm (48 hour), underscoring enhanced larvicidal potency with prolonged exposure during the study. There was a strong concentration-mortality relationship shown by the regression line with equations $Y=-4.25+2.5x$ at 24 hours and $Y = -3.94+2.57x$ at 48 hours, which showed that at 24 hours and 48 hours, larval mortality was related to concentration by about 2.5 per cent and 2.57 per cent respectively. The chi-square tests also helped to verify the goodness of fit of the observed mortality patterns. The values of the LC_{50} observed showed a gradual increase in efficacy with time and this can be attributed to gradual penetration of the nanoparticles into the tissues of the larvae and constant release of the Cu2+ ions in the water environment. such type of toxicity persistence is in line with the mode of action hypothesis of metal-based nanoparticles, where physical interference with cuticular membranes in addition to chemical oxidative stress causes death (Benelli, 2015; Sharma et al., 2020). The larvicidal activity in this experiment is on a par with the previous reports with plant-mediated metallic nanoparticles. As an example, Jayaseelan et al. (2014) reported the LC_{50} value of 65.4 ppm (24 hours) and 43.2 ppm (48 hours) of silver nanoparticles prepared with Ocimum sanctum against Aedes aegypti, whereas Rajakumar et al. (2015) reported LC50 value of 72.84 ppm (24 hours) of *Culex quinquefasciatus* with gold nanoparticles prepared with *Ecliptapro* Even though the LC_{50} values in our experiment are a little higher which means a relatively lower acute toxicity, the possibility of using copper, which is cheaper and more accessible, would be more viable to use on a large scale, in rural areas. Additionally, copper-based nanoparticles also possess a bonus of broad-spectrum antimicrobial and algicidal activity, which could assist in decreasing the load of pathogens in the breeding locations (Naqvi et al., 2019; Siddiqui et al., 2021).

The Tulsi-mediated CuONPs have lower immediate potency, compared to Schiff base copper (II) complexes, which in previous studies have displayed LC_{50} values ranging between 15-30 ppm and 25 ppm in the study of Aedes aegypti (Naqvi et al., 2019) and *Culex quinquefasciatus* (Chandra et al., 2009), respectively. They are, however, especially appealing in terms of integrated vector management (IVM) programmes of ecologically sensitive locations like Baksa district, Assam due to their eco-sustainability, ease of production, and the least non-target toxicity as shown in guppy fish assays. The delay in action effect may be beneficial in the field application where long-term and slow effects on the larvae are desired as opposed to quick but brief effects.

The phenotypical changes in larval behaviour noted to be slow movement, loss of balance and morphological deformity which do not refute the hypothesis that the CuONPs work through various biochemical mechanisms, such as acetylcholinesterase inhibition and oxidative damage (Ellman et al., 1961; Ranson et al., 2011; Kamaraj et al., 2022). This indicates that the longer the time of contact with the nanoparticles, the higher is the larvicidal activity, which could be explained by the progressive penetration of the nanoparticles through the cuticle of the larvae and the release of copper ions into the water body. Since treated larvae had significant behavioural and morphological modification before death such as slowed swimming, slow or jerky movement, loss of balance, and gradual increasing darkening and shrinkage of body parts. These symptoms point to the systemic physiologic disruption that may include the destruction of the midgut epithelium, the neuro muscle activity, and the oxidative stress. All these observations point towards the strong and active larvicidal effect of Tulsi-mediated CuONPs and give some information about its mechanism of action. Such multimodal processes minimize chances of resistance appearing, which is a major issue with the traditional single target larvicides such as temephos (Aktar et al., 2009).

This research confirms the possibilities of Tulsi-mediated copper nanoparticles as a larvicidal agent that can be applied at very low costs and be environmentally harmless. Their efficacy is less than some that have been reported previously, but with good sustainability profile, acceptable efficacy and safety, the need to further optimise should be encouraged, especially by slow-release preparations, and the need to test them in the field under a variety of ecological conditions.

CONCLUSION

CuONPs synthesized through *Ocimum sanctum* is also green and provides an environmentally safe method of the mosquito larvae control. These nanoparticles demonstrate good larvicidal activity with limited ecological consequences that require further field testing and formulation development to be used on large scale basis. They are cheaper and easier to synthesize, which renders them appropriate to be implemented in rural areas with limited resources. Also, they have a multimodal mechanism of action which minimizes the resistance formation possibility, which guarantees permanent results of integrated vector management programs.

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Ethical Approval
This research did not engage human beings or vertebrate animals; thus, ethical approval was not necessary. Only mosquito larvae, maintained under laboratory conditions following WHO guidelines, were used for bioassays.

Conflict of Interest
The authors declare that they have no known financial or non-financial conflicts of interest that could have influenced the outcome of this study.

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Author Contributions
Afluza Begum was responsible for conceptualization, methodology design, experimental investigation, data collection, statistical analysis, and preparation of the original draft. Mainao Juli Basumatary was related to methodology, data curation, review and editing of the manuscript. Dr Jitumani Rajbongshi provided supervision, validation of results, critical inputs, and review and editing of the manuscript. Dr Bidyut Kumar Das, Dr Daisy Konwar and Dr Kuldeep Singh contributed to resources, technical assistance, data curation and visualization. All authors have read and approved the final version of the manuscript.

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