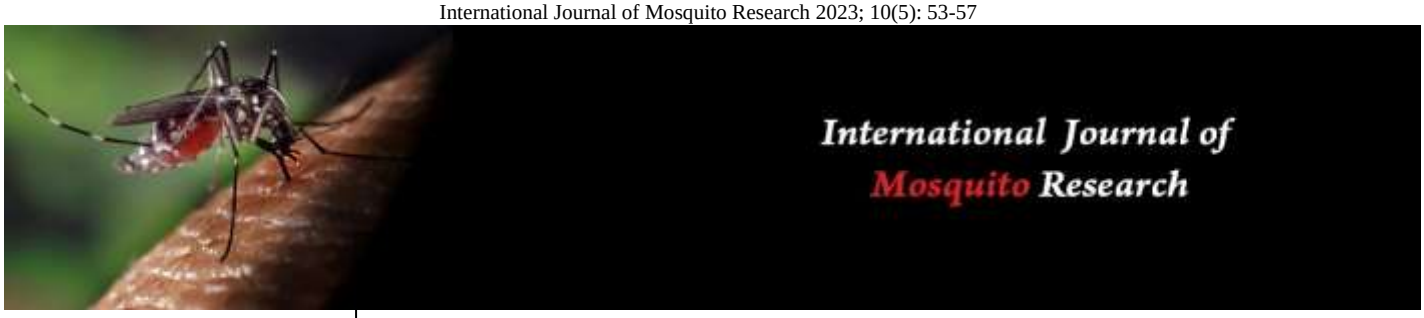

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2023; 10(5): 53-57

https://www.dipterajournal.com
Received: 12-07-2023
Accepted: 16-09-2023

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Green synthesis of copper oxide nanoparticles for assessment of larvicidal activity against *Aedes aegypti, Culex quinquefasciatus* mosquito vectors

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DOI: https://doi.org/10.22271/23487941.2023.v10.i5a.697

Abstract
The present research work describes the plant mediated synthesis of Copper oxide nanoparticles using *Pistia stratiotes* aqueous leaf extract and synthesized CuO NPs was used to assess the larvicidal activity against mosquito vectors *(Aedes aegypti, Culex quinquefasciatus)*. The phase purity of synthesized CuO NPs was confirmed using X-Ray diffraction analysis. The average crystallite size of CuO NPs was calculated around 12 nm. The optical absorption band was observed at 277 nm through the ultra-violet visible absorption (UV-Vis Abs) spectrometer. Functional group analysis of CuO NPs was performed through the Fourier Transform Infra-red spectrometer. The larvicidal activity of CuO NPs was carried out using *Aedes aegypti, Culex quinquefasciatus* larvae. Lethal concentrations LC_{50} and LC_{90} values of both mosquito vectors were calculated using SPSS software. The maximum mortality rate of *Culex quinquefasciatus* was found at a 15 ppm concentration of 100% and their LC_{50} values, LC_{90} values are 4.23 ppm and 13.67 respectively.

Keywords: *Aedes aegypti, Culex quinquefasciatus,* CuO NPs, *Pistia stratiotes* leaf extract

1. Introduction
Mosquito vectors are a serious threat to human health, causing diseases like dengue fever, chikungunya etc. In recent years, the death rate of peoples have gradually increased worldwide due to dengue fever [1]. The reason behind this type of human health problem is generated by the *Aedes aegypti (Ae. aegypti)* and *Culex quinquefasciatus (Cx. quinquefasciatus)*. These mosquitoes act as a carrier for dengue fever virus and filarial parasites, chikungunya, and zika infection [2-6]. Hence, to eradicate the growth rate of mosquito vectors a simple and cost effective method was adopted by the researchers. This force the researchers to come with a potential material having larvicidal activity against the *Ae. aegypti, Cx. quinquefasciatus* mosquito vectors.

Although, several work have been reported on biological activities of Gold, Silver, Copper nanoparticles [7-9]. The copper metal is a low-cost material and having a superior biological activity material equivalent to other metal and metal oxide nanoparticles. Therefore, the Cu based nanoparticles has been synthesized using various methods such as sol-gel method, electrochemical method, coprecipitation technique, and green synthesis method [10-13]. Among these methods, green synthesis method has been widely used to synthesize a nanoparticle to have an eco-friendly approach and uses the medicinal property of plants in effective manner.

Plant extract and its metabolites can act as an excellent source of reducing agent to synthesize a metal oxide nanoparticle for larvicidal activity and so on. So far, the copper oxide nanoparticles have been synthesized and reported using various leaf extracts, such as, *Aloe vera, Cissus quadrangularis, Spinacia oleracea,* and *Calotropis gigantean* etc. [14-17].

In this present work copper oxide nanoparticles (CuO NPs) were successfully synthesized using *Pistia stratiotes* aqueous leaf extract for larvicidal activity against *Ae. aegypti, Cu. quinquefasciatus* mosquito vectors. The results from larvicidal activity, it has to the potential of *Pistia stratiotes* aqueous leaf extract combined with copper oxide nanoparticles

which is a suitable material in addressing the problem of mosquito vectors.

2. Experimental Section

2.1 Preparation of leaf extract

The *Pistia stratiotes* leaves were collected from the local area, Tirunelveli. The collected leaves were washed with several times normal tap water and double distilled water to remove unwanted waste from leaves. Then, the washed leaves are subjected to a drying process under sunlight for one day. After that, the *Pistia stratiotes* leaves were cut and chopped into powder form. Subsequently, the 10 grams of leaf powder was immersed with 100mL of double distilled water. The *Pistia stratiotes* leaf powder solution was vigorously stirred using a magnetic stirrer under 80 °C temperature. Later on, the solution was filtered out to remove supernatant using Whatmann filter no.1 grade paper. The filtered leaf extract was stored at 4 °C temperature for further use.

2.2 Synthesis of CuO NPs

The 0.5M of copper sulphate precursor was dissolved in 100 mL of double distilled water. The precursor solution was rinsed using a magnetic stirrer. After, the 30 mL of prepared *Pistia stratiotes* leaf extract was added drop wise to the precursor solution. Then the precursor solution color was changed from blue to brown. Consequently, the solution was stirred using a magnetic stirrer under 80 °C temperature for one hour. Afterwards, a precipitate was formed at the bottom of the beaker. Next, the precipitate was washed with double distilled water to remove unreacted reagents. The collected precipitate was dried for one day and calcinated at 500 °C temperature. Finally, the CuO NPs were obtained.

2.3 Sample Characterization

The X-Ray Diffraction patterns of CuO NPs were obtained using a Bruker D8 Advance instrument with a 20°-80° angle. Optical absorption spectra of CuO NPs were recorded through the Shimadzu/UV 2600 Ultra-Violet Visible spectrophotometer. Functional groups of CuO NPs were ascertained to range from 400cm^{-1} to 4000cm^{-1} using the Thermo Nicolet FTIR instrument.

2.4 Bioassay fabrication

The larvicidal activity of *Pistia stratiotes* crude extract and CuO NPs was evaluated as per the method recommended by the World Health Organization (2005). The plant extract and CuO NPs were tested for larvicidal activity against *Ae. aegypti and Cx. quinquefasciatus*. Batches of 25 fourth instar larvae of *Ae. aegypti and Cx. quinquefasciatus* were transferred to small disposable test cups separately, each test cup containing 200 mL of distilled water. The appropriate volume of dilution was added to 200 mL of distilled water in the test cups to obtain the desired target dosage, starting with the lower concentration to higher concentration. The larval mortality was observed and recorded after 24 hours of post-treatment. Each test was replicated three times and an equal number of controls were set up simultaneously using tap water. The percentage of mortality was calculated using Abbott's formula [18].

2.5 Statistical calculation

The lethal concentration values of LC_{50} mortality in the population, LC_{90} mortality in the population, upper confidence limit (UCL), lower confidence limit (LCL) and Chi-Square (χ^2) values were analysed using statistical SPSS software version 20.

3. Results

To analyze a phase formation of synthesized CuO NPs using the X-Ray Diffractometer instrument. Figure 1 shows the XRD patterns of synthesized CuO NPs using *Pistia stratiotes* leaf extract. From this observed, diffracted peaks were evidenced of the phase pure CuO NPs with a monoclinic structure. The diffracted peaks of CuO NPs were well matched with the JCPDS card number (80-1916) [19]. The characteristic peaks of CuO NPs were located at 35.49° and 38.68°, which correspond to the (-111) and (111) hkl planes respectively. The average crystallite size of synthesized CuO NPs was calculated to be 12 nm using the Debye-Scherer relation.

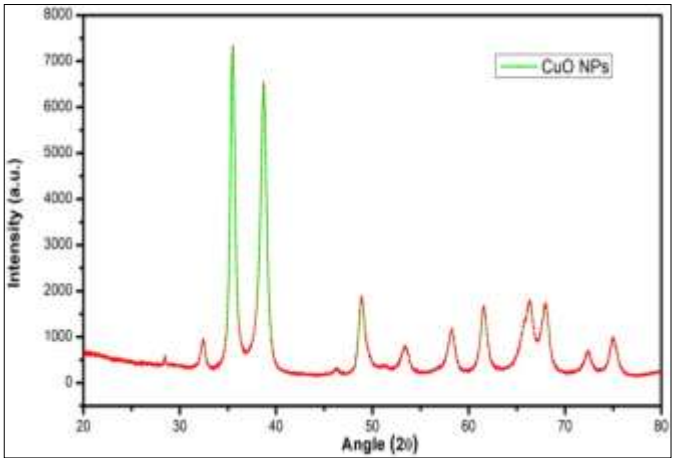

Fig 1: XRD peak of synthesized CuO NPs

The optical properties of synthesized CuO NPs were examined through the ultra-violet visible absorption (UV-Vis Abs) spectrophotometer which is shown in figure 2. The optical absorption band was present in the ultra-violet wavelength region around 277nm which is associated with the CuO NPs due to the reduction of copper sulphate precursor (Cu^{2+}) to CuO NPs [20].

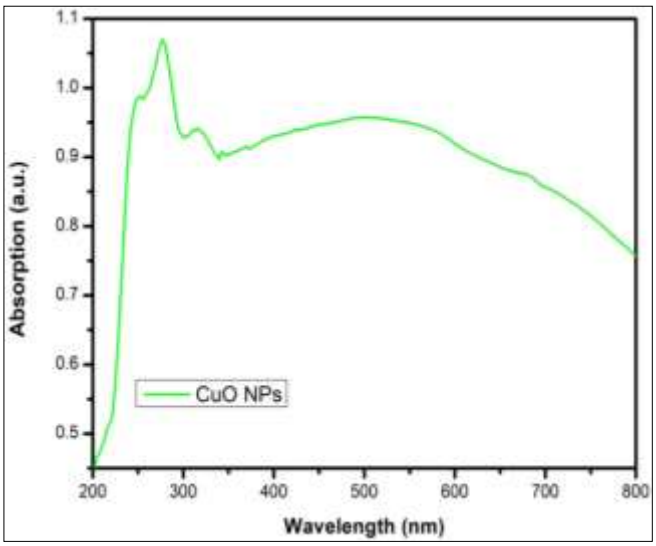

Fig 2: UV-Vis Absorption spectrum of synthesized CuO NPs

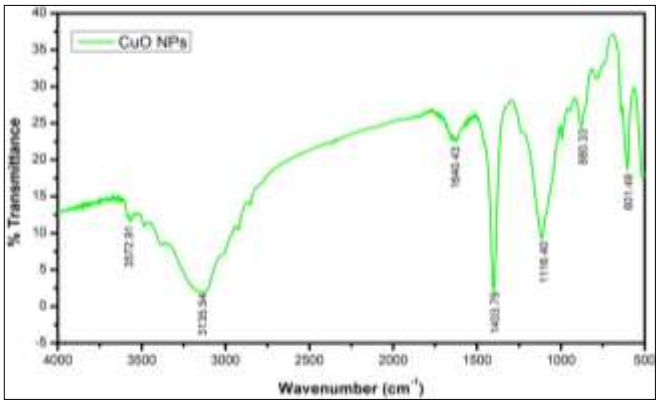

Fig 3: FTIR spectra of synthesized CuO NPs

Functional groups of synthesized CuO NPs were analyzed using Fourier Transform Infra-Red (FT-IR) spectroscopy displayed in figure 3. The metal-oxygen (Cu-O) bond of synthesized CuO NPs was confirmed around at 601.49 cm^{-1} [21]. The strong C=C bending, medium C=C stretching alkenes band of vibration corresponds to the 880.33cm^{-1} and 1640.43cm^{-1} respectively. The broad band of O-H stretching occurred at 3135.54cm^{-1}, responsible for the carboxylic acid group. Another O-H stretching vibration peak appeared at 3135.54 cm^{-1} associated with the alcohol group. The C-O stretching vibration and C-H bending vibration peaks arise at 1116.40 cm^{-1}, 1403.79cm^{-1} respectively.

The results of larvicidal bioassay carried out in the early fourth instar are larvae of *Ae. aegypti*, and *Cx. quinquefasciatus* treated with *Pistia stratiotes* leaf extract and synthesized CuO NPs. Initially, the *Ae. aegypti* and *Cx. quinquefasciatus* mosquito larvae were carried out using *Pistia stratiotes* leaf extract from 50 to 150 ppm; whereas, the same larvicidal activity was carried out using CuO NPs from 3 to 15 ppm concentration. From the observed results, lethal concentration values of LC_{50}, LC_{90}, and χ^2 values were calculated with corresponding leaf extract and CuO NPs concentration and tabulated in table 1-4. The data were significant at $p<0.05$

Table 1: Mortality rate of fourth instar larvae analysis using *Pistia Stratiotes* aqueous extract

Larvae	Concentration (ppm)	Mortality (% ±SD)	Lethal concentrations		χ^2 (df - 3)
			LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	
Ae. aegypti	Control	0±0.0	98.08 (88.69-109.68)	160.99 (154.97-195.01)	3.52
	50	11.2±0.71			
	75	29.6±0.91			
	100	51.2±2.1			
	125	70.4±0.71			
	150	88.88±0.56			
Cx. quinquefasciatus	Control	0±0.0	93.84 (88.60-99.27)	149.70 (137.97-166.43)	6.5
	50	12.00±1.68			
	75	26.40±1.07			
	100	51.20±2.2			
	125	72.00±1.63			
	150	90.40±1.7			

Table 2: The mortality rate of fourth instar larvae analysis using synthesized CuO NPs

Larvae	Concentration (ppm)	Mortality (% ±SD)	Lethal concentrations		X^2 (DF - 3)
			LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	
Ae. aegypti	Control	0±0.0	4.41 (1.48-6.01)	15 (13.71-20.53)	0.840
	3	36.8±1.0			
	6	53.8±1.5			
	9	65.8±1.3			
	12	80.2±2.2			
	15	93.01±1.8			
Cx. quinquefasciatus	Control	0±0.0	4.23 (1.48-6.01)	13.67 (13.71-20.53)	1.17
	3	41.5±1.6			
	6	60±1.9			
	9	75±0.5			
	12	86±0.9			
	15	100±0.0			

1. Each value (mean ± SD) represents mean value of three replicates
2. *Significant at $p<0.05$ level

Discussion

Figure 5 & 6 shows that the larvicidal activity using *Pistia stratiotes* leaf extract and CuO NPs with different concentrations against *Ae. aegypti, Cx. quinquefasciatus*. The mortality values of *Ae. aegypti, Cu. quinquefasciatus mosquito* vectors using leaf extract most probably equal (shown in figure 4). Hence, there are no major changes in mortality values that were observed in both mosquito vectors using *Pistia stratiotes* leaf extract. But, there is a considerable change in mortality values that were observed in both mosquito vectors using CuO NPs due to their average crystallite size about 12 nm.

The maximum mortality was found in synthesized CuO NPs for *Cx. quinquefasciatus* at a 15 ppm concentration of 100% and their LC_{50} values are 4.23 and LC_{90} values are 13.64. However, the *Ae. aegypti* mosquito vector had significant

mortality. The maximum mortality rate of *Ae. aegypti* was found at a 15 ppm concentration of 93% and their LC_{50} values, LC_{90} values are 4.41 ppm and 15 ppm respectively. Minimum mortality was noted in *Pistia stratiotes* aqueous leaf extract. In both, *Ae. aegypti* and *Cu. quinquefasciatus* their LC_{50} and LC_{90} values are (98.08, 160.09), (93.84, 149.70) respectively.

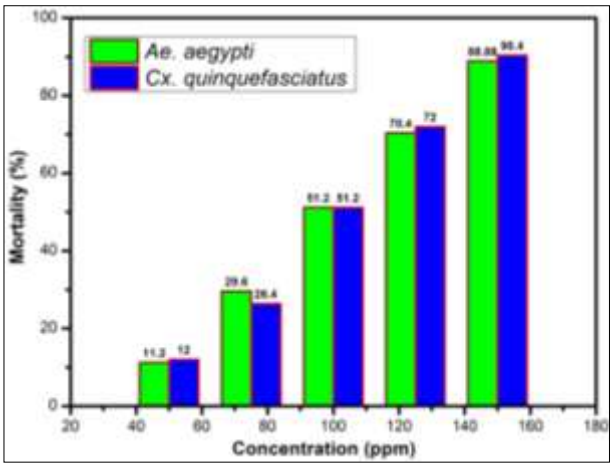

Fig 4: Larvicidal activity using leaf extract with different concentrations

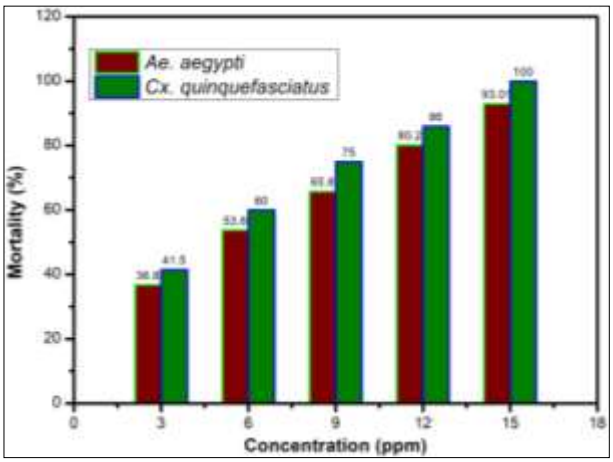

Fig 5: Larvicidal activity using CuO NPs with different concentrations

From these larvicidal activity results, CuO NPs has a maximum mortality value achieved at 15 ppm concentration comparative than the leaf extract for both *Ae. aegypti, Cu. quinquefasciatus* mosquito vectors. Moreover, the synthesized CuO NPs using *P. stratiotoes* has a potentially significant nanomaterial to control the growth rate of *Ae. aegypti* and *Cx. quinquefasciatus* mosquito vectors with the minimum amount of dosage compared with the previous reported literatures related to CuO NPs [22-25].

Conclusion

From this research work, it reports that the CuO NPs has been synthesized using *P. stratiotoes* aqueous leaf extract. The crystal profile and optical absorption band of CuO NPs were confirmed using an X-Ray Diffraction technique and UV-Vis absorption spectrometer instrument respectively. The evaluations of larvicidal activities were carried out using leaf extract and CuO NPs against *Ae. aegypti* and *Cx. quinquefasciatus* mosquito vectors. The significant mortality values of larvicidal activities were obtained. Therefore, the maximum mortality values can be achieved at low concentration (15 ppm) for both mosquito vectors using CuO NPs. These results show a notable mortality values obtained from CuO NPs.

Conflict of Interest

The authors declared that they have no known competing financial interests that could have appeared to influence the work reported in this research article.

Acknowledgement

We acknowledge the management of Sadakathullah Appa College (Autonomous) for their support during the conduct of the study.

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Appendix

PPM: Parts per million
Hrs: Hours
LC_{50}: Lethal Concentration that kills 50% of the exposed larvae
LC_{90}: Lethal Concentration that kills 90% of the exposed larvae
UCL: Upper Confidence Limit
LCL: Lower Confidence Limit
ºC: Degree Celsius
$\chi 2$: Chi-square