"BIO-NANO FUSION: *MUSA SAPIENTUM* SYNTHESIZED COPPER NANOPARTICLES FOR FILARIAL AND DENGUE VECTOR CONTROL"

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Abstract

In this work, copper nanoparticles (CuNPs) were synthesized through plant-mediated means using aqueous leaf extract from *Musa sapientum*. The larvicidal efficacy of the CuNPs against *Aedes aegypti* and *Culex quinquefasciatus* mosquito vectors was evaluated. The findings showed that the CuNPs with the highest death rate had the lowest LC_{50} and LC_{90} values for larvicidal activity against *C. quinquefasciatus* and *A. aegypti*. These values were for the following: second instar 1114.35 ppm and 263.82 ppm; third instar 4250.50 ppm and 502.04 ppm; fourth instar 9542.06 ppm and 863.73 ppm; second instar 17739.74 ppm and 1402.66 ppm; third instar 38709.82 ppm and 2729.19 ppm; fourth instar 67403.51 ppm and 5287.68 ppm, respectively. The results unequivocally demonstrated the potential larvicidal and environmentally benign use of biosynthesized CuNPs.

Keywords: *Musa sapientum,* CuNPs, *Aedes aegypti* , *Culex quinquefasciatus*

Introduction

Vector-borne infections are responsible for more than 17% of all infectious diseases. Each year, mosquito-borne illness infects up to 700 million people and kills more than a million [1]. Numerous mosquito-borne illnesses, including West Nile, dengue, Chikungunya, Zika, malaria, Japanese encephalitis, filariasis, and others, have claimed millions of lives globally and pose a threat to millions more(2).This kind of human health issue is caused by *C.*

quinquefasciatus (*C. quinquefasciatus*) and *A. aegypti* (*A. aegypti*). These mosquitoes transmit the dengue fever virus, chikungunya, zika, and filarial parasites [3-7].

Mosquitoes control chemical Insecticides have long been used to control insect vectors, particularly during outbreaks of vector-borne diseases. To suppress an outbreak of dengue and/or dengue hemorrhagic fevers, many insecticides have been employed to reduce the number of *A. aegypti,* the dengue virus's biological vector. Temephos is the most commonly used chemical insecticide in household water containers to combat *A. aegypti* larvae. Although temephos is highly efficient against mosquito larvae, it is low in toxicity to humans. However, it has been noted that continual intake may have harmful consequences for people. Furthermore, improper usage of this insecticide can lead to insecticide resistance in the vector [8].

Nanoparticles (NPs) are thought to be the essential building blocks of nanotechnology. The nanotechnology branch is interdisciplinary, encompassing physics, biology, chemistry, medicine, and material science. Currently, production of metal nanoparticles has been reported by numerous physical and chemical methods [9]. The green and/or bio-route to generating these nanoparticles (NPs) is the ideal approach for biological studies. For example, plant extracts that function as reducing agents for metal ions can remain on metal NPs as a capping agent, improving biological activity. There is a growing interest in the probable exploitation of metal nanoparticles in biomedical applications due to their exceptional biocompatibility and intrinsic antibacterial characteristics [10,11].

Copper nanoparticles with various structural characteristics and effective biological effects can be synthesized utilizing innovative green techniques [12]. Several studies have found that the bioactive properties of green-mediated copper nanoparticles increase environmental mobility while also allowing them to interact intimately with and permeate bacterial membranes. Copper nanoparticles were generated utilizing various plant extracts such as tea leaf [13]. Various plant extracts have been used to Synthesize copper nanoparticles [14]. In this study, we focused on the biosynthesis of copper nanoparticles to control mosquito vectors in an eco-friendly.

Since environmental issues are becoming a global issue, green, environmentally friendly solutions in green chemistry and Nanotechnologies are becoming more and more popular and necessary [15]. One of the herbaceous plants in the Musaceae family is *Musa sapientum*, popularly known as the "banana." According to [16], in India, it has been used to treat diabetes, stomach ulcers, hypertension, diarrhea, and dysentery. Studies by [17] and [18] have established the anti-diabetic effects of leaves, stem, fruit, root, and flower. According to studies [19,20], green fruits contain anti-ulcerative qualities. In addition, *Canna indica L. var. speciosa* is used with *Musa sapientum* to alleviate heavy menstruation [21]. The fruit of *M. sapientum* showed antimicrobial properties [22].

Therefore, the goal of this research is to synthesize CuNPs on a wide scale by green chemistry using crude banana peel (*Musa sapientum*) extraction. Then, using an X-ray diffraction (XRD), scanning electron microscopy (SEM), and UV-visible spectrophotometer, the physical characteristics of the Synthesized CuNPs and then evaluated their larvicidal effectiveness against *C. quinquefasciatus* and *A. aegypti*.

Materials and Methods

Collection and preparation of leaf broth

The leaves of *Musa sapientum* were gathered from the Steel Plant in Salem, Tamil Nadu, India. The leaves of *Musa sapientum* were cleaned with tap water, dried in the shade in a typical environment, and then ground into a powder. In order to make the broth solution, 5 g of leaf powder and 100 mL of sterile distilled water were combined in a 300 mL Erlenmeyer flask. The liquid was then boiled for 5 minutes before being eventually decanted. Within a week, they were used after being kept at 4^{o}C.

Synthesis of copper nanoparticles

At room temperature, 190 milliliters of an aqueous solution containing 5 milligrams of copper sulphate (CuSo4.5H2o). The solution mentioned above was mixed with an aqueous leaf extract of *Musa sapientum*. For the next twenty-four hours, this was left at room temperature. Characterization techniques were employed to examine the impact of temperature and reaction time on the synthesis rate, as well as the size and shape of the resulting copper nanoparticles. Using 5 mM Cu^{+}, the investigation was conducted. Purification of the resulting copper

nanoparticle solution involved redispersion of the pellet in deionized water after a series of centrifugations at 6000 rpm for 15 minutes.

Characterization of copper nanoparticles

A UV-3600 Shimadzu spectrophotometer running at a resolution of 1 nm was used to record UV-vis spectra. Energy dispersive X-ray spectroscopy and a 10 kV Ultra High Resolution Scanning Electron Microscope (FEI QUANTA-200 SEM) were used to study the structure and content of the purified Cu particles after they had been freeze dried. The crystalline structure of copper nanoparticles was ascertained by X-ray diffraction utilizing Cu kα radiation (PAN analytical X'pert Pro MPD diffractometer). A Philips Model PW 1050/37 diffractometer, operating at 40 kV and 30 mA with a step size of 0.02° (2θ), was used for the powder X-ray analysis.

Collection of Eggs of Mosquitoes

Water bodies and water-storage containers in and around Salem areas were used to gather *C. quinquefasciatus* and *A. aegypti* eggs for bioassay. Once these eggs arrived at the lab, they were placed in enamel trays with 500 milliliters of water and left until the larvae hatched.

Larval Toxicity Test

For larvicidal action, laboratory colonies of mosquito larvae were employed. At concentrations of 10,000 ppm, 5,000 ppm, 2,500 ppm, and 1,250 ppm, the copper nanoparticles were assessed. The control was distilled water that had not been treated. There were three sets of each treatment. To maintain batch uniformity, 25 actively swimming *C. quinquefasciatus* and *A. aegypti* larvae were sieved out of separate rearing trays and exposed to 100 ml concentrations of copper nanoparticles, with an untreated control held in a separate plastic container with a capacity of 250 ml (WHO, 1996). After a 24-hour exposure, the larvae were probed with a needle to determine the larvae's mortality rate; moribund larvae were tallied as dead [23]. Abbott's formula (1925) was used to adjust the control mortalities.

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical Analysis

An analysis of variance (ANOVA) was performed on all the data. By applying a profit regressing model to the observed relationship between the substance's logarithmic concentration and the percentage mortality of larvae, the LC_{50} and LC_{90} values as well as their 95% confidence limits were calculated. The 90% confidence limit for LC_{50} and LC_{90} was computed using a heterogeneity factor in the event of a large departure. Version 16.0 of the SPSS software was used for all analyses.

Results

An investigation was conducted into the biosynthesis of CuNPs through the reduction of CuSO4.5H2O utilizing plant extract from *Musa sapientum*. In order to prevent further agglomeration, the reducing agents can reduce $Cu2^{+}$ and cap to the copper nanoparticles. After the reaction vesicles reacted for 24 hours, a bright yellow to dark yellow tint appeared, indicating the synthesis of CuNPs.

Uv-Visible Absorption Spectroscopy Studies

The sample comprising 200 milliliters of plant extract exposed to 5 milligrams of $CuSO_4$ for a 24-hour reaction time was used to measure the UV absorption of copper nanoparticles (Figure 1). The sample's greatest absorption peak had an average wavelength of 641 nm and ranged from 300 to 700 nm. When compared to the other reaction durations, the impact of CuSO4 (5 mM) on the conversion and particle size of copper nanoparticles treated for 24 hours at room temperature in 200 ml of plant extract was shown to be optimal.

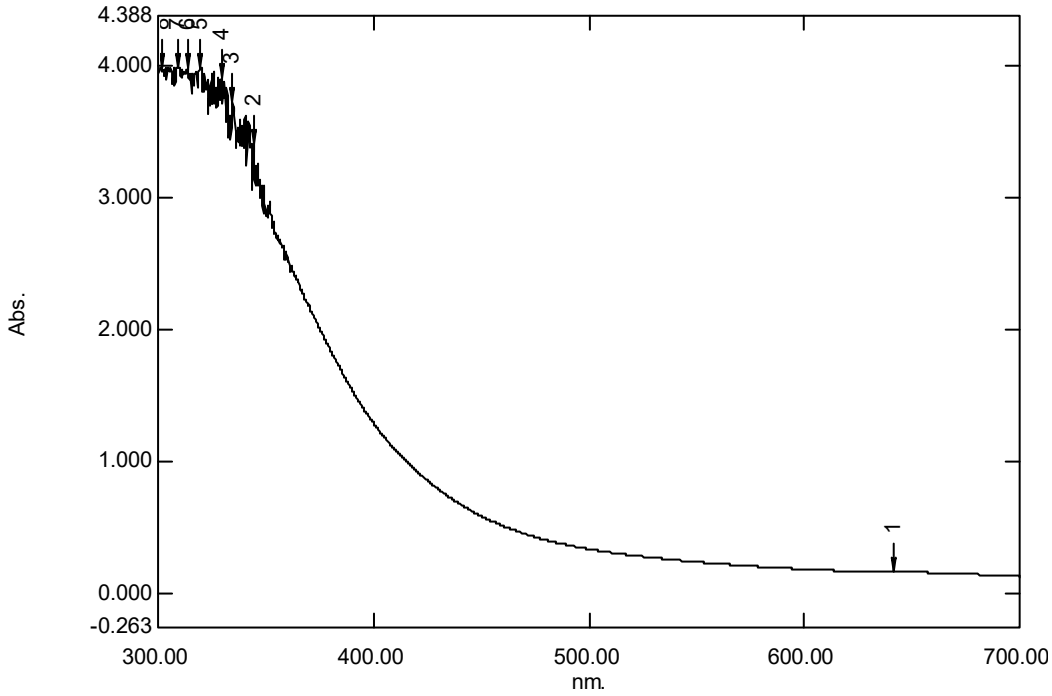

Fig.1. Ultraviolet-visible spectra recorded of copper nanoparticles synthesized using *Musa sapientum*.

X- Ray Differaction Studies

The x-ray diffraction technique was used to examine the biosynthesized copper nanoparticles using plant extract (Figure 2). The diffractometer was set to operate at 40kv and 30Ma, with a step size of 0.020. The scanning was carried out for 2/ in the range of 100 to 800. The X-ray diffraction examination verified that the cu nanoparticles are crystalline. The CU NPs caused the intense diffraction peaks at 2θ values of 23.0138, 34.0690, 35.8468, 38.8285, 53.7280, 54.7161, 56.4142, 61.7661, 62.8064, 63.6040, and 68.4804 degrees, which correspond to the face centered cubic crystal structure's (021), (002), (111), (022), (132), (061), (151), (200), (043), and (221) facets. The development of nanoparticles is shown by the broadening of the Bragg peaks. Apart from the Bragg peaks that symbolize the many aspects of the face. Additionally, unassigned peaks in the center of the copper nanocrystals were seen,

indicating that the bioorganic phase is crystallized on the copper nanoparticles' surface. XRD peaks that matched the JCPDS: 35-0505 of copper indicated that the copper nanoparticles were crystalline.

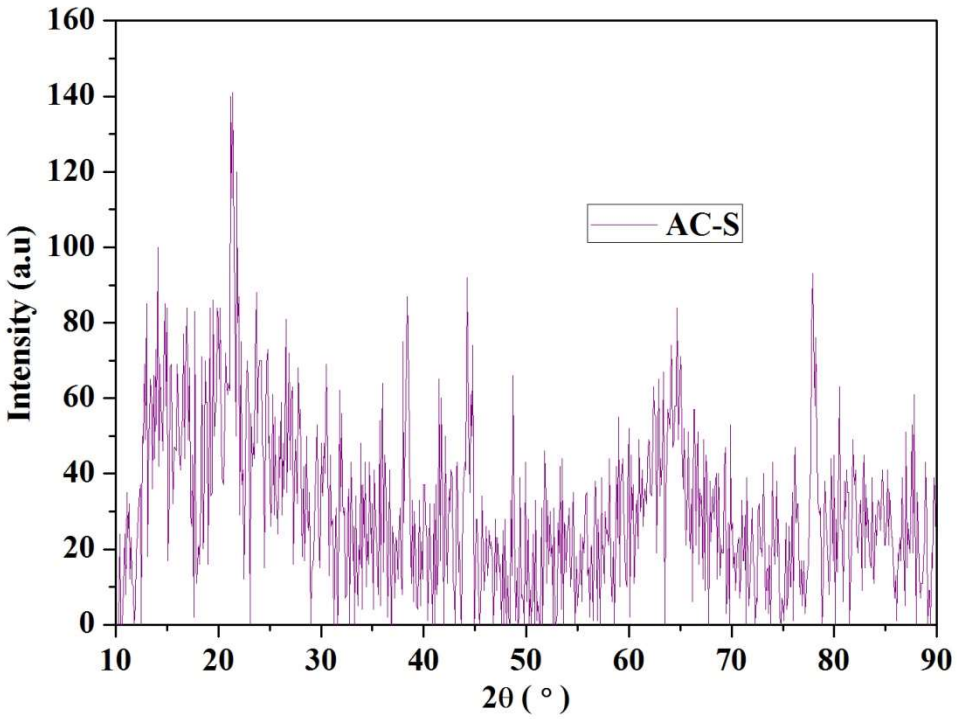

Fig. 2. XRD pattern of synthesized copper nanoparticles using *Musa Sapientum*.

Scanning Electron Microscopy (SEM) Studies

The size and form of the Cu nanoparticles were seen using the SEM method. SEM pictures of Cu^{2+}nanoparticles made from a room-temperature extract of the *Musa sapientum* plant with a 24-hour reaction time are displayed in Figures 3. The FEI QUANTA-200 SEM, a 10kV extremely high resolution scanning electron microscope, was employed. SEM grids were made by applying a drop of the particle solution to a copper grid covered with carbon and drying it under a lamp. The creation of copper nanoparticles and the SEM study's morphological measurements showed that the average size was between 500 and 700 nm, with

interparticle spacing **(Figure 3, 4µm view**). The copper nanoparticles that were seen had a spherical form.

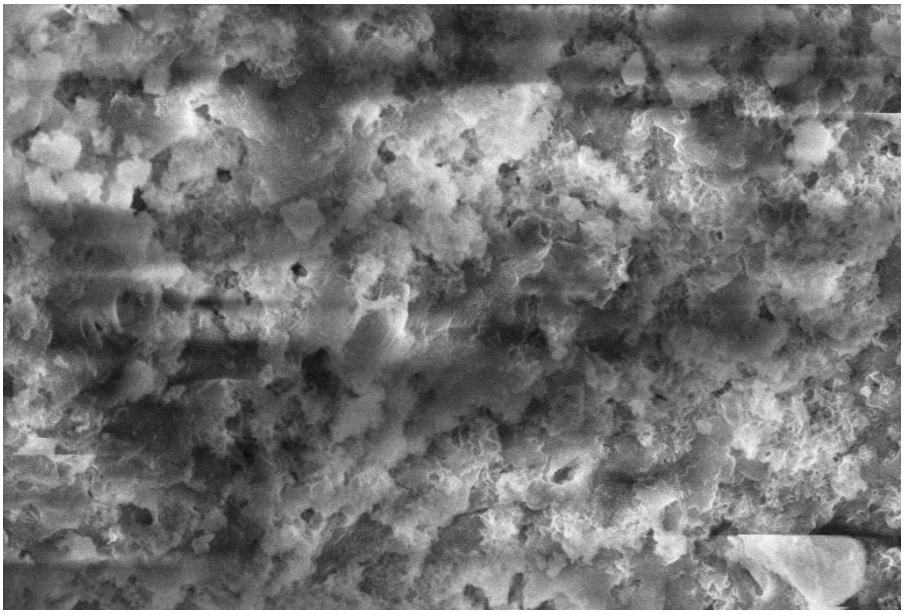

Fig.3. SEM image of copper nanoparticles (4µm view) synthesized using *Musa Sapientum.*

Larvicidal Activity of Copper Nanoparticles

The mosquito vectors, *C. quinquefasciatus* and *A. aegypti*, have larval (II, III, and IV) mortality statistics shown in Tables 1, 2, 3, 4, 5, and 6 following treatment with copper nanoparticles at varying doses (1,250 ppm, 2,500 ppm, 5,000 ppm, 10,000 ppm). All larval stages showed the highest mortality at 10,000 ppm concentration. When the concentration was lower, the observed death rate was mild. *A. aegypti* is less successful than *C. quinquefasciatus* when it comes to the synthesis of CuNPs utilizing plant extract from *Musa sapientum*. Insect toxicity is contingent upon the quantities of nanoparticles.

The mortality rate of *C. quinquefasciatus* larvae in their second, third, and fourth instars after they were subjected to varying concentrations of an aqueous solution containing a pure pellet of CuNPs (1,250 ppm, 2,500 ppm, 5,000 ppm, and 10,000 ppm ppm) is shown in Tables 1, 2, and 3. The LC_{50} values for *C. quinquefasciatus* after a 24-hour exposure were 1114.35 for the II instar, 4250.50 for the III instar, and 9542.06 for the IV instar; the LC_{90} values were 104156.52 for the II instar, 1090493.42 for the III instar, and 1058581.99 for the IV instar. The LC_{50} values at 48 hours of exposure were 263.82 (II instar), 502.04 (III instar), and 863.73 (IV

instar); the LC_{90} value indicates 1537.11 (II instar), 4480.71 (III instar), and 8774.78 (IV instar). There was no discernible mortality in the control group.

Table 1. Larvicidal efficacy of green synthesized copper nanoparticles on 2nd instar larvae of *C. quinquefasciatus.*

S. No	Exposure Time	Mortality (Mean ± SD)					LC_{50} (ppm)	LC_{90} (ppm)	$\chi 2$
		Copper nanoparticles concentrations (ppm)							
		Control	1250ppm	2500ppm	5000ppm	10000ppm			
1	24 hrs	0.00± 0.00	51.66± 7.63	56.66± 5.77	70.00± 5.00	71.66± 5.77	1114.35	104156.52	0.93
2	48 hrs	0.00± 0.00	88.33± 7.63	93.33± 2.88	98.33± 2.88	100.00± 0.00	263.82	1537.11	1.05

Table 2. Larvicidal efficacy of green synthesized copper nanoparticles on 3rd instar larvae of *C. quinquefasciatus*

S. No	Exposure Time	Mortality (Mean ± SD)					LC_{50} (ppm)	LC_{90} (ppm)	$\chi 2$
		Copper nanoparticles concentrations (ppm)							
		Control	1250ppm	2500ppm	5000ppm	10000ppm			
1	24 hrs	0.00±0.00	40.00±5.00	41.66±2.88	55.00±5.00	56.66±5.77	4250.50	1090493.42	1.08
2	48 hrs	0.00±0.00	73.33±2.88	80.00± 5.00	88.33± 5.77	98.33± 2.88	502.04	4480.71	3.25

Table 3. Larvicidal efficacy of green synthesized copper nanoparticles on 4th instar larvae of *C. quinquefasciatus*

S. No	Exposure Time	Mortality (Mean ± SD)					LC_{50} (ppm)	LC_{90} (ppm)	χ^2
		Copper nanoparticles concentrations (ppm)							
		Control	1250ppm	2500ppm	5000ppm	10000ppm			
1	24 hrs	0.00± 0.00	28.33± 2.88	36.66± 2.88	43.33± 2.88	50.00± 5.00	9542.06	1058581.99	0.07
2	48 hrs	0.00± 0.00	55.00± 5.00	76.66± 2.88	83.33± 5.77	90.00± 5.00	863.73	8774.78	1.58

According to Figure 4, the observed LC_{50} values indicate that the three larval stages of *C. quinquefasciatus* exhibit varying degrees of AgNP susceptibility, with the second instar being more susceptible than the third and fourth instars.

The larval mortality of *A. aegypti* (second to fourth instar larvae) following treatment at varying concentrations of copper nanoparticles is presented in Tables 4, 5, and 6. The LC_{50} and LC_{90} values of CuNPs against *A. aegypti* vector mosquitoes' larvae in their second to fourth instar were as follows: The LC_{50} values during a 24-hour exposure were 17739.74 ppm (II instar), 38709.82 ppm (III instar), and 67403.51 ppm (IV instar); the LC_{90} values were 1077121.24 ppm (II instar), 2734676.36 ppm (III instar), and 5811843.33 ppm (IV instar). At a 48-hour exposure, the LC_{50} values were 1402.66 ppm (II instar), 2729.19 ppm (III instar), and 5287.68 ppm (IV instar); the LC_{90} values were, in turn, 11854.72 ppm (II instar), 41350.18 ppm (III instar), and 96206.34 ppm (IV instar). CuNPs were reported to have a moderately high susceptibility in *A. aegypti* larval stages. There was no mortality seen in the control group. The observed LC_{50} values have demonstrated the degree of CuNP susceptibility among the three *A. aegypti* larval stages in the following order: second instar > third instar > fourth instar (figure.5).

Table 4. Larvicidal efficacy of green synthesized copper nanoparticles on 2nd instar larvae of *A. aegypti*

S. No	Exposure Time	Mortality (Mean ± SD)					LC_{50} (ppm)	LC_{90} (ppm)	$\chi 2$
		Copper nanoparticles concentrations (ppm)							
		Control	1250ppm	2500ppm	5000ppm	10000ppm			
1	24 hrs	0.00±0.00	20.00±5.00	26.66±2.88	36.66±2.88	41.66±2.88	17739.74	1077121.24	0.26
2	48 hrs	0.00±0.00	46.66±2.88	65.00±5.00	76.66±2.88	88.33±7.63	1402.66	11854.72	0.17

Table 5. Larvicidal efficacy of green synthesized copper nanoparticles on 3rd instar larvae of *A. aegypti*

S. No	Exposure Time	Mortality (Mean ± SD)					LC_{50} (ppm)	LC_{90} (ppm)	$\chi 2$
		Copper nanoparticles concentrations (ppm)							
		Control	1250ppm	2500ppm	5000ppm	10000ppm			
1	24 hrs	0.00±0.00	13.33±2.88	23.33±2.88	26.66±2.88	33.33±5.77	38709.82	2734676.36	0.77
2	48 hrs	0.00±0.00	33.33±2.88	51.66±2.88	61.66±2.88	71.66±2.88	2729.19	41350.18	0.76

Table 6. Larvicidal efficacy of green synthesized copper nanoparticles on 4th instar larvae of *A. aegypti*

S. No	Exposure Time	Mortality (Mean ± SD)					LC_{50} (ppm)	LC_{90} (ppm)	$\chi 2$
		Copper nanoparticles concentrations (ppm)							
		Control	1250ppm	2500ppm	5000ppm	10000ppm			
1	24 hrs	0.00±0.00	11.66±2.88	18.33±2.88	23.33±2.88	28.33±2.88	67403.51	5811843.33	0.22
2	48 hrs	0.00±0.00	26.66±2.88	36.66±2.88	48.33±2.88	61.66±2.88	5287.68	96206.34	0.05

The mortality rate of all larval forms dropped as the concentration of nanoparticles decreased. The positive association between the rate of mortality (Y) and the concentration (X) of the synthesized CuNPs indicates that a concentration-dependent mortality was clearly detected using the developed regression equations. The results of this investigation unmistakably show that copper nanoparticles may be able to effectively suppress *A. aegypti* and *C. quinquefasciatus* larvae. The precise processes underlying CuNPs larvicidal action remain unknown. However, it is likely that the delicate larvae die as a result of the CuNPs penetrating through the larval barrier.

Discussion

The study of designing, creating, and modifying the structure of particles smaller than 100 nm is known as nanotechnology. By combining biological concepts with physical and chemical methods, bio-nanotechnology creates nanoparticles that have particular uses. It also offers a cost-effective alternative to physical and chemical processes for the synthesis of nanoparticles[24]

According to Abdul Hameed et al. [25], one such method is biological synthesis, where reducing and capping agents like bacteria, fungi, actinomycetes, yeast, and plants are used. Compared to chemical synthesis, very little literature is available on the biosynthesis of Cu NPs. Formation of copper nanoparticles from copper sulfate carried by reaction with leaf broth was followed by color change, and the intensity of change was recorded by UV-vis spectroscopy.

In the UV range of 560–640 nm, Saranyaadevi et al. [26] discovered that the formation of Cu NPs was confirmed by the peak at 531 nm; thus, it is confirmed with the current finding showing a peak at the same range. With an increase in particle size, it was discovered that the peak value gradually declined. According to Hariprasad et al. [27], copper nanoparticle surface plasmon vibrations created a peak at or around 562 nm. The UV range in the current study was 641 nm on average, ranging from 300 to 700 nm.

CuNPs are confirmed to be amorphous by the large halo seen in the X-ray diffraction pattern at approximately 2θ = 20–40°. A previous study [28]discovered that the XRD spectrum at two distinct diffraction peaks at 39.1° and 68.3° region and diffraction peaks acquired at 2θ angle. The study reveals that CuNPs cause significant diffraction peaks at 2θ values of 23.0138, 34.0690, and 35.8468, 38.8285, 53.7280, 54.7161, 56.4142, 61.7661, 62.8064, 63.6040 and 68.4804 degree were corresponding to (021), (002), (111), (022), (132), (061), (151), (113), (200), (043), and (221).

AgNPs made from *M. oleifera* seed extract were found to be extremely effective in a previous study against *A. aegypti* juvenile instars, with LC_{50} values of 10.24 ppm (I instar), 11.81 ppm (II instar), 13.84 ppm (III instar), 16.73 ppm (IV instar), and 21.17 ppm (pupae). Likewise, an increasing quantity of biofabricated nanoparticles demonstrated similar toxicity against several mosquito vectors, such as *Aegypti* (Suresh et al., 2015). The study found that the LC_{50} values of *A. aegypti* were 17739.74 ppm (II instar), 38709.82 ppm (III instar), and 67403.51 ppm (IV instar) after 24 hours of exposure, and 1402.66 ppm (II instar), 2729.19 ppm (III instar), and 5287.68 ppm (IV instar) after 48 hours.

When exposed to aqueous solutions containing purified Ag NP pellets at varying concentrations (10, 15, and 20 ppm) for a 24-hour period, the mortality rate of third- and fourth-instar larvae of *C. vishnui* and *C. quinquefasciatus* was found to be 80% and 76%, respectively, for the 20 ppm concentration. Anjali [29] revealed that the death rates in the fourth instar of *C. vishnui* and *C. quinquefasciatus* were 88% and 82.68%, respectively, at a concentration of 20 ppm, which is higher than those of 10 and 15 ppm. According to the current study, *C. quinquefasciatus's* LC_{50} values at 24 hours of exposure was 1114.35 (II

instar), 4250.50 (III instar), and 9542.06 (IV instar); during 48 hours of exposure, the LC_{50} values were 263.82 (II instar), and 502.04 (IV instar).

The rate of mortality of 3rd and 4th instar larvae of *C. vishnui* and *C. quinquefasciatus* when they were exposed to grade concentrations (10, 15, 20 ppm) of aqueous solution of purified pellet of Ag NP for 24 hours the 20 ppm concentration caused 80% and 76% mortality in 3rd instar of *C. vishnui* and *C. quinquefasciatus* respectively. While 88% and 82.68% mortality in 4th instar *C. vishnui* and *C. quinquefasciatus* at 20 ppm respectively which is highest than 10 and 15 ppm concentration was reported by Anjali [29]. In the present study, at 24 hours exposure LC_{50} value of *C. quinquefasciatus* shows 1114.35 (II instar), 4250.50 (III instar), 9542.06 (IV instar) and at 48 h exposure the LC_{50} values were 263.82 (II instar), 502.04 (III instar), 863.73 (IV instar) ppm respectively. The comparison of these two results clearly shows that the mortality rate with increasing concentration and duration exposure. All larval forms had a lower mortality rate as the concentration of nanoparticles dropped. It is evident from this work that copper nanoparticles have the potential to effectively suppress *A.aegypti* and *C. quinquefasciatus* larvae.

Conclusion

The copper nanoparticles UV absorption, measured after 200 ml of plant extract was exposed to 5 mM $CuSO_4$ for a 24-hour reaction time, revealed a maximum absorption peak that ranged from 300 to 700 nm, with an average of 641 nm. The results of the X-ray diffraction investigation verified that the Cu nanoparticles are crystalline. The copper nanoparticles that were seen had a spherical form. Excellent larvicidal effectiveness against *C. quinquefasciatus* and *A. aegypti* is exhibited by the produced CuNPs. The mortality rate rose as the length of time that copper nanoparticles were exposed to. The concentration of the Cu nanoparticles was directly correlated with the death rate. In the control treatment, there was no mortality noted. We highly advise using CuNPs generated by *Musa sapientum* to inhibit the vectors of *C. quinquefasciatus* and *A. aegypti* mosquitoes.

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