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Biosynthesis of Copper Nanoparticles using *Artocarpus heterophyllus* against Dengue Vector *Aedes aegypti*

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ABSTRACT

In our present study, we accounted for eco-friendly biosynthesis of copper nanoparticles using aqueous leaves extract of *Artocarpus heterophyllus* against first to fourth instar larvae of *Aedes aegypti.* The synthesized CuNPs were characterized by UV, XRD, FTIR and SEM analyses were clearly distinguishable. The four different immature mosquito larval stages of *A. aegypti* were exposed to varying concentrations of aqueous leaf extract of *A. heterophyllus,* copper sulphate ($CuSO_4$) and synthesized copper nanoparticles (CuNPs) for 24 h. The mortality was observed at aqueous extract (LC_{50}= 48.40, 60.55, 70.36 and 82.79 mg/ml), CuSO4 (LC_{50}=21.81, 26.92, 41.38 and 55.12 mg/ml) synthesized CuNPs against first to fourth instars of *A. aegypti* (LC_{50}= 3.85, 4.24, 4.66 and 5.08 mg/ml), respectively. The novel properties created not only improve the quality of human's life; it also helps in saving energy and environment.

Key-words: *Aedes aegypti, A. heterophyllus,* Aqueous leaf extract, Copper nanoparticles, Jackfruit

INTRODUCTION

Dengue is a viral infection transmitted between humans by *Aedes* mosquitoes as a vector [1] Dengue is prevalent in more than 100 countries as it threatens the health of approximately 2.5 billion people [2] and around eighty million people are infected annually at an attacking rate of 4% worldwide. In India, 7–16 thousand cases of dengue are reported annually. Thus, it led to development of new vector-control strategies, anti-virals and vaccines that can positively impact on dengue control and prevention [1].

In later years, synthetic insecticides in mosquito control programme has been reduced due to lack of novel insecticides, concern for environmental sustainability, high cost, harmful effect on human health, other non-target populations, their non-biodegradable nature, higher rate of biological magnification through the food chain, and increasing insecticide resistance on a global scale [3]. Fusion of the herbal extracts into novel systems have certain advantages, such as their bulk dosing and less absorption can be reduced, which is a major problem being faced, enticing the attention of major pharmaceutical corporations.

Medicinal plant products are proposed to be more efficient and rapid in extracellular biosynthesis of nanoparticles. Recently, elsewhere reported plant fabricated nanoparticles have been studied for their highly effective mosquitocidal properties. The green biosynthesis of nanoparticles is advantageous over chemical and physical methods since it is cheap; it is a single-step process and does not require high pressure, energy, temperature, and the use of highly toxic chemicals [4].

Jackfruit (*A. heterophyllus*) belongs to the family Moraceae. They grow abundantly in India, Bangladesh and in many parts of Southeast Asia. *A. heterophyllus* is

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an important evergreen tree in tropical areas and widely grown in Asia including India [5]. It has also several chemical constituents such as artocarpesin, artocarpin, cynomacurin, isoartocarpin and norartocarpetin [6]. The medicinal properties of jackfruit include anti-inflammatory, anti-asthmatic, antioxidant, antimicrobial, antiviral, anti-tubercular, anticancer and anti-malarial activities [7]. The present study dealed with biosynthesis of CuNPs by using jackfruit leaf aqueous extracts against dengue vector.

MATERIALS AND METHODS

Preparation of Aqueous leaves extract of *A. heterophyllus*- The jackfruit leaves were collected from our university campus and an identity authenticity by plant taxonomists. The leaves were washed thoroughly to remove impurities and under shade dried for about one week to remove the moisture. The leaves powdered in a mixer and then sieved using 20 mesh size sieves to get uniform size range. The aqueous extract was prepared by mixing 10 g of dried leaf powder with 100 ml distilled water at 60°C for 10 min and filtered through Whatman No. 1 filter paper.

Synthesis of copper nanoparticles- To synthesize CuNPs, an Erlenmeyer flask containing 100 ml of 5 mM $CuSO_4$ was magnetically stirred for 3 hrs. Following this, 60 ml of the aqueous extract of *A. heterophyllus* was added with 40 ml of 5 mM $CuSO_4$ at room temperature and was subsequently stirred for 24 hrs [8].

Characterization of Copper nanoparticles- The bioreduction was observed using UV spectra, which was done at regular intervals at 1 nm resolution. The solution mixture was subjected to centrifugation at 5,000 rpm for 15 min; resulting pellet was dissolved in distilled water and filtered through 0.22 µm millipore filter. The synthesized CuNPs were used for X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM).

Mosquito rearing- The rearing and collection of *A. aegypti* larvae were done based on the method performed by Kamaraj *et al.* [9]. *A. aegypti* larvae were collected from water bottles, tanks, containers and small water courses. They were kept at a temperature of 28±2°C and 80±10% RH (Relative Humidity) under the 12-hour light and dark photoperiod cycle. The larvae were fed dog biscuit and a brewer's yeast powder mixture 3:1 ratio is used in the laboratory. After five days, adult male mosquitoes were fed with 10% sucrose solution. The emerging female mosquitoes obtained blood meal from a white albino rat for 2-3 hrs for eggs production.

Larvicidal bioassay- Larvicidal activity was assessed by the procedure of WHO [10] with slightly few modifications as per the method of Rahuman *et al*. [11].

Statistical Analysis- Mean percent larval mortality data were subjected to analysis of variance and compared with Duncan's multiple range tests to determine any differences between plant species and within species and concentration (SPSS, 2007). LC_{50} and their associated confidence intervals were estimated from 24 h concentration mortality data using probit analysis [12]. There was a strong relationship between the doses and death rate of parasites showed in the linear correlation (r^2) and all differences were considered significant if P≤0.05.

RESULTS

UV–Vis spectrum analysis- Absorption spectrum of synthesized CuNPs with leaf aqueous extract of *A. heterophyllus* at different wave lengths ranging from 450 to 900 nm revealed a peak at 640 nm.

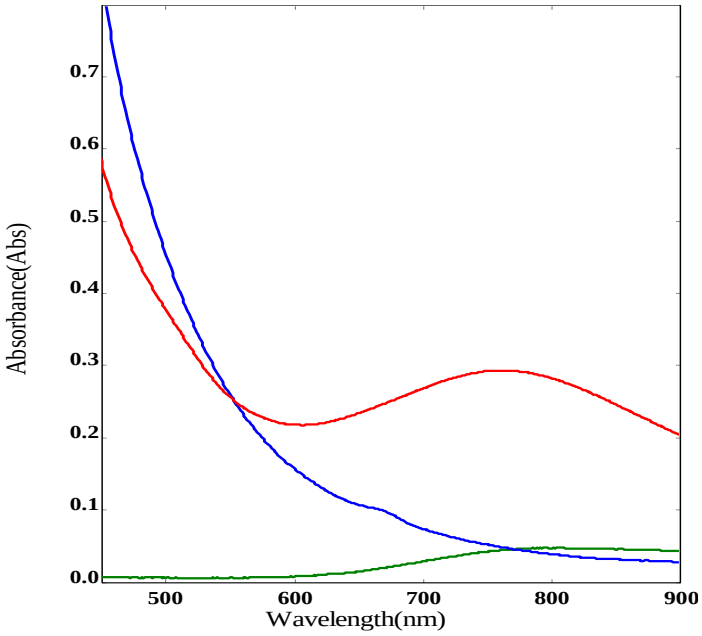

Fig. 1: UV–Vis spectra of synthesized CuNPs using aqueous leaf extract of *A. heterophyllus*

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X- ray diffraction- The sample demonstrated a high crystallinity level with diffraction angles of 26.35°, 35.02° and 40.24°, which corresponded to the characteristic face centred cubic of copper lines indexed at 111, 200 and 222, respectively (Fig. 2).

FTIR Analysis- FTIR spectra of copper nanoparticles exhibited prominent peaks at 3428, 2925, 1614, 1382, 1103 and 655.8 cm^{-1} (Fig. 3).

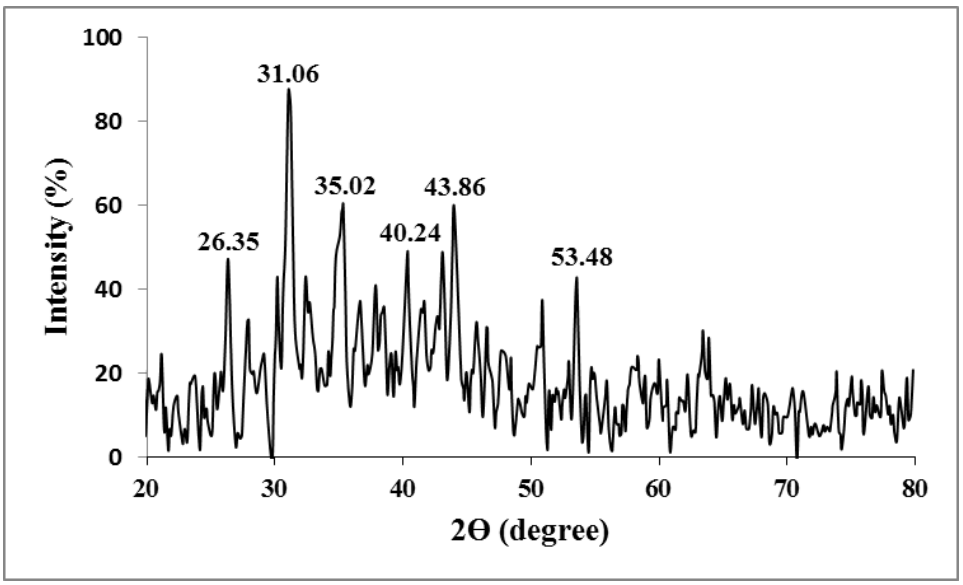

Fig. 2: XRD analysis of synthesized CuNPs using aqueous leaf extract of *A. heterophyllus*

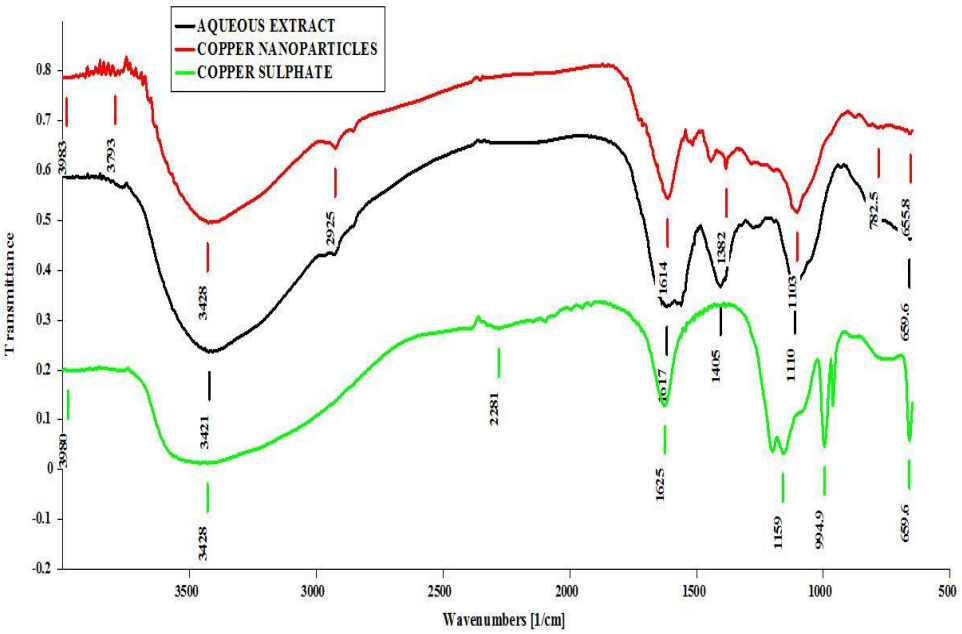

Fig. 3: FTIR spectrum of color variations (Black indicating Aqueous extract, Green indicating $CuSO_4$ and Red indicating Synthesized CuNPs)

SEM analysis- SEM determination of the samples has shown the formation of cupper nanoparticles. SEM analysis of the synthesized CuNPs was clearly distinguishable, which measured in size 132 nm (Fig. 4). CuNPs are asymmetrically dispersed and aggregated infrequently to form free crystals structures.

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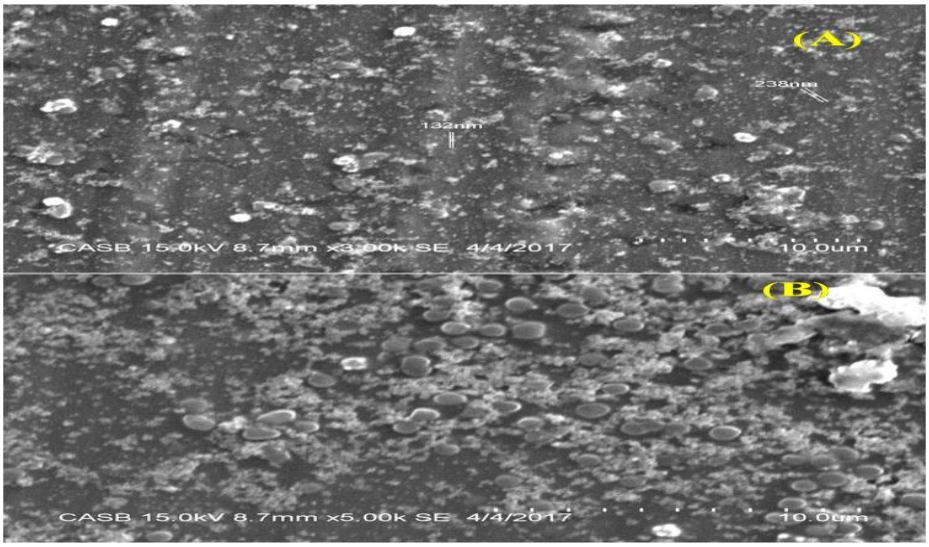

Fig. 4: Scanning electron micrographs of CuNPs synthesized with *A. heterophyllus* leaf extract and magnified **(A)** 8.7mm×3.00 and **(B)** 8.7mm×5.00 inset bar represents 10 µm

Toxicity studies

Synthesized CuNPs tested against first to fourth instar larvae of *A. aegypti*- In the present study, the mosquito larvicidal activity of aqueous leaf extracts, $CuSo_4$ solution (5 mM) and synthesized CuNPs of *A. heterophyllus* were noted; however, the synthesized CuNPs showed 100% mortality in first to fourth instars larvae of *A. aegypti* at the concentration of 10 mg/L.

The percentage mortality of aqueous leaf extracts of *A. heterophyllus* against first to fourth instar larvae of *A. aegypti* showed the values of 98, 82, 76, 64 & 48, 86, 76, 66, 58 and 44, 78, 66, 58, 48 & 36, 65, 58, 51, 43 and 26 at 100, 80, 60, 40 and 20 mg/L after 24 h (Fig. 5 & Table 1). The control (distilled water) showed nil mortality in the concurrent assay. The lethal effect first to fourth instars, larvae *A. aegypti* showed the values of LC_{50}= 21.81, 26.92, 41.38 and 55.12 mg/ml and r^2= 0.993, 0.992, 0.997 and 0.965.

Values of the efficacy of 5mM $CuSo_4$ solution against first to fourth instar larvae of *A. aegypti* reported the LC_{50} values of 48.40, 60.55, 70.36, 82.79 mg/ml with regression of 0.986, 0.969, 0.959, 0.965, respectively (Fig. 6 & Table 1).

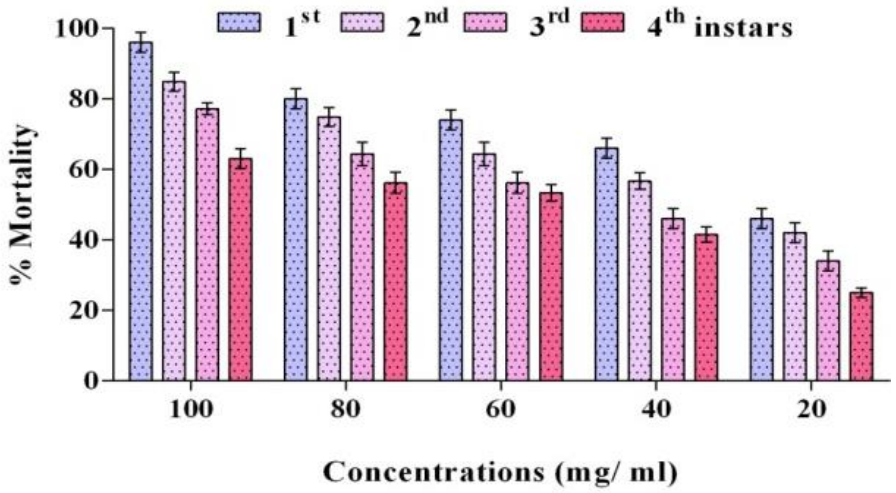

Fig. 5: Larvicidal activity of aqueous leaf extracts of *A. heterophyllus* against first to fourth instar larvae of *A. aegypti*

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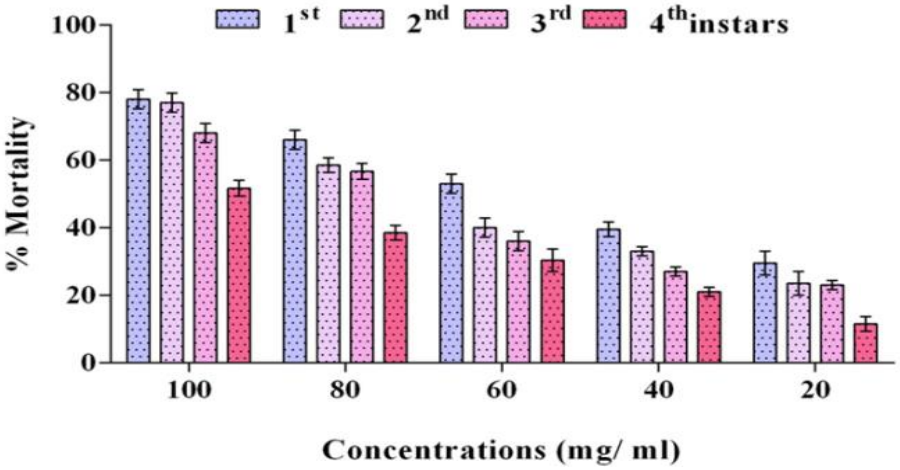

Fig. 6: Larvicidal activity of 5 mM $CuSO_4$ against *A. aegypti*

Synthesized CuNPs by *A. heterophyllus* extract against the first to fourth instar larvae of *A. aegypti* showed LC_{50} values of 3.85, 4.24, 4.66 and 5.08 mg/L and r^2 values of 0.992, 0.996, 0.990 and 0.990 respectively (Fig. 7 & Table 1). In the present result's study, the mosquito larvicidal activity showed the highest mortality in synthesized CuNPs than the aqueous leaf extract of *A. heterophyllus.* Each test included a control group (distilled water) with three replicates for each individual concentration. All the tested components that showed lethal effect and mortality were positively dose dependent. The results showed that the optimal hours for measuring the percent mortality of first to fourth instar larvae of *A. aegypti* synthesized CuNPs were 36%, 48%, 65%, 86% and 100%, 30%, 45%, 61%, 80% and 100%, 28%, 41%, 56%, 78% and 96%, 24%, 38%, 52%, 75% and 92% at 2, 4, 6, 8 and 24 h, respectively.

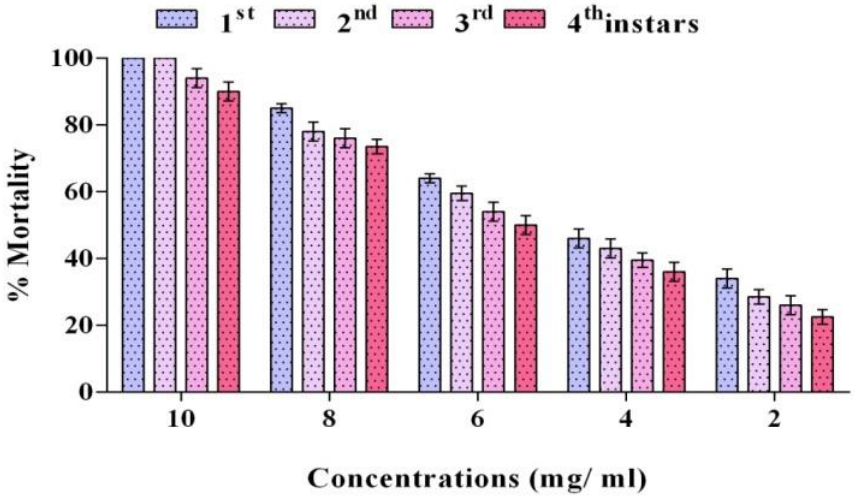

Fig. 7: Larvicidal activity of synthesized CuNPs against *A. aegypti*

Table 1: Mosquito larvicidal activity of aqueous leaf extracts of *A. heterophyllus*, $CuSO_4$ (5 mM) and synthesized CuNPs against *A. aegypti*

Extracts/ Products	Instars	LC_{50} mg/ml	LCL–UCL (mg/ml)	r^2
Aqueous leaf extracts	1st	21.81	14.88–31.97	0.993
	2nd	26.92	18.96–38.21	0.992
	3rd	41.38	31.98–53.65	0.997

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	4th	55.12	44.36–68.47	0.965
$CuSO_4$ (5 mM)	1st	48.40	39.51–59.28	0.986
	2nd	60.55	51.88–70.66	0.969
	3rd	70.36	62.99–78.58	0.959
	4th	55.12	82.79–108.97	0.965
Synthesized CuNPs	1st	3.85	3.03–4.87	0.992
	2nd	4.24	3.56–5.05	0.996
	3rd	4.66	3.96–5.47	0.990
	4th	5.08	4.43–5.82	0.990

DISCUSSION

Mosquitoes are quite commonly held responsible for distribution of major diseases like malaria, filariasis, dengue, chikungunya and japanese encephalitis etc. Mosquito borne diseases are distributed widely across the globe. When a mosquito bites a human it injects its saliva containing arbovirus, which is responsible for infecting a normal healthy human being.

UV- Analysis- The visual observation of aqueous leaf extract of *A. heterophyllus* was pale yellow color before the addition of $CuSO_4$ solution the color of aqueous leaf extract of *A. heterophyllus* was pale yellow color, which in turn changed into light brownish color (Fig. 8). The color of the extract further changed to light brown and later it changed to dark brown after two hours of incubation period after which there was no significant color change. Therefore, the plant extracts can act both as reducing agents and stabilizing agents in the biosynthesis of nanoparticles. Different plant materials contain different organic reducing agents at various concentrations and combinations [13] pertaining to the synthesis of nanoparticles. It has been reported that plant- mediated bioreduction of aqueous extract is combined with relevant metal salt [14]. The reaction occurs at room temperature and is generally completed within few minutes and the process is relatively complex. The reducing agents such as alkaloids, flavonoids, which were presented in the leaves extracts of various plants responsible for the reduction of copper ions. The reduction of Cu^{2+} ions to CuNPs was observed using UV-Vis spectroscopy where the surface plasma resonance showed a distinct peak at 640 nm (Fig. 1). A gradual increase in the characteristic peak with increase in reaction time and the concentration of aqueous extracts with salt ions is a clear indicator of nanoparticle formation.

Fig. 8: Visual observation of color change after addition of $CuSO_4$

X-Ray Diffraction- The average size of copper nanoparticles was found to be 65 nm using Debye-Scherrer equation. XRD is a technique mainly used to establish the metallic nature of particles gives information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities [15]. XRD analysis of the present study reveals the crystallographic structure of CuNPs and it also demonstrated a high crystallinity level with diffraction angles of 26.35°, 35.02° and 40.24° which correspond to the characteristic face-centred cubic of copper lines indexed at 111, 200 and 222, respectively.

FTIR Analysis- The peak at 3428 cm^{-1} overlaps with O-H stretching and alcohol group. The sharp absorption peak

at 2925 cm^{-1} is assigned to C–H stretching. The peak at 1614 cm^{-1} overlaps with N-H bending. The peak at 1382 cm^{-1} overlaps with C-H stretching. The sharp absorption peak at 1103 cm^{-1} overlaps is assigned to C–O stretching and alcohol group and the peak at 655.8 cm^{-1} overlaps with C-Cl stretching strong of alkyl halide. The peaks of CuNPs and aqueous extract also exhibited at 655.8 and 659.6 cm^{-1}, which overlap with C-Cl stretching strong of alkyl halide. Therefore the phenolic compounds and flavonoids present in the extract have high affinity to bind towards CuNPs and help in the stabilization of CuNPs. Thus, possibly forming CuNPs and also prevents possible aggregation.

SEM analysis- SEM determinations of the sample showed the formation of nanoparticles. SEM analysis of the synthesized CuNPs was clearly distinguishable, which measured in size 132 nm. SEM picture indicated the size of polycrystalline particles with more or less uniform in size and shape. Generally, on the nanometer scale, metals tend to nucleate and grow into twinned and multiply twinned particles with their surfaces bounded by the lowest-energy facets (Fig. 4). Alike, results were observed from the SEM micrographs of nanoparticles obtained in the filtrate, which showed that the CuNPs produced by *Penicillium citrinum* were spherical shaped [16].

Synthesized CuNPs tested against first to fourth instar larvae of *A. aegypti*- In the present study, the mosquito larvicidal activity of aqueous leaf extracts, $CuSO_4$ solution (5 mM) and synthesized CuNPs of *A. heterophyllus* were noted; however, the synthesized CuNPs showed 100% mortality first to fourth instars larvae of *A. aegypti* at the concentration of 10 mg/L.

The percentage mortality of aqueous leaf extracts of *A. heterophyllus* against first to fourth instar larvae of *A. aegypti* showed the values of 98 %, 82 %, 76%, 64% and 48%; 86%, 76%, 66%, 58% and 44%; 78%, 66%, 58%, 48 % and 36 %; 65 %, 58 %, 51 %, 43 % and 26 % at 100 %, 80 %, 60 %, 40 % and 20 % mg/L after 24 h (Fig. 6 & Table 1). The control (distilled water) shown nil mortality in the concurrent assay. The lethal effect first to fourth instars larvae *A. aegypti* showed the values of LC_{50} were 21.81, 26.92, 41.38 and 55.12 mg/ml and r^2 were 0.993, 0.992, 0.997 and 0.965. Values of the efficacy of 5 mM $CuSO_4$ solution against first to fourth instar larvae of *A. aegypti* reported the LC_{50} values of 48.40, 60.55, 70.36, and 82.79 mg/ml with regression of 0.986, 0.969, 0.959, and 0.965, respectively (Fig. 7 & Table 1). Ramyadevi *et al.* [17] had reported copper acetate solution against the larvae of *Anopheles subpictus* and *C. quinquefasciatus* (LC_{50} values of 23.47 and 15.24 mg/L).

CuNPs were synthesized by *A. heterophyllus* extract against the first to fourth instar larvae of *A. aegypti* showed LC_{50} values were 3.85, 4.24, 4.66 and 5.08 mg/L and r^2 values were 0.992, 0.996, 0.990 and 0.990 respectively (Fig. 8 & Table 1). All the tested components showed that lethal effect and mortality were positively dosed dependent. The results showed that the optimal hours for measuring the percent mortality of first to fourth instar larvae of *A. aegypti* synthesized CuNPs were 36%, 48%, 65%, 86% and 100%; 30%, 45%, 61%, 80% and 100%; 28%,41%, 56%, 78% and 96%; 24%, 38%, 52%, 75% and 92% at 2, 4, 6, 8 and 24 h, respectively. Our results showed that biosynthesized CuNPs using *A. heterophyllus* against the first to fourth instar larvae of *A. aegypti* showed the LC_{50} values of 3.85, 4.24, 4.66 and 5.08 mg/L. The control showed no mortality of larvae Santhoshkumar *et al.* [18] studied the aqueous and synthesized AgNPs, which used *Nelumbo nucifera* plant extract against third instar larvae of *A. subpictus* (LC_{50} values of 11.82 and 0.69 ppm and third instar larvae of *C. quinquefasciatus* (LC_{50} values of 13.65 and 1.10 ppm). This implied that biosynthesized CuNPs has good penetration capacity due to its small size and high surface to volume ratio which results in disruption of organelles and enzymes in young juvenile instars as they are easily susceptible to the action of CuNPs, when compared to higher instars.

CONCLUSIONS

In this study, the biosynthesis of CuNPs using *A. heterophyllus* was studied. The physical property of synthesized nanoparticle was characterized using appropriate techniques. It was the first report on evaluating larvicidal activity against *A. aegypti* using CuNPs. Further research on CuNPs could bring a very promising avenue for vector control.

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CONTRIBUTION OF AUTHORS

Dr. R. Ramanibai- Research Concept, Research Design, Supervision, Funding and Materials;

E. Agnita Sharon- Data collection and processing, Data Analysis, Literature search and Writing Article;

K. Velayutham- Critical review, Article editing and Final approval.

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