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Synthesis of silver nanoparticle with *Colocasia esculenta* (L.) stem and its larvicidal activity against *Culex quinquefasciatus* and *Chironomus* sp

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ABSTRACT

Objective: To synthesize silver nanoparticles with *Colocasia esculenta* as a reducing agent and to evaluate their effect against *Culex quinquefasciatus* and *Chironomus* sp.

Methods: The aqueous extract of *Colocasia esculenta* stem was used for nanosynthesis. The synthesized nanoparticles were characterized by UV-Vis spectrophotometry, Fourier-transform infrared spectroscopy, scanning electron microscope, transmission electron microscopy, energy-dispersive X-ray spectroscopy, X-ray diffraction and Zeta potential studies. The toxicity of *Colocasia esculenta* stem extract and the synthesized silver nanoparticles was evaluated against the larval stages of target human filarial vector *Culex quinquefasciatus* and non-target *Chironomus* sp.

Results: Scanning electron microscopy and transmission electron microscopy studies revealed almost spherical shape of the synthesized silver nanoparticles with size ranging from 13-50 nm. After 24 hours of exposure, the LC_{50} and LC_{90} of the plant extract against 4th instars larvae of *Culex quinquefasciatus* were 745.56 mg/L and 1 258.28 mg/L, respectively, which were higher than those of synthesized silver nanoparticles (5.17 mg/L and 17.32 mg/L after 24 h; 1.58 mg/L and 13.01 mg/L after 48 h). In addition, the LC_{50} and LC_{90} of silver nanoparticles against *Chironomus* sp. were 9.71 mg/L and 23.15 mg/L after 24 h as well as 2.38 mg/L and 19.49 mg/L after 48 h, respectively.

Conclusions: The aqueous stem extract of *Colocasia esculenta* is a good agent for synthesis of silver nanoparticles, which are almost spherical with size less than 30 nm. The synthesized nanoparticles show good larvicidal activity without any harmful effect on non-target species.

1. Introduction

Mosquitoes are disease-causing vectors that are responsible for transmitting human filariasis, malaria, and many other viral diseases like dengue, Japanese encephalitis, Zika and West Nile virus[1,2]. Mainly three genera of mosquitoes, namely, *Culex* sp. *Anopheles* sp. and *Aedes* sp. are widely distributed all over the world including India, and cause millions of fatalities. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is a vector of *Wuchereria bancrofti*, the parasite of filarial disease. Moreover, *Cx. quinquefasciatus* transmits causative agent of western equine, encephalitis, avian malaria, St. Louis

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encephalitis and West Nile fever[3]. According to the report of National Vector Borne Disease Control Programme, in 83 countries all over the world, almost 120 million people are infected with human filariasis and it is predicted that 1.1 billion are at risk[4]. About 40 million people are disabled by the infection of filarial parasite and 76 million people have hidden internal lymphatic infection with apparently normal appearance. According to the World Health Organization report, among the infected people worldwide, 70% are from India, Indonesia, Bangladesh and Nigeria. At present, many chemically synthesized insecticides such as dichlo rodiphenyltrichloroethane, dieldrin, organophosphorus, fenitrothion and synthetic pyrethroids are used for control of mosquitoes. But the residues of these insecticides have extremely harmful impact on the whole biosphere[5].

To overcome the above problem, various bio-control methods have been adopted such as fish, application of bit granules to larval habitat, plant-based ovicides and larvicides[6-8]. None of these agents provide mosquito control to a satisfactory level. Therefore, an alternative method is required which should give the desired result, and should be safer for the environment. Numerous recent literatures highlighted that mosquito larvae can be controlled by nanoparticles[9-12]. The biologically synthesized nanoparticles have many advantages over chemical and physical methods. Plant-mediated nanoparticles yield dual benefits, as reducing and capping agent due to the presence of different biomolecules. Therefore, the synthesis of plant-based nanoparticles has generated tremendous interest due to its green, rapid and nonpolluting nature[13,14]. Various plant parts such as leaf, root, shoot, flower and latex are being used for the synthesis of nanoparticles[15].

Colocasia esculenta (*C. esculenta*) is a tropical plant and widely distributed throughout Southern India and Southeast Asia. This particular plant has high level of protein (520 mg/100 g), carbohydrate (34.6 mg/100 g) and minerals such as potassium (484 mg/100 gm) as the major components[16].

According to our information, *C. esculenta* has not been used by any previous researcher for the synthesis of silver nanoparticles. This information gave us an impetus to synthesize the particular silver nanoparticles. The subsequent characterization of synthesized nanoparticles was detected by various analytical instruments [UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) and Zeta potential]. Then, the efficacy of the synthesized silver nanoparticles was evaluated as a larvicidal agent against *Cx. quinquefasciatus*, and the toxicity of AgNPs was determined by using *Chironomus* sp. as a non-target organism.

2. Materials and methods

2.1. Reagents and chemicals

All glass goods were purchased from Borosil Pvt. Ltd. The chemical silver nitrate ($AgNO_3$) was purchased from Merck Chemical Reagent Co. Ltd. India. Before starting the experiment, all glassware was washed with tap water followed by distilled water and dried in hot air oven before use.

2.2. Collection and preparation of plant extract

C. esculenta (L.) Schott (voucher number: 198055, authenticated by Dr. Chittaranjan Das) plants (Supplementary Figure S1) were collected from the Burdwan University campus (latitude 23°15'22''N, longitude 87°50'58''E). Prior to the experiment, *C. esculenta* stem was rinsed thoroughly by double distilled water. A total of 25 g of stem were cut into fine pieces and mixed with 100 mL of mili Q water in 250 mL beaker, and then the mixture was boiled for 10 min before decanting.

2.3. Silver nanoparticle preparation

For the synthesis of silver nanoparticles, according to the methods of Lade and Patil[17] with modifications, 90 mL of 5 Mm/L $AgNO_3$ was reacted with 10 mL of aqueous stem extract of *C. esculenta* in the Erlenmeyer flask. Then the mixture was placed in the water bath for 30 min at 70 ℃. Synthesized nanoparticles were preserved at room temperature for further use.

2.4. Characterization of nanoparticles

The synthesized nanoparticles were observed by the color change of the solution to yellowish brown and the spectral signature was measured by UV-Vis spectra at wavelength of 240-700 nm (UV-Vis Spectrophotometer, Optimizenpop, Korea)[18]. Furthermore, the size, morphology and composition of AgNPs were determined by TEM (JEOL JEM 1400 plus) and SEM with EDX (JEOL JSM-6390LV, USA). Then XRD (Bruker D8, Germany) was used for understanding whether these particles were crystalline or amorphous in structure, and from this study, average nanoparticles size was also calculated through the Debye scherrer equation. The Zeta potential was performed to determine the surface charges on synthesized AgNPs using zetasizer ver. 7.12 (Malvern Instruments Ltd, UK). FTIR (Perkin Elmer Frontier, and Germany) was used for understanding the surface chemistry of the nanoparticles[19].

2.5. Mosquito collection and rearing

Cx. quinquefasciatus were collected from stagnant water in discarded tyre from an adjoining area of Burdwan town (latitude 23°15'22''N, longitude 87°50'58''E). Then the mosquito larvae were transferred to enamel trays (18 cm × 14 cm × 4 cm) containing 500 mL mineral water, where they were allowed to hatch and were maintained as per methods of Arasu *et al*[20]. Mosquito larvae were fed 5 g brewer's yeast and dog biscuit in a 1:3 ratio daily. Then the larvae were transferred to a container containing 500 mL water. Pupae were collected in small bowls and were placed into

mosquito rearing cages (90 cm × 90 cm × 90 cm) and emerging adult mosquitoes were fed with 10% sucrose solution. After the emergence of adults, the mosquitoes were identified according to the identification key of Service, 1967[21]. The fourth instars larvae of *Cx. quinquefasciatus* were taken for larvicidal study. The insectary condition was maintained at 70%–80% humidity, 24–28 ℃ and at 16-h light, 8-h dark cycle[22].

2.6. Larvicidal activity of aqueous plant extract and AgNPs against Cx. quinquefasciatus

In the larvicidal bioassay study, twenty-five fourth instar larvae were kept for each replicate in different polystyrene bowls, which contained 250 mL dechlorinated tap water for standard and for different concentrations of AgNPs ranging from 0.5 mg/L to 10 mg/L in triplicate. Simultaneously, aqueous plant extract was used at the concentrations of 85, 170, 255, 340 and 425 mg/L. The larvicidal experiment was done according to previous work of Laird[23] and Govindarajan *et al*[24] after 24 and 48 hours of exposure. The number of dead larvae was counted and the statistical analysis was done. Moreover, dead larvae were observed under microscope (LEICA DM 2000) at 40× magnification for morphological study.

2.7. Effect on non-target organism

According to the method by Govindarajan *et al*, pure plant extract and AgNPs were tested against non-target organism *Chironomus* sp.[25]. *Chironomus* larvae were selected as non-target organisms because both *Chironomus* sp. and *Cx. quinquefasciatus* are sympatric. The species were collected from the field and maintained in a cement tank containing water at 27 ℃ with an artificial environment. The *C. esculenta* aqueous extract and AgNPs were tested at same concentrations as on mosquito larvae. The experiment at each concentration was performed in triplicate. The mortality of the larvae was recorded after 24 and 48 hours of exposure. Reduction of swimming power and moribund larvae were also observed during the same period.

2.8. Dose response bioassay

Mosquitocidal bioassay experiment was conducted at different concentrations (0.5, 1, 3, 8 and 10 mg/L) of AgNPs under 24 and 48 hours of exposure. The mortality of mosquito larvae was calculated by following the Abbott's formula:

$$\text{Mortality}(\%) = \frac{N_0}{N_{total}} \times 100$$

Where N_0 was number of dead larvae, N_{total} number of larvae introduced.

2.9. Statistical analysis

The larval mortality was recorded after 24 and 48 h. AgNPs treatment and their corresponding lethal doses (LC_{50} and LC_{90} values), upper and lower confidence limits (UCL and LCL), *chi*-square values, standard deviation and regression equations were calculated according to Probit Analysis, using the SPSS software 20 and Minitab 17. Corrected percentage mortality was calculated using Abbott's formula. $P < 0.05$ was used to determine the significance of differences among the means.

3. Results

3.1. UV–Vis spectral analysis

The yellowish brown color of AgNPs was assessed by UV-Vis spectra (Figure 1). The reduction of Ag ions by the *C. esculenta* stem extract was easily analyzed by UV-Vis spectroscopy (Figure 1). Neither silver salt nor plant extract showed absorption spectrum in the range of 380-460 nm but in case of synthesized AgNPs, a new peak appeared between 380 and 480 nm. At 426 nm, a broad absorption peak appeared without any unwanted noise, which represented the characteristic of surface plasmon resonance of spherical and aggregated AgNPs.

3.2. TEM, SEM, XRD and EDX studies

TEM clearly exhibited the cubical, triangular and spherical shape of AgNPs, having an average size less than 100 nm (Figure 2). The present distribution of AgNPs demonstrated that the majority of particles were spherical in shape. Moreover, the SEM micrograph (Figure 3A) depicts the morphology of lyophilized nanoparticle, which also provided a tentative idea about the size and shape of the nanoparticles. Figure 3A clearly revealed that the AgNPs were cubical, triangular and spherical in shape with uniform distribution. XRD study indicated the existence of clear and significant peak ranging from 23.68° to 77.21° (Figure 4). This XRD pattern was again nicely utilized for determination of an average size of the synthesized AgNPs through Debye-Scherrer equation. The EDX spectrum (Figure 3B) of the synthesized nanoparticles indicated the presence of elemental silver.

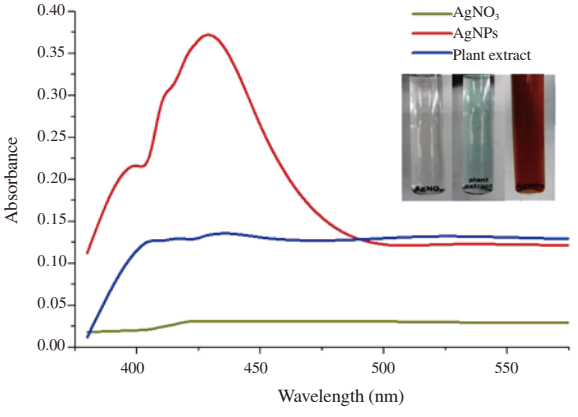

Figure 1. UV-Vis spectra of silver nanoparticles (AgNPs), plant extract and silver salt.

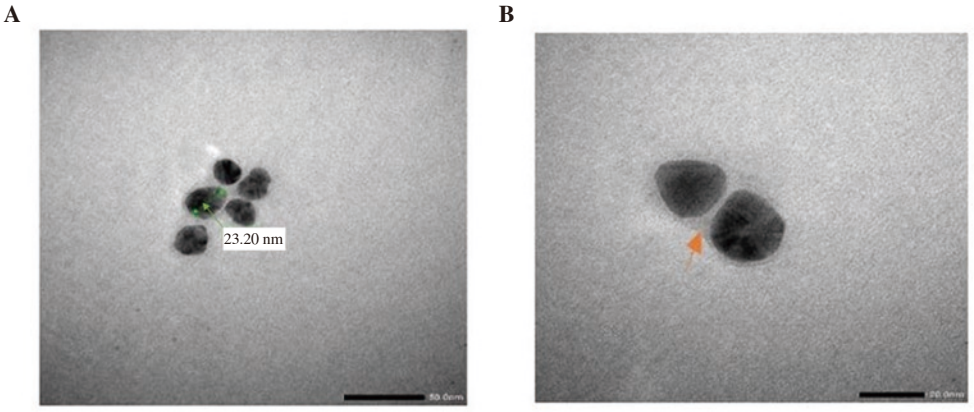

Figure 2. TEM (A & B) image of silver nanoparticles.

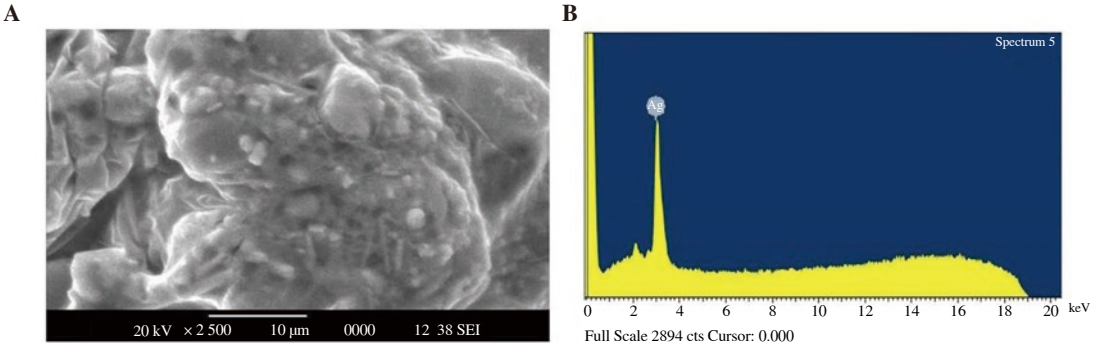

Figure 3. SEM image of silver nanoparticles (A), and EDX spectrum of silver nanoparticles (B).

3.3. FTIR studies

The FTIR spectrum of biosynthesized AgNPs using *C. esculenta* plant extract was shown in Figure 5. This figure depicts the prominent bands of absorbance at 3 417.54, 3 039.88, 2 889.42, 1 599.45, 1 435.74, 1 360.16 and 1 054.83 cm^{-1}, respectively.

3.4. Zeta potential study

The magnitude of Zeta potential (Supplementary Figure S2) normally expressed the degree of repulsion between the similar charges of the nanoparticles. Higher repulsion prevents agglomeration, which is the cause of stability of the nanoparticles. The magnitude of electrical double layer repulsion can be expressed either in a positive or negative value with the unit mili volte.

3.5. Mosquito larvicidal bioassay

After 24 hours of exposure, LC_{50} and LC_{90} for AgNPs were 5.17 mg/L and 17.32 mg/L while for plant extract 745.56 mg/L and 1 258.28 mg/L, respectively. After 48 hours of exposure, LC_{50} and LC_{90} were 1.58 mg/L and 13.01 mg/L for AgNPs, showing better larvicidal activity than plant extract with LC_{50} and LC_{90} of 619.10 mg/L and 1 047.49 mg/L, respectively. This result suggested that *C. esculenta* extract by itself has no power to kill the mosquito larvae. However, this extract can be efficiently used for nanosynthesis.

Table 1 also revealed that mortality of AgNPs was concentration dependent at 24 h and 48 h. Moreover, the *Chi*-square test result revealed that there was no significant difference in mortality among different concentrations of AgNPs at 24 h and 48 h (P>0.05). Similarly, no significant difference was observed in mortality among different concentrations of plant extract at 24 h and 48 h (P>0.05). The larval mortality was higher when exposed to AgNPs.

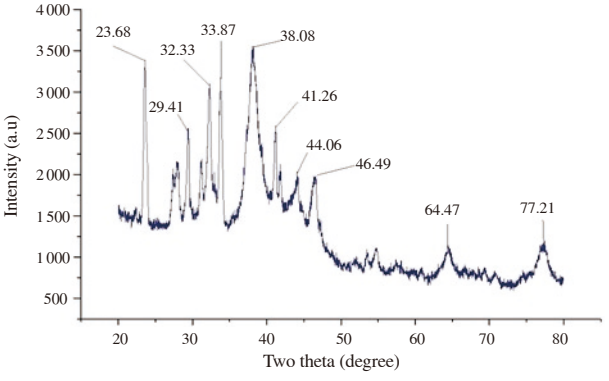

Figure 4. XRD pattern of silver nanoparticles synthesized using *Colocasia esculenta* stem extract.

Table 1. Larvicidal activity of silver nanoparticles and plant extract against the fourth instar *Culex quinquefasciatus* larvae.

Time	Treatment	Percent of mortality (%)	No. of dead mosquito larvae (mean±SD)	LC_{50} (95% LCL-UCL) (mg/L)	LC_{90} (95% LCL-UCL) (mg/L)	*Chi*-square (*df*=3)	*P* value
24 h	Silver nanoparticles (mg/L)						
	0.5	28	7.0 ± 1.0	5.17 (3.83-7.17)	17.32 (13.36-32.51)	1.815	0.612
	1.0	36	9.0 ± 2.6				
	3.0	44	11.0 ± 1.0				
	8.0	52	13.0 ± 2.6				
	10.0	76	19.0 ± 3.0				
	Plant extract (mg/L)						
	85.0	4	1.0 ± 1.0	745.56 (516.18-923.26)	1 258.28 (927.45-1 476.72)	0.104	0.991
	170.0	8	2.0 ± 1.7				
	255.0	12	3.0 ± 1.0				
	340.0	16	4.0 ± 2.0				
	425.0	20	5.0 ± 1.0				
48 h	Silver nanoparticles (mg/L)						
	0.5	40	10.0 ± 1.7	1.58 (0.09-3.65)	13.01 (9.36-24.97)	2.086	0.555
	1.0	52	13.0 ± 2.0				
	3.0	60	15.0 ± 1.7				
	8.0	68	17.0 ± 2.6				
	10.0	88	22.0 ± 1.7				
	Plant extract (mg/L)						
	85.0	4	1.0 ± 1.0	619.10 (456.83-905.26)	1 047.49 (702.45-1 325.69)	0.465	0.926
	170.0	12	3.0 ± 1.0				
	255.0	12	3.0 ± 1.7				
	340.0	20	5.0 ± 1.0				
	425.0	28	7.0 ± 1.0				

UCL: upper confidence limit, LCL: lower confidence limit, DF: degree of freedom, SD: standard deviation.

Table 2. Biotoxicity of synthesized silver nanoparticles and *Colocasia esculenta* plant extract against non-target organism *Chironomus* sp. larvae.

Time	Treatment	Percent of mortality (%)	No. of dead mosquito larvae (mean±SD)	LC_{50} (95% LCL-UCL) (mg/L)	LC_{90} (95% LCL-UCL) (mg/L)	*Chi*-square (*df*=3)	*P* value
24 h	Silver nanoparticles (mg/L)						
	0.5	12	3.0 ± 1.7	9.71 (6.94-19.14)	23.15 (15.78-55.95)	1.589	0.662
	1.0	24	6.0 ± 2.0				
	3.0	32	8.0 ± 2.6				
	8.0	40	10.0 ± 1.7				
	10.0	52	13.0 ± 2.6				
	Plant extract (mg/L)						
	85.0	4	1.0 ± 1.0	1 354.59 (1 087.27-1 647.89)	2 345.58 (1 969.59-2 636.73)	0.254	0.968
	170.0	8	2.0 ± 1.0				
	255.0	8	2.0 ± 1.0				
	340.0	8	2.0 ± 1.0				
	425.0	12	3.0 ± 1.0				
48 h	Silver nanoparticles (mg/L)						
	0.5	36	9.0 ± 2.0	2.38 (0.56-5.51)	19.49 (12.42-58.57)	1.325	0.723
	1.0	52	13.0 ± 1.7				
	3.0	56	14.0 ± 1.0				
	8.0	64	16.0 ± 2.6				
	10.0	72	18.0 ± 2.6				
	Plant extract (mg/L)						
	85.0	0	0.0 ± 0.0	914.75 (675.69-1 246.24)	1 398.46 (1 083.48-1 638.41)	0.807	0.848
	170.0	4	1.0 ± 1.0				
	255.0	4	1.0 ± 1.7				
	340.0	8	2.0 ± 2.0				
	425.0	8	2.0 ± 1.0				

UCL: upper confidence limit, LCL: lower confidence limit, DF: degree of freedom, SD: standard deviation.

3.6. Microscopic view of nano-treated mosquito larvae

The mechanism of interaction between nanoparticles and mosquito is still unclear. But during this study, dead mosquito larvae which were treated with five concentrations (0.5, 1, 3, 8 and 10 mg/L) of AgNPs were examined under high-resolution light microscope (Olympus SZ51). The larvae under exposure of lower concentrations did not show any distortion of the cuticular layer, inner structure or body hair of mosquito larvae. On the other hand, at higher concentrations, the silver nanoparticles treated dead larvae showed severe signature of disruption of its cuticular surface and reduced level of body hair. From Supplementary Figure S3, it was clearly observed that the chitin layer of the larval body was severely damaged by treated nanoparticles.

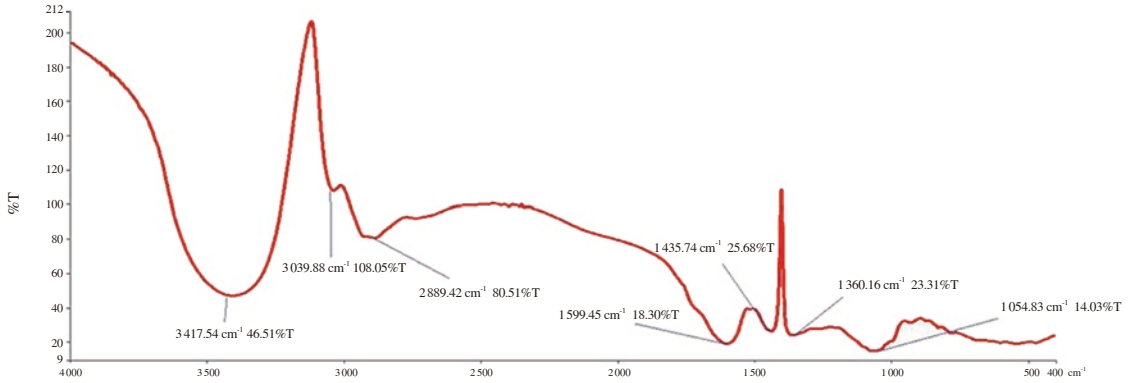

Figure 5. FTIR spectra of silver nanoparticles synthesized from *Colocasia esculenta*.

3.7. Toxicological impact on non-target organisms

The results showed that after 24 hours of exposure, LC_{50} and LC_{90} values were 9.71 mg/L and 23.15 mg/L, respectively for AgNPs with mortality ranging from 12% to 52% (Table 2). On the other hand, LC_{50} and LC_{90} values were 1 354.59 mg/L and 2 345.58 mg/L, respectively for plant extract with 4% to 12% of mortality. After 48 hours of exposure, LC_{50} and LC_{90} values were 2.38 mg/L and 19.49 mg/L for AgNPs, respectively against *Chironomus* sp, while they were 914.75 mg/L and 1 398.46 mg/L for plant extract, respectively.

4. Discussion

The addition of *C. esculenta* stem extract to the aqueous solution of $AgNO_3$ resulted in the yellowish brown color due to the surface plasmon resonance that strongly depended on the synthesis rate, particle size, dielectric constant on the medium and chemical nature around the nanoparticles[26]. In the present study, the sharp spectroscopic signature at 426 nm indicated the formation of surface plasmon resonance of AgNPs due to the collective oscillation of the valence electron[27-29]. Previous research highlighted that the symmetry of nanoparticles is related to the number of surface plasmon resonance peaks[30]. Different instrumental analyses like XRD pattern indicated the existence of clear and significant peak ranging from 23.68˚ to 77.21˚ value, which clearly confirmed the crystalline nature of synthesized silver nanoparticles[31]. The average size of the synthesized AgNPs was calculated by Debye-Scherrer equation and it was recorded 16.16 nm. Moreover, both XRD and TEM results were justified with the nano range of synthesized silver nanoparticles. The semi-clear thin layer around the particles, which was supposed to be the mixture of biomaterials acting as a capping agent to prevent the agglomeration of those particles[32]. Similarly, the signature of FTIR study clearly indicated the functional groups of –OH, –N-H and –C-N and all these functional groups are directly or indirectly responsible for the biological reduction of silver ion to its zero valent state. The biomolecules are also responsible for stabilizing the particles in aqueous media[1,16,33]. A peak at 3 KeV was observed in the study which demonstrates the presence of silver in its nano form which has been reduced from ionic silver[34]. Patil *et al*[35] suggested that low Zeta potential (20 mV) is sufficient to stabilize the nanoparticles. In the present study, Zeta potential value of synthesized AgNPs was recorded as -25.1 mV. Therefore, it may be assumed that the present nanoparticles showed moderate stability.

After 24 hours of exposure, both AgNPs and plant extract showed higher lethal concentration than 48 hours of exposure, which was probably due to the fact that, at longer duration, nano particles get enough chance to interact with mosquito larvae. Almost similar results reflected in higher concentrations of treated nanoparticles. These findings are in accordance with the earlier reports[31,34]. Similar researches with higher mortality at very low concentrations were reported by Kumar *et al*.[30] and Soni and Prakash[36]. On the other hand, the effects of nanoparticles on *Chironomus* larvae (*Chironomus* sp.) were very negligible compared to target mosquito larvae (*Cx. quinquefasciatus*). Further microscopic observation clearly indicated that the surface of target mosquito larval bodies was deformed, probably due to the interaction of nanoparticles with the cuticular layer of larvae. The same phenomenon was also reported by Hajra and Mondal[37], who also suggested that AgNPs may penetrate into the cell membrane of mosquito larvae. Similarly, they also reported that synthesized AgNPs cross the cell membrane by the attraction between cell membranes and silver ions, which may be probable cause of larval death. Therefore, it can be suggested that larval mortality of *Cx. quinquefasciatus* can be achieved with a much lower concentration of AgNPs compared to previously synthesized AgNPs[10,21,38,39,40].

The synthesized nanoparticles were mostly spherical with size between 13-50 nm and the nanoparticles were stable over a long period of time. The synthesized nanoparticles showed efficient larvicidal activities without any detrimental effect on the non-target organism. Therefore, synthesized silver nanoparticles could be an effective tool towards control of mosquito larvae without affecting

non-target organisms like *Chironomus* sp. However, more researches may be necessary to further confirm its efficacy.

Conflict of interest statement

We declare no conflict of interest.

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Authors' contributions

AM conducted the entire lab experiment including materials collection, process, mosquito larvae mortality test, data collection, article writing, and articles revision. Initial characterizations such as UV-Visible spectroscopy, XRD, and TEM were also conducted by AM. AH was mainly involved in conducting the experiment related to the impact of silver nanoparticles on non-target species, and WAS involved in the characterizations of nanoparticles by using various instruments such FTIR, SEM, EDX and zeta potential. SC was mainly responsible for conducting some of the lab experiments for reproduction of the experimental results including manuscript correction. The entire research design was framed by NKM. Moreover, NKM drafted the main manuscript and corrected the manuscript.

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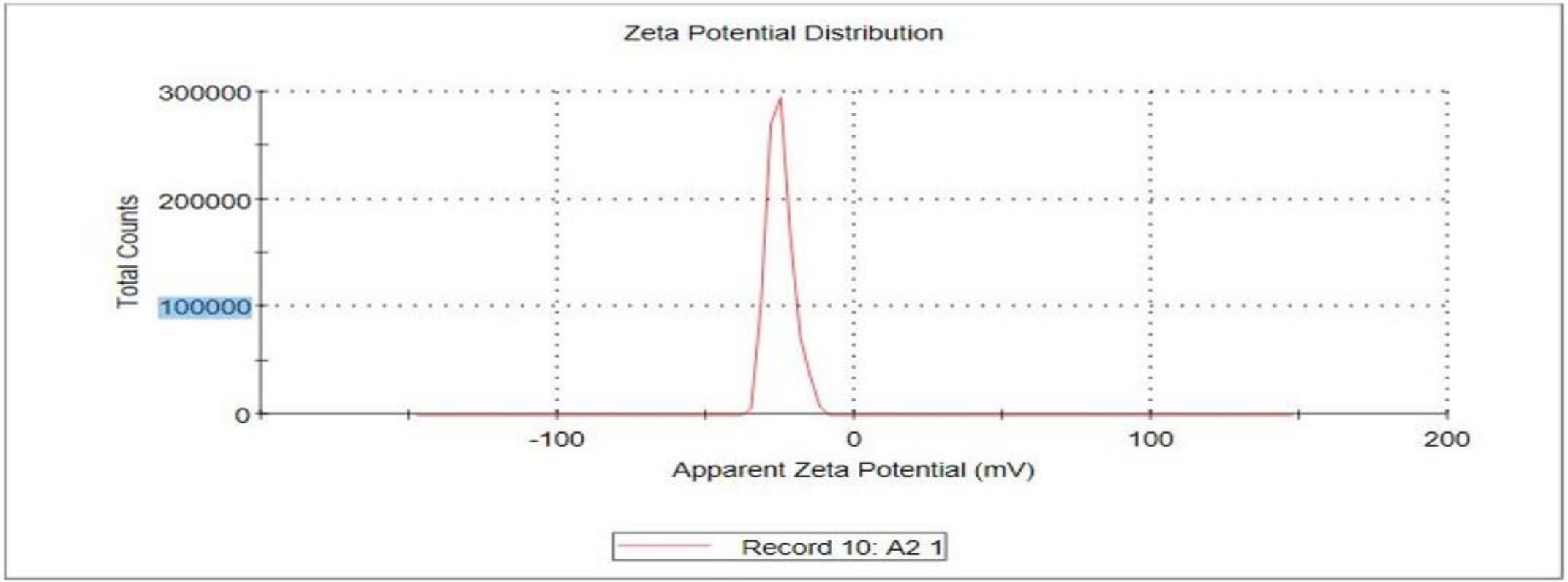
Supplementary Figure S1: Stem of *Colocasia esculenta* plant.

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-25.1	**Peak 1:**	-25.1	100.0	4.35
Zeta Deviation (mV):	4.35	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0916	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



Supplementary Figure S2: Zeta potential study of synthesized AgNPs.

Supplementary Figure S3: Microscopic view of dead *Culex* larvae treated at (a) 0.5 mg/L, (b) 1 mg/L, (c) 3 mg/L, (d) 8 mg/L, and (e) 10 mg/L concentration of synthesized silver nanoparticles.