



Original article

A facile and rapid method for green synthesis of *Achyranthes aspera* stem extract-mediated silver nano-composites with cidal potential against *Aedes aegypti* L.

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ARTICLE INFO

Article history:

Received 11 August 2017

Revised 6 October 2017

Accepted 1 November 2017

Available online 14 November 2017

Keywords:

Silver nano-composites

*Achyranthes aspera**Aedes aegypti*

SEM

TEM

EDX

XRD

FT-IR

ABSTRACT

Aedes aegypti L. is the primary vector associated with transmission of globally concerned diseases; Zika, yellow fever, dengue and Chikungunya. Present study investigates an efficient, alternative and comparative approach for mosquito control which is safe to environment and non-target organisms. The silver nano-composites (AgNCs) were synthesized from the aqueous stem extract of *Achyranthes aspera* (AASE) using different concentration of aqueous silver nitrate (AgNO₃). The synthesis was tracked by UV-vis spectrophotometer and particle size analyser (DLS). The evaluation of their larvicidal potential against early fourth instars of *Ae. aegypti* showed significant potency, the toxicity increasing with the concentration of silver nitrate. The 24, 48 and 72 h bioassays resulted in respective LC₅₀ values of 26.693, 1.113 and 0.610 µg/mL (3 mM AASE-AgNO₃) 9.119, 0.420 and 0.407 µg/mL (4 mM AASE-AgNO₃) and that of 4.283, 0.3 and 0.248 µg/mL (5 mM AASE-AgNO₃). Keeping in view the significantly high larvicidal efficiency at lower concentration of silver nitrate, the 4 mM nano-composites were selected over 5 mM composites for further biophysical characterization carried out by X-ray Diffraction (XRD), Fourier transform infrared spectrometer (FTIR), Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX) spectroscopy and Transmission electron microscopy (TEM). SEM and TEM confirmed the synthesis of spherical poly-dispersed AgNCs with average size ranging from 1–30 nm. Characterization through XRD showed the crystalline face-centered-cubic (fcc) structure of AgNCs with the highest intense peak obtained at 2θ value of 31.82°. FT-IR data suggests complex nature of AgNCs showing clearly defined peaks in different ranges. The present investigations recommend AgNCs of *A. aspera* stems as a low-cost and eco-friendly alternative to chemical insecticides for mosquito control.

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1. Introduction

Aedes aegypti, the principal and worldwide vector of Yellow fever, Zika, Dengue and Chikungunya viruses, has enthralled extensive interest among researchers because of quick and rampant spread of these diseases at global level. The tropical and subtropical regions of the world are experiencing increased incidences

of dengue each year resulting in almost 30-fold increase in its occurrence since past five decades (WHO, 2016). It is important to note that though almost half of the world population lives in dengue-prevalent countries; nearly 75% of global load is borne by countries in Asia-Pacific regions alone. The data compiled by Union Health Ministry of India showed 12% rise in dengue cases in India in just one year. The reports revealed 99,913 cases of dengue and 220 deaths in 2015 which increased to 111,880 incidences and 227 losses of human lives in 2016. The highest figure of 17,702 dengue cases was recorded in West Bengal, located in Eastern part of India. Concurrently, India suffered from 27,553 Chikungunya cases in 2015 which almost doubled in 2016 resulting in 58,136 sufferers (NVBDCP, 2017).

The mosquito management strategies have always been reliant on the application of various chemicals in the breeding sites. However, these strategies have raised major concerns of resistance to

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Peer review under responsibility of King Saud University.



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insecticides, harmful impact on the non-target organisms, environment pollution and bio-magnification in food chain (Kumar et al., 2002, 2004; Borase et al., 2013). These complications have created an instant need to develop new control strategies which could be used indoor, outdoor and at mosquito breeding sites without causing any harm to human beings, environment and beneficial organisms.

Since past few years, researchers have revitalized their interests on herbal products as mosquito control agents. The attempts have been made to explore the phytochemicals with control potential with diverse mode of actions against different stages of mosquito vectors; such as ovicidal, larvicidal, adulticidal, growth regulatory, repellency and oviposition deterrence (Maia and Moore, 2011; Ghosh et al., 2012, Kumar et al., 2012a, 2012b; Warikoo and Kumar 2013; 2014). Reports advocate that eco-friendly and biodegradable phytochemicals possessing multiple mechanisms of action may symbolize noteworthy molecules and efficient alternatives to synthetic insecticides. Furthermore, it is suggested that these molecules of significance if blended with nanotechnology make them more efficient for effectual management of mosquitoes because of easier dispersion and penetration (Benelli et al., 2016).

The dire need to control mosquitoes and necessity to build environment-friendly technologies has augmented focus of researchers on biological synthesis of nano-composites as probable control agents. Noble metallic nanoparticles such as those of silver, gold, titanium and platinum are already used in nano-medicinal applications. Particularly, silver nanoparticles have been found to possess potential antifungal, antibacterial, antiplasmodial and mosquito larvicidal properties (Elumalai et al., 2016). The nano-composites have been recognized as potentially effective replacement of chemical insecticides imposing much less ecological damage. Mehlhorn, (2016) has summarized the latest developments in the field of nanobiotechnology and advocated that nanobiotechnology technique does not allow direct contact between hosts of the parasites and the chemicals used. As a result, it leads to development of quite low resistance levels and minimal effects on the host health.

Over the past several years, various biological agents have been investigated for production of low-cost, simple and non-toxic metallic nanoparticles (Thakkar et al., 2009; Salunkhe et al., 2011; Tran et al., 2013; Shah et al., 2015; Ahmed et al., 2016). Several plants, such as Neem (*Azadirachta indica*), green tea (*Camellia sinensis*), leguminous shrub (*Sesbania drummondii*), natural rubber, starch, *Aloe vera* plant extract, lemongrass leaves extract and various leaf and stem broth have been exploited for the synthesis of silver nanoparticles (Vijayaraghavan et al., 2012). The silver nanoparticles synthesised from aqueous leaf extract of *Mimosa pudica* were found to exhibit noticeable larvicidal efficacy against fourth instars of *Culex quinquefasciatus* Say and *Anopheles subpictus* Grassi (Marimuthu et al., 2011). Similar efficacy of nanoparticles against mosquito vectors was reported by Santoshkumar et al. (2011) when synthesised from *Nelumbo nucifera* aqueous leaf extract. Plant-mediated silver nano-composites have been reported as a rapid and simple method and comparatively advantageous because of its cost-effectiveness, environment-friendly approach and controlled synthesis resulting in requisite shape, size and sensitivity (Gnanadesigan et al., 2011; Elumalai et al., 2016).

Achyranthes aspera L. (Amaranthaceae); native to Africa and Asia, and found throughout India; is an erect, annual or perennial herb found on the road sides, field boundaries and waste lands. Commonly known as Prickly chaff flower or devil's horsewhip, the plant is used in several medical conditions such as asthma, cough and even pneumonia (Sharma et al., 2015). The flowering spikes have been used to treat venomous bites of snakes and reptiles whereas aqueous root paste of the plant is used in ophthalmia and corneal opacities. The extensive medicinal properties of the

plant have also been exploited for the treatment of stomach ache, piles, boils and skin eruptions (Amaladhas et al., 2013; Elumalai et al., 2016). Occurrence of several organic compounds such as achyranthine, ecdysterone, betaine, pentatriacontanone, pentatriacontane, hexatriacontane and tritriacontane has been reported in the stem extract of *A. aspera* (Peddi and Sadeh, 2015).

Based on the literature study and necessity to control mosquitoes using environment-friendly approach, current investigations were undertaken with the nano-composites (NCs) synthesised from aqueous stem extracts of *A. aspera*. The presence of NCs in the colloid was confirmed by UV spectral analysis and Dynamic Light Scattering (DLS). The larvicidal efficacy of silver nano-composites (AgNCs) was assessed against early fourth instars of *Ae. aegypti* and most efficient NC was characterized by different techniques; SEM, EDX, TEM, XRD and FT-IR. The present study suggests about the employment of *A. aspera* nano-composites in the breeding sites of mosquitoes which could prove as a model eco-friendly approach to combat mosquitoes as well as to utilize beneficial aspects of harmful weeds.

2. Materials and methods

2.1. Rearing of *Ae. aegypti*

The present investigations were conducted on the early fourth instars of dengue vector, *Ae. aegypti* maintained in an insectary under controlled conditions of 28 ± 1 °C, $80 \pm 5\%$ RH and 14:10 L/D photoperiod as per protocol of Sharma et al. (2016).

2.2. Plant collection

Fresh, young and healthy stems of *A. aspera* L. were collected from local area of New Delhi, India. The plant was taxonomically identified with the help of Dr. Arun K. Pandey, Professor, Department of Botany, University of Delhi. The voucher specimen was deposited in the University of Delhi Herbarium (DUH) with accession No. 14376.

2.3. Preparation of plant extract

The stems of *A. aspera* were washed thoroughly with tap water followed by double distilled water to remove dust and dirt. The stems were carefully scrutinized for any disease, cut into small pieces, weighed and grinded in an electric blender. The 10 g of blended stems were transferred into a 250 mL glass beaker containing 100 mL double distilled water. A stem broth was prepared by boiling the contents at 60 °C for about 15–20 min (Elumalai et al., 2016). The aqueous extract was kept undisturbed for 2–3 h and filtered through muslin cloth followed by filtering through Whatman No. 1 filter paper to remove particulate matter. The clear extract obtained was refrigerated (4 °C) in amber colour culture bottles for further investigations.

2.4. Synthesis and optimization of silver nano-composites

Preliminary investigations were held with varying volumes of stem extract (ranging from 0.8 mL to 2.0 mL), added to 10 mL of 3 mM silver nitrate (AgNO_3). Based on the results, 1.8 mL of *A. aspera* extract was selected to synthesise nano-composites. Subsequently, nano-composites were formulated by adding selected volume of stem extract (1.8 mL) with different concentrations of silver nitrate (1, 2, 3, 4 and 5 mM). The mixtures were incubated at 85–95 °C for 30 min, allowed to cool down and monitored periodically to evaluate the bioreduction of Ag^+ in aqueous solution. The

change of colour from light green to dark brown was used as indicator of the biosynthesis of nano-composites.

2.5. Selection of silver nano-composites

The synthesised nano-composites were selected for further investigations based on intensity and size analysed by UV-visible spectrum and Dynamic light scattering.

2.5.1. UV-Vis spectral analysis

The intensity of the nano-composite mixtures was scanned and recorded in UV-visible spectra ranging from 200 to 700 nm wavelengths, using a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) with a resolution of 1 nm. The aqueous solution of AgNO₃ was used as control during the spectrophotometry.

2.5.2. Dynamic Light Scattering (DLS)

Particle size of silver nano-composites of *A. aspera* was analysed on particle size analyser system (Zeta sizer, Malvern Instruments Ltd., USA). The average distribution of nano-composites was based on intensity, volume and number.

2.6. Mosquito larvicidal bioassay

The standard WHO protocol (2005) was followed with slight modification to estimate the larvicidal activity of the AgNCs of *A. aspera* against the early fourth instars of *Ae. aegypti*. The concentration of AgNCs assessed for efficacy ranged from 2 µg/mL to 50 µg/mL. A total of 20 active early fourth instars of *Ae. aegypti* were transferred into a thoroughly stirred mixture of 1 mL of nano-composites and 149 mL of dechlorinated water. Five replicates were carried out simultaneously for each concentration. Control sets were exposed to the aqueous AgNO₃ solution. Larval mortality was recorded after every 24 h till 3 days.

2.7. Data analysis

The data obtained was subjected to probit analysis using SPSS Statistical Software Package (Version: 19.0). The LC values were estimated at 30, 50 and 90 levels. Other statistical parameters including 95% confidential limits, chi-square, standard deviation and regression coefficient were calculated in order to compute the significance and evaluate the difference between test samples.

2.8. Characterization of effective silver nano-composites

The most effective nano-composites with maximum larvicidal efficiency were characterized by different bio-physical techniques; Scanning Electron Microscopy (SEM), Energy dispersive X-ray (EDX) spectroscopy, Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD) and Fourier Transform Infrared Radiation (FTIR) spectroscopy.

2.8.1. Scanning Electron Microscopy (SEM)

The aqueous solution containing silver nano-composites was centrifugated repeatedly and placed on the carbon-coated copper grids to prepare thin films. Excess solution was absorbed by blotting sheet and the film was dried keeping under a mercury lamp for 5 min. The morphology of the composites was characterized using HRSEM (High Resolution Scanning Electron Microscopy) Zeiss Model: V5.05 (Sigma) at accelerating voltage of 20 keV.

2.8.2. Energy dispersive X-ray (EDX) spectroscopy

The sample was analysed using Bruker instrument at accelerating voltage HV: 20.0 kV for qualitative and quantitative estimation

of the elements possibly involved in formation of nano-composites.

2.8.3. Transmission Electron Microscopy (TEM)

A drop of the silver nano-composites was placed on a piece of parafilm of the carbon-coated copper grid and was allowed to settle for 5–10 min. The excess solution was soaked with the help of blotting sheet and the grid was left undisturbed for 10–20 min for absorption. The composites were then observed under Transmission Electron Microscope (FEI Tecnai G² 30 S-TWIN) operated at an accelerating voltage of 300 kV.

2.8.4. Fourier Transform Infrared Radiation (FT-IR) analysis

FT-IR analysis was performed using Bruker Optik, model number Verter 70V to evaluate the functional groups present on the surface area of nano-composites probably responsible for reduction and stabilization of silver ions in silver nano-composites.

2.8.5. X-ray diffraction (XRD) analysis

XRD spectrum was recorded using Rigaku Smart Lab[®] X-ray Diffractometer to ensure crystalline nature of synthesized silver nano-composites.

3. Results

Current investigations were carried out to assess the potential use of silver nano-composites, formulated with aqueous stem extracts of *A. aspera*, as a control agent of *Ae. aegypti*. The NCs were synthesised with different concentrations of silver nitrate solutions, evaluated for larvicidal potential and characterized by different techniques.

3.1. AgNCs synthesis: Spectroscopic confirmation

The formation of silver nano-composites (AgNCs) was carried out by adding different volumes of aqueous stem extract of *A. aspera* (AASE) in 10 mL of 3 mM silver nitrate. The formation of NCs was analysed and confirmed by UV-visible spectra, the results of which are presented in Fig. 1. Peaks obtained at different wavelengths are summarized in Table 1. Our results revealed the most prominent; narrow and highest peak at 427 nm obtained with NCs formed with 1.8 mL of stem extract (Fig. 1). It also shows

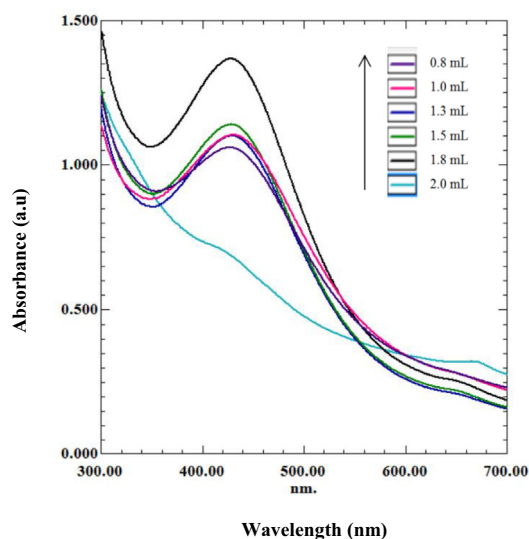


Fig. 1. UV-Vis spectra of silver nano-composites synthesized using different volumes of AASE and 3 mM silver nitrate.

Table 1

Screening of different ratios of aqueous stem extract of *Achyranthes aspera* and silver nitrate for the optimum synthesis of silver nano-composites using spectrophotometer (Shimadzu, 1800).

Aqueous Stem Extract: AgNO ₃	AgNO ₃ Conc. (mM)	Volume of AASE (mL)	Wavelength (nm)	Absorbance (a.u)
Varied volumes of AASE: 3 mM of AgNO ₃	3.0	0.8	426	1.060
		1.0	430	1.104
		1.3	429	1.101
		1.5	428	1.410
		1.8	427	1.368
		2.0	665	0.319
	1.0	1.8	671	0.195
		2.0	426	1.273
		3.0	431	1.814
		4.0	432	1.887
5.0	1.8	435	2.001	

AgNO₃: Silver Nitrate.

AASE: *Achyranthes aspera* stem extract.

shifting of the SPR (Surface Plasmon Resonance) bands to lower wavelengths with increase in the volume of plant extract demonstrating the formation of smaller sized nanoparticles. Based on the above results, 1.8 mL of aqueous AASE was selected for synthesis of NCs with different concentration of AgNO₃ (1–5 mM). The mixtures showed change in the colour within 30–45 min depending on the AgNO₃ concentration. The solutions containing 3, 4 and 5 mM of silver nitrate exhibited significant colour change in colour from light green to dark green followed by dark brown indicating the formation of AgNCs (Fig. 2). Rest two solutions did not exhibit major colour change though addition of 2 mM silver nitrate could cause moderate alterations.

The biosynthesis of silver nano-composites at 2 mM, 3 mM, 4 mM and 5 mM concentrations could be traced at 426 nm, 431 nm and 432 nm and 435 nm, respectively (Table 1; Fig. 3). However, synthesis with 1 mM and 2 mM of silver nitrate could not

form stable NCs as evident by their absorption spectra. The results also showed a slight red shift and increase in the spread and intensity of absorption bands with an increase in concentration of AgNO₃. The average distribution of nano-composites formulated with at 3 mM, 4 mM and 5 mM silver nitrate was assessed through Dynamic Light Scattering (DLS) based on their intensity, volume and number. Results showed the respective average particle size distribution of silver synthesized nanoparticles as 54.96 nm, 73.78 nm and 69.84 nm with dispersal index ranging from 0.33 to 0.38 (Table 2; Fig. 4).

3.2. Mosquito larvicidal assay

AgNCs synthesized from *A. aspera* stem extract when assayed against early fourth instars of *Aedes aegypti*, showed efficient larvicidal activity. The efficacy increased exponentially with AgNO₃



Fig. 2. Colour change in *Achyranthes aspera* stems extract (AASE)-Silver nitrate mixtures at different concentration (1–5 mM) indicating synthesis of silver nano-composites; (A) Initial mixtures; (B) Final mixtures.

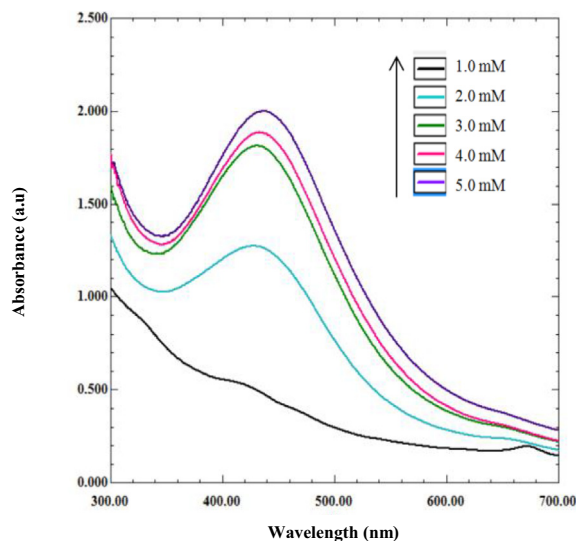


Fig. 3. UV-Vis spectra of silver nano-composites synthesized using 1.8 mL of AASE and different concentration of silver nitrate (1–5 mM).

concentration resulting in respective LC_{50} values of 26.693 $\mu\text{g/mL}$ (3 mM AASE- AgNO_3) 9.119 $\mu\text{g/mL}$ (4 mM AASE- AgNO_3) and LC_{50} value of 4.283 $\mu\text{g/mL}$ (5 mM AASE- AgNO_3) at 24 h (Table 3). Control assays conducted with silver nitrate solution, however, caused no mortality.

Our results also revealed the increased larvicidal toxicity of AgNCs when assay was continued further for two days. The assays with 4 mM AASE-AgNCs reduced the LC_{50} value by 95.39% (0.420 $\mu\text{g/mL}$) at 48 h and by 95.53% (0.407 $\mu\text{g/mL}$) at 72 h (Table 4). Keeping in view the noticeable larvicidal response with 4 mM AASE- AgNO_3 at 24 h and lower concentration AgNO_3 , these AgNCs were characterized further.

3.3. Characterization of selected silver nano-composites

The characterization of 4 mM AgNCs by XRD analysis showed the main peaks of Bragg reflections at 2θ degree; 27.50°, 31.82°, 45.82°, 54.49°, 57.01°, 67.04°, 73.99° and 76.33° corresponding to the lattice planes (111), (200), (220), (311), (222), (400), (331) and (420) respectively (Fig. 5). The peak with maximum intensity was observed at (200) lattice plane with 2θ value of 31.82°. The XRD pattern of AASE-AgNCs suggested the crystalline and face-centered-cubic (fcc) structure of composites. When nano-composites were analysed by high resolution scanning electron microscopy, the particles with an average size of 25 nm were observed (Fig. 6). The scanning electron micrograph also revealed almost spherical shapes of AgNCs found mostly in aggregated form. The elemental composition of synthesized AgNCs was identified by

EDX pattern which confirmed the presence of Ag, Cl, C and O in their order of concentration in weight%. The element silver (58.35%) being found in *A. aspera*-mediated synthesised AgNCs with a strong signal and in the highest concentration in weight% evidenced the reduction of silver nanoparticles to elemental silver (Fig. 7). Nevertheless, the spectrum also showed weak signals of chlorine, oxygen and carbon peaks. The morphology and size of the AgNCs further analysed using Transmission Electron Micrographs reinforced the fact that the *A. aspera*-mediated synthesised AgNCs were spherical in shape, uniformly distributed and without agglomeration with an average size of 30 nm (Fig. 8). The FT-IR spectrum of AgNCs synthesized using the *A. aspera* stem extract is presented in Fig. 9. Main peaks were identified at 3023, 2400, 2287, 1585, 1484, 1427, 1314, 918, 607 and 488 cm^{-1} which indicated the probable existence of different phytochemicals in the *A. aspera* stem extract, involved in reducing as well as stabilizing the AgNCs. The peak obtained at 3023 cm^{-1} was probably due to the medium intensities of alkene C–H and aromatic C–H stretch vibrations. The peaks at 2400 and 2287 cm^{-1} may be assigned to O=C=O and Si–H silane stretching (Fig. 9). On the other hand, peak at 1585 cm^{-1} may correspond to bending vibrations of N–H while peaks at 1484 and 1427 cm^{-1} may be linked to the presence of medium-weak, multiple bands of aromatic C=C stretch. The spectrum also revealed a peak at 1314 cm^{-1} which may be because of strong acidic stretch of C–O, sulfone S=O stretching, strong C–N stretching of aromatic amines and medium bending corresponding to phenol (O–H). The peak observed at 918 cm^{-1} could be attributed to strong bending vibrations of alkene C=C and strong stretching of alkyl halide, while the peak identified at 607 cm^{-1} could correspond to C–Cl and C–Br halo compounds.

4. Discussion

Tropical and sub-tropical countries of the world are facing continued rise in mosquito-borne diseases. Use of chemical-based control measures to counter the mosquito nuisance has not only impacted our environment adversely, but also caused increased chemical resistance in the mosquito population and lethal effects on non-target organisms and humans. Consequently, as suitable alternate to the chemical insecticides, plant-based products are catching attention of researchers. Various plants have been explored as the potential mosquito control agents against their different life stages (Kumar et al., 2012b; Warikoo et al., 2013). In recent years, nanoparticles, particularly silver nanoparticles reported to possess anti-fungal, anti-inflammatory and anti-viral activity, have engrossed substantial attention owing to their extensive selection for applications other than drug delivery, diagnostics, imaging, sensing, gene delivery, etc. (Poopathi et al., 2015). Nowadays, researchers have been exploring phytochemicals and microbes in the form of nanoparticles against mosquitoes (Li et al., 2011; Kuppusamy et al., 2016). The larvicidal activities of cobalt nanoparticles synthesised from *Bacillus thuringiensis* have

Table 2
Dynamic light scattering (DLS) of AgNCs synthesized from aqueous stem extract of *Achyranthes aspera* indicating the average particle size and distribution of nano-composites.

S. No.	Conc.	Average size (d.nm)	Poly-dispersity index (Pdl)	Count rate (kcps)	Size (d.nm)	% Intensity	St. Dev. (d.nm)
1	3 mM	54.96	0.355	260.6	Peak 1	54.79	77.4
					Peak 2	255.2	19.9
					Peak 3	4546	2.7
2	4 mM	73.78	0.382	212.1	Peak 1	92.29	97.2
					Peak 2	5378	1.7
					Peak 3	11.30	1.1
3	5 mM	69.84	0.332	305.9	Peak 1	89.95	97.5
					Peak 2	4861	2.5
					Peak 3	0.00	0.00

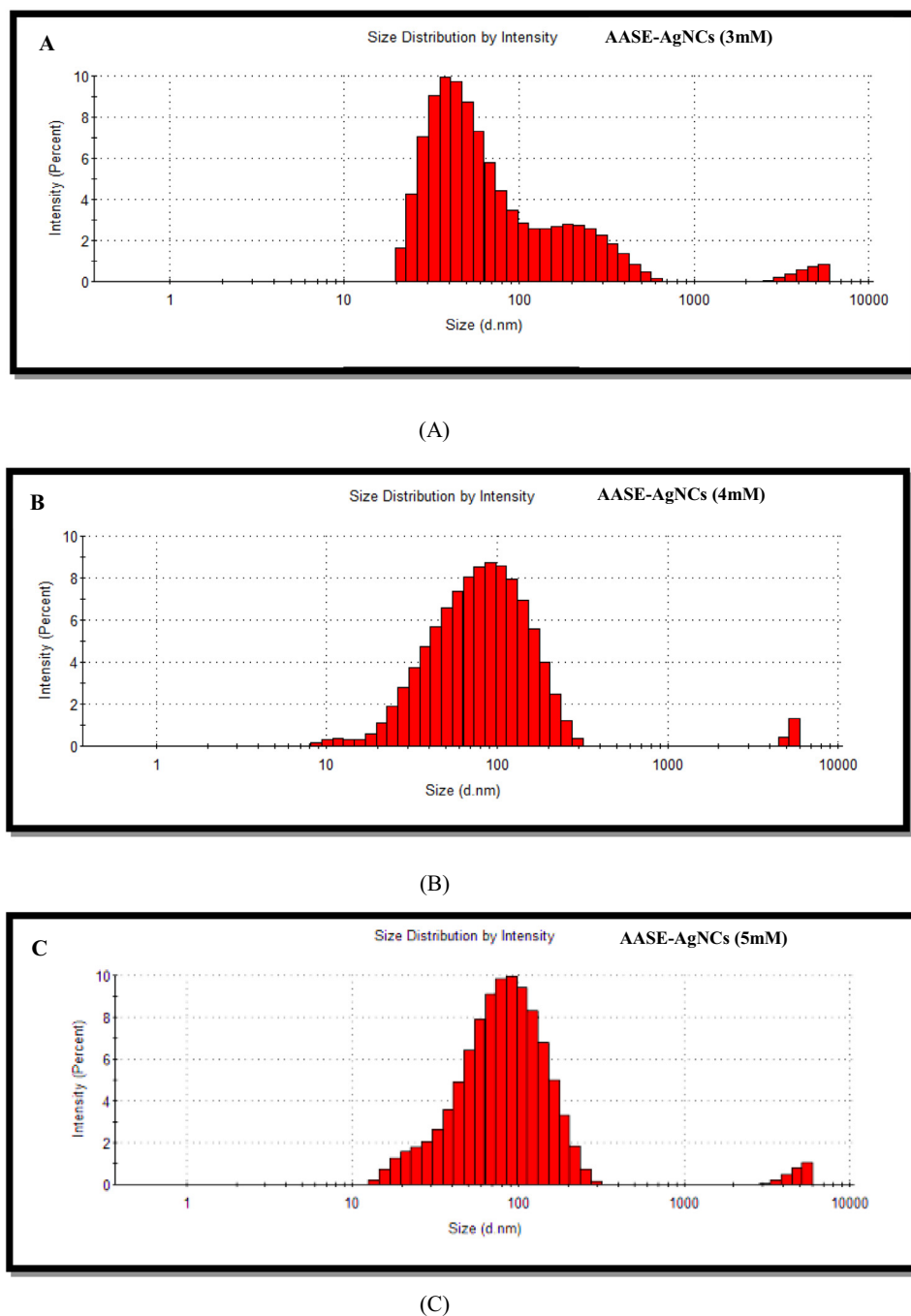


Fig. 4. Dynamic Light Scattering of silver nano-composites (AgNCs) synthesized with AASE showing size distribution at different concentrations of silver nitrate; (A) 3 mM, (B) 4 mM, and (C) 5 mM.

Table 3

Larvicidal activity of silver nano-composites synthesized from stems of *Achyranthes aspera* against early fourth instars of *Aedes aegypti* when exposed for 24 h.

Sample	LC ₅₀ (μg/mL)	S.E.	χ ²	df	RC
AASE-AgNCs (3 mM)	26.693 (14.437–113.459)	0.217	1.296	7	0.713
AASE-AgNCs (4 mM)	9.119 (3.785–17.082)	0.216	2.736	7	0.731
AASE-AgNCs (5 mM)	4.283 (2.702–5.863)	0.276	1.575	7	1.701

No mortality was observed in the control.

AASE-AgNCs: *Achyranthes aspera* Stems Extract-Silver Nano-composites.

LC₅₀ lethal concentration that kills 50% of the exposed larvae, Values in parentheses indicate the lower and upper 95% fiducial limits, S.E. = Standard error, χ² = chi-square, df = degree of freedom, RC = Regression Coefficient, Test samples were transformed into log covariant (log₁₀), p < .05, level of significance, Values are mean of five replicates.

Table 4
Larvicidal activity of the silver nano-composites synthesized from stems of *Achyranthes aspera* against early fourth instars of *Aedes aegypti* when exposed for 48 h and 72 h.

AgNCs	LC ₅₀ (µg/mL) at 48 and 72 h							
	48 h	S.E.	χ^2 (df)	RC	72 h	S.E.	χ^2 (df)	RC
AASE-AgNCs (3 mM)	1.113 (0.023–2.981)	0.239	1.773 (7)	0.743	0.610 (0.014–1.735)	0.309	1.342 (7)	1.017
AASE-AgNCs (4 mM)	0.420 (0.000–2.209)	0.232	2.590 (7)	0.510	0.407 (0.000–1.699)	0.260	3.693 (7)	0.704
AASE-AgNCs (5 mM)	0.3 (0.000–1.348)	0.296	3.383 (7)	0.806	0.248 (0.000–1.137)	0.364	1.231 (7)	0.958

No mortality was observed in the control.

AASE-AgNCs: *Achyranthes aspera* Stems Extract-Silver Nano-composites.

LC₅₀ lethal concentration that kills 50% of the exposed larvae. Values in parentheses indicate the lower and upper 95% fiducial limits, S.E. = Standard error, χ^2 = chi-square, df = degree of freedom, RC = Regression Coefficient, Test samples were transformed into log covariant (\log_{10}), $p < .05$, level of significance, Values are mean of five replicates.

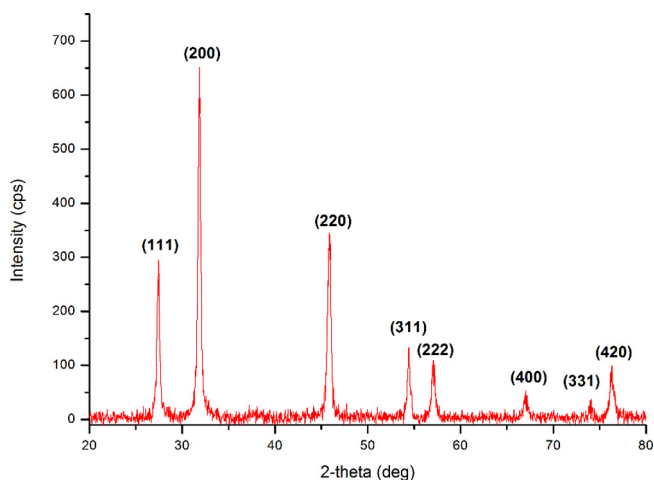


Fig. 5. X-ray Diffraction (XRD) pattern of silver nano-composites synthesized from aqueous stem extract of *Achyranthes aspera*.

been tested against *Ae. aegypti* and *An. Subpictus* (Martimuthu et al., 2013). Similar larvicidal activity against *Ae. aegypti* has been reported when silver nanoparticles were synthesized from

B. thuringiensis (Banu et al., 2014). In recent times, a number of plants have been explored for the green synthesis of silver nanoparticles (AgNPs). It has been showed that the combination of nanoparticles with bioactive components of plant extracts imparts improved efficiency (Benelli, 2016). The larvicidal potential of leaf extract-mediated synthesis of AgNPs from *Mukia maderaspatana* (Chitra et al., 2015), *A. aspera* (Elumalai et al., 2016), *Gmelina asiatica* and *Chomelia asiatica* (Muthukumar et al., 2015a, 2015b), *Malva sylvestris*, *Quisqualis indica* and *Carissa carandas* (Govindarajan and Benelli, 2016a; Govindarajan et al., 2016a, 2016b) has been explored against different species of mosquitoes. The increased potential of plant extracts has been observed when used as silver nano-composites which are suggested for use as a low-cost, highly effective, eco-friendly and promising option in the fight against Zika, malaria and filariasis vectors. AgNCs derived from leaf of *Catharanthus roseus* (Mukunthan et al., 2011), *A. aspera* (Amaladhas, 2013), olive extract (Khalil et al., 2014) and *Parkia speciosa* Hassk pods (Fatimah, 2016) have also been reported to possess desirable cytotoxicity towards bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*).

Present study investigates the potential of a common weed, *A. aspera* as mosquito control agent. Our earlier studies have revealed the larvicidal potential of hexane extracts prepared from the leaves

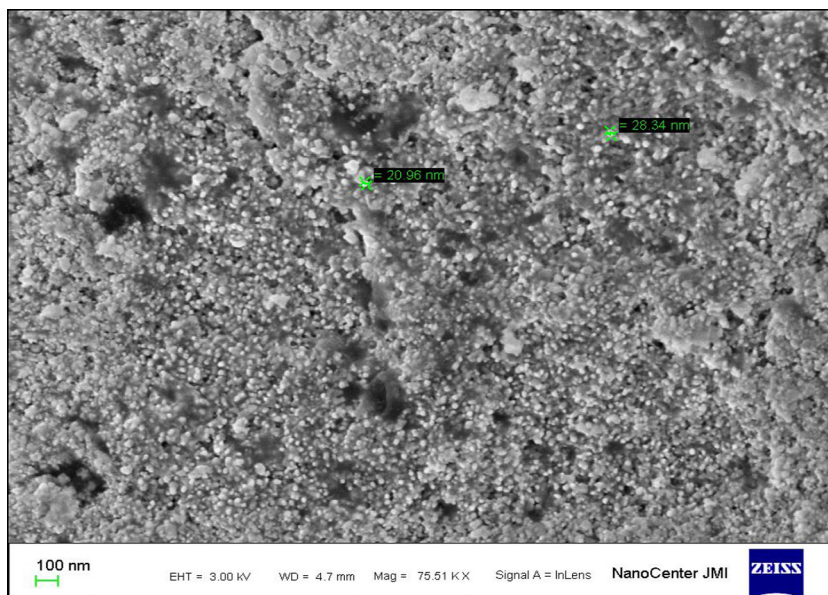
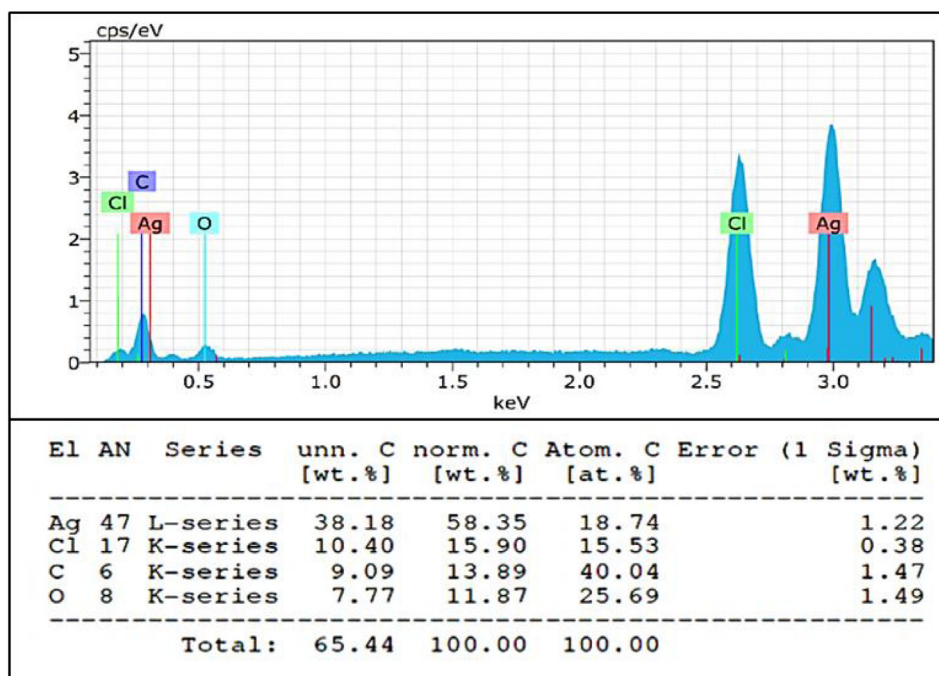


Fig. 6. Scanning Electron Microscopy (SEM) image of silver nano-composites synthesized from *Achyranthes aspera* stem extract and 4 mM AgNO₃. Image magnification: 100 nm.



EL = Element; AN = Atomic Number; Series = characteristic X-ray lines; unn. C [wt. %] = the unnormalised concentration in weight percent of the element; norm. C [wt. %] = the normalised concentration in weight percent of the element; C Atom. [at. %] the atomic weight percent; C Error (1 Sigma) [wt. %] = error in the weight percent concentration at the 1 sigma level.

Fig. 7. Energy dispersive X-ray (EDX) spectrum of silver nano-composites synthesized from aqueous stem extract of *Achyranthes aspera*. EL = Element; AN = Atomic Number; Series = characteristic X-ray lines; unn. C [wt.%] = the unnormalised concentration in weight percent of the element; norm. C [wt.%] = the normalised concentration in weight percent of the element; C Atom. [at.%] the atomic weight percent; C Error (1 Sigma) [wt.%] = error in the weight percent concentration at the 1 sigma level.

and stems of the plant (Sharma et al., 2015). The laboratory assays with hexane stem extracts resulted in LC_{50} value of 68.133 ppm while exposure with leaf extracts revealed LC_{50} value of 82.555 ppm. It has been suggested that plant-borne molecules are often effective at few parts per million against *Aedes*, *Anopheles* and *Culex* young instars and can be used for the rapid fabrication of mosquitocidal nano-formulations which could be employed to formulate low-priced repellents with low human toxicity (Benelli and Mehlhorn, 2016). Keeping this in view, the attempts were made to synthesize nano-composites from *A. aspera* stem extracts and investigated for their larvicidal potential.

It has been already reported that selection of botanical agent and its incorporation as reducing and stabilizing agent during the synthesis of NCs is the key step and the deciding factor for the formulation, biophysical parameters and efficacy of NCs (Govindarajan et al., 2016b). Furthermore, use of aqueous extracts of plants for the green synthesis of nano-composites instead of organic solvents made this biosynthetic process an eco-friendly route for nano-formulations (Reddy et al., 2015). Therefore, present study involves synthesis of nano-composites from aqueous extracts of *A. aspera* stem extracts. The effective NCs based on their larvicidal efficacy were then characterized using different techniques; UV-vis spectrum, DLS, XRD, SEM, EDX, TEM, FT-IR.

Our investigations showed a gradual colour change of the solution from light green to dark green and then to dark brown within 30–45 min during the synthesis of AgNCs. It is suggested that the change in colour of the solution is because of the excitation of Surface Plasmon Vibration in the silver nano-composites and is directly correlated with $AgNO_3$ concentration in the solution (Elumalai et al., 2016). When the surface plasmon resonance peak

in the UV-vis absorption spectra of the synthesized silver nano-composites were analysed, the narrow, most prominent and highest peak was observed at 427 nm indicating maximum reduction of the silver nitrate. Synthesis of AgNCs from *A. aspera* stem extract at exactly same wavelengths has been reported by Peddi and Sadeh (2015). Our results also showed that with increased concentration of $AgNO_3$, the intensity and breadth of absorption bands also increased with a slight red shift in absorption bands. The broadening of the peak signifies the formation of polydispersed large nanoparticles due to slow reduction rates (Peddi and Sadeh, 2015; Fatimah, 2016). Similar results have been reported by Gude et al. (2012), who advocated that increased intensity and broadness of absorption bands indicates the increase in percentage of formation of AgNCs. It has also been revealed that SPR bands are influenced by size, shape, composition, morphology and dielectric environment of the synthesized AgNCs (Govindarajan and Benelli, 2016b).

When assayed against early fourth instars of *Ae. aegypti*, the *A. aspera* NCs showed increased efficacy increasing exponentially with $AgNO_3$ concentration. The 24 h assays with NCs synthesised with 4 mM $AgNO_3$ resulted in LC_{50} value of 9.119 $\mu g/mL$, the toxicity increasing when assays were continued for next 48 h. Earlier, Patil et al. (2012) reported the larvicidal potential of silver NPs formulated with *Pergularia daemia* plant latex exhibiting LC_{50} value of 6.18 ppm and 6.47 ppm against *Ae. aegypti* and *An. Stephensi*, respectively. The larvicidal activity of *Pongamia pinnata* and *Lucas aspera* NCs synthesised from the leaf extracts have been reported against *Ae. aegypti* (Naik et al., 2014; Suganya et al., 2014). Subarani et al. (2013) revealed efficacy of *C. roseus* leaves extract AgNPs against *An. stephensi* with LC_{50} value of 12.47 and 16.84

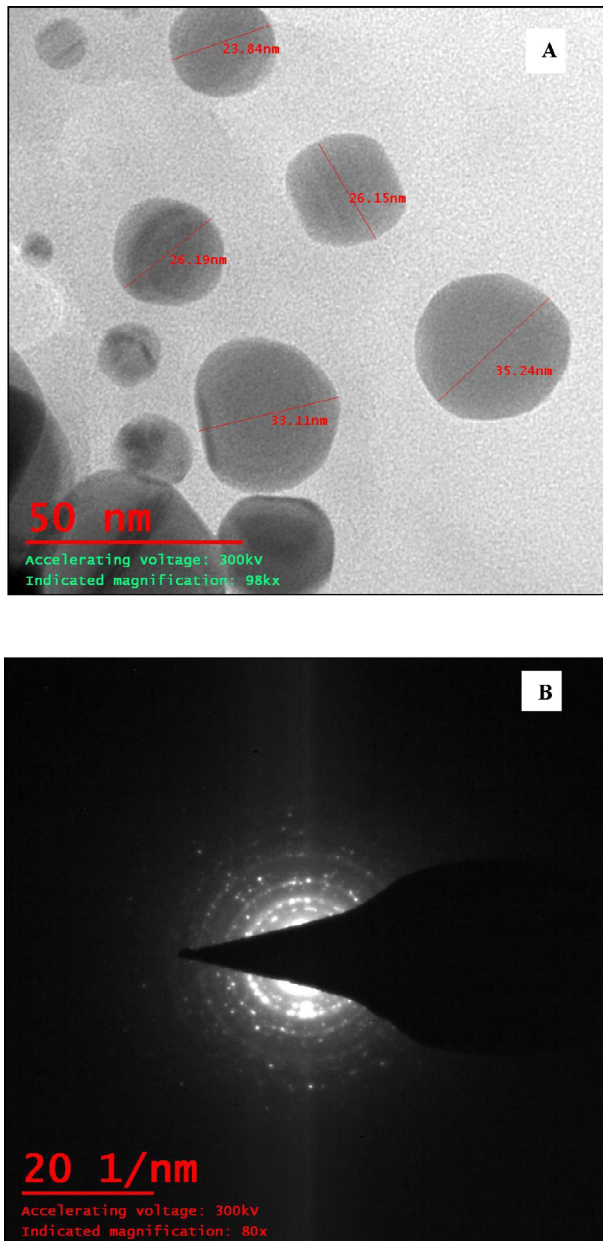


Fig. 8. Transmission Electron Microscopy (TEM) images of Silver nano-composites derived from stem extracts of *Achyranthes aspera*; [A] Entire image; [B] SAED [Selected area (electron) diffraction] image.

mg/mL after 48 and 72 h of exposure and 43.80 mg/mL against *Cx. quinquefasciatus* after 72 h of exposure. Moreover, they also stated that AgNPs were safe for non-target organisms and did not impart any adverse impact on *Poecillia reticulata* even after 48 h to 72 h of exposure. Likewise, silver nano-crystals synthesized from *Hymenodictyon orixense* also exhibited efficient larvicidal potential against *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus* with LC_{50} value of $833 \mu\text{g ml}^{-1}$ (Govindarajan and Benelli, 2016b). They also reported the nontoxicity of AgNPs to the non-target mosquito predator *Diplonychus indicus*. Similar efficacy of *Nicandra physalodes*-mediated synthesis of AgNPs has been reported against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* while were found safe against the non-target aquatic organism *Diplonychus indicus* sharing the mosquito larval habitats (Govindarajan et al., 2016c).

Our investigations also revealed the main peaks of Bragg reflections of NCs at lattice planes (111), (200), (220), (311), (222), (400),

(331) and (420) when analysed by XRD suggesting the crystalline and face-centered-cubic (fcc) structure of composites. Similar reflections at (111), (200), (220) and (311) of silver metal confirming the face centred cubic symmetry have been identified in the silver nanoparticles synthesized from stems of *A. aspera* (Peddi and Sadeh, 2015). They also reported the formation of predominantly spherical nanoparticles with uniform shape of AgNPs of size range 30–80 nm which is in conformity with our results revealing the particles of almost spherical shapes with an average size of 25 nm.

It is known that the shape of metal nanoparticle substantially varies with their optical and elemental properties which can be analysed by EDX technique (Xu and Käll 2002; Suman et al., 2013). The EDX pattern of *A. aspera* NCs in present study confirmed the presence of Ag, Cl, C and O in their order of concentration in weight%. Elumalai et al. (2016) observed the weak signals of oxygen and carbon peaks in EDX of silver nanoparticles synthesized from leaf extract of *A. aspera* and suggested that they might have originated from the biomolecules bound to the surface of the nano-composites. The presence of carbon in nanoparticles is suggested to be possibly due to the carbon tape used to mount the samples (Govindarajan et al., 2016b).

The TEM micrograph of *A. aspera* NCs further confirmed the presence of polydispersed AgNCs with an average size of 30 nm. It was also noted that AgNCs were polydispersed without direct contact and were stable for long period of time. This may be due to the coating of thin layer of biomolecule on their surface which act as stabilizing agent (Vignesh et al., 2013). The FT-IR spectrum of AgNCs synthesized in the present study showed a total of 10 peaks, stretches and vibrations indicating the existence of different phytochemicals in the *A. aspera* stem extract, involved in reducing as well as stabilizing the AgNCs (Silverstein et al., 1981; Reusch, 2013). The analysis of FT-IR spectral suggests the presence of diverse organic molecules such as alkenes, alkanes, thiol, amines, aromatic amines, phenols, and halo compounds in the nano-composites which can majorly be contributed by flavonoids, saponins, proteins and glycosides.

Our studies showed the higher efficacy of silver nano-composites synthesized from aqueous extracts of *A. aspera* stems against early fourth instars of *Ae. aegypti* when compared to the efficacy of only extracts. The increased potential of AgNCs fabricated from plant extracts may be attributed to the interaction between nano-composites and the extracellular lipoprotein matrix increasing the penetrability of the plasma membrane; the possible mechanism(s) of action (Govindarajan et al., 2016a). Moreover, it has also been suggested that the denaturation and loss of the cellular function of organelles and enzymes due to the probable interaction between AgNPs and phosphorous from DNA and/or sulphur from proteins or any other compounds may reduce ATP synthesis and ion exchange causing reduced membrane permeability eventually causing cell death (Sap-lam et al., 2010).

5. Conclusion

The synthesis of AgNCs is presently considered an eco-friendly, cost-effective and efficient alternative to the chemical and microbial insecticides. In the present study, AgNCs synthesized from the aqueous stem extracts of *A. aspera* were found to be polydispersed, spherical in shape, crystalline in nature, with face-centered cubic (fcc) geometry and average size ranging from 1 to 30 nm. AgNCs showed excellent larvicidal potential against major dengue vector *Ae. aegypti*. The research highlighted that *A. aspera*-synthesized AgNCs are easy and rapid to produce, stable over time, and can be employed at low dosages against *Ae. aegypti* larvae and thereby could be considered as advance, newer and safer mosquito larvicides.

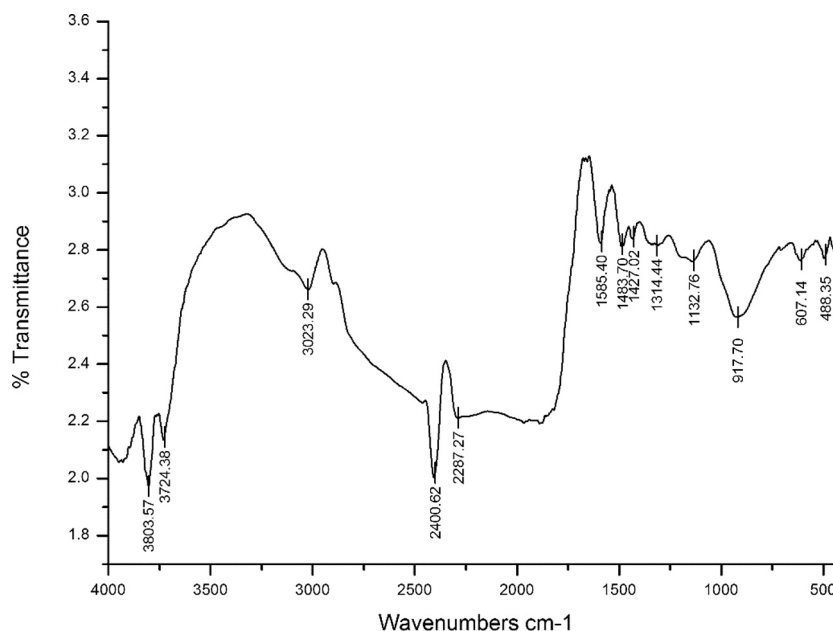


Fig. 9. Fourier Transform Infrared Radiation (FTIR) spectrum of silver nano-composites synthesized from aqueous stem extract of *Achyranthes aspera*.

Acknowledgements

Authors are highly thankful to Dr. Savithri Singh, Principal, Acharya Narendra Dev College, University of Delhi and School of Sciences, Indira Gandhi National Open University for providing infrastructure and research facilities.

Conflict of interest

The authors declare no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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