Pesticidal activity of eco-friendly synthesized silver nanoparticles using *Aristolochia indica* extract against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

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ABSTRACT

In the present study, the antifeedant, larvicidal and cytotoxic activities of synthesized silver nanoparticles (Ag NPs) using aqueous leaf extract of *Aristolochia indica* against third instar larvae of *Helicoverpa armigera* and HeLa cell lines were evaluated. The synthesized Ag NPs were characterized by UV-vis spectrum, XRD, FTIR, SEM and TEM analyses. Pesticidal and cytotoxic activities of aqueous crude extracts and synthesized Ag NPs were studied using leaf disc no choice, leaf dipping methods and MTT assay, respectively. Synthesis of Ag NPs was confirmed by analyzing the excitation of surface Plasmon resonance (SPR) using UV–vis spectrophotometer at 420 nm. TEM analysis showed aggregates and some were scattered with an average size of 16.54 nm. The FTIR result clearly showed that the extracts containing strong absorption band at 2926.56 cm^{-1} corresponding to aldehydic C-H stretching. The maximum antifeedant and larvicidal efficacy was observed in crude aqueous and synthesized Ag NPs against *H. armigera* larvae (LC_{50} = 127.49, 84.56 mg/L; 766.54, and 309.98 mg/mL), respectively. The extract of *A.indica* and Ag NPs elicited low cytotoxic effect with TC_{50} values of >100 and 89 µg/mL, respectively. Gas chromatography–mass spectrometry of aqueous extract confirmed the presence of 3-O-Methyl-d-glucose which may have influenced in the

reduction and stabilization process of Ag NPs synthesis. It is a novel, eco-friendly and cost-effective approach to synthesis Ag NPs using *A. indica* against *H. armigera*. It represents a new direction in developing pesticidal nanomaterials to satisfy the requirement of large-scale industrial production.

Key words: *Aristolochia indica*; Silver nanoparticles; *Helicoverpa armigera*; Antifeedant; Larvicidal; Cytotoxicity.

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Introduction

The American bollworm, *Helicoverpa armigera* is one of the key pests and distributed in most of Asia, Australia, Africa and Southern mediterranean region, including cotton producing countries such as India, China, Pakistan and Egypt [5]. *H. armigera* is a polyphagous pest distributed worldwide inflicting annual crop damage in India worth US$ 1 billion. In India, the pest affected cotton, pigeon pea, chickpea, tomato, okra, and black gram [38]. It has developed resistance to many synthetic pesticides [16]. Hence, search for viable and sustainable alternatives to synthetic pesticides is vital [39].

Botanical pesticides tend to have broad-spectrum activity are relatively specific in their mode of action, safe to living organisms and environment [40]. Antifeedant and insecticidal activities of many plant extracts and their bioactive compounds against several insect pests have been demonstrated [31, 7, 2, 25]. One of the challenges faced by pest control is the choice and availability of safe, effective and cheap insecticides. The disadvantages associated with synthetic pesticides, includes development of pesticide resistant strains, ecological imbalances and harm to non-target organisms. There is a renewed effort to develop substances of plant origin which are considered to be more environment eco-friendly due to their innate biodegradability and lower toxicity to most of the organisms [6]. Many pesticides

are poorly soluble in water due to which large amounts of organic solvents are required to solubilise them. These limitations therefore necessitate the search for new control method which may replace the synthetic insecticides. The synthesized nanoparticles, which are less likely to cause ecological damage have been identified as potential replacement of synthetic chemical insecticides. The current study aimed in evaluating the antifeedant and larvicidal efficacy of synthesized Ag NPs against *H. armigera*.

Nanoparticles exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed based on specific characteristics such as size, distribution, and morphology [43]. The Ag NPs were reported to possess anti-bacterial [36], anti-viral [34] and anti-fungal activities [27]. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for low-cost, energy-efficient, and non-toxic production of metallic nanoparticles [41]. Use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as it eliminates the elaborate process of maintaining cell cultures. Recently green synthesis Ag NPs have been reported using *Euphorbia prostrata* and used to control the adult of *Sitophilus oryzae*, *Haemaphysalis bispinosa* and *Hippobosca maculata* [44,45] and using *Nelumbo nucifera* exhibited effective control against *Anopheles subpictus* and *Culex quinquefasciatus* [35]. Magnetite octadecylsilane nanoparticles were synthesized and used for pest control [19]; the bioefficacy of developed β-cyfluthrin formulations synthesized poly (ethylene glycols) based amphiphilic copolymers were evaluated against *Callosobruchus maculatus* [18] and the feeding deterrent activity of synthesized Ag NPs using leaf aqueous extract of *Manilkara zapota* was evaluated against *Musca domestica* [12].

In this study, the antifeedent, larvicidal and cytotoxic effect of synthesized Ag NPs using the leaf extract of *A. indica* were assessed. Here we demonstrate the potential use of green synthesized Ag NPs were applied to prevent crop damage caused by insects. We believe that

this method can be developed into a simple way to synthesize Ag NPs using *A. indica* extract indicating the industrial prospects for its large scale and low cost synthesis to control pest.

Materials and methods

Plant material

Aristolochia indica L. (Aristolochiaceae) leaves were collected from Malaiyur Hills, Dharmapuri district, Tamil Nadu, South India, in June 2011.The taxonomic identification was made by Dr. A. Akbar, Department of Botany, C. Abdul Hakeem College, Melvisharam Vellore, India. The voucher specimen (ZD/AB/K127) was deposited in our research laboratory for further reference. Silver nitrate ($AgNO_3$, analytical grade) was purchased from Sigma–Aldrich, USA (99.9% pure).

Preparation of *A. indica* leaf aqueous extract

Aqueous extract was prepared by mixing 100 g of dried leaf powder with 500 mL of water (boiled and cooled Milli Q water) under constant stirring using a magnetic stirrer for 3 h [21]. The suspension of dried leaf powder in water was filtered through Whatman no. 1 filter paper. The filtrate was stored in amber coloured air tight bottle at 10°C and used within a week.

Synthesis of silver NPs

A. indica leaves were washed thoroughly in tap water for 10 min in order to remove the dust particles and rinsed briefly in deionized water. The plant leaf broth solution was prepared by taking 10 g of washed and finely cut leaves in a 250 mL Erlenmeyer flask along with 100 mL of deionized water and then boiling the mixture at 60°C for 5 min. After boiling, the solution was decanted, and 15 mL of this broth was added to 85 mL of 3 mM aqueous $AgNO_3$ solution and the resulting solution became brown in colour. This extract was filtered through nylon mesh (spectrum), followed by Millipore hydrophilic filter (0.22 µm) and used for further experiments [28]. The colour intensity of the extract was measured at 420 nm of

different intervals of time (5, 10, 15, 20, 25 and 30 min) and the control was maintained without plant extract.

Insect culture

Larvae of *H. armigera* Hübner (Lepidoptera: Noctuidae) were collected from the infested field of Kannamangalam, Tiruvannamalai district, Tamil Nadu, India, and cultured at room temperature (27 ± 2°C) in the insectary [11]. The pest was identified by Dr. V. Rajagopal, Zonal Entomological Research Centre, Vellore, Tamil Nadu. The larvae were fed with standard artificial diet [13]. The laboratory reared third instar larvae were used for bioassay test.

Antifeedant activity of *H. armigera*

Antifeedant activity of the crude extracts was studied using leaf disc no choice method (Isman et al., 1990). Fresh cotton leaf discs of 4 cm in diameter were punched using cork borer and dipped in 5, 10, 20, 30, 40 and 50 mg/mL concentrations of aqueous and synthesized Ag NPs extracts. The leaf discs treated with $AgNO_3$ (3 mM solution), Milli Q water was used as negative control and Azadirachtin was used at 5, 10, 20, 30, 40 and 50 mL/L (40.86% purity, obtained from Madras Fertilizers Ltd., Chennai) as positive control. In each petri dish, (1.5 cm X 9 cm) a wet filter paper was placed to avoid the early drying of the leaf discs and one third instar larva was introduced into each petri dish. Progressive consumption of leaf area by the treated and control larvae after 24 h was recorded using leaf area meter. Leaf area, eaten by larvae in treatment was corrected from the negative control. Five replicates were maintained for each treatment. The experiment was conducted at laboratory conditions (27 ± 2°C) with 14:10 photoperiod and 75 ± 5% relative humidity.

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{treated leaf}}{\text{Leaf area consumed in control} + \text{treated leaf}} \text{ X } 100$$

Larvicidal activity of *H. armigera*

Larvicidal activity was studied using leaf dip method [2]. The cotton leaf discs were dipped in different concentrations of aqueous extract, synthesized Ag NPs, $AgNO_3$ solution and azadirachtin. After 24 h of treatment, the larvae were continuously maintained on the non-treated fresh cotton leaves. Diet was changed every 24 h. Larval mortality was recorded after 96 h of treatment. Three replicates were maintained for each treatment with 10 larvae per replicate. LC_{50} and LC_{90} values were calculated using probit analysis [33].

Gas chromatography and mass spectroscopy (GC–MS) analysis of A. indica leaf aqueous extracts

The chemical compositions of leaf aqueous extract of *A. indica* were analyzed using GC–MS (GCD-HP1800A system, Hewlett-Packard, USA) equipped with a split/splitless capillary injection port. For GC–MS detection, an electron ionization system (quadruples analyzer; mass range, 10–425 amu) with ionization energy of 70 eV was used. Each of these steps was carried out under high vacuum (10^{-4} to 10^{-8} Torr). Helium gas was used as a carrier at a constant flow rate of 1 mL/min. Injector and mass transfer line temperatures were set at 250°C and 280°C, respectively. The components of *A. indica* leaf constituents were identified after comparison with available data in the computer library (NIST) attached to the GC–MS instrument and reported.

Characterization of synthesized Ag NPs

The bioreduction of Ag NPs was monitored by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV-vis spectra, at the wavelength of 200-700 nm in Schimadzu 1601 spectrophotometer operated at a resolution of 1 nm. The Ag NPs exhibited unique and tunable optical properties on account of their surface plasmon resonance (SPR), dependent on shape and size distribution of the nanoparticles [8].The reduction of Ag^+ ions was monitored by measuring the UV-visible spectra of the solutions

after diluting 20 times of aliquot (0.2 mL) with Milli Q water. The solution mixture was subjected to centrifugation at 10,000 rpm for 45 min and the resulting pellet was dissolved in deionized water which was filtered through 0.22 μm Millipore filter. X-ray diffraction (XRD) measurements of the *A. indica* leaf broth reduced to Ag NPs were carried out on films of the respective solutions drop-coated onto glass substrates on a Phillips PW 1830 instrument operating at a voltage of 40 kV and a current of 30 mA with Cu Kα1 radiation.

Fourier transform infrared (FTIR) spectroscopy measurements were obtained dry powders of the nanoparticles. The Ag NPs synthesized after 30 min reaction with *A. indica* leaf broth were centrifuged at 10,000 rpm for 15 min, following which the pellet was redispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The process of centrifugation and redispersion in sterile distilled water was repeated thrice to ensure better separation of free entities from the metal nanoparticles. The purified pellets were then dried and the powders were subjected to FTIR spectroscopy measurement. Characterization involved FTIR analysis of the dried powder of Ag NPs by scanning it in the range of 350-3000 cm^{-1} at a resolution of 4 cm^{-1}. These measurements were carried out on a Perkin elmer spectrum one instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets and the pellets were mixed with KBr powder and pelletized after drying properly. The pellets were later subjected to FTIR spectroscopy measurement.

For electron microscopic studies, 25 μL of sample was sputter-coated on copper stub, and the images of nanoparticles were studied using Scanning electron microscopy (SEM; JEOL, Model JFC-1600) and Transmission electron microscopy (TEM; JEOL, model 1200EX) measurements were operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder.

Cytotoxic activity on HeLa cells using MTT assay

The cytotoxic effects of aqueous extract and synthesized Ag NPs of *A. indica* on host cells were assessed by functional assay as described [22]using HeLa cells cultured in RPMI containing 10 % fetal bovine serum, 0.21 % sodium bicarbonate (Sigma) and 50 µg/mL gentamycin (complete medium). Briefly, cells (104cells/200 µL/well) were seeded into 96-well flat bottom tissue culture plates in complete medium. Test solutions were added after 24 h of seeding and incubated for 48 h in a humidified atmosphere of 37°C and 5 % CO_2. DMSO (positive inhibitor) was added at 10 %. 20 µL of the stock solution of MTT (5 mg/mL in 1X phosphate buffered saline) was added to each well, gently mixed and incubated for another 4 h. After spinning the plate at 1500 rpm for 5 min, supernatant was removed and 100 µL of DMSO (stop agent) was added. Formation of formazon was read using microtiter plate reader (Versa max tunable multi-well plate reader) at 570 nm. The 50 % cytotoxic concentration (TC_{50}) of test extracts was determined by analysis of dose-response curves.

Statistical analysis

The average antifeedant and larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the software developed by [33]. Results with $p<0.05$ were considered to be statistically significant.

Results

Antifeedant activity

The leaf aqueous extract of *A. indica* and synthesized Ag NPs showed good antifeedant activity with 8.45, 13.16, 27.38, 41.33, 62.44, 72.22 %; 22.20, 28.96, 33.87, 51.60, 78.61 and 92.40 % at 5, 10, 20, 30, 40 and 50 mg/mL and exhibited the LC_{50} and LC_{90} values of 623.45, 1725.37 mg/mL and 365.72 and 968.33 mg/mL, respectively against *H. armigera* (Table 1). Synthesized Ag NPs showed strong antifeedant activity compared with aqueous extract

against *H. armigera*. The positive control azadirachtin showed potential antifeedant activity with 97.28% against *H. armigera* with LC_{50} and LC_{90} values of 348.98 and 955.39 mL/L, respectively (Table 2)

Table 1

Antifeedant and larvicidal activity of *A. indica* aqueous extract, synthesized Ag NPs, Azadirachtin and Ag NO_3 solutions against III instar larvae of *H. armigera*.

Sample/Extract	Concentrations (mg/mL)	% Mortality* (mg/mL) ± SD	
		Antifeedant	Larvicidal
Aqueous extract of *A. indica*	5	08.45 ± 3.19	16.60 ± 2.34
	10	13.16 ± 1.36	27.07 ± 3.39
	20	27.38 ± 4.48	36.53 ± 2.38
	30	41.33 ± 2.85	54.82 ± 4.56
	40	62.44 ± 1.94	68.16 ± 3.10
	50	72.22 ± 4.61	87.69 ± 3.17
Synthesized Ag NPs	5	22.20 ± 2.97	27.19 ± 1.34
	10	28.96 ± 3.50	31.33 ± 2.85
	20	33.87 ± 4.63	36.53 ± 3.38
	30	51.60 ± 5.95	64.04 ± 4.18
	40	78.61 ± 5.73	86.38 ± 3.05
	50	92.40 ± 3.17	100.0±0.00
Azadirachtin (mL/L)	5	38.12 ± 1.88	45.87 ± 4.63
	10	43.03 ± 2.85	53.24 ± 4.06
	20	54.46 ± 2.51	66.45 ± 3.94
	30	62.16± 3.10	74.81 ± 3.17
	40	75.30 ± 2.10	84.07 ± 2.06
	50	97.28 ± 4.91	100.0 ±0.00
Ag NO_3 (mg/mL)	50	04.28±0.83	02.63±0.42

Control (Distilled water); Nil mortality, * Mean value of three replicates ± SD

Table 2

LC_{50} and LC_{90} of antifeedant and larvicidal activity of aqueous and synthesized Ag NPs using the extract of *A. indica* and Azadirachtin against III instar larvae of *H. armigera*.

Activities and Extracts	LC_{50}±SE (mg/mL)	95 % Confidence limit		LC_{90}±SE (mg/mL)	95 % Confidence limit		$\chi 2$ (df=4)
		Lower limit (LCL)	Upper limit (UCL)		Lower limit (LCL)	Upper limit (UCL)	
Antifeedant							
Aqueous	623.45±9.34	458.43	673.02	1725.37±24.33	1285.64	2248.14	15.36
Ag NPs	365.72±8.24	268.52	513.76	968.33±16.52	784.93	1090.24	6.82
Azadirachtin	348.98±9.15	321.84	668.20	955.39±15.34	778.31	1036.17	16.64
Larvicidal							
Aqueous	766.54±12.04	864.46	1296.92	1859.06±18.35	1377.30	2189.80	15.67
Ag NPs	309.98±8.98	282.77	547.20	980.81±28.33	788.08	1273.52	12.65
Azadirachtin	297.03±11.37	281.43	433.62	855.98±16.48	685.26	1136.17	13.58

LC_{50} Lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, $\chi 2$ Chi-square, df degree of freedom, SE-Standard error, significant at $P<0.05$ level.

Larvicidal activity

Larvicidal activity of aqueous extract of *A. indica* and synthesized Ag NPs showed 54.82, 68.16, 87. 69 % and 64.04, 86.38 and 100% against *H. armigera* at 30, 40 and 50 mg/mL, respectively and the activity was statistically significant compared with control (Table 1). The LC_{50} and LC_{90} values were 766.54 and 1859.06 mg/mL for aqueous extract and 309.98 and 980.81 mg/mL for synthesized Ag NPs against *H. armigera*, respectively (Table 2). 100 % larval mortality was observed in azadirachtin with LC_{50} value of 297.03 and LC_{90} value of 855.98 mL/L.

Synthesis of Ag NPs using leaves aqueous extract of *A. indica*

The aqueous $AgNO_3$ solution turned into yellowish brown colour within 1 h of the addition of leaf aqueous extract of *A. indica*. Intensity of brown colour increased in direct proportion to the incubation period. It was due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$. The silver SPR was observed at 420 nm with steady increase in intensity as a function of time of reaction (ranging from 5 to 30 min) without showing any shift of the wavelength (Fig.1 A).

GC–MS analysis

In the results pertaining to the GC–MS analysis, six compounds were detected in the aqueous leaf extract of *A. indica* (Fig. 1 B). The major chemical constituent was identified as 3-O-methyl-d-glucose (peak area 11.0 %) (**1**) by comparing with mass spectral data and retention times (Fig. 1C).The other constituents present in the aqueous extract were -4H-pyran-4-one, 2, 3- dihydro-3, 5- dihydroxy- 6 - methyl-(4.4%) (**2**), Ethanone, 1- (2-hydroxy-5 methyl phenyl)-(6.7%) (**3**), Sucrose (8.1%) (**4**), Oxalic acid, hexyl 2-methylphenyl ester (18.3%) (**5**) and Tricylo [7.4.0.0.(3,8)] tridec-12-en-2-one,5,6-epoxy-4-methyl-(18.5%) (**6**).

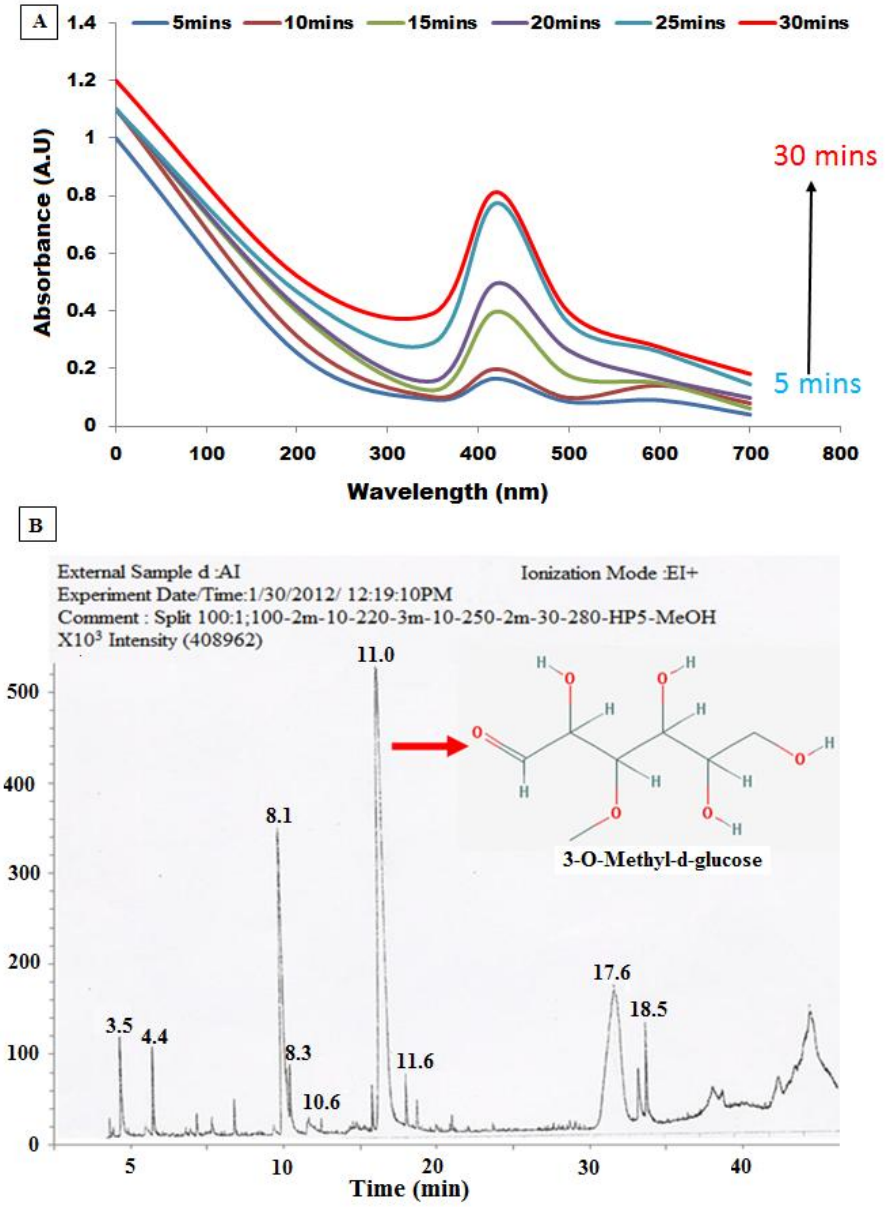

Figure 1 (**A)** UV–vis spectra of aqueous $AgNO_3$ with *A. indica* leaf extract at different time intervals and (**B)** GC-MS peak result of *A. indica* leaf aqueous extract and (**C)** Structure of major chemical constituent (3-O-Methyl-d-glucose

XRD analysis

XRD showed intense peaks in the whole spectrum of 2θ value ranging from 10 to 80 and this pattern was similar to the Braggs' reflection of silver nanocrystals. The Bragg reflections samples were observed in the nanoparticle sample. The peaks were assigned to diffraction from the 38.22˚ (111), 44.54˚ (200), 64.55˚ (220) and 77.40˚ (311) planes of face centered cubic (fcc) silver, which were in good agreement with reference to the unit cell of the fcc structure with a lattice parameter of 2θ value of 4.077 Å (Fig. 2 A). It revealed that the prepared Ag NPs were biphasic in nature. The slight shift in the peak positions indicated the presence of strain in the crystal structure which is a characteristic of nanocrystallites.

FTIR analysis

FTIR spectroscopy analysis was carried out to identify the biomolecules responsible for the reduction of Ag^+ ions and capping of the bioreduced Ag NPs synthesized by using plant extract. FTIR spectra of our vacuum dried *A. indica* leaf extract powder showed strong absorption band at 2926.56 cm^{-1} corresponding to around aldehydic C-H stretching. Another band observed at 1541 cm^{-1} is assigned an around of C=C group. After reduction of $AgNO_3$, the decrease in intensity at 2922.56 cm^{-1} signify the involvement of the around aldehydic CH stretching in the reduction process. On the other hand, the shift of the band from 1541.14 cm^{-1} to 1597.35 cm^{-1} is assigned an around of C=C group with nanoparticles. A number of bands was formed due to O–H stretching (around 3424.16 cm^{-1}), C–C and C–N stretching (1379.96 cm^{-1}) and C–O stretch (around 1058.30 cm^{-1}). When the metal nanoparticles form in solution, they must be stabilized against the van der Waals forces of attraction which may otherwise cause coagulation. Physisorbed surfactant and polymers may cause steric or electrostatic barriers or purely electrostatic barriers around the particle surface and may

thereby provide stabilization (Mulvaney, 1996). The FTIR spectrum of *A. indica* leaf aqueous extract and Ag NPs synthesized using *A. indica* are shown in Fig.2 B

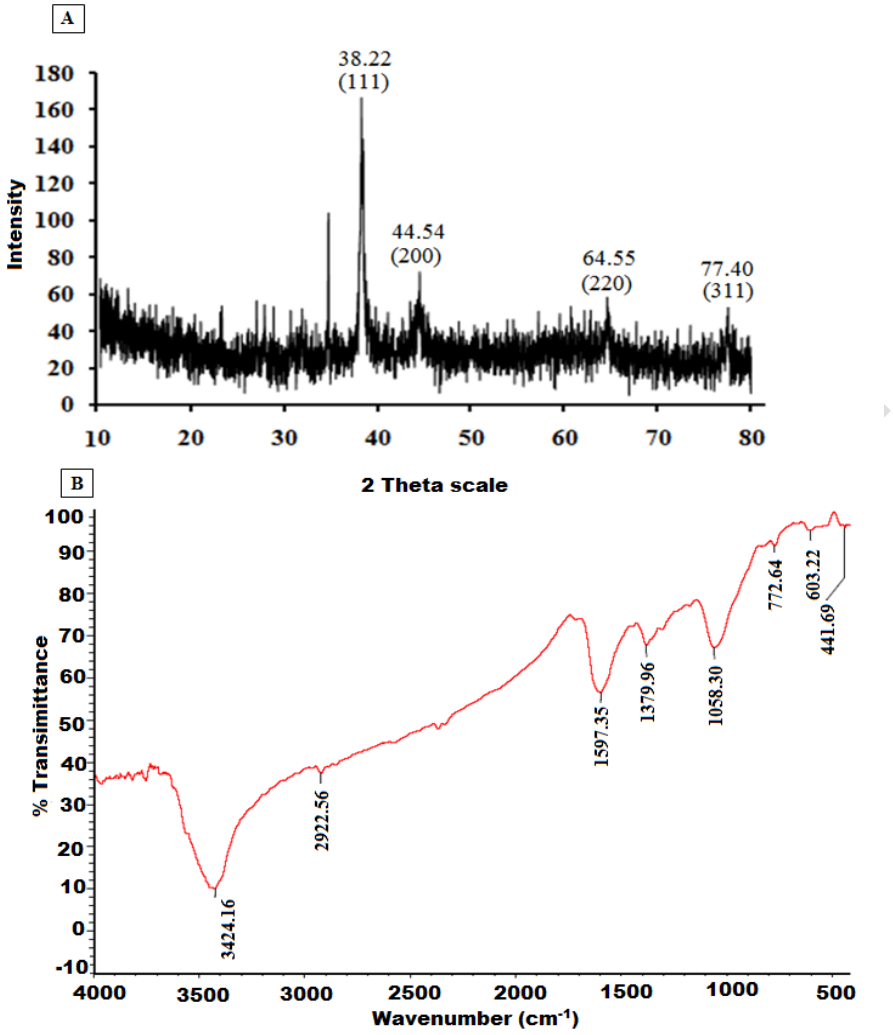

Figure 2 (A) XRD pattern of synthesized Ag NPs using *A. indica* leaf aqueous extract, **(B)** FTIR spectrum of synthesized Ag NPs using *A. indica* leaf aqueous extract

SEM analysis

SEM determinations of the sample ($AgNO_3$) showed the formation of nanoparticles, which were confirmed to be the silver by EDX. SEM analysis of the synthesized Ag NPs was clearly distinguishable with an average size of 112.35 nm (Fig. 3 A and B). The NPs were asymmetrical dispersed and mostly aggregated. **EDX analysis**

EDX attachment present with the SEM is known to provide information on the chemical analysis of the fields that are being investigated or the composition at specific locations (spot EDX). The representative profile of the spot EDX analysis was obtained by focusing on Ag NPs (Fig.3 C).

TEM analysis

The shapes and sizes of Ag NPs were measured after 24 h of incubation by using TEM analysis (Fig. 4 A and B). The nanoparticles were spherical in shape with varying size distribution ranging from 5.19 to 24.91 nm with an average size of 16.54 nm (Fig.4 C). TEM analysis showed that nanoparticles were aggregates and a few of them were scattered. XRD absorption spectra provided solid evidence of NPs formation and their growth kinetics, the shape and size of the resultant particles were elucidated with the help of TEM.

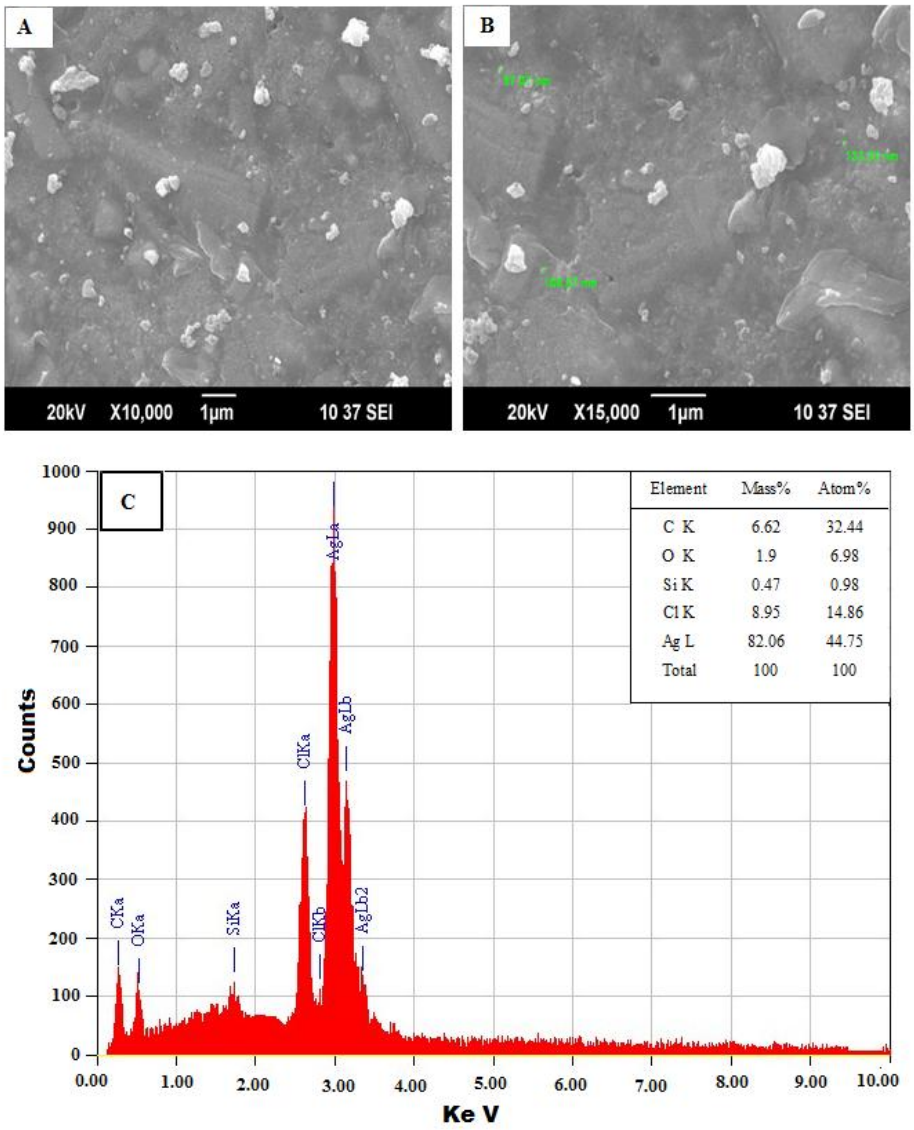

Figure 3 (A) SEM image of Ag NPs from A. indica leaf extract magnification at 10,000X, inset bar: 1 μm, (**B)** SEM image magnification at 15,000X, inset bar: 1 μm and **(C)** Image of Energy dispersive X-ray (EDX) observation of synthesized Ag NPs showing their chemical composition of synthesized Ag NPs.

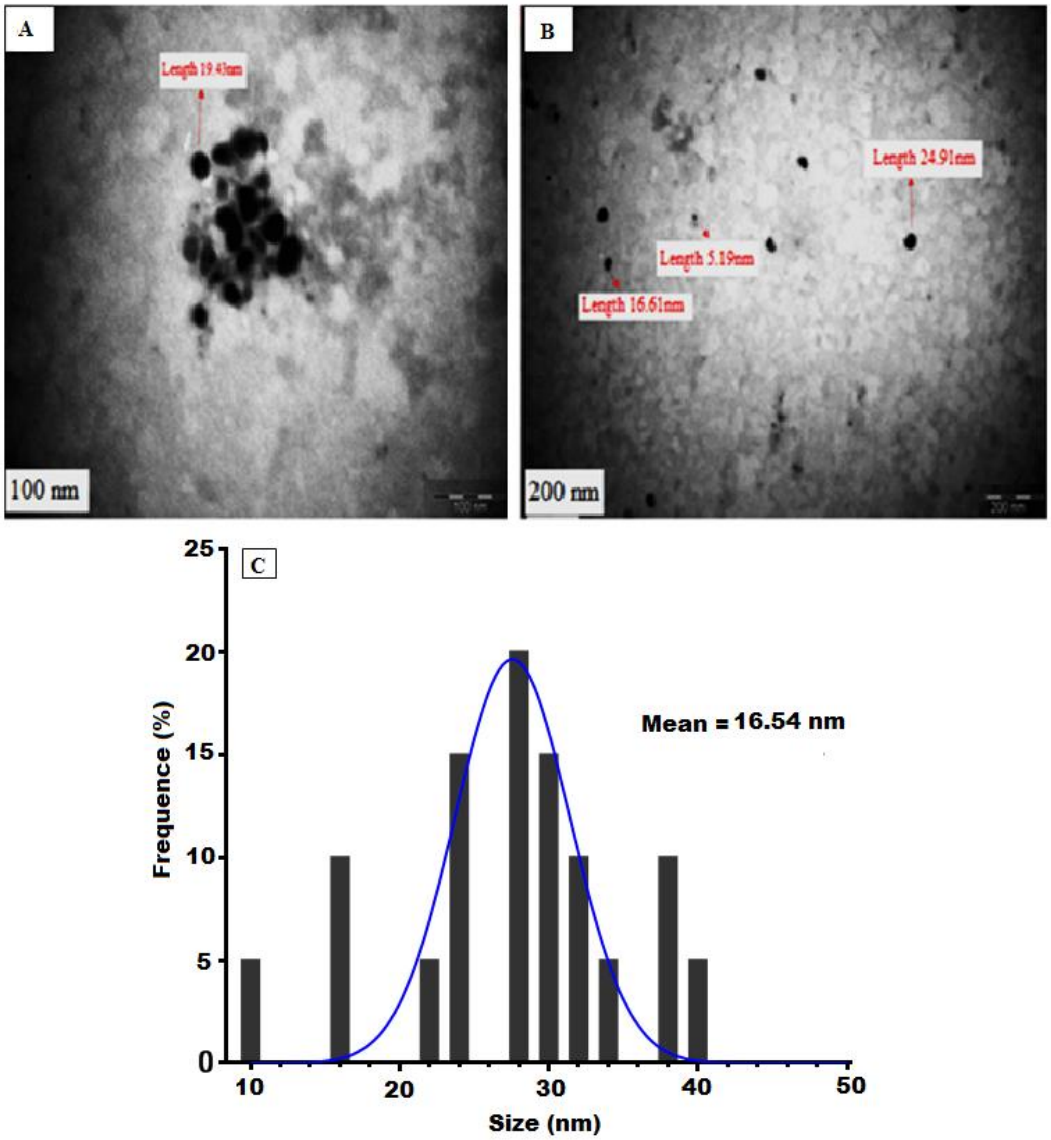

Figure 4 (A) and (**B)** Transmission electron microscopy images of Ag NPs derived from *A .indica* leaf extract and **(C)** particle size distribution.

Cytotoxic activity

The aim of this study was to analyse the antifeedant and larvicidal property of *A. indica* aqueous and synthesized Ag NPs against *H. armigera* and also to analyse its safety tested on human HeLa cells. The aqueous extracts of *A. indica* and synthesized Ag NPs exhibited least cytotoxic effects on HeLa cells. Cytotoxic effect of aqueous and synthesized Ag NPs was evaluated by MTT assay using HeLa cells and the results are shown in Figs. 5 A, B, C and D. The percentages of cell viability are tabulated in Table 3. The viability of HeLa cells incubated with the aqueous and synthesized Ag NPs for 48 h were more than 90 % in the concentrations ranging from 1 to 500 µg/mL. No significant morphological changes were observed in cells treated with aqueous and synthesized Ag NPs. 50 % cytotoxic concentration (TC_{50}) of aqueous and synthesized Ag NPs were determined by analysing the of dose–response curves (Table 3). HeLa cells treated with the aqueous and synthesized Ag NPs showed a concentration-dependent cytotoxic effect. As the concentration increased from 1 to 500 µg/mL, the percentage of inhibition was found to be increasing. 88.56 % and 72.25 % of cell viability were observed at the concentration of 500 µg/mL in aqueous and synthesized Ag NPs, respectively (Figs. 5 A and B). The cytotoxic concentration (TC_{50}) values were found to be >100 and 80.5 µg/mL, respectively. The azadirachtin compound showed lesions of cells when increasing the concentrations with TC_{50} value of <10 µL/mL (Fig. 5 C). Normal HeLa cell lines are shown in Fig. 5D.

Table 3

Cell viability (%) of *A. indica* extract, Ag NPs and azadirachtin treated against human cervical cancer cell lines (HeLa)

Concentrations µg/mL	*A. indica* (%)	Ag NPs (%)	Azadirachtin (%)
500	88.56± 0.23	72.25±1.74	6.08 ±0.46
250	93.96±0.52	60.58±0.37	18.56±1.54
125	98.58±1.65	54.23±1.39	22.14±1.36
100	98.64±0.61	56.32±0.92	35.69±1.37
50	100±0.00	60.35±1.83	42.25±0.24
25	100±0.00	68.75±1.53	48.77±1.94
10	100±0.00	72.58±1.57	51.23±0.48
5	100±0.00	81.24±0.35	59.63±1.43
1	100±0.00	86.32±0.74	63.25±0.84
Cell control	100	100	100
Cytotoxicity (TC_{50} µg/ml)	>100	80.5	< 10

The values are expressed in mean ± SE. values are analyzed by Graph Pad prism 5 Software

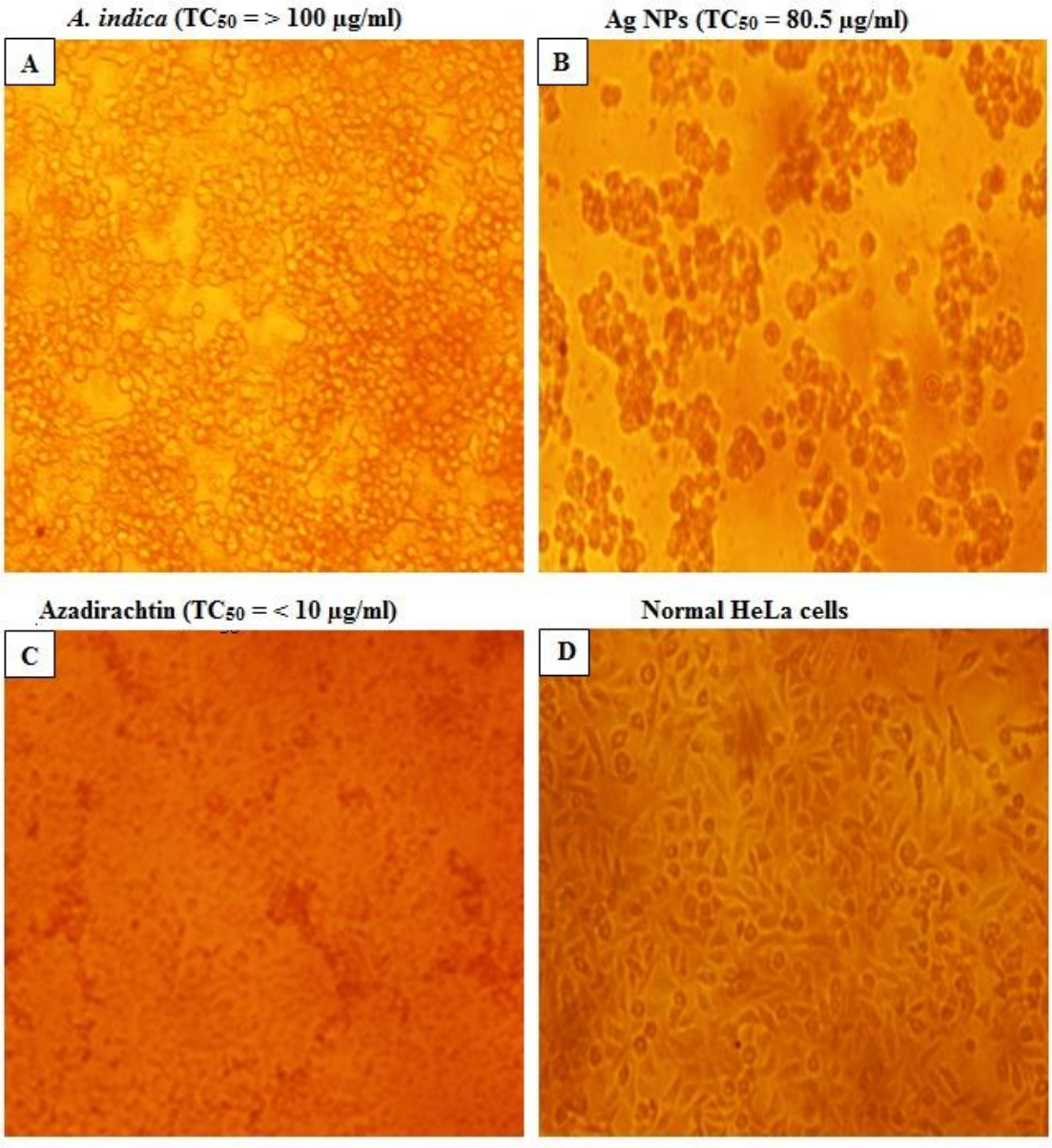

Figure 5 A, B, C and **D** Photograph showing cytotoxicity (TC_{50}) effect of *A. indica*, synthesized Ag NPs, Azadirachtin and normal HeLa cells.

Discussion

Plants or their extracts can be efficiently used in the synthesis of Ag NPs as a greener route. Control over the shape and size of nanoparticles seems to be very easy with the use of plants. Such plant synthesized NPs have been used in various applications for human benefit. Plant-mediated synthesis of nanoparticles is a very promising area of research [17]. Nanoparticles help to produce new pesticides, insecticides and insect repellents [26]. The synthesized Ag NPs from *A. indica* showed more than 80% antifeedant and larvicidal activities against *H. armigera* at the concentration of 50 mg/mL. The present finding corroborated [30]. Who reported that natural compound 6-(4,7-hydroxyhyptyl) quinone isolated from ethyl acetate extract of *Pergularia daemia* showed more than 65% feeding deterrent activity against *H. armigera* at 1000 ppm. The rhein compound derived from ethyl acetate extract of *Cassia fistula* showed 76 % antifeedant activity at 1000 ppm against *H. armigera* [4]. [3], reported that the leaf and root extracts of *Aristolochia tagala* showed higher antifeedant activity (56.06%), lethal concentration for feeding inhibition (3.69%), larvicidal (40.66%), pupicidal (28%), total mortality (68.66%) and prolonged larval and pupal duration (12.04–13.08 days) against *S. litura*. [42] reported that fractions from ethyl acetate extract of *Hydnocarpus alpina* showed good antifeedant activity against *H. armigera*, the fractions might have affected the mouth parts or chemoreceptors since the insects were deterred from feeding. The synthesized Ag NPs of the present study showed effective larvicidal activity compared to antifeedant activity against *H. armigera*. [14] observed that the aglaroxin A inhibited the food intake resulting in the reduction of *H. armigera* larval growth.

The results of the present study showed that synthesized Ag NPs using *A. indica* extract in relation to $AgNO_3$ exhibited various levels of reduction of larvae of *H. armigera* and offered various degrees of protection to plants compared with *A. indica* based aqueous extract

formulations. Various studies have been taken in use for the detection of the usage of botanicals as control in the pest. The pesticidal activity showed that the synthesized Ag NPs using *Euphorbia prostrata* was more effective than the aqueous extract and $AgNO_3$ solution, the complete mortality (100 %) was observed on 7th day for the synthesized Ag NPs but in aqueous extract and the $AgNO_3$ solutions the same mortality was observed after 14 days against *S. oryzae* [44]. The present study azadirachtin showed lesions in cells with increasing concentration (TC_{50} <10µg/ml) and 50 % cytotoxic concentration (TC_{50}) of aqueous and synthesized Ag NPs was determined by analysing the dose–response curves. Patel et al. (2009) reported that the *Solanum nigrum* methanolic extract showed significant inhibitory action on HeLa cell line in concentration range between 10 mg/ml to 0.0196 mg/ml by using MTT assay. Aqueous extract of mature fruit of *Capparis spinosa* caused less inhibition activity on the growth HeLa tumour cell lines with cytotoxic concentration (CC 50 %) for more than 10000 µg/ml [1].

The aqueous $AgNO_3$ solution turned into yellowish brown colour within 1 h of addition of *A. indica* leaf extract. In UV spectral analysis, the generation of color was due to excitation of surface plasmon in metal nanoparticles [24]. The synthesized Ag NPs using aqueous leaf extract of *A. indica* and the XRD showed the number of Bragg reflections with 2θ values of 38.22˚, 44.54˚, 64.55˚ and 77.40˚ sets of lattice planes were observed at 111, 200, 220 and 311 fcc of silver, respectively. [20] reported that the Ag NPs synthesized by leaf aqueous extract of *Lawsonia inermis* showed the XRD pattern at four intense peaks in the whole spectrum of 2θ values of 38.34°, 44.59°, 65.04°, and 77.77° assigned to the (111), (200), (220), and (311) planes of a face centred cubic lattice of silver.

In the present study, the FTIR analysis of Ag NPs free from proteins and water soluble compounds was done in this direction. There was a shift in the following peak and the spectra showed sharp and strong absorption band at 3424.16 to 2922.56 cm^{-1} double in case of NH_2

group of a primary amine (N-H Stretch).The presence of the medium peak at 1597.35 to 1379.96 cm^{-1} very broad often looks like distorted baseline at presence of aromatics rings (C–C stretch (in–ring). The strong bands 1058.30 to 772.64 cm^{-1} were assigned to =C–H bend, alkenes and the medium bands 603.22 to 441.69 cm^{-1} were assigned to C–Br stretch alkyl halides. The FTIR peak located at around 2359 cm^{-1} was attributed to the N–H stretching vibrations or the C=O stretching vibrations. A broad intense band at 3402 cm^{-1} in the spectra can be assigned to the N–H stretching frequency arising from the peptide linkages present in the proteins of the extract [23].

SEM micrographs of the reaction mixtures containing 10 mg of *A. indica* leaf extract powder and 1.0 mM of $AgNO_3$ solution were incubated for 15 minutes and magnified to 10,000X and 15,000X which revealed particle size of 97.07-133.33 nm. For the SEM studies, reaction mixtures were air-dried on silicon wafers. As a result, a coffee ring phenomenon was observed. [10] reported the SEM micrographs of the synthesized Ag NPs of *Tinospora cordifolia* and its size was measured to be in rage of 55–80 nm.

In the present study, the chemical purity and composition was analysis with Energy-dispersive X-ray spectroscopy (EDX). The EDX attachment present with the SEM is known to provide information on the chemical analysis of the fields that are being investigated or the composition at specific locations. EDX chemical analysis provide information on the fields that are being investigated or the composition at specific locations (spot EDX) was obtained by focusing on Ag NPs in *Manilkara zapota* [32].

In the present study, the TEM analysis revealed that the nanoparticles were spherical in shape. TEM analysis showed that the nanoparticles were aggregated and a few of them were scattered. The size of particles ranged from 5.19 nm to 24.91 nm and the average size was calculated as 16.54 nm. The absorption spectra provide solid evidence of nanoparticles formation and their growth kinetics, the shape and size of the resultant particles were

elucidated with the help of TEM. The particle shape of plant-mediated Ag NPs were mostly spherical in shape with exception of neem (*Azadirachta indica*) which yielded polydisperse particles both with spherical and flat plate-like morphology with 5–35 nm in size [37].

Conclusion

We propose an eco-friendly method for Ag NPs synthesis by the green chemistry approach using the aqueous leaf extract of *A. indica*. The leaf extract of *A. indica* is environmentally benign and renewable and is capable of acting as both reducing and stabilizing agent. SEM analysis revealed that the particles were mostly aggregated and spherical in shape with an average of 112.35 nm. Synthesized Ag NPs showed potential antifeedant (92.40 %) and larvicidal (100 %) activities against *H. armigera* with LC_{50} values of 365.72 and 309.98 mg/mL; 623.45 and 766.54 mg/mL, respectively. The aqueous and synthesized Ag NPs extracts of *A. indica* exhibited least cytotoxic effects on human HeLa cells. In the present approach, we avoid the use of hazardous, toxic solvents. This nanostructure showed excellent antifeedant and larvicidal activity against *H. armigera*. Therefore, the synthesized Ag NPs using plant extract provides a promising approach for the large-scale industrial production of nanomaterials for pest control.

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