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Evaluation of mosquito larvicidal activity of green synthesized crystalline silver nanoparticles using leave and fruit extracts of *Phyllanthus acidus* L. (Phyllanthaceae)

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Abstract

The present study evaluates the potentiality of green silver nanoparticles from the leaves and fruit extracts of *Phyllanthus acidus* L. against third instar larvae of two vector mosquitoes namely *Culex quinquefasciatus* and *Culex vishnui*. Various spectroscopic techniques were used to characterize the synthesized silver nanoparticles (AgNPs). Synthesized silver nanoparticles from both the leaf and fruits of *P. acidus* were spherical to quasi-spherical in shape and showed Surface Plasmon Resonance (SPR) bands at 420 and 409 nm respectively. In larvicidal bioassay with synthesized AgNPs from the leave of *P. acidus*, 100% mortality was observed at 20 ppm against third instar larvae of both the mosquito species with LC_{50} values of 1.64 and 0.87 ppm respectively. 100% mortality was observed in 5 ppm concentration against both *Cx. quinquefasciatus* and *Cx. vishnui* in synthesized AgNPs from fruits of *P. acidus* with LC_{50} values of 1.62 and 1.24 ppm respectively. The above findings suggest that the AgNPs synthesized from *P. acidus* leaves and fruit extracts have the potential to be employed in vector mosquito population control.

Keywords: *Culex quinquefasciatus*; *Culex vishnui*; larvicidal activity; *Phyllanthus acidus*; probit analysis; silver nanoparticles

Introduction

The prevalence of mosquito-transmitted diseases, such as dengue, dengue hemorrhagic fever, Japanese encephalitis, malaria, and filariasis, is rising globally, especially in tropical and subtropical regions. Lymphatic filariasis, which affects 120 million people globally and could impact 400 million more, is spread by *Culex quinquefasciatus*, causing a yearly economic loss of 1.5 billion dollars (WHO, 2002). India accounts for one-third of the world's lymphatic filariasis cases, making it a severe public health issue (WHO, 1997). Bancroftian filariasis is currently a risk for 863 million people across 47 countries, and to stop the parasite's chain of transmission, chemotherapy is necessary as a preventative measure (WHO, 2022). One of the principal vectors of the Japanese encephalitis virus (JEV) is the *Culex vishnui*. In 24 nations in the Asia-Pacific area, this virus is of critical medical relevance because it can cause fatal severe encephalitis and other complications, especially in

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young children. Potentially, *Cx. vishnui* could act as a bridge vector for the transmission of viruses from natural and wild hosts to people. This underappreciated species of mosquito may pose a severe health danger in one of the world's most densely inhabited areas due to its zoo- and anthropophagy, ubiquity in the Oriental region, and strong resistance to various pesticide families (Maquart *et al*., 2022). Due to the vectors' favourable ecological conditions, intimate contact with humans, and reproductive biology, mosquito-borne diseases are endemic to India. Mosquito control is getting better in many ways, but there are still several obstacles, such as rising insecticide resistance and a lack of suitable substitutes that are affordable and safe (Potera, 2008). Due to the rise in pesticide resistance, methods for extending the use of highly effective vector control agents must be developed. One such approach that might work for controlling mosquitoes is the use of mixtures of several pesticides and phytochemicals.

Biological methods for producing nanoparticles using microbes, enzymes, plants, or plant extracts have been presented as promising environmentally friendly alternatives to chemical and physical processes (Mohanpuria *et al*., 2008). Different plant parts have been identified in traditional medical systems as being effective against a range of ailments. The development of ecologically acceptable processes for synthesizing nanoparticles is a key area in nanotechnology. Nanoparticles are crucial for the delivery of medications, diagnostics, imaging, sensing, gene transfer, artificial implants, and tissue engineering (Matthew *et al*., 2006). Due to their mosquito larvicidal effectiveness against dangerous vectors and medically challenging infections, the green synthesis of silver nanoparticles has recently drawn a lot of attention (Soni and Prakash, 2014; Madanagopal *et al*., 2017; Morejon *et al*., 2018; Pilaquinga *et al*., 2019). The development of a trustworthy green method to make silver nanoparticles is a significant focus of current nanotechnology research. Within the last decade, a significant volume of articles has been published on the biogenic synthesis of silver nanoparticles utilizing different plant parts, which has potential mosquito larvicidal activity and remains more advantageous over other conventional methods as it is very effective in minimal dose, less toxic to the environment, and shows target specificity (Haldar *et al*., 2013; Rawani *et al*., 2013; Rawani, 2017; Ghosh *et al*., 2022; Veerakumar and Govindarajan, 2014; Vivekanandhan *et al*., 2017).

In the present study, an attempt has been done to fabricate silver nanoparticles using aqueous extract of leaves and fruit of *Phyllanthus acidus* L. According to the most recent literature review, this is the first such report that has efficacy as mosquito larvicide.

Phyllanthus acidus L, known as the star gooseberry, is one of the trees in the family Phyllanthaceae with edible small yellow berries fruits. It is an intermediary between a shrub and a tree, reaching 2 to 9 m (6½ to 30 ft) height (Centre for New Crops and Plants, 2008). The leaves are 2–7.5 cm long and thin, they are green and smooth on the upper side and blue-green on the underside with pointed ends. The fruits are numerous, oblate, with 6 to 8 ribs, and densely clustered. They are pale yellow or white, waxy, crisp and juicy, and quite sour. 4 to 6 seeds are contained in a stone at the centre of each fruit (Edible: An Illustrated Guide to the World´s Food Plants, 2011). The peppered leaves are used to make a poultice to treat sciatica, lumbago, and rheumatism, while the seeds are used as a cathartic and the root, if prepared with care, as a purgative (Leeya *et al*., 2010). The syrup is used in the medication of the stomach, and India, the fruit is eaten as a blood-enhancer for the liver (Jain *et al*., 2011). *P. acidus* retains 4-hydroxybenzoic acid, caffeic acid (Miller and Bailey, 2011), adenosine, kaempferol, and hypogallic acid (Sousa *et al*., 2006). In traditional medicine, the plant was used to cure a variety of diseases like inflammation, bronchitis, asthma, respiratory disorders, hepatic illness, diabetes, and hypertension.

The objective of the present study was to synthesize green silver nanoparticles from leaves and fruit extracts of *P. acidus*, to characterize the synthesized AgNPs of both the plant parts, and to evaluate the larvicidal efficacy of synthesized AgNPs against 3rd instar larvae of two mosquito species, namely *Culex quinquefasciatus* and *Culex vishnui*.

Materials and Methods

Plant material collection and identification

From different regions of village-Nalagola (25.26° N, 88.42° E), Malda district, West Bengal, India, mature leaves and fruits of the *P. acidus* plant have been collected during the study period from March and April, 2023 and brought to the laboratory. Authentication of the plant was done with the help of the taxonomist, Dr. Manoranjan Chowdhury, Department of Botany, University of North Bengal, Siliguri, West Bengal. The voucher specimen was given a number and deposited in the authors' research laboratory of Parasitology, Vector Biology and Nanotechnology, Department of Zoology, University of Gour Banga.

Collection of larvae

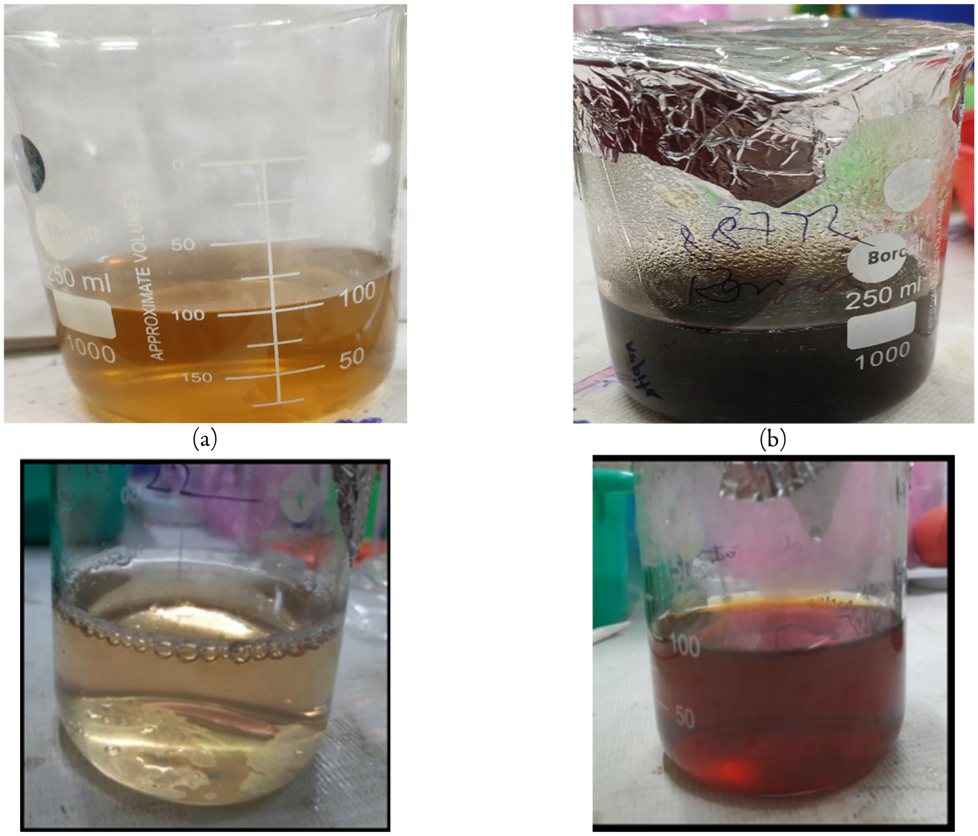
Larvae of *Culex quinquefasciatus* were collected from the cemented drain surrounding the campus of the University of Gour Banga, Malda. While larvae of *Cx. vishnui* collected from the fresh pond waters and rice fields. Collected larvae of both the mosquito species were brought to the laboratory and transferred into separate plastic trays (18 cm L × 13 cm W × 4 cm) containing 500 mL of water and maintained following the protocol of Kamaraj *et al*. (2009). Larvae were fed with a small amount of powdered dog biscuits. Mosquito larvae were reared under 28 ± 2 °C and 75-85% relative humidity in a 14:10 (L: D) photoperiod and protected from infections, pesticides, and repellent exposure. For the identification of the mosquito species, larvae of each mosquito species were reared to the adult stage and then identified according to the key of Chandra (2000), which was arranged following Christopher (1944) and Barraud (1934).

Preparation of aqueous extract

Fresh and mature leaves and fruits of *P. acidus* were collected, rinsed well with running tap water, and dehydrated at room temperature. A plant leaf and fruit broth were prepared by placing ten grams of thinly incised leaves and fruits in 250-mL flask with 100 mL of sterile distilled water. The mixtures of both the plant parts were then kept over the vapour of boiling water for 10 min with intermittent shaking then filtered and stored at 4 °C, and used within 15 days.

Green synthesis of silver nanoparticles

The methodology adopted here is according to the protocol of Rawani et al. (2013). In a sterile conical flask, 12 mL of leaves and fruits filtered aqueous extracts were combined with 88 mL of silver nitrate (10^{-3} M) solution separately and exposed to heat at 60 °C for 10 min. The change of colour takes place within a few minutes from colourless to reddish brown. The change of coloration to a brown solution in both the samples indicates the formation of AgNPs (Figure A and Figure B). It was discovered that aqueous silver ions can be reduced by aqueous extract of the plant parts to produce exceptionally stable silver nanoparticles in water, resulting in a brownish solution that indicates the creation of AgNPs. Each sample was undergoing repeated centrifugation (twice) at 10,000 rpm for 15 mins using a centrifuge machine (Remi R – 24) to get rid of any un-interacted biological molecules to the surface of the synthesized nanoparticles and the supernatants were decanted. The pellets of both the samples were collected, dried in vacuum desiccators and stored for future use.



(a) (b) (c) (d)

Figure 1. Initial colour of leaf aqueous extract (a); Colour changes after addition of $AgNO_3$ solution (b); Initial colour of fruit aqueous extract (c); Colour changes after addition of $AgNO_3$ solution (d)

Larvicidal bioassay

The toxicity of synthesized silver nanoparticles from both the plant parts of *P. acidus* on 3rd instar larvae of both the mosquito species were conducted according to guidelines of WHO (2005). Different test concentrations (1, 5, 10, 15 and 20 ppm of leaves AgNps; 1, 2, 3, 4 and 5 ppm of fruit AgNPs) were prepared by mixing distilled water according to a suitable methodology of Rawani *et al.* (2013) and Ghosh *et al.* (2022). Randomly early third instar larvae of *Cx. quinquefasciatus* and *Cx. vishnui* were taken and placed into 100 mL of each tested concentration along with the control setup with 25 larvae in 100 mL of distilled water. The experiments were conducted in an environmental chamber at 27 °C with a photoperiod of 16:8-h light/dark cycle. Mortality of mosquito larvae was assessed after exposure of 24 hours. The experiments were replicated three times along with control. The numbers of dead larvae were counted in each experiment and the percentage of mortality was recorded from the average of three replicates.

Characterization of silver nanoparticles (AgNPs)

The formation of synthesized silver nanoparticles was confirmed by sampling the reaction mixture at regular intervals and the Surface Plasmon Resonance (SPR) band was observed by ultraviolet-visible (UV-vis) spectra at the wavelengths of 350-600 nm in a UV-3600 Shimadzu spectrophotometer at 1 nm resolution at room temperature at the Department of Physiology, University of Gour Banga, Malda, West Bengal, India.

X-ray Diffraction (XRD) analysis was carried out to examine nature of obtained AgNPs formation using Siemens X-Ray diffractometer (Japan), operated at 30 kV and 20mA current with CuKa (I=1.54A°) at IIT Bhilai, Chhattisgarh, India. The scanning range during the characterization was selected between 10° to 80°

(Pal and Sharma, 2015). It provides detailed information about the crystallographic structure of a material (Pandya *et al*., 2022).

The facility of the Scanning Electron Microscope (SEM) was obtained to determine the surface topography of developed nanoparticles at IIT Bhilai, Chhattisgarh, India. Samples of AgNPs were deposited in dried form on a double conductive tape which was sticked on a sample holder at room temperature (27 °C ± 1). A thin layer of gold-platinum was coated to make samples conductive. Then the samples were imaged at 80 kV operation voltage.

Transmission Electron Microscopy (TEM) was used to magnify the lattice arrangements of atoms; shape and size of the synthesized AgNPs. The samples were casted on carbon coated copper TEM grids of 300 mesh size. The TEM micrographs of synthesized AgNPs were obtained by JEOL Ltd.; JEM 1400 plus (120 KeV) TEM at The University of Burdwan, West Bengal, India.

The powder nanoparticles obtained after centrifugation were used for Fourier Transformed Infrared Spectrophotometer (Perkin Elmer) at IIT Bhilai, Raipur, Chhattisgarh, India. The scanning range was 40 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. FTIR was used to qualitatively confirm the surface groups of the As- formed silver nanoparticles (Stuart, 2004).

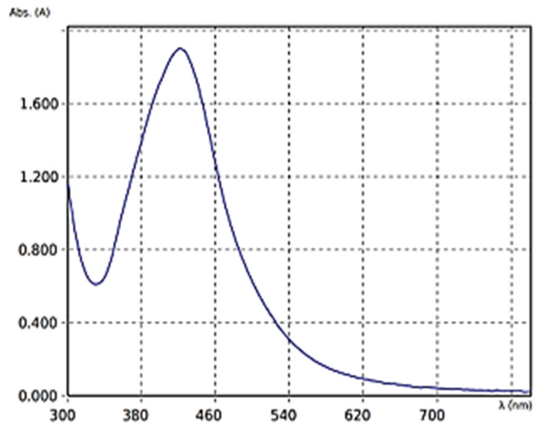
Statistical analysis

The correction of percentage of larval mortality was carried by Abbott's formula (1925). Average larval mortality data were calculated using MS EXCEL 2017. The larval mortality was subjected to probit analysis by the method of Finney (1971) using Statplus v5. Three-way factorial ANOVA carried out using the software SPSS 17.0 version.

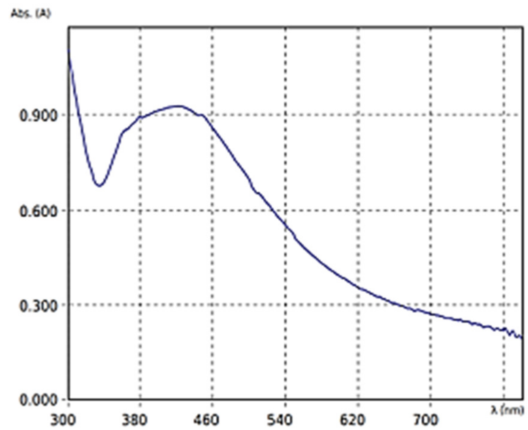
Results

UV-Vis spectroscopy study

The aqueous extract of leaves and fruits of *P. acidus* were light yellow and white in colour respectively before immersion in $AgNO_3$ solutions. The colour of leaves and fruit aqueous extract changed into deep brown colour when mixed with $AgNO_3$ solutions. The synthesized silver nanoparticles were confirmed by change of colouration due to reduction of Ag^+ to Ag^o and AgNPs were formed. The nanoparticle formation kinetics was monitored by sampling of extract diluted to 3 mL by deionized water taken in a 10 mm optical path length quartz cuvette. Parthiban et al., 2019 reported that AgNPs formation was revealed by taking the UV – absorption spectra between 200 to 800 nm and the obtained absorption spectrum of the synthesized nanoparticles from leaves of *Annona reticulata* obtained at 416 nm. In the present study formation of AgNPs was established by observing the characteristic Surface Plasmon Resonance (SPR) band at 420 and 409 nm for leaves and fruits of *P. acidus* respectively using a UV-Vis spectrophotometer (Figure 2a and b).



(a)



(b)

Figure 2. The formation of peak at 420 nm (a) and 409 nm (b) confirms the formation of nanoparticles in leaves and fruit extract of *P. acidus* respectively

SEM analysis

The spherical morphology of silver nanoparticles was confirmed from the SEM image. The morphology of the green synthesized AgNPs were found to be in polymorphic shapes, some of them were irregularly granulated and highly aggregated (Rai *et al*., 2020). Similarly, the SEM images of synthesized AgNPs of leaves and fruits clearly showed the surface of the synthesized silver nanoparticles were rough (Figure 3a, b, c and d) and crystalline in nature. Most of the nanoparticles were aggregated, and few individual particles were also present. The magnified SEM images (Figure 3b and d) also showed the agglomeration of the particles formed.

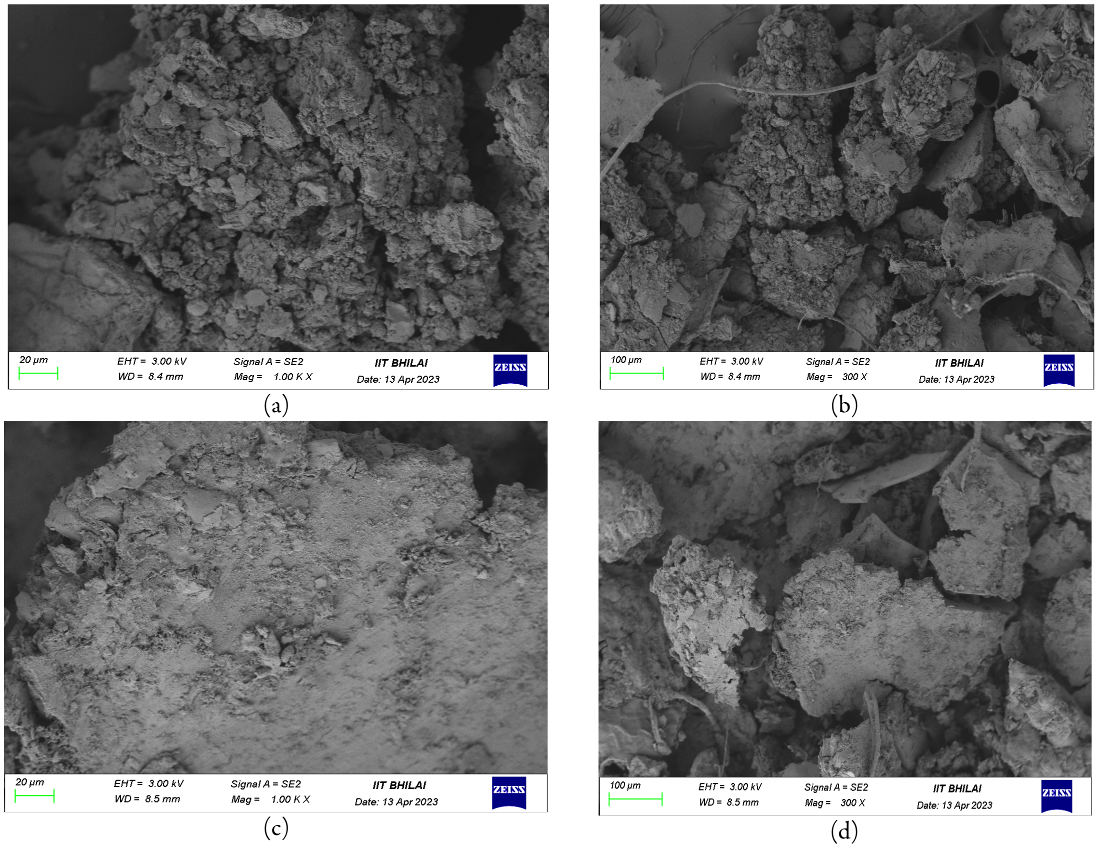


Figure 3. Field emission scanning electron microscope (FESEM) image of silver nanoparticles synthesized from the leaves (a and b) and fruits (c and d) of *P. acidus*

TEM analysis

TEM Analysis was done to visualize the shape as well as to measure the diameter of the Ag nanoclusters. The study reveals that most of the nano-crystals formed are spherical or oval in shape (Figure 4a and b shows a representative TEM photograph of the synthesized AgNPs from fresh leaves). Figure 4c and d shows TEM photograph of the synthesized AgNPs from fresh fruits of *P. acidus* where synthesized AgNps were spherical and some nanoparticles are cylindrical in shape. The average size of the synthesized AgNps of both the plant parts were about 50-75 nm in diameters.

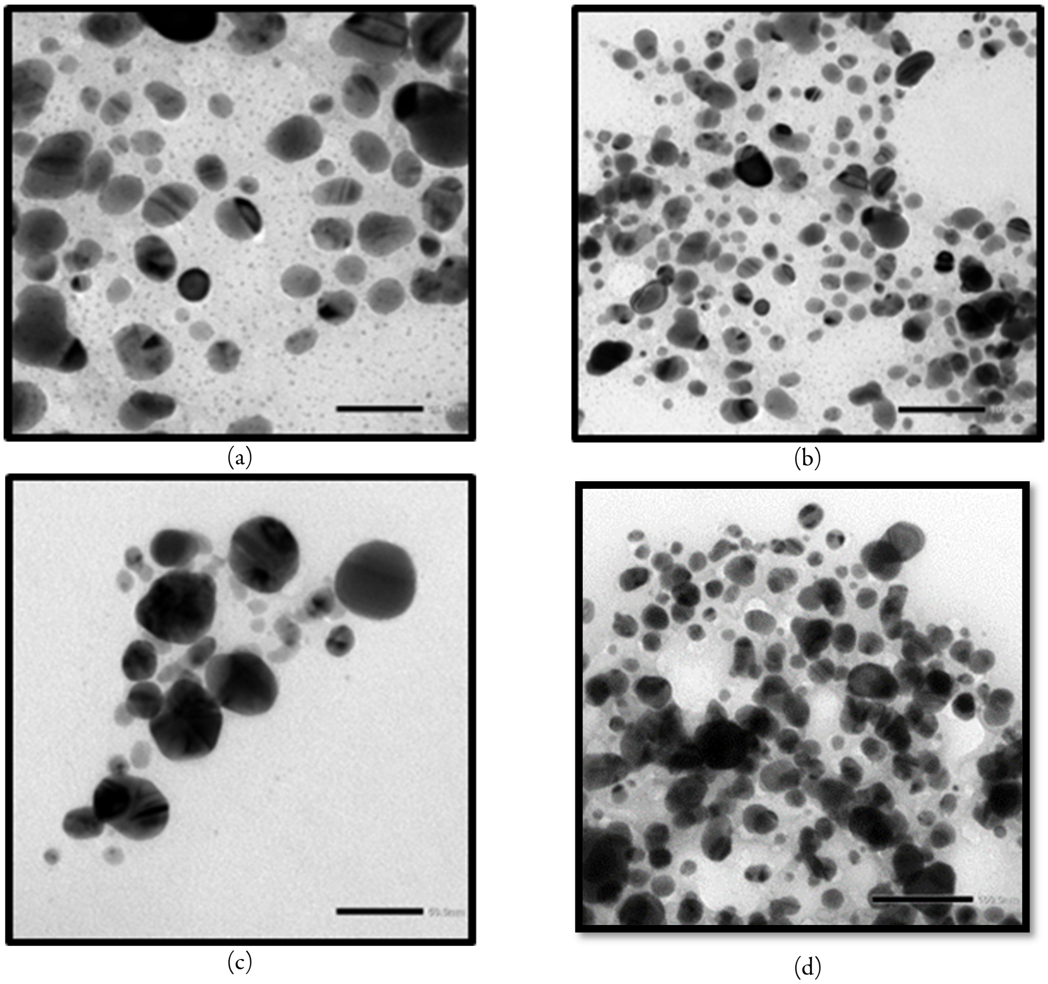
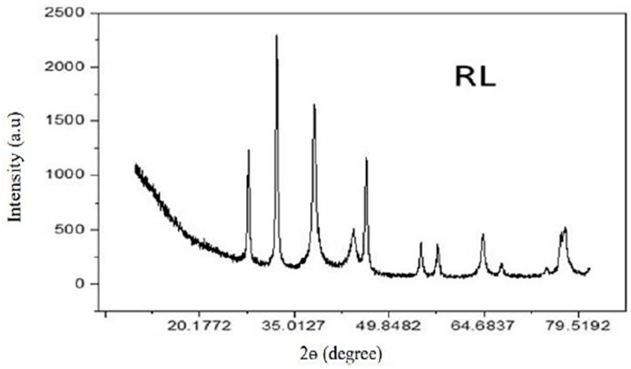


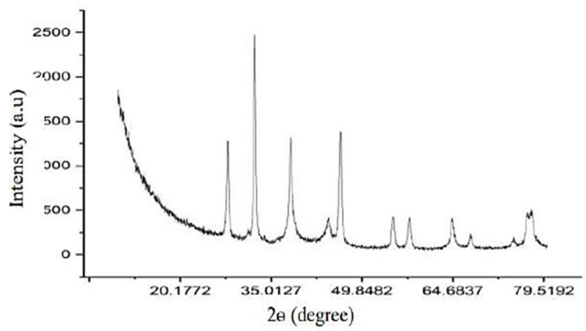
Figure 4. TEM images of synthesized silver nanoparticles from leaves (a and b) and fruit (c and d) of *P. acidus*

X-ray diffraction analysis

The phyto reduced AgNPs of both the plant parts were analyzed by the XRD pattern that showed the resemblance with the literature values of face centered-cubic (fcc) crystal structure of silver. Figure 5 a showed the XRD pattern observed from the synthesized nanoparticles of fresh leaves of *P. acidus*. The four distinct diffraction peaks at 2ϴ values of 38.05°, 44.23°, 64.41°, and 76.66° could be assigned to the four main facets known for zero-valent fcc silver crystal planes due to Bragg's reflections and also stated the reflection planes of face centered cubic structure of silver (Das *et al*., 2014; Bhat *et al*., 2013). Whereas, Figure 5 b showed the XRD pattern observed from the synthesized nanoparticles of fresh fruits of *P. acidus* was in general agreement with the XRD pattern of *P. acidus* leaf AgNPs. Presence of these Bragg's peaks were due to leaves and fruits extract which contains organic compounds that are responsible for the reduction of silver ions and stabilization of resultant nanoparticles (Ibrahim *et al*., 2015; Rawani *et al*., 2013; Ghosh *et al*., 2022). A few unassigned peaks were also noticed in the XRD patterns of both plant parts in the vicinity of characteristic peaks.



(a)

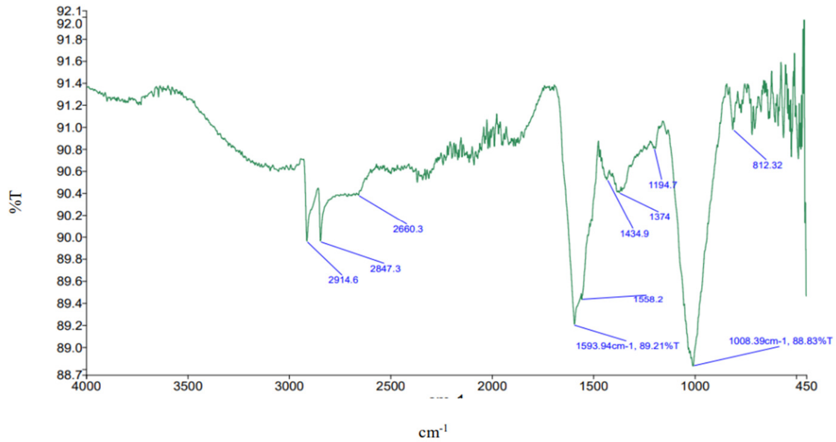


(b)

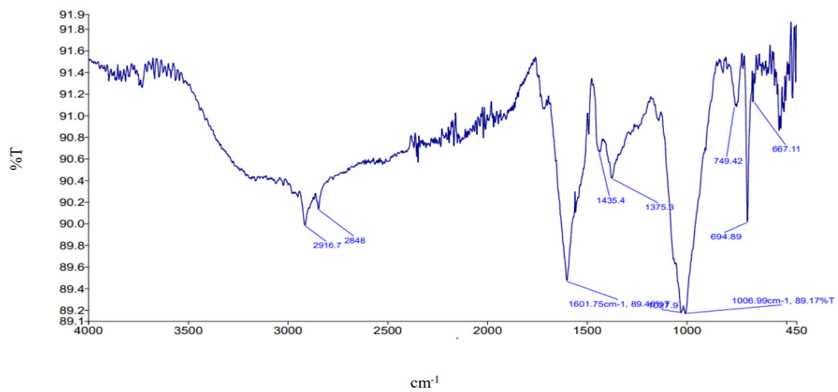
Figure 5. X-Ray diffraction (XRD) analysis of AgNPs from the leaves of *P. acidus* (a); X-Ray diffraction (XRD) analysis of AgNPs from the fruits of *P. acidus* (b)

FTIR study

FTIR measurements were carried out to study the core-shell functional groups present in the leaves and fruits extracts and their possible involvement in the synthesis and stabilization of silver nanoparticles. The majority of the peaks corresponds to the phenolic groups of the polyphenols, triterpenoids, alkaloids, steroids and tannins adequately present in the leaves extract, which help in the formation of AgNPs . The spectrum of synthesized AgNPs from leaves and fruits of *P. acidus* is represented in Figure 6 a and b. The extracts showed several peaks indicating the complex nature of the biological material. In Figure 6a, the bands appearing at 2914.6, 2847.3, 1593.94, and 1008.39 cm^{-1} were characteristics of the presence of C-H, CHO, N-H and C-O groups respectively. Figure 6b showed bands at 2916.7, 1601.75, 1375.3, 1006.99 and 694.89 cm^{-1} represent the presence of C-H, N-H, N=O, C-O and aromatic C-H functional groups respectively. There was a shift in the peaks of synthesized silver nanoparticles which suggests that functional groups of plant part extracts participate in the formation of AgNPs. The interpretation of the band is given below in Table 1.



(a)



(b)

Figure 6. (a) IR spectra of AgNps of fresh leaves of *P. acidus;* (b) IR spectra of AgNps of fresh fruits of *P. acidus*

Table 1. FTIR analyses, probable functional group of NPs of fresh leaves and fruits extracts of *P. acidus*

Plant part	Frequency (cm^{-1})	Probable Functional Groups
Fresh leaves	2914.6	Alkanes
	2847.3	Aldehydes
	1593.94	Amides
	1008.39	Alkoxy group
Fresh fruits	2916.7	Alkanes
	1601.75	Amines
	1375.3	Nitro compound
	1006.99	Alkoxy
	694.89	Aromatic compound

Mosquito larvicidal bioassay

In the present study, the efficacy of the AgNPs synthesized from the leaves and fruit extract of *P. acidus* was tested against the third instar larvae of *Cx. quinquefasciatus* and *Cx. vishnui*. Figure 7 denotes the results of the rate of mortality of 3rd instar larvae when exposed to graded concentrations (1, 5, 10, 15 and 20 ppm) of leaf extract and Figure 8 represents the rate of mortality of 3rd instar larvae when exposed to graded concentrations (1, 2, 3, 4 and 5 ppm) of fruits extract of the purified pellet of AgNPs after 24 hours of exposure.

The highest mortality (100 %) of 3rd instar larvae of both the mosquito species were observed in 20 ppm and 5 ppm of leaf and fruit synthesized silver nanoparticles after 24 hours of exposure. Between the two mosquito species *Cx. vishnui* showed greater mortality in each tested concentration of leaf and fruit-synthesized AgNPs. Table 2 and Table 3 represent the LC_{50} and LC_{90} values (Finney, 1971), regression equations along with R values of *Cx. quinquefasciatus* and *Cx. vishnui* 3rd larval stages against synthesized AgNPs of both the plant parts. Strong dose-dependent mortality was observed from the established regression equations, which is evident from the positive correlation between the rate of mortality (Y) and the concentration (X) of the synthesized AgNPs, and in each case, the regression coefficient value is close to one. Completely randomized three-way factorial ANOVA was also carried out related to the mortality of two mosquito species (*Cx. quinquefasciatus, and Cx. vishnui)* using different concentrations of synthesized silver nanoparticles of mature fruits of *P. acidus* and different hours of exposure as three variables (Tables 4 and 5).

To find out the reason behind the mortality of mosquito larvae, dead larvae were observed under a 10× view of the compound microscope. As shown by the regression equation, there is a linear correlation between the dose of AgNPs and the mortality rate in 3rd larval instar of both mosquito species. This suggests that higher doses of AgNPs permit greater uptake of nanoparticles by the larval body and consequently result in more mortality. However, Figure 9b and 10a showed that the *P. acidus* leaf AgNPs penetrated through the cuticle of the 3rd instar larva of *Cx. quinquefasciatus* and *Cx. vishnui* and causes the death of the delicate larvae. While in the case of *P. acidus* fruit AgNPs, penetration of AgNPs through the treated larval cuticle of 3rd instar larva of *Cx. quinquefasciatus* and *Cx. vishnui* (Figure 9a and 10b) caused an interaction with midgut epithelial cells resulting in the death of larvae (Sundaravadivelan and Nalini, 2012). However, further experimental studies are required to support our findings.

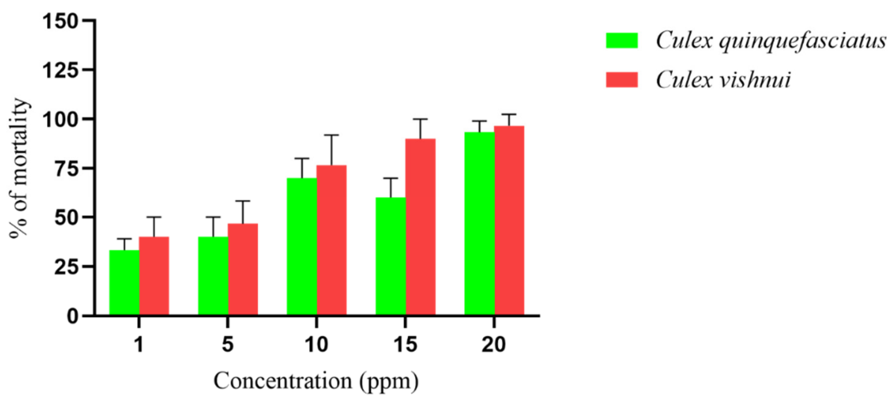


Figure 7. Column diagram showing comparative larvicidal mortality of silver nanoparticles synthesized from mature leaves of *P. acidus* in 1, 5, 10, 15, and 20 ppm of concentration against 3rd instar of *Cx. quinquefasciatus and Cx. vishnui*

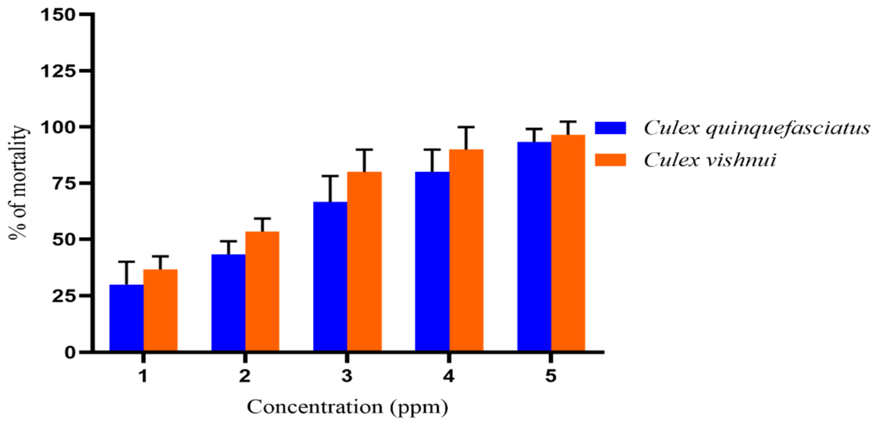
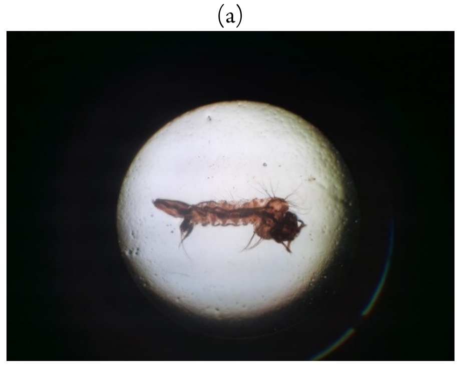


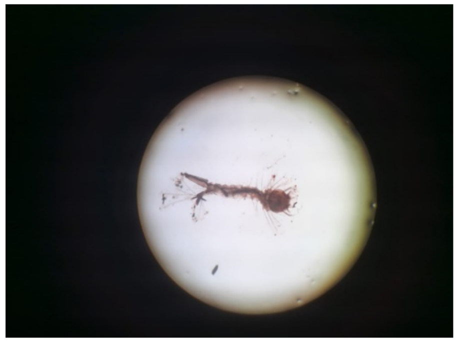
Figure 8. Column diagram showing comparative larvicidal mortality of silver nanoparticles synthesized from mature fruits of *P. acidus* in 1-5 ppm of concentration against 3rd instar of *Cx. quinquefasciatus and Cx. vishnui*

(a)



(b)

Figure 9. *P. acidus* fruit AgNPs treated larvae of *Cx. quinquefasciatus* (a); *P. acidus* leaf AgNPs treated larvae of *Cx. quinquefasciatus* (b)



(a)

(b)

Figure 10. (a) *P. acidus* leaf AgNPs treated *Cx. vishnui*; (b) *P. acidus* fruit AgNPs treated *Cx. vishnui*

Table 2. Probit analysis and regression analysis of mortality rates of *Cx. quinquefasciatus* and *Cx. vishnui* from synthesized silver nanoparticles from mature leaves of *P. acidus*

Mosquito species	Hours of exposure	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	R value
Cx. quinquefasciatus	24	2.90	21.55	Y=0.34x+3.36	0.93
	48	1.64	13.39	Y=0.29x+4.63	0.91
Cx. vishnui	24	1.41	13.20	Y=0.26x+5.14	0.89
	48	0.87	7.46	Y=0.21x+6.30	0.86

Table 3. Probit analysis and regression analysis of mortality rates of *Cx. quinquefasciatus* and *Cx. vishnui* from synthesized silver nanoparticles from mature fruits of *P. acidus*

Mosquito species	Hours of exposure	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	R value
Cx. quinquefasciatus	24	1.87	5.75	Y=1.63x+1.36	0.95
	48	1.62	4.25	Y=1.70x+1.83	0.96
Cx. vishnui	24	1.50	4.17	Y=1.57x+2.43	0.94
	48	1.24	3.42	Y=1.40x+3.53	0.91

Table 4. Completely randomized three-way factorial ANOVA related to mortality of two mosquito species (*Cx. quinquefasciatus, and Cx. vishnui)* using different concentrations of synthesized silver nanoparticles of mature fruits of *P. acidus* and different hours of exposure as three variables

Source of variation	Type III Sum of squares	df	Mean squares	F value	P value
Mosquito species (MS)	10.42	1	10.42	15.63	0.001 **
Hours (H)	6.02	1	6.02	9.03	0.001 **
Concentration (C)	308.40	4	77.10	115.65	0.001 **
MS *H	0.017	1	.017	.025	0.875
MS*C	2.00	4	0.50	0.75	0.564
H*C	0.40	4	0.10	0.15	0.962
MS*H *C	1.07	4	0.27	0.40	0.807
Within group	26.67	40	0.67		
Total	354.93	59	4.83		

** denotes significant value

Table 5. Completely randomized three-way factorial ANOVA related to mortality of two mosquito species (*Cx. quinquefasciatus, and Cx. vishnui)* using different concentrations of synthesized silver nanoparticles of mature leaves of *P. acidus* and different hours of exposure as three variables

Source of variation	Type III Sum of squares	df	Mean squares	F value	P value
Mosquito species (MS)	12.15	1	12.15	17.36	0.001 **
Hours (H)	8.82	1	8.87	12.59	0.001 **
Concentration (C)	227.07	4	56.77	81.09	0.001 **
MS *H	0.150	1	0.150	0.22	0.646
MS*C	6.60	4	1.65	2.36	0.070
H*C	1.60	4	0.40	0.57	0.685
MS*H *C	0.60	4	0.15	0.21	0.929
Within group	28.00	40	0.70		
Total	284.98	59	4.83		

** denotes significant value

Discussion

Numerous strategies have been used to improve the biosynthesis of nanoparticles, which is preferred to chemical and physical processes since it is economical, environmentally safe, and doesn't involve the use of harmful chemicals, high pressure, energy, or temperature (Sinha *et al*., 2009; Goodsell, 2004). In the present study, the synthesis of silver nanoparticles was confirmed by a change of coloration from colorless to deep brown color. The formation of AgNPs is further supported by the characteristic Surface Plasmon Resonance (SPR) bands at 420 and 409 nm for leaves and fruits of *P. acidus* respectively. Approximately similar observations are recorded in some earlier studies (Haldar *et al*., 2013; Rawani *et al*., 2013; Rawani, 2017). TEM images confirm spherical or oval shapes of the synthesized nanoparticles and an average size of 50 to 75 nm. Rawani *et al*. (2013) reported that the majority of the silver nanoparticles are found within 50 to 100 mm in diameter. Ghosh *et al*. (2022) also reveal that most of the nano-crystals formed are quasi-spherical (or polyhedral) in shape and have an average diameter of 50 nm. FESEM images of synthesized AgNPs of both the plant parts show rough and crystalline nanoparticles which is also evidenced by the XRD results, confirmed that the synthesized AgNPs of the plant parts were crystalline. (Ibrahim *et al*., 2015; Mulvancy, 1996), and showed the resemblance with the literature values of the face-centered-cubic (fcc) crystal structure of silver

(Das *et al*., 2014; Bhat *et al*., 2013). Bragg's peak presence in XRD reveals the reduction of silver ions and stabilization of formed nanoparticles (Ibrahim *et al*., 2015; Rawani *et al*., 2013; Ghosh *et al*., 2022). Stabilization of synthesized AgNPs of both the plant parts was also supported by the study of the core-shell functional groups through FTIR analysis. FTIR spectra show characteristic bands that include functional groups like alkanes, amines, alkoxy, aromatic compounds, and nitro compounds. Phytochemicals present in the leaves and fruit extracts of *P. acidus* participated in the formation of core-shell morphology of synthesized silver nanoparticles (Rodriguez *et al*., 2021). These findings were also consistent with the results of Rawani *et al*. (2013); Rawani (2017); Haldar *et al*. (2013) and Ghosh *et al*. (2022).

The present study's data unambiguously show that silver nanoparticles synthesized from leaves and fruits extract of *P. acidus* have the potential to effectively control *Cx. vishnui* and *Cx. quinquefasciatus* larvae. Silver nanoparticles cause 100 % mortality against 3rd instar larvae of both *Cx. quinquefasciatus* and *Cx. vishnui* mosquito at 20 ppm and 5 ppm concentration of leaf and fruit nanoparticles respectively after 24 hours of exposure. Lowest LC_{50} values calculated were 1.64 ppm (*Cx. quinquefasciatus)* and 0.87 ppm (*Cx. vishnui*) in leaf AgNPs while 1.62 ppm *Cx. quinquefasciatus)* and 1.24 ppm (*Cx. vishnui)* in fruit AgNPs after 48 h of exposure. Some researchers also found out the efficacy of synthesized silver nanoparticles against the larval forms of vector mosquitoes. Rawani *et al*. (2013) synthesized AgNPs from fresh leaves, dry leaves, and green berries of *S. nigrum* and tested them against larvae of *Cx. quinquefasciatus* and *Anopheles stephensi*. The highest mortality was observed at 10 ppm against *An. stephensi* with LC_{50} values of 1.33, 1.59, and 1.56 ppm and LC_{90} values of 3.97, 7.31, and 4.76 ppm for dry leaves, fresh leaves, and berries respectively. Rawani (2017) also synthesized AgNps from the bud of *Polianthus tuberosa* and evaluated its potency against *Cx. vishnui* and *Cx. quinquefasciatus*. Synthesized AgNPs showed the highest mortality at 20 ppm against *Cx. vishnui* with LC_{50} values of 8.25 and 7.46 ppm 3rd and 4th instars respectively; whereas in *Cx. quinquefasciatus* the LC_{50} values were 9.65 and 7.94 against 3rd and 4th instars respectively. The aqueous solution of the purified pellet of AgNPs synthesized from fruits of *Drypetes roxburghii* when exposed to 2nd, 3rd, and 4th instar larvae of *Cx. quinquefasciatus* and *An. stephensi* in graded concentrations for 24 hours. 10 ppm concentration caused 100% mortality in all instars of both the mosquitoes whereas at 5 ppm concentration, 100% mortality occurs in 2nd instar larvae of *An. stephensi* (Halder *et al*., 2013). The pellet of AgNPs prepared by using fresh fruits of *C. diurnum* was subjected to mosquito larvicidal bioassay on the 2nd and 3rd instars larvae of *Cx. quinquefasciatus* and *An. stephensi* by Ghosh *et al*. (2021). 100% mortality was observed in 10 ppm concentration in the 2nd and 3rd larval instar of *An. stephensi* while 95 and 100% mortality were recorded in 2nd and 3rd instar larvae of *Cx. quinquefasciatus*. The present study shows that the nanoparticles synthesized from leaves and fruit extract of *P. acidus* are much more effective in terms of LC_{50} value for the 3rd instar larvae of *Cx. quinquefasciatus* and *Cx. vishnui*. Present findings also evaluate that these synthesized nanoparticles might be penetrated through the cuticle of delicate larvae and cause interaction with midgut epithelial cells. This penetration might be possible due to the attraction of positive silver ions and the cell membrane (Kvitek *et al*., 2008). Earlier studies from Sundaravadivelan and Nalini (2012) support the view that penetration of AgNPs through larval cuticle caused an interaction with cell molecules resulting in the death of larvae. Further studies are required on the evaluation of the toxic effect of nanoparticles proved to be non-hazardous to non-target species and would be harmless, unlike other commercially available insecticides.

Conclusions

The present study of green synthesis shows that the environmentally benign and renewable source of *P. acidus* can be used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silver nanoparticles would be a boon for the development of a clean, nontoxic, and environmentally acceptable green approach to producing green AgNPs, involving organisms extending even to higher plants. The synthesized AgNPs are highly stable and significant mosquito larvicides. Further research is needed to improve the efficiency of the production of silver nanoparticles using *P. acidus* and other plant parts as reducing and stabilizing agents to increase the biological activity to levels appropriate for commercial products. The mechanisms of the mode of action are also in need to combat the effective vector control program. Based on these findings, the development of methods and techniques may lead to valuable discoveries necessary for the green synthesis of silver nanoparticles by using microorganisms, such as bacteria and fungi, for mosquito control.

Authors' Contributions

AG prepared the sample and analysed the experimental assays. AR supervised the whole research work and AR and AG prepared the manuscripts. PC helped in TEM studies. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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