Toxicology Reports 8 (2021) 1248–1254

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

A novel approach for synthesis of silver nanoparticles using *Pila virens* shell and its mosquito larvicidal activity

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ARTICLE INFO

Handling Editor Dr. Aristidis Tsatsakis

Keywords:
Pila virens shell
Silver nanoparticles
SEM
TEM
Larvicidal activity

ABSTRACT

Mosquito act as a vector for variety of deadly diseases. In this study, larvicide activity was investigated in relation to *Aedes aegypti* (*A. aegypti*) and *Culex quinquefasciatus* (*C. quinquefasciatus*) of synthesised silver nanoparticles (AgNPs) of the *Pila virens* (*P.virens*) shell extract. The characterization techniques UV–vis spectral, Fourier transforms infrared spectroscopy (FTIR),High Resonance Scanning electron microscope (HR-SEM) analysis, X-ray diffraction studies (XRD), High Resonance-Transmission electron microscopy (HR-TEM) used to characterize biosynthesized AgNPs. UV–vis, absorption showed peaks of 450 nm for the biosynthesised AgNPs, SEM observed spherical shaped particles of 25.9–28.9 nm in size and the XRD pattern shows the synthesized AgNPs fcc structure. FTIR investigation shown that the esters, carboxylic acid and ether as functional groups have been intricate in the reduction of metal ions. The larvicidal efficacy of synthesized AgNPs towards a larvae of *A. aegypti* $LC_{50}$and LC_{90} value of (37.87 and 132.86 ppm) and *C. quinquefasciatus* was (14.70 and 28.96 ppm) respectively. The synthesized AgNPs of *P. virens* confirmed highest mortality towards larvae of and *A. aegypti* and *C. quinquefasciatus.*

1. Introduction

Mosquitoes are the most common sources of the spread of lethal diseases, such as malaria, chikungunya, filariasis and yellow fever. In recent years, Dengue fever has become the epidemic in tropical and subtropical regions about 390 million dengue infected worldwide, 96 million of which will be clinically manifested [1]. Dengue fever has become a key public health task for health officials and healthcare professionals in the world [2–4]. Actually, there is no specific dengue treatment even though a vaccine is being developed [5,6]. *C. quinquefasciatus*is a major lymphatic filariosis mosquito vector, more than 120 million people infected worldwide [7,8]. Efforts to control higher outbreak in mosquitos generally are focused on the usage of synthetic insecticides, particularly pyrethroids and organophosphates [9].Despite attempts to research various chemicals, there are just a few insecticides [10]. This led to the quest for new, sustainable solutions, such as botanical insecticides [11].Nanoparticles are currently attracting the attention of scientists because of their comprehensive application to enhance the latest applied science in the fields of electronics, materials science, catalysts at nanoscale. Nanotechnology advances the development of green processes using aqueous extracts for the nanoparticles synthesis [12]. Nanoparticles have a significant role to play in diagnosis, drug delivery, sensing, gene transfer, imaging, tissue engineering and artificial implants [13]. In current consideration given to biosynthesis method for nanoparticle synthesis [14]. Nanoparticles' biosynthesis will reduce toxicity effectively and make the nanoparticles more stable. AgNPs possessing larvicidal [15],antibacterial [16], antifungal [17] and anticancer activity [18]. The use of biomaterials for metallic nanoparticles synthesis ensures that the pharmaceutical and other biomedical applications are environmentally friendly because the synthesis protocols prevent the use of toxic chemicals.

Nowadays, biological synthesis of nanoparticles has been proposed as an emerging technology, and offers several advantages compared to conventional methods. Several studies reported the synthesis of nanoparticles using biological materials, such as microorganisms, plant extracts, milk, and panchakavya [19–25]. Among metal nanoparticles

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https://doi.org/10.1016/j.toxrep.2021.06.018
Received 15 October 2020; Received in revised form 6 June 2021; Accepted 14 June 2021
Available online 16 June 2021

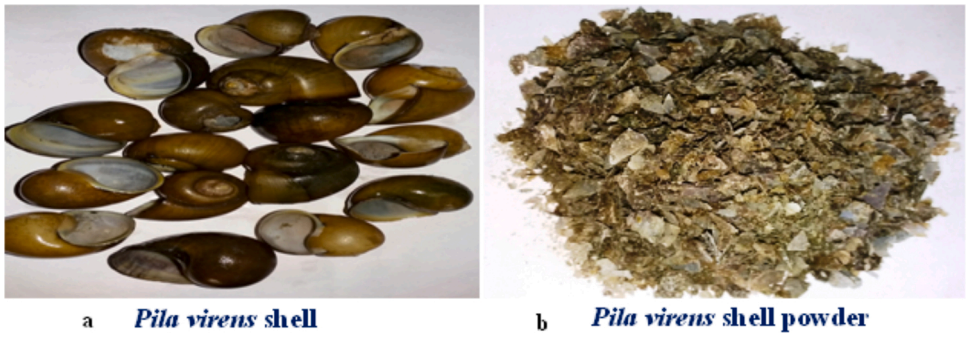

Fig. 1. a) *Pila virens* shell, b) *Pila virens* shell powder.

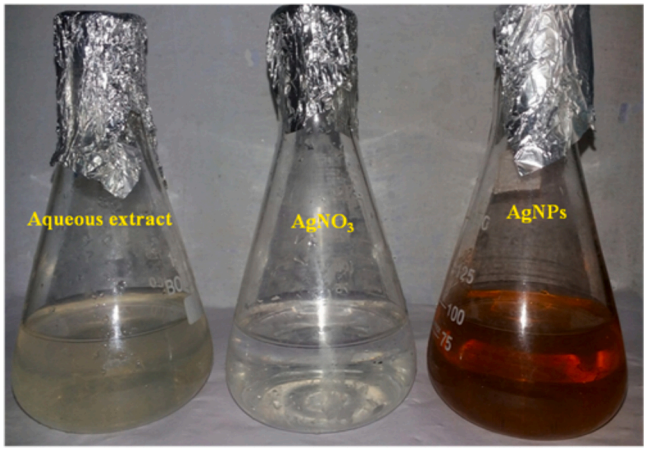

Fig. 2. Shows colour change from light yellow to brown before and after the process of Ag + to AgNPs of *P.virens* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

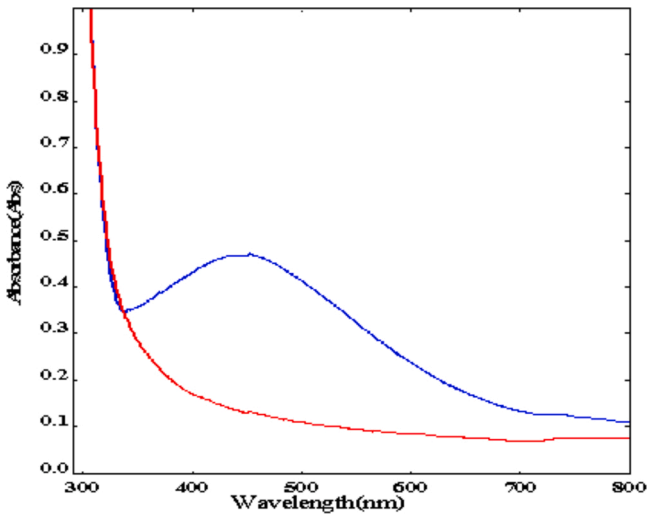

Fig. 3. UV–vis spectrum of shell aqueous extract and synthesized AgNPs.

AgNPs have been frequently used in different fields including biotechnology, biomedicine, veterinary medicine, pharmacy, food and electronics with further possible uses in agriculture, plant pathology, ecology, construction, textiles, cosmetics, and other industries [26–29]. Numerous studies reported the potential application of these biologically synthesized AgNPs [30–33]. The bacteria, cellulose, enzymes, plant leaves used as templates for the synthesis of nanometer [34]. However, other inexpensive, non-toxic biomaterials for synthesizing NPs for organic applications remain to be explored.

Molluscs are especially diverse due to their size and anatomical structure [35]. Phylum members exist in a wide variety of ecosystems, including aquatic, freshwater and terrestrial environments. A number of research publications have been reviewed concerning antimicrobial activity in molluscs such as haemolymph and egg mass or extracts of whole corpses [36] and are key characteristics for the rational development of compound against cancer and neurology [37–39]. Among the molluscs, oysters and mussels are very fine sources of bioactive compounds given their importance and lack of data was undertaken in this line to confirm the larvicidal activity of *Pila virens* extracts. In this study we have evaluated the toxicity of biosynthesized AgNPs using *P. virens* shell extracts.

2. Materials and methods

2.1. Pila shell collection and preparation of extract

P.virens was collected from Oragadam lake, Kanchipuram District, Tamil Nadu, India and identified by Dr. R. Venkitesan, Scientist-D, Zoological Survey of India (ZSI), 130, Santhome High Road, Chennai-600 028. For ten minutes, the shell of *P.virens* (Fig. 1) washed thoroughly in deionized water to eliminate dust particles. The solution of aqueous was formulated with 8 g of cleaned and fine parts soaked in Erlenmeyer 250 mL flask boiled for 20 min at 60◦C with 100 mL of deionized water [40]. This sample processed by a nylon mesh, (0.22 m) and used for further studies.

2.2. Mosquito culture

Mosquito larvae culture *A. aegypti* and *C. quinquefasciatus* obtained from the Entomological Research Centre, Loyola College, Chennai, Tamil Nadu, India. All larvae were put with tap water in plastic trays and placed in the laboratory. The experiment conducted at relative humidity (75–85 %) temperature (27 ± 2°-C). 3 (Dog Biscuit):1(yeast powder) ratio was fed to larvae. Cultures were established and grown in the lab using previous study [41].

2.3. Synthesis of silver AgNPs

The 15 mL of pila shell extract added in 85 mL of silver nitrate 1 mM solution added and incubated at 60 min for 28 °C. UV–vis spectroscopy analysed bio-reduced AgNPs [42].

2.4. Characterization of AgNPs

At time intervals, 1 mL of the sample was collected and measured using a spectrophotometer (Shimadzu 1601 model, Japan) between 200 and 800 nm with 1 nm resolution. Furthermore, synthesised nanoparticles have been centrifuged (twice) 20 min at 10,000 rpm. The final

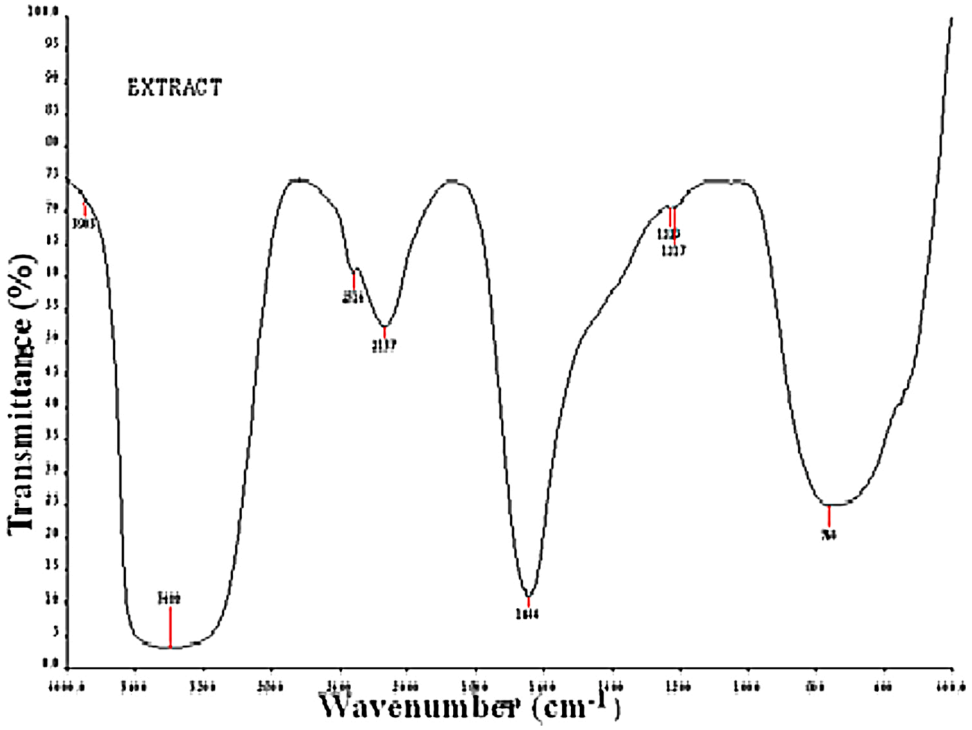

Fig. 4. FTIR spectra of *P. virens* shell extract solution.

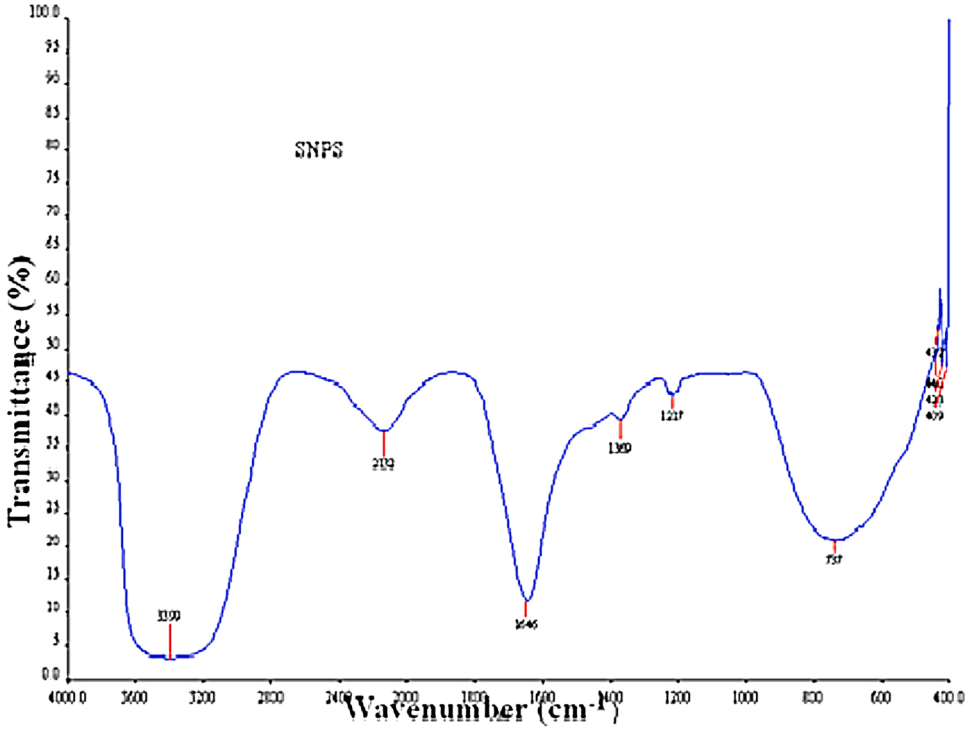

Fig. 5. FTIR spectra of *P. virens* shell extract solution of AgNPs.

pellet was processed, vacuum-dried and preserved for future use. Using an FT-IR Spectrometer (PERKIN ELMER-SPECTRUM ONE), the dried powder obtained is used to analyse the chemical compostion around the nanoparticles. The scanning range was 4000−450 $cm^{-1}$with 4 cm^{-1}resolutions.The X-ray diffractometer obtained using (D / Max 2500, Rigaku, Japan) at 40 kV voltage and a current of 50 mA with Cu-Kα radiation. HR-SEM (Hitachi SU6600) was used for morphological analysis. Thin films were prepared with a solution on a carbon-coated copper grid and the residual liquid removed using blotter paper. The filmdried for 5 min under a mercury lamp before examination. The TEM pictures were captured using JEOL model 3010 TEM operated at an accelerating voltage of the 300 kV. The samples of the nanoparticles were prepared by placing the solution drops over the copper grid and it was possible to dry and capture images at a different magnification.

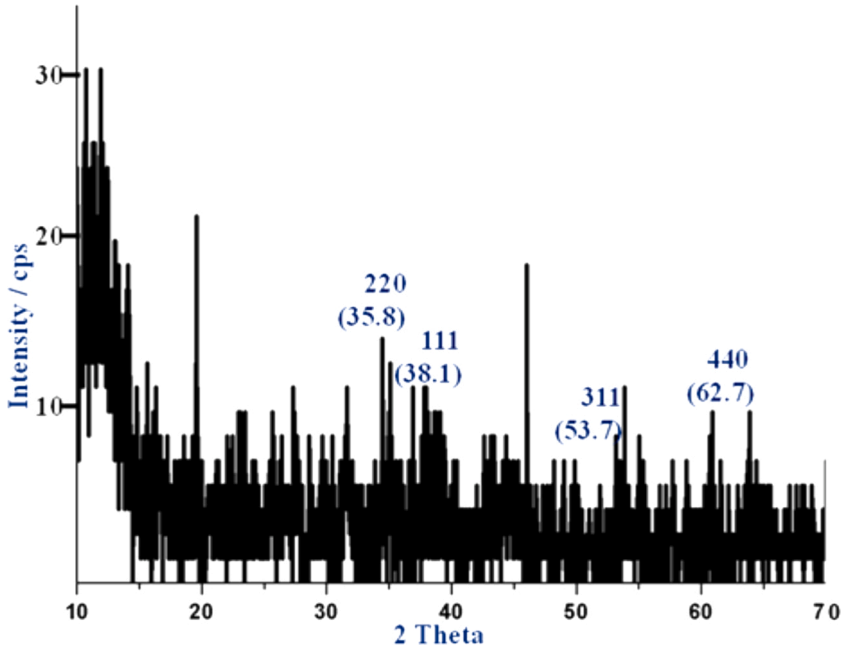

Fig. 6. Represents the XRD pattern of the synthesized AgNPs.

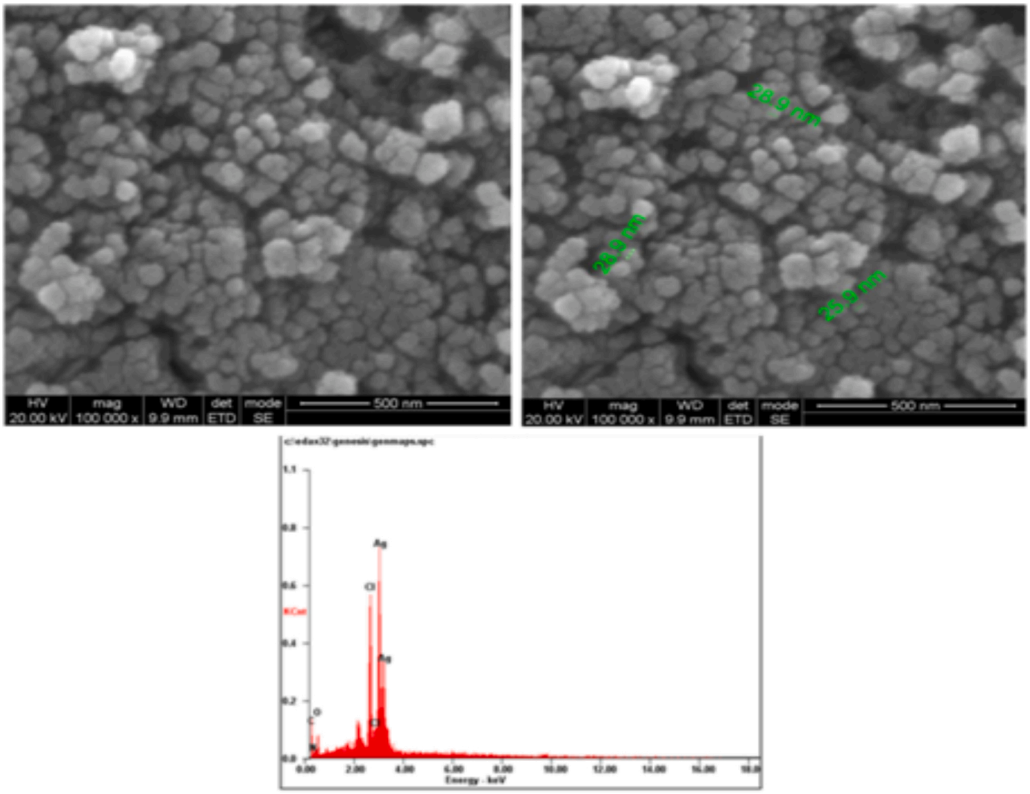

Fig. 7. FE-SEM images and EDX spectrum of synthesized AgNPs.

2.5. Larvicidal activity

Larvicidal activity with some modifications on shell extracts used to be conducted in line with [43]. 20 numbers of 4th instar larvae placed into a 500 mL glass beaker containing 249 mL of dechlorinated water and necessary amounts of aqueous shell extract. After 24 h of exposure, the amount of dead larvae was determined, and LC_{50} and LC_{90} were estimated. A multi-concentration test was used to assess the synthesized AgNPs toxicity and consists of controls and different biosynthesized AgNPs concentrations. Twenty mosquito larvae were tested each time with 250 mL of sterilised dual distilled waters including required concentration of aqueous extract from the *P. virens* shell (100, 200, 300, 400 and 500 ppm) and *P.virens* shell synthesized AgNPs (10, 20, 30, 40 and 50 ppm) were prepared. The experiments were performed with a control group (distilled water) in three replicates. The acute toxicity of the 4th instar mosquito larvae has been measured after 24 h. On average, the

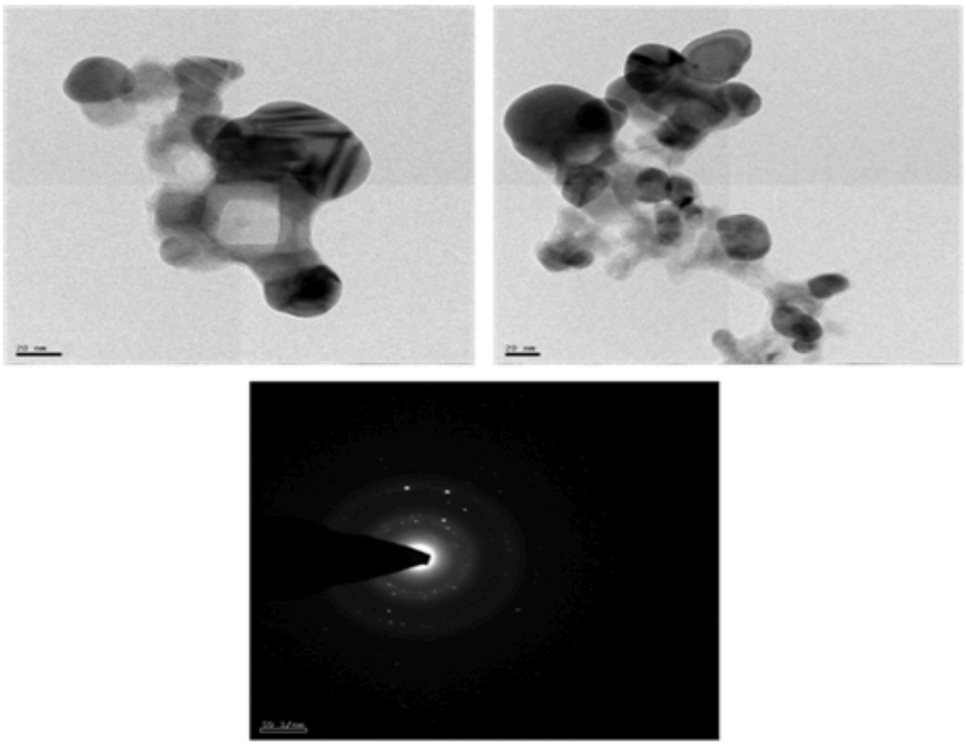

Fig. 8. Transmission electron microscopy and SAED images of AgNPs derived from *P.virens*.

Table 1
Larvicidal activity of shell extracts of *P.virens* against fourth instar larvae of *A. aegypti* and *C.quinquefasciatus*.

Extract/species	Concentration(ppm)	24 h % Mortality	LC_{50} (UCL-LCL) (ppm)	LC_{90} (UCL-LCL) (ppm)	Chi
Aqueous	500	76			
	400	64			
	300	50	261.02 (417.48–199.34)	280.75 (469.62–175.6)	2.21
A.aegypti	200	20			
	100	11			
AgNPs	100	100			
	75	80			
	50	57	37.87 (158.15–4.91)	132.86 (204.6–61.30)	37.51
A.aegypti	25	35			
	10	20			
Aqueous	100	83			
	75	57			
	50	36	149.81 (83.17–61.96)	234.33 (420.48–170.56)	2.44
C.quinquefasciatus	25	23			
	10	9			
AgNPs	10	100			
	08	87			
	06	65	14.70 (8.31–1.11)	28.96 (84.25–6.36)	28.31
C.quinquefasciatus	04	38			
	02	21			

Control –Nil Mortality.
LC50- Lethal concentration of 50 %.
LC 90 –Lethal Concentration of.90 %.
LCL Lower confidence limit, UCL- upper confidence limit.

values LC_{50} and LC_{90} were determined for three triplicates.

2.6. Statistical analysis

For each analysis of LC_{50}, LC_{90} values results due to a probit study. The SPSS (Statistical Package of the Social Sciences) package 11.5 has been used to measure the chi-square. The $p < 0.05$ results are regarded as significant.

3. Results and discussion

3.1. Characterization of biosynthesized AgNPs

Naturally, invertebrates are important natural products with a range of biological activities. *P.virens* shells are used to provide a valuable biological entity including peptides,glycosaminoglycans, carbohydrate and lectins [44,45]. During exposure to the shell extract, silver nitrate solution turninto brown colour (Fig. 2). The Fig. 3 shows a strong absorption band with a maximum of 450 nm. The FTIR analysis was

conducted to detect the biomolecules, which reduce and cap NPs. (Fig. 4). The FT-IR bands of the shell extract have been at 3400, 2137, 1644 and 760 cm^{-1} which related to hydroxyl groups [46–48], alkyene group [49], and amino [50]. The biosynthesized AgNPs confirmed peak at 3399, 2132, 1646 and 737 cm^{-1}. When compared to (Figs. 4 and 5) there is a slight change in peak shift in FTIR spectrum values of hydroxyl, amino and alkyene group which capable for silver nitrate reduction to AgNPs.The XRD Fig. 6 shows the usual AgNPsfcc structure. The diffraction peaks at $2\theta = 35.8°$, 38.1°, 53.7° and 62.7° respectively for (220), (111), (311), and (440) pure fcc phase reflection of AgNPs (JCPDS. × 6−00837) respectively. In addition there are unassigned peaks that may be due to the crystallization of others phases [51].SEM observed the synthesized NPs in the range of 25.9–28.9 nm in size. EDX showed a strong signal for silver Fig. 7. TEM image at different magnifications have been presented in (Fig. 8). The AgNPs were relatively well distributed in the medium with uniform spherical shape. It can also be seen that the NPs are well differentiated, suggesting the appropriate capping and lack of aggregation. The analysis of SAED results shows that diffraction rings indicating the polycrystalline nature of the AgNPs. The positions can be identified with the (111) planes reflected (JCPDS.00837). The SAED picture also demonstrates the single crystal structure of AgNPs [52]. This is in line with the SAED effects achieved [52], unassigned peak was observed can sometimes also be triggered by the different crystallization phases.

3.2. Mosquito larvicidal activity of silver nanoparticles

The impact of AgNPs on *C. quinquefasciatus* and *A. aegypti* shown in (Table 1). Unlike AgNPs towards the *A. aegypti* and *C. quinquefasciatus*, the toxic effects of aqueous extract were less. The two tested mosquito species were particularly sensitive to AgNPs against fourth instar larvae. 100 % mortality was reported after 24 h of post-treatment at 10 ppm. Values of LC_{50} and LC_{90} for *Pila* shell aqueous extract treated with fourth instar *A. aegypti* larvae were 280.75 and 261.02 ppm, and 149.81 and 234.33 ppm against *C. quinquefasciatus* respectively. LC_{50} and LC_{90} value from synthesized AgNPs were 37.87 and 132.86 ppm for *A.aegypti* and 14.70 and 28.96 ppm against *C. quinquefasciatus* fourth instar larvae. These findings were comparable to previous reports from Priyadarshini*et al.* [53] that AgNPs synthesized with *Eurphorbiahirta* leaves were toxic to larvae and pupae of malarial vector from first to fourth instar *A. stephensi*. The higher doses of AgNPs make it easier to take nanoparticles by mouth and trigger their death respectively. The mechanism that causes larval death may be the nanoparticles' ability to penetrate the larval membrane and can encourage positive silver ion attraction on the membrane of the cell [54,55].

4. Conclusion

Locally available fresh water *Pila* selected for the biosynthesized AgNPs at room temperature. Using UV visible spectroscopy, the bioreduced AgNPs were analysed the wide bands at 450 nm were obtained. The FTIR spectroscopy reveals that proteins and carboxylic acid, esters and ether which act as capping and stabilizing agent for synthesized nanoparticles. Studies by XRD demonstrated the crystalline structure of AgNPs. In the EDX analysis the strong signals in the silver regions were revealed and silver nanoparticles were confirmed. The size of the nanoparticles 25.9–28.9. The larvicidal activity of synthesized AgNPs towards a larvae of *A. aegypti* LC_{50} and LC_{90} value of (37.87 and 132. 86 ppm) and *C. quinquefasciatus* was (14.70 and 28.96 ppm) respectively.

Conflict of Interest

The authors declare that they have no known competing financial interestsor personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Our thanks to The Management and Principal of Pachaiyappas College for Men, Kanchipuram, for providing infrastructure and research facilities.

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