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Evaluation of larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector

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PEER REVIEW

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Comments

The present line of research quoted in this research article could be focusing an insight in biogenic nanoparticle research and their possible role towards the control of mosquitoes. Thus this biogenic nanoparticle can be an alternative role in integrated vector control programme.

(Details on Page 178)

ABSTRACT

Objective: To evaluate the larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector. **Methods:** The synthesized AgNPs were characterized by UV–vis. spectrum, Fourier transform infrared and X–ray diffraction. Larvae were exposed to varying concentrations of aqueous extract of synthesized AgNPs for 10 min. The different concentrations of 5, 2.5, 1.25, 0.625 and 0.312 mg/L silver nanoparticles were tested against the *Culex* larvae. **Results:** The mortality rate of *Agaricus bisporus* biogenic nanoparticles against *Culex* larvae are 5 mg/L (100%), 2.5 mg/L (81%), 1.25 mg/L (62%), 0.625 mg/L (28%) and 0.312 mg/L (11%). **Conclusions:** These results suggest that the synthesized biogenic AgNPs have the potential to be used as an ideal eco–friendly approach for controlling *Culex* larvae.

KEYWORDS

Silver nanoparticles, Larvicidal activity, *Culex*, Biological control**1. Introduction**

Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and ecosystem; it neither eliminates pathogen nor disease, but brings them into natural balance^[1]. Mosquitoes which transmit a number of diseases such as malaria (*Anopheles*), filariasis (*Culex*, *Mansonia*), and dengue (*Aedes aegypti*) etc., causing millions of deaths every year, are the most important group of insects in term of public health. Lymphatic filariasis, whose vector is *Culex quinquefasciatus* (*C. quinquefasciatus*), is a widely distributed tropical disease with around 120 million people infected worldwide and 44

million people having common chronic manifestation^[2]. Hence, to prevent mosquito borne diseases especially lymphatic filariasis, it is necessary to control the *C. quinquefasciatus* mosquito population.

The rise of insecticide resistance soon dashed such hopes. In recent years, repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance^[3], undesirable effects on non target organisms, fostered environmental and human health concerns^[4], which initiated a search for alternative control measures.

Nanoparticles have attracted considerable attention,

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owing to their various applications particularly silver nanoparticles, which are reported to possess antifungal, anti-inflammatory and anti-viral activity[5]. Additionally, AgNPs' larvicidal activity was proved by Rajkumar *et al*[6]. Due to the above mentioned reasons, the use of biologically synthesized silver nanoparticles for the control of *Culex* mosquito larvae will fulfill the disadvantages of the synthetic insecticides. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable size, shapes, chemical composition and controlled dispersity for human benefits. The most predominantly studied about nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd.

Among the four metals, silver nanoparticles play a significant role in the field of biology and medicine. There is a growing need to develop clean, nontoxic and environmentally friendly (green chemistry) procedures for synthesis and assembly of nanoparticles, biosynthesis of silver nanoparticles using plants, bacteria, fungi and yeast are known to reduce silver ions into silver nanoparticles by both extra and intra cellular. Dhanasekaran *et al.* found that the actinobacteria isolates had strong larvicidal activity against *Anopheles* mosquito larvae[7]. However, actinomycetes were effectively used against *C. quinquefasciatus*[8]. Nikhil *et al.* presented an eco-friendly process for synthesis of silver nanoparticles using a fungus *Penicillium brevicompactum* WA2315[9]. In another study by Parikh *et al.*, the bacterium isolated from an insect gut *Morganella* sp. (family Enterobacteriaceae) was also able to synthesize crystalline silver nanoparticles[10]. Basavaraja *et al.* and Ingle *et al.* demonstrated extracellular biosynthesis of silver nanoparticles by *Fusarium semitectum* and *Fusarium solani* (USM-3799)[11,12]. In the present study, we described about evaluation of larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector.

2. Materials and methods

2.1. Cleaning of glassware

Clean Borosil glassware's were used. They were soaked in tap water for a few minutes and then thoroughly washed in tap water. They were soaked in dichromate solution for a few hours to remove tough residues, and washed again in tap water.

2.2. Sterilization of glassware and chemicals

All types of glassware such as conical flask, petri plates, test tubes, pipettes, centrifuge tubes, tip boxes, saline bottles *etc.*, and the different types of bacterial and fungal

medium such as nutrient agar, Sabourod dextrose agar and lactose broth were sterilized at 121 °C for 15 min in autoclave.

2.3. Collection of mosquito larvae

The stagnant water samples were collected from roadside ditches, irrigation canals, drainage canals, temporary water pools and ponds around Tiruchirappalli, Tamil Nadu, India, using sterile wide mouth container.

2.4. Surveillance of mosquito larvae

Sampling sites include various water bodies that are roadside ditches, irrigation canals, drainage canals, temporary water pools and ponds *etc.*, surveys of larval habitats at five sampling sites in Tiruchirappalli district were recorded.

2.5. Characterization and identification of mosquito larvae

The filariasis causing *Culex* sp. mosquito larvae were collected from stagnant water samples and identified morphologically by using Atlas of Mosquito manual. The identified *Culex* sp mosquito larvae were kept in plastic and enamel trays containing tap water, maintained and reared in laboratory as per the method of Kamaraj *et al*[13].

2.6. Collection of microbial isolates

About 10 microbial (bacterial as well as fungal) isolates were obtained from Bioprocess Technology Lab, Department of Microbiology, Bharathidasan University, Tiruchirappalli.

2.7. Biosynthesis of silver nanoparticles using bacterial and fungal cultures

Microbial cultures were grown in appropriate medium until the growth reached stationary growth phase, bacterial cultures were centrifuged for 10000 rpm for 5 min, supernatant were discarded and fungal cultures were filtered. Bacteria and fungi were resuspended with minimal medium and incubated in shaker at optimum temperature. The cultures were centrifuged at 10000 rpm for 5 min or filtered after the incubation period. The culture supernatant/filtrates restrained microbial products were added to appropriate molarities solution of AgNO₃ and incubated.

2.8. Screening of larvicidal activity of silver nanoparticles

Larvicidal activities of silver nanoparticles were analyzed as per the standard procedures recommended by World Health Organization[14]. The silver nanoparticles solutions were diluted using double distilled water according to

desired concentrations. Each test included a set control (silver nitrate and distilled water) with five replicates for each individual concentration. Mortality were assessed every 3 h to determine acute toxicities on fourth instar larvae of *Culex* sp.

2.9. Larvicidal activity of nanoparticles test

Potential larvicidal activity of AgNPs using bacterial and fungal cultures was visibly observed by changes of the medium color, aliquots were subjected to UV–visible spectroscopy to measure the peak, then the particles were purified by density gradient centrifugation. Releases of silver ions in the solution were measured. The bioreduction of silver nanoparticles 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 Shimadzu 1601 spectrophotometer operated at a resolution of 1 nm.

2.10. Dose–response bioassay

Based on the preliminary screening results, synthesized AgNPs were subjected to dose–response bioassay for larvicidal activity against the larvae of *Culex* sp. Different concentrations ranging from 0.1 to 0.5 g/L (for synthesized AgNPs and silver nitrate) were prepared for larvicidal activity of parasites. The numbers of dead larvae were counted every 3 h interval of exposure and the percentage of mortality were reported from the average of five replicates. However, at the end of 24 h, the selected test samples turned out to be equal in their toxic potential.

3. Results

3.1. Morphological characterization of mosquito larvae

Culex larvae usually hang on water surface. They have well–developed head with mouth brushes, large thorax and eight segmented abdomen. In larval phase, they have no legs. Siphon (breathing tube) are found on the eighth abdominal segment. The body of *Culex* larvae is stout and wings are unspotted as shown in Figure 1.

3.2. Survey of mosquito larvae in Tiruchirappalli district

A total of 1000 larvae representing *Culex* species from five sampling location Mathur, Thuraiyur, Palkaliperur, Thuvakudi and Sempettu in Tiruchirappalli district area. The *Culex* larvae population was dominant in Palkaliperur. *Culex* mosquito larvae depended on hydrological types and various plant communities in water bodies. Present investigation showed that the *Culex* mosquito larvae were present in ditches and ponds of Tiruchirappalli (Table 1).

Table 1

Mosquito surveillance in aquatic habitats of Tiruchirappalli district.

Aquatic habitat	Mosquito genera		
	<i>Anopheles</i> sp.	<i>Culex</i> sp.	<i>Aedes</i> sp.
Roadside ditches	++++	+++	+++
Irrigator cannal	++	+++	++++
Drainage cannal	–	+++	–
Temporary water pools	+++	++	–
Pond	++	–	–

++++: predominantly present; +++: moderately present; ++: minimum of mosquito larvae; –: absence of mosquito larvae.

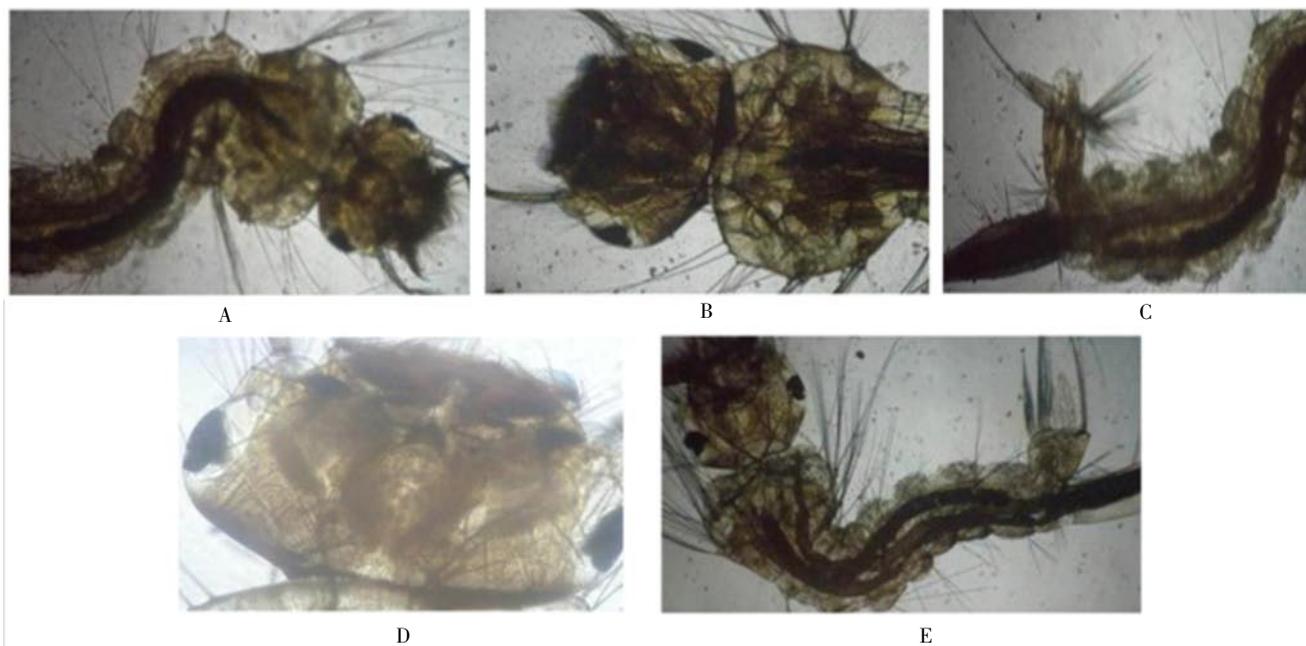


Figure 1. Morphological characterization of *Culex* mosquito larva.

A: Abdominal region; B: Thorax region; C: Siphon; D: Head; E: Morphology of larvae.

3.3. Potential bacterial and fungal isolates

Total ten bacterial and fungal isolates were screened for synthesis of silver nanoparticles. Synthesized nanoparticles were screened for larvicidal activity. The four isolates such as *Agaricus bisporus* (*A. bisporus*), *E. coli*, *Penicillium* sp. and *Vibrio* sp. showed the significant larvicidal activity (Table 2).

3.4. Biosynthesis of silver nanoparticle

Silver nitrate solution (1 mmol/L) was treated with microbial extracts and incubate for 48 h. The colour of broth changed to dark brown, indicating silver nanoparticles formed.

3.5. *A. bisporus* synthesized nanoparticles

Silver nanoparticles exhibit unique and tunable optical properties on account of their surface plasmon resonance, dependent on shape, size and distribution of the nanoparticles. The reduction of Ag^+ ions was monitored by measuring the UV–visible spectra of the solutions after diluting a small aliquot (0.2 mL) of the sample 20 times (Figure 2).

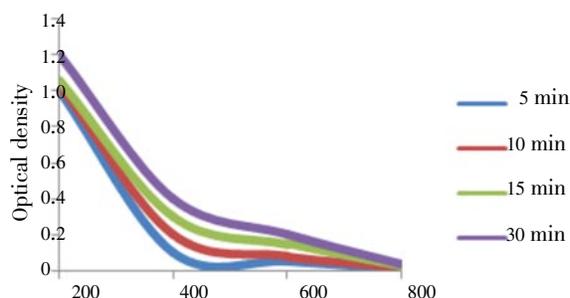


Figure 2. UV spectra of silver nanoparticle synthesized by *A. bisporus*.

3.6. Functional groups of nanoparticles from *A. bisporus*

The spectrum shows the band at 1650 cm^{-1} corresponds to a primary amine NH band; similarly, bands at 1540 and 1060 cm^{-1} correspond to a secondary amine NH band and a primary amine CN stretch vibration of the protein, respectively. The positions of these bands were close to those reported native proteins; the FTIR results indicate

that the secondary structures of proteins were not affected as a consequence of reaction with Ag ions or binding with silver nanoparticles. The band at 1425 cm^{-1} is assigned to a methylene scissoring vibration from the protein in the solution. This evidence suggests that the release of extracellular protein molecules could possibly perform the function for formation and stabilization of silver nanoparticles in aqueous medium (Figure 3).

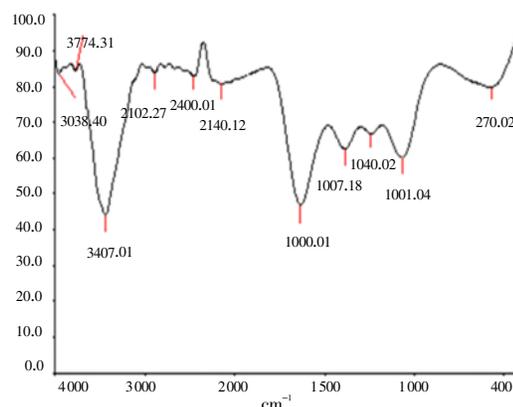


Figure 3. FTIR analysis silver nanoparticle synthesized by *A. bisporus*.

3.7. X-ray diffraction of nanoparticles from *A. bisporus*

The crystalline natures of silver nanoparticles were confirmed from the X-ray diffraction analysis. The X-ray diffraction pattern of freeze-dried nanoparticles exhibited peaks at 38.01° , 44.30° , 64.40° and 77.40° , which correspond to the (111), (200), (220) and (311) reflection of fullerene containing carbon material, respectively. The X-ray results show that the silver nanoparticles formed are crystalline in nature (Figure 4).

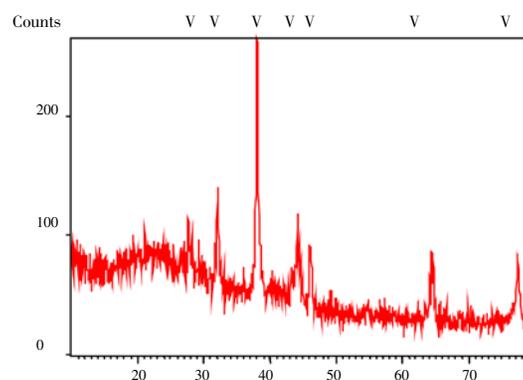


Figure 4. X-ray diffraction analysis of biogenic silver nanoparticles from *A. bisporus*.

Table 2

Primary screening of larvicidal activity of biogenic nanoparticles against fourth instar *Culex* mosquito larvae.

Microorganisms	Nanoparticles synthesized from the microorganisms	No. of mosquito larvae inoculated	Larvicidal activity against <i>Culex</i>		
			No. of mosquito larvae died	Mortality time (h)	% of larvicidal activity
<i>Agaricus bisporus</i>	Silver	5	5	18	100
<i>E. coli</i>	Silver	5	5	24	100
<i>Penicillium</i> sp.	Silver	5	5	24	100
<i>Vibrio</i> sp.	Silver	5	5	24	100

4. Discussion

Despite advances in medical science, mosquitoes in almost all tropical and subtropical countries are responsible for the transmission of pathogens causing some of the most life threatening and debilitating diseases, like malaria, yellow fever, dengue fever, chikunguniya, filariasis, encephalitis, etc. Repeated use of synthetic insecticides for mosquito control disrupts natural biological systems. The use of biological control agents is therefore essential. Microbes have been reported to reduce metal ions and stabilize nanoparticles with a wide size range. In the present investigation, ten isolates were tested, four of them were silver nanoparticles synthesizer. Silver nanoparticles were prepared successfully when the cell-free extracts of *A. bisporus* were used as a reducing agent as well as stabilizer and silver nitrate as a substrate. The microbial isolate, *A. bisporus* seems to be a good candidate for the extracellular biosynthesis of silver nanoparticles. Their formation proceeds via an extracellular mechanism, but there are still some questions regarding the details of the process. Nanoparticles were characterized by Fourier transformed infra-red spectrophotometer that protein might have played important role in the stabilization of silver nanoparticles. The X-ray diffraction pattern confirmed the fullerene containing carbon material, crystalline structure of metallic silver. The X-ray diffraction pattern of pure silver ions is known to display peaks at 2^{θ} values of 07.9° , 11.4° , 17.8° , 30.38° and 44° [15]. The line broadening in the X-ray diffraction graph is due to smaller particle size. Thus, X-ray diffraction spectrum confirmed the formation of silver nanoparticles[16]. Therefore, X-ray diffraction results also suggest that crystallization of bioorganic phase occurs on the surface of silver nanoparticles. Silver products have long been known to have strong bactericidal effects, which has been used for centuries to prevent and treat various diseases, most notably infections[17]. Mouchet *et al.* reported that a high mortality rate (85%) was noted at the highest double walled carbon nanotubes concentration against the larvae of *Xenopus laevis*[18]. Recent studies demonstrated that silver nanoparticles induce embryonic injuries and reduce survivals in zebrafish[19–25].

In conclusion, biogenic nanoparticles from bacteria and fungus can be an alternative source for present chemical larvicides, because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Further research is extended to find out the mass production, purification, physiochemical nature of biogenic nanoparticles and their *in vivo* efficacy in laboratory and field trials.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

C. quinquefasciatus is a major vector of filariasis and various encephalitis in India and worldwide. Vector control remains the most successful strategy for the suppression of mosquito borne diseases. The genetic structure of vector populations in terms of insecticide resistance and susceptibility or refractoriness to infection may possibly vary. To exploit the genetic variability in vector population could pave the path for the alternative strategies in vector management. In this context, the present study was aimed to evaluate the larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector.

Research frontiers

The data obtained from the present experiments are in close agreement with the earlier reports of Rajkumar *et al* (2011) and Kamaraj *et al* (2009).

Related reports

Pioneering of reliable and eco-friendly process for synthesis of metallic nanoparticles is biologically an important step in the field of nanobiotechnology applications. This paper reports the extracellular biosynthesis of silver nanoparticles using bacterial as well as fungal isolates. These findings are in close with the earlier findings.

Innovations & breakthroughs

Since there is no previous record of literature available about the larvicidal activity of biogenic nanoparticles of the bacterial and fungal culture filtrates, the present report serve as first hand information on larvicidal activities biogenic nanoparticles against the larvae of the *Culex* mosquito larvae.

Applications

Synthesis of nanoparticles using biological entities has great interest due to their unique shape dependent optical, electrical and chemical properties have potential application in nanobiotechnology. In these circumstances, an improvised method using the biologically synthesized silver nanoparticles were evaluated for the destruction of the mosquito larvae. The synthesis and assembly of nanoparticles would benefit from the development of clean,

nontoxic and environmentally acceptable “green chemistry” approaches for nanoparticles.

Peer review

The present line of research quoted in this research article could be focusing an insight in biogenic nanoparticle research and their possible role towards the control of mosquitoes. Thus this biogenic nanoparticle can be an alternative role in integrated vector control programme.

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