

# Antimicrobial and mosquitocidal activity of microbial synthesized silver nanoparticles

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**Abstract** Microbial synthesis of nanoparticles is a green approach that interconnects nanotechnology and microbial biotechnology. Here, we synthesized the silver nanoparticles (AgNPs) using bacterial strains of *Listeria monocytogenes*, *Bacillus subtilis* and *Streptomyces anulatus*. We tested the efficacy of AgNPs against the larvae, pupae and adults of *Anopheles stephensi* and *Culex quinquefasciatus*. We have also investigated the antifungal activity of AgNPs against the soil keratinophilic fungus of *Chrysosporium keratinophilum*. The efficacy tests were then performed at different concentrations and varying numbers of hours by probit analysis. The results were obtained using a UV-visible spectrophotometer, and the images were recorded with a transmission electron microscope (TEM). The synthesized AgNPs were in varied shape and sizes. The larvae and pupae of *Cx. quinquefasciatus* were found highly susceptible to AgNPs synthesized using the *L. monocytogenes*, *B. subtilis* and *S. anulatus* than the *An. stephensi*, while the adults of *An. stephensi* were found more susceptible to the AgNPs synthesized using the *L. monocytogenes*, *B. subtilis* and *S. anulatus* the *Cx. quinquefasciatus*. Further, these nanoparticles have also been tested as antifungal activity against the entomopathogenic fungus *C. keratinophilum*. The higher zone of inhibition occurred at the concentration level of 50 µl. This study gives an innovative approach to develop eco-friendly AgNPs which act as an effective antifungal agent/fungicide and insecticide.

**Keywords** Microbial synthesis · Silver nanoparticles · Mosquito control · Antimicrobial

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## Introduction

Mosquitoes are found across the globe, with the exception of Antarctica and Iceland. They are also a well-known vector for many diseases, including malaria, dengue fever, yellow fever and viral encephalitis. Among these, malaria, spread by the bite of a female *Anopheles* mosquito, and filariasis, spread by *Culex* mosquito are the two vector-borne diseases of the tropical region and are considered as major public health concerns.

According to WHO, there were about 219 million cases of malaria in 2010 (with an uncertainty range of 154 million to 289 million) and an estimated 660,000 deaths (with an uncertainty range of 490,000 to 836,000). Malaria mortality rate has fallen by more than 25 % globally since 2000 and by 33 % in the WHO African region. Most deaths occur among children living in Africa, where malaria claims the life of a child every minute. Country-level burden estimates available for 2010 show that an estimated 80 % of malaria deaths occur in just 14 countries and about 80 % of cases occur in 17 countries. Together, the Democratic Republic of the Congo and Nigeria account for over 40 % of the total estimated malaria deaths globally (World Health Organization 2013a).

On the other hand, nearly 1.4 billion people in 73 countries worldwide are threatened by lymphatic filariasis, commonly known as elephantiasis. Over 120 million people are currently infected, with about 40 million disfigured and incapacitated by the disease (World Health Organization 2013b). Control or eradication of the mosquito population could significantly restrict the spread of disease.

Nanoparticles attract greater attention due to their various applications in different fields. Microbes are also being used in nanotechnology for producing nanoparticles. Therefore, the present green synthesis has shown that environmentally benign and renewable sources of microbes can be used as an effective reducing agent for the synthesis of silver and gold

nanoparticles and mosquito control also. Biosynthesis of nanoparticles by using the bacteria *Bacillus* (Dhandapani and Supraja 2012; Das et al. 2013; Malarkodi et al. 2013; Priyadarshini et al. 2013; Omolbanin Shivai et al. 2013; Janardhanan et al. 2013), *Streptomyces* (Alani et al. 2012), *Pseudomonas* (Radhika Rajasree and Suma 2012; Silambarasan and Jayanthi 2013), *Vibrio* (Rajeshkumar et al. 2013) and *Idiomarina* (Seshadri et al. 2012) has been reported.

The antimicrobial activity of synthesized nanoparticles by using the *Bacillus* (Prakash et al. 2011; Kannan and Subblaxmi 2011; Sunkar and Valli Nachiyar 2012; Vijayaraghavan et al. 2012; El-Batal et al. 2013; Gopinath and Velusamy 2013), *Streptomyces* (Selvakumar et al. 2012; Subashini and Kannabiran 2013; Chauhan et al. 2013) and *Lactobacillus* (Salman 2013) has been investigated.

The larvicidal activities of synthesized cobalt nanoparticles (CoNPs) using biocontrol agent, *Bacillus thuringiensis* against malaria vector, *Anopheles subpictus*, and dengue vector, *Aedes aegypti* (Diptera: Culicidae), have been reported (Marimuthu et al. 2013). Similarly, the larvicidal activity of silver nanoparticles (AgNPs) synthesized by *Bacillus thuringiensis* (Bt) has been tested against *Ae. aegypti* (Banu et al. 2014).

In the present investigation, the AgNPs were synthesized using bacterial strains of *Listeria monocytogenes*, *Bacillus subtilis* and *Streptomyces anulatus*. We tested the efficacy of AgNPs against the larvae, pupae and adults of *Anopheles stephensi* and *Culex quinquefasciatus*. We have also investigated the antifungal activity of AgNPs against the soil keratinophilic fungus of *Chrysosporium keratinophilum*.

## Materials and methods

### Microbes and culture

The bacterial strains of *L. monocytogenes* (J0161), *B. subtilis* (ATCC 11774) and *S. anulatus* (MTCC 2528) were obtained from the Agriculture Research Services (ARS), US Department of Agriculture (USDA) and Microbial Type Culture Collection, Chandigarh, India.

The bacterial strains of *L. monocytogenes*, *B. subtilis* and *S. anulatus* were cultured on tryptone soy broth at 37 °C. After 24–48 h of incubation, biomass developed on the medium.

### Synthesis of silver nanoparticles

After 24–48 h of incubation, culture solution was centrifuged at 7500 rpm for 15 min. Then, the supernatant was taken in a clean 250-ml conical flask and added 1 M silver nitrate into 100 ml of supernatant. Then, the mixture was incubated in the orbital shaker for the synthesis of silver nanoparticles for 24 h. The change in colour was observed visually.

### Characterization of silver nanoparticles

Synthesized silver nanoparticles were confirmed by sampling the reaction mixture at regular intervals, and the absorption maxima were scanned by UV-Vis spectra, at the wavelength of 350–750 nm in a UV-3600 Shimadzu spectrophotometer at 1-nm resolution. The micrographs of silver nanoparticles were obtained by a TECHNAI 200 Kv TEM (Fei, Electron Optics) transmission electron microscope. For transmission electron microscopy analysis, samples were prepared on carbon-coated copper TEM grids.

### Vector rearing

The larvae of *Cx. quinquefasciatus* and *An. stephensi* were collected from various localities including urban, rural and semi-urban regions of Agra (27° 10' N, 78° 05' E), India. The larvae were reared in deionized water containing glucose and yeast powder. The colonies of *Cx. quinquefasciatus* and *An. stephensi* were maintained in the laboratory at a temperature of 25 °C with relative humidity of 75±5 % and 14 h of photoperiod. The larvae of *Cx. quinquefasciatus* and *An. stephensi* were maintained in separate enamel containers as per the standard method (Gerberg et al. 1994). The pupae were collected from the culture tray and transferred to Petri dishes containing 50 ml of water. The Petri dishes were placed inside a screened cage (25 cm in length×15 cm in width×5 cm in depth) to retain emerging adults, for which 5 % sucrose solution in water was provided to the adults.

### Bioassays, data management and statistical analysis

AgNPs synthesized by *L. monocytogenes*, *B. subtilis* and *S. anulatus* were tested for their killing activities against the larvae, pupae and adults of *Cx. quinquefasciatus* and *An. stephensi* and were assessed by using the standard method (World Health Organization 2005). All larvae and pupae of *Cx. quinquefasciatus* and *An. stephensi* were separated and placed in a container in microbe-free deionized water. After that, different test concentrations of silver nanoparticles in 100 ml deionized water were prepared in 250-ml beakers. Bioassays were conducted separately for each instar and pupae at five different concentrations of aqueous silver nanoparticles (2, 4, 6, 8 and 10 ppm for larvae and 20, 40, 60, 80 and 100 ppm, for pupa). To test the larvicidal and pupicidal activity of our silver nanoparticles, 20 pupae and larvae of each stage were separately exposed to 100 ml of test concentrations. Thereafter, we examined their mortality after different times of treatment during the experimental periods.

The adulticidal bioassays were carried out with laboratory-reared *Cx. quinquefasciatus* and *An. stephensi* as per standard procedures recommended by the World Health Organization with some modifications (World Health Organization 2006).

The freshly emerged 3-day-old sugar-fed adults were used for the assay. The five different volumes 0.16, 0.33, 0.66, 1.33 and 2.66  $\mu\text{l}/\text{cm}^2$  of aqueous silver nanoparticles of *L. monocytogenes*, *B. subtilis* and *S. anulatus* synthesized were sprayed in a cage (25 cm long  $\times$  15 cm wide  $\times$  5 cm deep) containing 100 mosquitoes. The exposed mosquitoes were kept under observation, and the dead mosquitoes were discarded every day. Each bioassay including the control was conducted in triplicates on different days. Similarly, the control (extract of *L. monocytogenes*, *B. subtilis* and *S. anulatus* without  $\text{AgNO}_3$ ) was run to test the natural mortality. The data on the efficacy were subjected to probit analysis (Finney 1971). The control mortality was corrected by Abbott's formula (Abbott 1925).

#### Antifungal activity of AgNPs

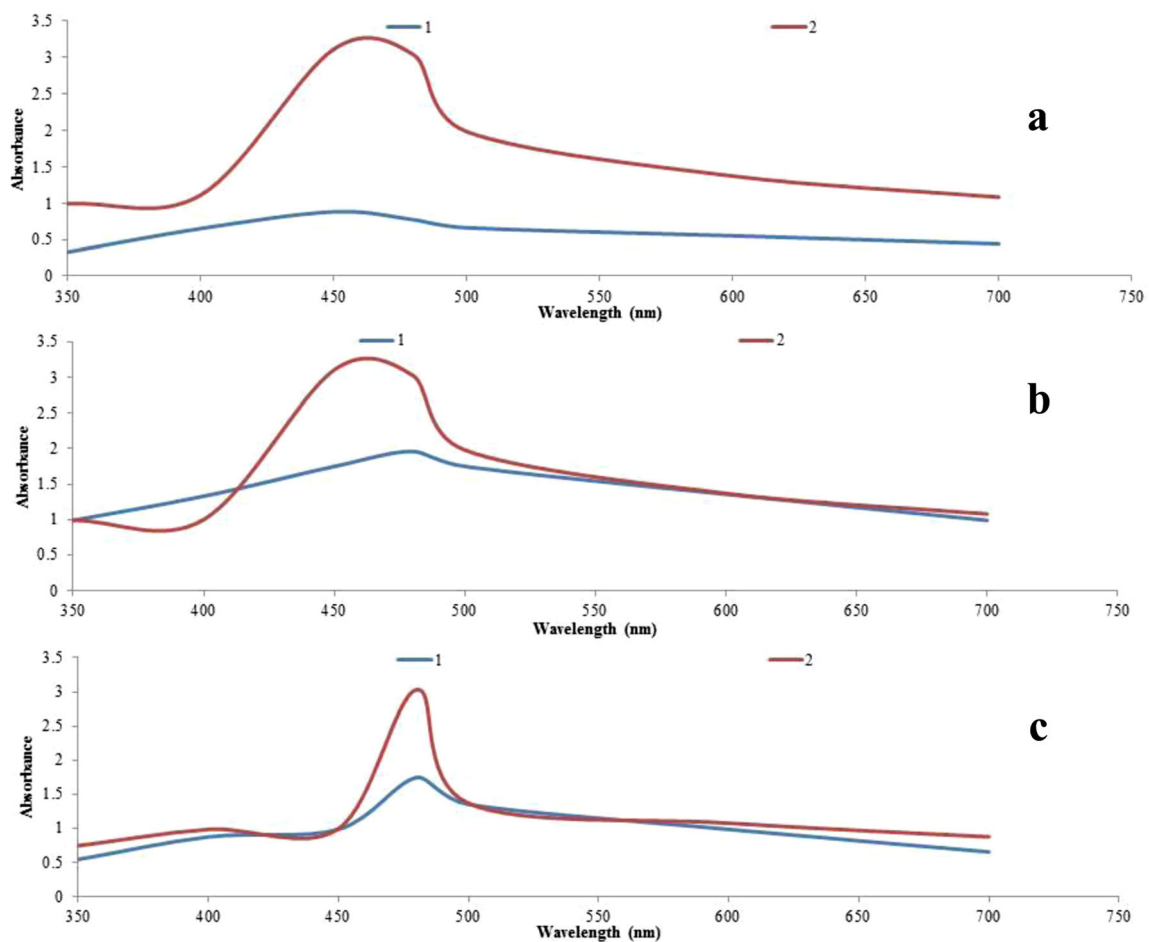
The fungicidal activity of AgNPs synthesized by using the *L. monocytogenes*, *B. subtilis* and *S. anulatus* was carried out against the entomopathogenic fungus *C. keratinophilum* (MTCC 2828). The fungicidal activity was done by using well diffusion method. The potato dextrose agar (PDA)

medium was prepared and poured into plates, and wells were made in the agar medium. Then, 24-h-old test fungicidal cultures were swabbed in PDA medium to form a confluent lawn of fungal cultures. Different concentrations (20, 30 and 50  $\mu\text{l}$ ) of AgNPs were loaded into the wells. Then, the plates were incubated at 25 °C for 24–84 h. After 24–48 h of inhibition, the plates were observed for zone of inhibition.

#### Results

##### UV-Vis spectroscopy and TEM analysis of synthesized AgNPs

By mixing the culture broth of *L. monocytogenes*, *B. subtilis* and *S. anulatus* with the aqueous solution of Ag ions, the colour of culture broth of *L. monocytogenes*, *B. subtilis* and *S. anulatus* changed to dark brown colour after 24 h of incubation. The change in colour is a signal for the formation of silver nanoparticles. Silver nanoparticle formation was further confirmed by the UV-Vis spectroscopy. Figure 1a–c



**Fig. 1** a–c UV-Vis spectra of silver nanoparticles synthesized by using the *Listeria monocytogenes*, *Bacillus subtilis* and *Streptomyces anulatus* recorded from reaction medium before (1) and after immersion of  $\text{AgNO}_3$  (2) after 24 h

shows the UV-Vis spectra of silver nanoparticles synthesized by using the *L. monocytogenes*, *B. subtilis* and *S. anulatus* recorded from reaction medium before (1) and after immersion of AgNO<sub>3</sub> (2) after 24 h. Absorption spectra of silver nanoparticles formed in the reaction medium have a broad absorption band centred at 480 nm ca.

Figure 2a–c shows the TEM micrographs of *L. monocytogenes*, *B. subtilis* and *S. anulatus*-synthesized silver nanoparticles. The silver nanoparticles were of varying sizes and shapes (Table 1).

Efficacy study of AgNPs synthesized by using the *B. subtilis* against the *Cx. quinquefasciatus* and *An. stephensi*

The different concentration doses (2, 4, 6, 8 and 10 ppm; 20, 40, 60, 80 and 100 ppm and 0.16, 0.33, 0.66, 1.33 and 2.66 µl/cm<sup>2</sup>, respectively) of silver nanoparticles synthesized by using the *B. subtilis* were tested against the larvae, pupae and adults of *Cx. quinquefasciatus* and *An. stephensi*. The larvae and pupae of *Cx. quinquefasciatus* and *An. stephensi* were found to be more susceptible to the synthesized silver nanoparticles than the adults. The mortality was observed after a span of different times of exposures.

The larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized silver nanoparticles. The first and second instar larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized silver nanoparticles and have shown 100 % mortality within 15 min. While the third and

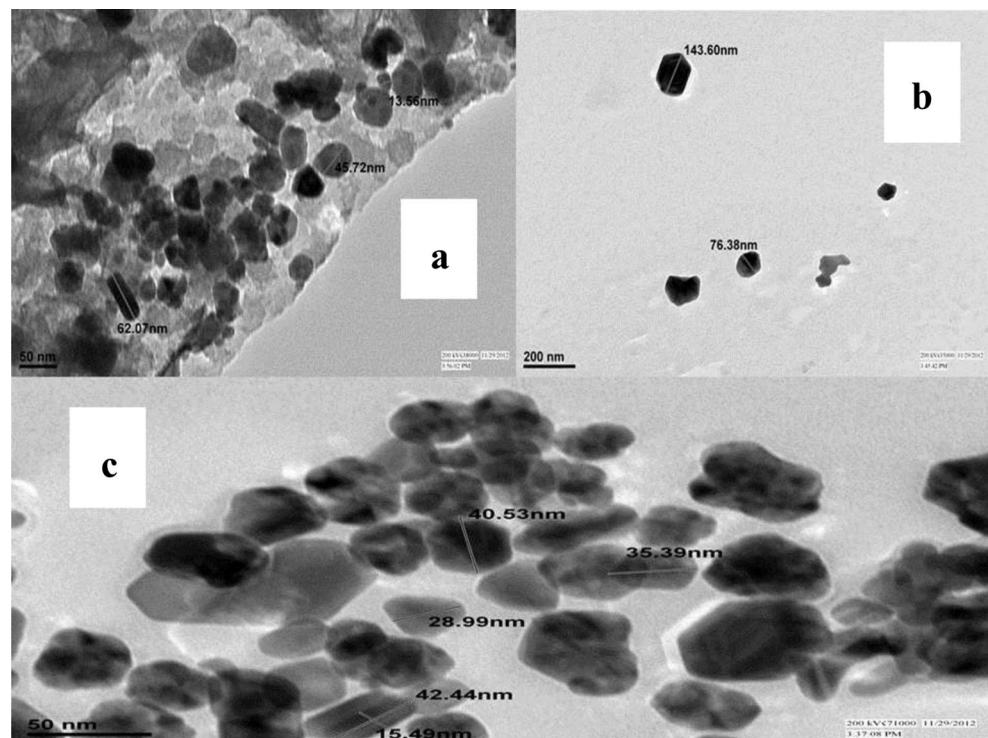
**Table 1** Shape and size of silver nanoparticles synthesized by *Listeria monocytogenes*, *Bacillus subtilis* and *Streptomyces anulatus*

Species	Shape	Size (nm)
<i>L. monocytogenes</i>	Rod	62.07
	Spherical	29.64
<i>B. subtilis</i>	Hexagonal	143.60
	Spherical	76.38
<i>S. anulatus</i>	Rod	42.44
	Hexagonal	37.96
	Triangular	28.99
	Spherical	11.53

fourth larvae have shown 100 % mortality after 45 min with their confidential limits. The pupae of *Cx. quinquefasciatus* were found more susceptible to the synthesized silver nanoparticles than the adults. The pupae have shown 100 % mortality after 5 h, while no mortality could be observed in adults after 5 h. In the control group, no mortality could be recoded (Table 2 (a)).

The larvae of *An. stephensi* were found susceptible to the silver nanoparticles synthesized by using *B. subtilis*. The first and third instar larvae of *An. stephensi* have shown 100 % mortality after 72 h of exposure. The second instars (lethal concentration 50 (LC<sub>50</sub>) 1, LC<sub>90</sub> 8 and LC<sub>99</sub> 10.5 ppm) have shown mortality after 72 h. The fourth instars (LC<sub>50</sub> 1, LC<sub>90</sub> 8

**Fig. 2** a–c TEM micrographs of *Listeria monocytogenes*, *Bacillus subtilis* and *Streptomyces anulatus*-synthesized silver nanoparticles



and LC<sub>99</sub> 10.5 ppm) have shown mortality after 72 h and were observed with their confidential limits. In the control group, no mortality could be observed. The pupae of *An. stephensi*

have shown 100 % mortality after 24 h. The adults have shown (LC<sub>50</sub> 0.16, LC<sub>90</sub> 3 and LC<sub>99</sub> 4 µl/cm<sup>2</sup>) after 2 h and 30 min with their confidential limits (Table 2 (a)).

**Table 2** Efficacy of silver nanoparticles synthesized by (A) *Listeria monocytogenes*, (B) *Bacillus subtilis* and (C) *Streptomyces anulatus* against the larvae, pupae and adults of *Cx. quinquefasciatus* and *An. stephensi* with their LC values and 95 % confidential limits (CLs) after different times of exposure

Species	Instar (minute/hours)	Time	LC <sub>50</sub> (95 % CL)	LC <sub>90</sub> (95 % CL)	LC <sub>99</sub> (95 % CL)
<b>A</b>					
<i>Cx. quinquefasciatus</i>	First	15 min	**	**	**
	Second	15 min	**	**	**
	Third	45 min	**	**	**
	Fourth	45 min	**	**	**
	Pupa	5 h	**	**	**
	Adult	5 h	—	—	—
<i>An. stephensi</i>	First	72 h	**	**	**
	Second	72 h	**	**	**
	Third	72 h	1 (0.86–2.14)	8 (6.83–9.17)	10.5 (9.27–11.73)
	Fourth	72 h	1 (0.86–2.14)	8 (6.83–9.17)	10.5 (9.27–11.73)
	Pupa	24 h	**	**	**
	Adult	2 h and 30 m	0.16 (0.14–0.18)	3 (1.88–4.12)	4 (2.86–5.14)
<b>B</b>					
<i>Cx. quinquefasciatus</i>	First	24 h	**	**	**
	Second	24 h	**	**	**
	Third	24 h	2 (0.86–3.14)	10.5 (9.27–11.73)	11.5 (10.12–12.88)
	Fourth	24 h	2.66 (1.52–3.8)	5.33 (4.10–6.56)	5.86 (4.40–7.32)
	Pupa	4 h	**	**	**
	Adult	4 h	—	—	—
<i>An. stephensi</i>	First	4 h	**	**	**
	Second	4 h	**	**	**
	Third	4 h	**	**	**
	Fourth	4 h	**	**	**
	Pupa	24 h	**	**	**
	Adult	5 h	0.08 (0.07–0.09)	3.99 (2.85–5.13)	5 (3.83–6.17)
<b>C</b>					
<i>Cx. quinquefasciatus</i>	First	15 m	**	**	**
	Second	3 h	**	**	**
	Third	8 h	**	**	**
	Fourth	1 h and 30 m	**	**	**
	Pupa	4 h	**	**	**
	Adult	4 h	—	—	—
<i>An. stephensi</i>	First	5 h	**	**	**
	Second	5 h	**	**	**
	Third	5 h	**	**	**
	Fourth	5 h	**	**	**
	Pupa	3 h and 20 m	20 (18.86–21.14)	120 (118.73–121.27)	130 (128.51–131.49)
	Adult	2 h and 30 m	0.06 (0.05–0.07)	1.33(0.21–2.45)	3 (1.86–4.14)

‘\*\*\*’ 100 % mortality

‘—’ No mortality

Efficacy study of AgNPs synthesized by using the *L. monocytogenes* against the *Cx. quinquefasciatus* and *An. stephensi*

The different concentration doses (2, 4, 6, 8 and 10 ppm; 20, 40, 60, 80 and 100 ppm and 0.16, 0.33, 0.66, 1.33 and 2.66  $\mu\text{l}/\text{cm}^2$ , respectively) of silver nanoparticles synthesized by using the *L. monocytogenes* were tested against the larvae, pupae and adults of *Cx. quinquefasciatus* and *An. stephensi*. The larvae and pupae of *Cx. quinquefasciatus* and *An. stephensi* were found to be more susceptible to the synthesized silver nanoparticles than the adults. The mortality was observed after a span of different times of exposures.

The larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized silver nanoparticles. The first and second instar larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized silver nanoparticles and have shown 100 % mortality within 24 min. For third instar larvae ( $\text{LC}_{50}$  2,  $\text{LC}_{90}$  10.5 and  $\text{LC}_{99}$  15.5 ppm) and fourth instar ( $\text{LC}_{50}$  2.66,  $\text{LC}_{90}$  5.33 and  $\text{LC}_{99}$  5.86 ppm) the susceptibility was recorded after 24 h with their confidential limits. The pupae of *Cx. quinquefasciatus* were found more susceptible to the synthesized silver nanoparticles than the adults. The pupae have shown 100 % mortality after 24 h, while no mortality could be observed in adults after 5 h. In the control group, no mortality could be recorded (Table 2 (b)).

The larvae of *An. stephensi* were found susceptible to the silver nanoparticles synthesized by using *L. monocytogenes*. The larvae and pupae of *An. stephensi* have shown 100 % mortality after 4 and 24 h. In the control group, no mortality could be observed. The adults have shown ( $\text{LC}_{50}$  0.08,  $\text{LC}_{90}$  3.99 and  $\text{LC}_{99}$  5  $\mu\text{l}/\text{cm}^2$ ) after 5 h with their confidential limits (Table 2 (b)).

Efficacy study of AgNPs synthesized by using the *S. anulatus* against the *Cx. quinquefasciatus* and *An. stephensi*

The different concentration doses (2, 4, 6, 8 and 10 ppm; 20, 40, 60, 80 and 100 ppm and 0.16, 0.33, 0.66, 1.33 and 2.66  $\mu\text{l}/\text{cm}^2$ , respectively) of silver nanoparticles synthesized by using the *S. anulatus* were tested against the larvae, pupae and adults of *Cx. quinquefasciatus* and *An. stephensi*. The larvae and pupae of *Cx. quinquefasciatus* and *An. stephensi* were found to be more susceptible to the synthesized silver nanoparticles than the adults. The mortality was observed after a span of different times of exposures.

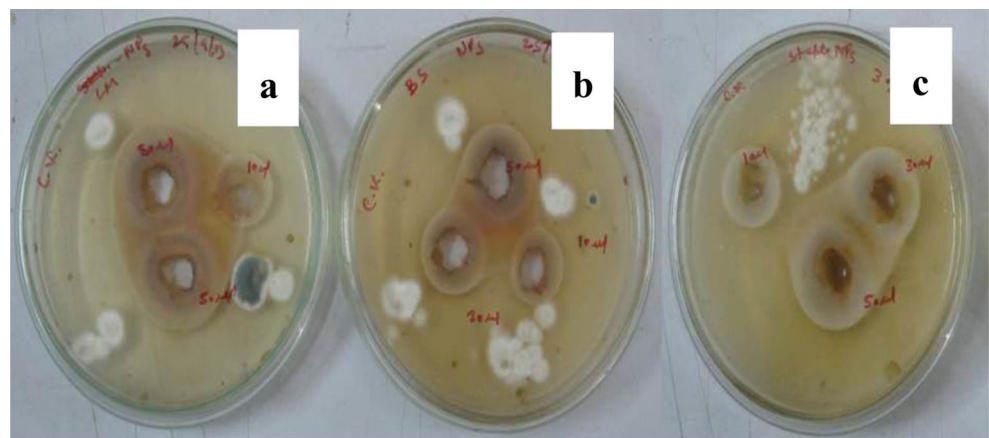
The larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized silver nanoparticles. The larvae and pupae of *Cx. quinquefasciatus* have shown 100 % mortality after different hours of exposure, while no mortality could be observed in adults after 4 h. In the control group, no mortality could be recorded (Table 2 (c)).

The larvae of *An. stephensi* were found susceptible to the silver nanoparticles synthesized by using *S. anulatus*. The larvae of *An. stephensi* have shown 100 % mortality after 5 h of exposure. In the control group, no mortality could be observed. The pupae of *An. stephensi* have shown mortality ( $\text{LC}_{50}$  20,  $\text{LC}_{90}$  120 and  $\text{LC}_{99}$  130 ppm) after 3 h and 20 min. The adults have shown mortality ( $\text{LC}_{50}$  0.06,  $\text{LC}_{90}$  1.333 and  $\text{LC}_{99}$  3  $\mu\text{l}/\text{cm}^2$ ) after 2 h and 30 min with their confidential limits (Table 2 (c)).

Antifungal activity of synthesized silver nanoparticles

Well diffusion method was used to provide the evidence for and validate the antifungal activity of synthesized silver nanoparticles against *C. keratinophilum*. Different concentrations (10, 30 and 50  $\mu\text{l}$ ) of AgNPs were used to confirm the antifungal efficiency of AgNPs. The antifungal activity of silver nanoparticles was indicated by the formation of the

**Fig. 3 a–c** Antifungal activity of silver nanoparticles synthesized by *Listeria monocytogenes*, *Bacillus subtilis*, and *Streptomyces anulatus* against the *Chrysosporium keratinophilum*



zone. The diameter of the inhibition zone was measured in millimetres. The maximum zone of inhibition was observed in *C. keratinophilum* against the AgNPs synthesized by *L. monocytogenes* (5, 19 and 20 mm). However, the zone of inhibition by *S. anulatus* (6, 9 and 11 mm) and *B. subtilius* (5, 5, and 11 mm) synthesized nanoparticles was observed (Fig. 3a–c). The maximum zone of inhibition occurred at 50- $\mu$ l concentration of synthesized AgNPs.

## Discussion

In the present investigation, the AgNPs were synthesized using bacterial strains of *L. monocytogenes*, *B. subtilius* and *S. anulatus*. We tested the efficacy of AgNPs against the larvae, pupae and adults of *An. stephensi* and *Cx. quinquefasciatus*. We have also investigated the antifungal activity of AgNPs against the soil keratinophilic fungus of *C. keratinophilum*.

The larvicidal activities of synthesized cobalt nanoparticles using the biocontrol agent, *B. thuringiensis*, have been investigated against the malaria vector *An. subpictus* and dengue vector, *Ae. aegypti* (Marimuthu et al. 2013). Furthermore, the larvicidal activity of silver nanoparticles synthesized by using *B. thuringiensis* has been revealed against the *Ae. aegypti* (Banu et al. 2014), whereas in the present study, we have tested the larvicidal, pupicidal and adulticidal activity of silver nanoparticles synthesized by *L. monocytogenes*, *B. subtilius* and *S. anulatus* against the *An. stephensi* and *Cx. quinquefasciatus*.

The efficacy of fungus-mediated silver and gold nanoparticles has been tested against the larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* (Soni and Prakash 2012a, b, c). Furthermore, the larvicidal and pupicidal activities of silver and gold nanoparticles synthesized by fungi have also been investigated against the *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* (Soni and Prakash 2013a, b, c). However, in the present study, the silver nanoparticles have been synthesized by *L. monocytogenes*, *B. subtilius* and *S. anulatus* and tested as larvicides, pupicides and adulticides against the *An. stephensi* and *Cx. quinquefasciatus*.

The antibacterial activity of silver nanoparticles synthesized by *B. cereus* has been evaluated against the pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* (Sunkar and Valli Nachiyar 2012). Antimicrobial activity of silver nanoparticles synthesized by *Streptomyces rochei* has been screened against common human pathogen *P. aeruginosa*, *E. coli*, *K pneumoniae*, *Enterobacter faecalis* and *S. aureus* (Selvakumar et al. 2012). The antifungal effect of silver nanoparticles synthesized by *B. subtilius* has been screened against the *Candida albicans* and *Aspergillus niger* (Vijayaraghavan et al. 2012).

The antimicrobial activity of *Streptomyces* sp. VITBT7 and its synthesized silver nanoparticles has been evaluated against the medically important fungal and bacterial pathogens (Subashini and Kannabiran 2013). The antifungal activity of silver nanoparticles synthesized by *Bacillus* sp. GP-23 has been screened towards the *Fusarium oxysporum* (Gopinath and Velusamy 2013), whereas in the present investigation, the antifungal activity of silver nanoparticles synthesized by *L. monocytogenes*, *B. subtilius* and *S. anulatus* has been screened against the entomopathogenic fungus *C. keratinophilum*.

## Conclusion

Here, the silver nanoparticles have been synthesized by bacterial strains of *L. monocytogenes*, *B. subtilius* and *S. anulatus*. The synthesized silver nanoparticles have been tested as larvicides, pupicides and adulticides against the larvae, pupae and adults of *An. stephensi* and *Cx. quinquefasciatus*. The antifungal activity of synthesized silver nanoparticles has also been evaluated against the entomopathogenic fungus *C. keratinophilum*. The higher zone of inhibition occurred at the concentration level of 50  $\mu$ l. This study gives an innovative approach to develop eco-friendly AgNPs which act as an effective antifungal agent/fungicide and insecticide. In the future, the nanoparticles could be useful in food packing, food preservation, food borne-disease, etc.

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