Research Article

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Efficacy of chitosan silver nanoparticles from shrimp-shell wastes against major mosquito vectors of public health importance

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Abstract: Mosquito-borne diseases are causing serious damage to public health worldwide, and control of these deadly mosquito vectors is a major thrust area for epidemiologists and public health workers. Therefore, the present research reports an eco-friendly solution with multipotency of silver nanoparticle fabricated from shrimp shell biowaste in controlling mosquitoes and bacterial pathogens. The biofabricated chitosan silver nanoparticles (Cs-AgNPs) were confirmed by UV-visible spectroscopy, transmission electron microscopy (TEM), Fourier transform infrared spectroscopy, energy dispersive X-ray spectroscopy, X-ray powder diffraction and zeta potential analysis. The TEM studies showed that the obtained Cs-AgNPs were mostly spherical in shape. Low doses of chitosan and Cs-AgNPs showed high mosquitocidal properties against both larvae and adult of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. The LC₅₀ (lethal concentration 50%) of Cs-AgNPs was 10.240 ppm (fourth instar larvae) and 9.671 ppm (adult) for *An. stephensi*; 11.349 ppm (fourth instar) and 12.015 ppm (adult) for *Ae. aegypti* and 12.426 ppm (fourth instar) and 12.965 ppm (adult) for *Cx. quinquefasciatus*. The concerning part of antibacterial studies showed that Cs-AgNP had significant inhibition on tested bacterial pathogens. Overall, this study shows that chitosan extracted

from the shrimp shell wastes can be used as a potential source for controlling major mosquito vectors.

Keywords: shrimp shells, larvicidal, adulticidal, green synthesis, antibacterial activity

1 Introduction

The shellfish industry is a major sector of fisheries that has huge economic value. During the processing of shrimps, mainly the flesh is used for consumption; thus shell and head portions are generated as wastes. This huge amount of biowaste is a serious environmental concern, which leads to unhygienic atmosphere along with severe obnoxious smell. These shrimp shells mainly contain protein, minerals and chitin. Therefore, by deproteinization and demineralization of shell wastes we can obtain chitin. Further, this chitin can be deacetylated to produce chitosan – a polysaccharide with multiple uses [1]. For instance, chitosan can increase host plant defences thereby helping in plant protection [2,3]. It is also used as a potential antitranspirant to conserve water in agriculture [4]. Chitosan and its derivatives are used for improving production and quality of orchids [5]. Also, the application of chitosan solutions significantly increased rice yields [6]. Furthermore, chitosan has an important optical characteristic that is useful for photography. Its resistance to abrasion and film-forming ability is also a noticeable property. Chitosan being a biodegradable, biocompatible, and non-toxic polymer has varied biomedical applications. The low immunogenicity and antimicrobial activities of chitosan make it ideal for biomedical use. Biomedical applications of chitosan include tissue engineering [7,8], gene delivery [9], wound healing [10] and drug delivery [7,11]. Quick healing of wounds is necessary particularly for patients suffering from diabetes. In addition, many reports on the insecticidal properties of chitosan and its fabricated nanomaterials on insect pests have been reported [12–15]. Anand et al. [16] observed that crab- and squilla-derived chitosan nanoparticles were

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highly potent against dengue vector *Aedes aegypti* with the LC₅₀ (lethal concentration 50%) values of 30.30 (crab) and 26.13 (squilla) mg L⁻¹. The chitosan nanoparticles–wrapped pesticidal protein Beauvericin (csnp-Bv) enhanced the insecticidal activity on *Spodoptera litura* (Fab.) [17].

Several researchers have investigated the role of chitin and chitosan as potential wound healing accelerators [18,19]. In dentistry, chitosan is used for dressing oral mucous wounds. It is also revealed that chewing of gum containing chitosan inhibits the growth of cryogenic bacteria [20]. Shrimp shells-derived chitosan showed better antibacterial activity on Gram-positive and Gram-negative bacteria [21]. Chitosan silver nanoparticles (Cs-AgNPs) exhibited good antifungal effect on *Neoscytalidium dimidiatum* [22]. Further, the modified chitosan nanoparticles were used for cancer therapy [23]. Cs-AgNPs are of interest because of their unique properties that can be incorporated into antifungal and antimicrobial applications [24,25]. Very recently, Alebouyeh et al. [26] noticed that *Penaeus semisulcatus*-extracted chitosan nanoparticles highly inhibit the growth of *Listeria monocytogenes* and *Salmonella typhi*. Chitosan is used as a flocculant to purify water (drinking as well as pool water). It is also used for removal of metals from water [27–31]. It also forms protective, antibacterial, and fungistatic coating for fruits and vegetables [32,33]. Chitosan increases quality and shelf life of foods [34]. Chitosan and its derivatives have novel applications in food sciences [35]. Chitosan and chitosan derivatives have immense applications in nanotechnology. Low toxicity, high solubility, and versatile routes make it an ideal material for nanotechnology.

Mosquito-borne diseases are causing havoc worldwide, and the control of the mosquito vectors is among the topmost agenda to control the spread of these deadly diseases. *Anopheles stephensi* is a major malaria vector capable of transmitting both *Plasmodium falciparum* and *Plasmodium vivax*. These two species of *Plasmodium* poses the maximum threat for global malaria. *Aedes* mosquitoes are vectors for Zika virus that evolved as a major global epidemiological issue after its recent detection in Brazil [36]. Occurrence of microcephaly in infants and Guillain–Barré syndrome have further complicated the situation. Because there is no vaccine for Zika virus, control of these mosquitoes is the best available alternative. *Aedes* mosquitoes are also the vectors for Dengue virus, another major threat that has spread in more than 128 countries, every year infecting 390 million people, with a quarter of them becoming severe [37]. *Culex quinquefasciatus* is the vector that is transmitted through nematodes causing filariasis.

In this study, we used chitosan obtained from shrimp shell wastes to produce chitosan nanoparticles and tested

its efficacy against the larvae and adults of three major diseases causing mosquito species. Also, antibacterial assay was performed against three bacterial species, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *S. typhi*. Finally, the chitosan-fabricated AgNPs were confirmed by UV-visible (UV-Vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), X-ray powder diffraction (XRD) and zeta potential.

2 Materials and methods

2.1 Collection of shrimp shell and sample preparation

The shrimps were obtained from a local fish market, Tabuk, Kingdom of Saudi Arabia. The shrimp's exoskeletons collected were washed thoroughly, frozen overnight, and then chopped into small pieces using a meat tenderizer. Wet samples of 10 g of chopped shrimp shells were weighed and then oven-dried for 4 days at 65°C to obtain a constant weight. The dry weight and moisture content of the samples were calculated based on the difference between wet and dry weights.

2.2 Chitosan recovery from shrimp shell

The oven-dried shrimp shells were washed in double distilled water. Then the shrimp exoskeletons were boiled in 500 mL beakers containing (2 and 4% w/v) sodium hydroxide (NaOH) for 1 h to remove the protein content from the shrimp shell. After boiling the samples with NaOH, the beaker was removed from the hot plate and was allowed to cool down for 30 min at room temperature [38]. The treated shrimp shells were powdered to obtain size ranging from 0.5 to 5.0 mm using a meat tenderizer.

2.3 Demineralization

The treated shrimp shell powder was further soaked in 1% HCl with four times its quantity for 24 h to remove the minerals from shells [39]. The processed shrimp shell powder was treated with 50 mL of a 2% sodium hydroxide

solution for about 1 h to decompose the albumen into water soluble fractions. The obtained chitin was thoroughly washed with milli-Q water and the supernatant was totally removed. Chitosan was obtained from this chitin by the process of deacetylation [40].

2.4 Deacetylation

The obtained chitin was boiled in 50% NaOH for 2 h at 100°C. The samples were allowed to cool down at room temperature. The samples were washed repeatedly with 50% NaOH followed by filtration to obtain the solid chitosan. The samples were dried in hot-air oven at 110°C for 6 h to obtain creamy-white powder [41].

2.5 Biosynthesis and characterization of Cs-AgNPs

Two grams of obtained powdered chitosan was placed in a 250 mL Erlenmeyer flask containing 100 mL double distilled water (DDH₂O), and then the mixture was boiled for 20 min. The extract was filtered using Whatman filter paper n.1, stored at -4°C. The filtrate was treated with 1 mM silver nitrate (AgNO₃ 99%, Sigma-Aldrich, USA) aqueous solution in an Erlenmeyer flask and incubated at room temperature. A pale-yellow solution indicated the formation of Cs-AgNPs, because aqueous silver ions were reduced by the chitosan extract producing stable Cs-Ag nanocomposite in H₂O.

A synthesized Cs-AgNP was confirmed by UV-Vis spectral studies. The absorption spectra of synthesized Cs-NPs were recorded in 200–800 nm using UV-3600 Shimadzu spectrophotometer. The size and morphology of the fabricated Cs-AgNP were studied using JEOL 1200 EX TEM operating at 120 kV voltage. FTIR was performed through Perkin-Elmer Spectrum 2000 FTIR spectrophotometer to identify possible biomolecules that were present in chitosan extract [42]. In addition, XRD and EDX were also examined to detect the presence of metal elements in the sample.

2.6 Toxicity on mosquito larvae

Mosquitoes were reared as described by Suresh et al. [43]. Following the methods of Murugan et al. [44], 25 numbers of IV instar mosquito larvae were introduced for 24 h

in 250 mL sterilized DDH₂O containing glass beaker with chosen concentration of 50, 100, 150, 200, and 250 µg/mL (chitosan) and 5, 10, 15, 20, and 25 µg/mL (Cs-Ag nanoparticles). In control, 25 numbers of IV instar mosquito larvae were placed in 250 mL sterilized DDH₂O containing glass beaker for 24 h. Each dose was replicated thrice on IV instar larvae. Percentage mortality was calculated as follows:

$$\begin{aligned} & \% \text{ mortality} \\ &= \left(\frac{\text{number of dead individuals}}{\text{number of treated individuals}} \right) \times 100. \end{aligned} \quad (1)$$

2.7 Toxicity on mosquito adults

Bioassay of adult mosquitoes was performed according to the procedure outlined by Ref. [45] with minor changes as suggested by Ref. [46]. The chitosan extract was tested at 100, 200, 300, 400, and 500 µg/mL, whereas chitosan nanoparticles were assessed at 10, 20, 30, 40, and 50 µg/mL. The chitosan extract and Cs-AgNPs were applied on Whatman no. 1 filter paper (size 12 × 15 cm) lining a glass holding tube (diameter 30 mm; length 60 mm; $n = 20$ mosquitoes per test tube). In the controls, filter paper was treated with DDH₂O and AgNO₃, respectively.

2.8 Antibacterial activity

Shrimp-synthesized Cs-AgNPs were applied on *B. subtilis*, *K. pneumoniae*, and *S. typhi*. Tested bacteria species were procured from Microbial Type Culture, Collection and Gene Bank Institute of Microbial Technology, Sector 39-A, Chandigarh-160036 (India). For testing culture 18–24 h old bacterial culture was used. The nutrient broth used to culture the bacteria strain was composed of peptone (5 g/L), hydrolysed yeast extract (1.50 g/L), beef extract (1.50 g/L), and sodium chloride (5 g/L), and the final pH was 7.4. Nutrient broth (13 g) was suspended in 100 mL of distilled water. Twenty-five millilitres of nutrient broth were transferred in each of the four conical flasks, and then the flasks were autoclaved at 121°C for 15 min (15 psi). Later, all tested strains were inoculated and incubated at 37°C for 24 h. After 24 h incubation, 2×10^{-6} cfu/mL of the culture was used for antibacterial assays.

The antibacterial activity of Cs-AgNPs was determined by agar disk diffusion method [47]. The tested bacteria were evenly swabbed on solidified Muller Hinton agar medium plates, and then three wells were made in each plate, treated with various concentrations of

Cs-AgNPs (30, 60, and 90 $\mu\text{g/mL}$). The plates were incubated for 24 h at 37°C. After 12 h, the zone of inhibition (mm) was monitored by photomicroscopic method (Leica ES2, Germany) [48].

2.9 Data analysis

All analyses were performed using SPSS software package (16.0 version). Data from bioassay (mosquitocidal) were analysed by probit analysis [49]. Bacteria growth inhibition data were analysed by two-way ANOVA with two factors (i.e. tested dose and species). Means were separated using Tukey's HSD (honestly significant difference) test ($P < 0.05$).

3 Results and discussions

3.1 Characterisation of Cs-AgNPs

We observed synthesized Cs-AgNPs after incubation of chitosan extract in aqueous AgNO_3 solution. The colour turned from pale yellow to dark brown indicating the production of chitosan nanocomposites from chitosan. Figure 1 shows the absorption peak at 446 nm confirming the presence of Cs-AgNPs. Similarly, Murugan et al. [15] noticed that the *Xenograpsus testudinatus*-derived Cs-AgNPs express UV absorption peak at 426 nm. Further, Sanpui et al. [50] observed the surface plasmon resonance (SPR) peak of Cs-AgNPs at 410 nm, whereas Murugan et al. [51] reported the confirmation peak for Cs-AgNPs at 441 nm. In addition, Chen et al. [52] studied the UV absorption pattern against various dose of γ -ray irradiation on chitosan nanoparticles. They reported that a single narrow peak is produced at low irradiation dose (27 kGy) thereby indicating narrow size distribution pattern of chitosan nanoparticles. At higher dose, the peak intensity became higher indicating more production of nanoparticles, and with even higher dose of 170 kGy the peak was broadened with low intensity thereby indicating the degradation of chitosan nanoparticles at very high γ -ray irradiation.

The results of the TEM image shows that Cs-AgNPs were mainly spherical shaped (Figure 2), which coincides with the observation of Govindan et al. [53] that the field emission scanning electron microscope (FE-SEM) revealed that chitosan nanomaterials were uniformly spherical.

Figure 1: The UV-Vis absorbance spectra of synthesized Cs-AgNPs.

Furthermore, it was reported that the agglomeration can be prevented by dispersing Ag in chitosan for longer period. Also, Murugan et al. [15] noticed that Cs-AgNPs extracted from crab shells were spherical with size ranging from 20 to 50 nm. Recently, Parthasarathy et al. [54] highlighted that the cubic shape and the size of Cs-AgNPs were ranged from 8 to 48 nm. Similarly, TEM and SEM analyses of synthesized Cs-AgNPs were spherical in shape and size ranged from 17 to 50 nm [55]. EDX spectrum of Cs-AgNP emitted prominent peak in the silver region, strongly indicating the presence of Ag and corresponding to an 8.95% atomic percentage of silver (Figure 3). Metallic silver nanomaterials exhibited characteristic optical absorption

Figure 2: TEM image of synthesized Cs-AgNPs.

Figure 3: EDX spectrum of synthesized Cs-AgNPs.

spectra at around 3 keV because of SPR [44]. EDX image also indicated the presence of weak O_2 , suggesting that silver nanoparticles were stabilized by the organic metabolites contained in the shrimp shell extract, as evidenced through FTIR studies [56]. Also, the presence of Cu in EDX spectra could be because of the preparation of sample using copper grits for TEM studies.

XRD diffraction of synthesized Cs-AgNPs shows the strong diffraction peaks at 38.11° , 44.21° , 64.42° , and 77.42° corresponding to lattice planes (111), (200), (220), and (311) respectively. The XRD result of Cs-AgNPs has significantly matched with JCPDS card no. 04-0783. Thus, XRD showed that AgNPs were produced by the reduction of $AgNO_3$ with shrimp extract (Figure 4), where crystal structure well matched with earlier reports [15,51,57]. Furthermore, the crystalline nature of shrimp-derived

Figure 4: XRD pattern of synthesized Cs-AgNPs.

chitosan nanoparticles was determined by the most intense diffraction peaks at values of 11.7 and 20.2° [58]. FTIR studies were conducted to reveal the probable chemicals contributing to the reduction of silver nanoparticles (Figure 5). The FTIR peaks in Cs-AgNPs at $3,477$, $2,910$, $1,570$, $1,025$, and 675 cm^{-1} . Comparably, the FTIR peaks at approximately $3,400\text{ cm}^{-1}$ in the pure Cs-AgNPs spectrum, which corresponds to the $-OH$ stretching and $CO-NH$ bending at $1,570\text{ cm}^{-1}$. The band at $2,910\text{ cm}^{-1}$ represents $C-H$ and $C-N$ groups, which clearly indicates the absorption spectrum of chitosan [53]. The band at nearly 1025.33 cm^{-1} stretches $C-O-C$ groups [59]. Furthermore, other researchers have also reported FTIR spectrum for chitosan nanoparticles revealing some variations in the obtained data [14,15,51,60,61].

The zeta potential of the Cs-AgNPs results are presented in Figure 6. The result obtained is comparable to Cu-CsNPs [57]. Also, the zeta potential result agreed with Parthasarathy et al. [54] who have reported that the zeta potential of Cs-AgNPs was -21.4 Mv . The characteristics of nanoparticles, particularly the surface morphology, is related to the zeta potential mainly because of optic properties of external polysaccharides and biomolecules [62].

3.2 Larvicidal and adulticidal toxicity of chitosan and chitosan-fabricated AgNPs

In the laboratory conditions, chitosan was toxic to the fourth instar larvae at low doses. The LC_{50} values after 24 h exposure for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were 114.603 , 127.681 , and $141.266\text{ }\mu\text{g/mL}$, respectively (Table 1). Comparably, larvicidal effect of *X. testudinatus*-extracted chitosan exhibited significant mortality on coastal malaria vector *Anopheles sundaicus* with LC_{50} values ranged from 50.75 ppm (I instar) to 100.05 (pupa) [15]. Further, Perez et al. [63] reported that *Ranina ranina*-derived chitosan had greater larvicidal effect against *Ae. aegypti*. Said et al. [64] highlighted the insecticidal effect of chitosan against *Galleria melleonella*. Finally, Anand et al. [65] studied that the chitosan from shrimp shell caused less mortality when compared to Cs-NPs on third instar larvae of *Ae. aegypti*.

Chitosan-derived AgNPs were highly toxic with LC_{50} values after 24 h exposure for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were 10.240 , 11.349 , and 12.426 , respectively (Table 2). Similarly, crab shell-fabricated nano-silver structures were highly toxic to *An. stephensi* with LC_{50} values ranging from 3.18 to 6.54 ppm [14]. The larvicidal effect of bio-encapsulated Cs-AgNPs exhibit high larval mortality

Figure 5: FTIR spectrum of synthesized Cs-AgNPs.

against *An. stephensi* with the LC_{50} values ranged from 4.432 (first instar) to 7.641 ppm (pupa) [51]. Further, chitosan nanoparticles from crab and squilla showed significant toxicity on third larval instar of *Ae. aegypti* with LC_{50} values of 30.30 (crab) and 26.13 (squilla) mg/L [66]. Furthermore, α -chitin nanomaterials from *Penaeus monodon* shells exhibited good antibacterial, antifungal, and mosquito larvicidal activity on *Ae. aegypti* [67]. In addition, Namasivayam et al. [68] highlighted that *Nomura earileyi*-fabricated chitosan nanomaterials greatly reduced macromolecules and high larval mortality against *S. litura*. Arjunan et al. [69] found out that Cs-Ag nanoparticles exhibited highest antimicrobial and anticancer effect with IC_{50} value $29.36 \mu\text{g mL}^{-1}$.

In the adult mosquitoes, LC_{50} values after 24 h exposure to chitosan for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were 296.040, 351.102, and 388.397 $\mu\text{g/mL}$, whereas to Cs-AgNPs against key mosquito vectors it was 9.671 $\mu\text{g/mL}$ for *An. stephensi*, 12.015 for *Ae. aegypti*, and 12.965 for *Cx. quinquefasciatus*, respectively (Tables 3 and 4). To the best of our knowledge, reports on mosquito adulticidal assay using chitosan and its nanoparticles are scarce. The results obtained from chitosan nanoparticles are quite comparable with plant-mediated nanoparticles. Indeed, Aziz et al. [70] studied that *Artemisia herba-alba*-fabricated AgNPs exhibited moderate adulticidal activity against *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*, and *Cx. pipiens* with LC_{50} values of 8.22, 8.71, 13.03, and 27.39 $\mu\text{g/mL}$, respectively.

3.3 Antibacterial assay

The results of bacterial inhibition experiments involving Cs-AgNPs indicated high antibacterial activities against *B. subtilis*, *K. pneumoniae*, and *S. typhi* (Table 5). We noted that at 90 ppm concentration of AgNPs inhibited the growth of *B. subtilis* (inhibition zone 26.76 mm), *K. pneumoniae* (inhibition zone 22.66 mm), and *S. typhi* (inhibition zone 24.33 mm). It is also noted that at the concentration of 30 ppm the zone of inhibition exceeded 13 mm, and at the concentration of 60 ppm the zone of inhibition exceeded 18 mm for all the three bacteria

Figure 6: Zeta potential spectrum of synthesized Cs-AgNPs.

Table 1: Larval toxicity of chitosan against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquito species	LC ₅₀ (µg/mL) (LCL–UCL)	LC ₉₀ (µg/mL) (LCL–UCL)	χ^2 (d.f. = 4)
<i>An. stephensi</i>	114.603 (73.512–144.638)	226.159 (187.258–318.146)	8.404 n.s
<i>Ae. aegypti</i>	127.681 (116.130–138.530)	240.174 (222.398–263.934)	4.909 n.s
<i>Cx. quinquefasciatus</i>	141.266 (129.821–152.457)	258.254 (238.768–284.571)	3.563 n.s

Control – no mortality, LC₅₀ – lethal concentration killing 50% of the insects, LC₉₀ – lethal concentration killing 90% of the insects, χ^2 – chi-square, d.f. – degrees of freedom, n.s. – not significant ($\alpha = 0.05$).

Table 2: Larval toxicity of Cs-AgNPs against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquito species	LC ₅₀ (µg/mL) (LCL–UCL)	LC ₉₀ (µg/mL) (LCL–UCL)	χ^2 (d.f. = 4)
<i>An. stephensi</i>	10.240 (4.929–13.498)	21.684 (17.626–32.296)	9.438 n.s
<i>Ae. aegypti</i>	11.349 (10.173–12.413)	22.024 (20.413–24.158)	4.494 n.s
<i>Cx. quinquefasciatus</i>	12.426 (11.295–13.482)	23.252 (21.570–25.480)	4.841 n.s

Control – no mortality, LC₅₀ – lethal concentration killing 50% of the insects, LC₉₀ – lethal concentration killing 90% of the insects, χ^2 – chi-square, d.f. – degrees of freedom, n.s. – not significant ($\alpha = 0.05$).

Table 3: Adulticidal toxicity of chitosan against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*.

Mosquito species	LC ₅₀ (µg/mL) (LCL–UCL)	LC ₉₀ (µg/mL) (LCL–UCL)	χ^2 (d.f. = 4)
<i>An. stephensi</i>	296.040 (260.565–331.064)	678.578 (597.073–810.072)	0.540 n.s
<i>Ae. aegypti</i>	351.102 (312.191–397.791)	789.280 (677.595–984.319)	1.682 n.s
<i>Cx. quinquefasciatus</i>	388.397 (347.503–443.241)	830.362 (709.509–1043.895)	1.597 n.s

Control – no mortality, LC₅₀ – lethal concentration killing 50% of the insects, LC₉₀ – lethal concentration killing 90% of the insects, χ^2 – chi-square, d.f. – degrees of freedom, n.s. – not significant ($\alpha = 0.05$).

Table 4: Adulticidal toxicity of Cs-AgNPs against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquito species	LC ₅₀ (µg/mL) (LCL–UCL)	LC ₉₀ (µg/mL) (LCL–UCL)	χ^2 (d.f. = 4)
<i>An. stephensi</i>	9.671 (26.493–19.172)	40.117 (30.645–76.021)	8.918 n.s
<i>Ae. aegypti</i>	12.015 (16.951–21.000)	44.444 (34.433–80.890)	7.995 n.s
<i>Cx. quinquefasciatus</i>	12.965 (6.555–17.304)	50.119 (44.389–59.235)	2.477 n.s

Control – no mortality, LC₅₀ – lethal concentration killing 50% of the insects, LC₉₀ – lethal concentration killing 90% of the insects, χ^2 – chi-square, d.f. – degrees of freedom, n.s. – not significant ($\alpha = 0.05$).

Table 5: Antibacterial activity of Cs-AgNPs on *B. subtilis*, *K. pneumoniae*, and *S. typhi*

Bacterial species	Inhibition zone (mm)			
	Control	30	60	90
<i>B. subtilis</i>	8.36 ± 0.73 ^a	16.43 ± 0.32 ^b	21.73 ± 0.58 ^c	26.76 ± 0.35 ^d
<i>K. pneumoniae</i>	7.66 ± 0.20 ^a	13.13 ± 0.55 ^b	19.23 ± 0.30 ^c	22.66 ± 0.37 ^d
<i>S. typhi</i>	7.93 ± 0.25 ^a	15.60 ± 0.45 ^b	18.13 ± 0.28 ^c	24.33 ± 0.65 ^d

Values are means ± SD of three replicates.

Within a row, different letters indicate significant differences (ANOVA, Tukey's HSD, $P < 0.05$).

tested, thereby showing high antibacterial activity. Similarly, Senthilkumar [71] reported that hybrid Cs-AgNPs synthesized from *Talinum portulacifolium* exhibited significant antibacterial activities on both *Escherichia coli* and *Serratia marcescens*. Murugan et al. [49] studied antibacterial activities of crab shells-fabricated Cs-AgNPs on *B. subtilis*, *E. coli*, *S. typhi*, and *P. vulgaris*. Further, Said et al. [51] reported that combination of three nanoparticles, silver, chitosan, and curcumin, exhibited highest anti-parasitic effect on *Giardia lamblia*. Furthermore, research has shown that chitosan can be used as a stabilizer during γ -ray irradiation of AgNPs thereby increasing the longevity of the Ag nanomaterials in aqueous solutions [43].

4 Conclusions

Overall, our study shows that chitosan-synthesized AgNPs can be effectively used for managing key mosquito vectors. We confirmed the proper synthesis of Cs-AgNPs by UV-Vis spectroscopy, XRD, TEM, EDX, FTIR, and zeta potential. Furthermore, we showed that shrimp-derived Cs-AgNPs had a higher growth inhibition potential against tested microbes, *B. subtilis*, *K. pneumoniae*, and *S. typhi*. The chitosan extracted from the biowaste (prawn shell wastes) can serve as an excellent tool in controlling deadly mosquitoes and microbial pathogens, thereby mitigating two main public health issues related to waste recycling and control of major mosquito vectors.

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Compliance with ethical standards: All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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