Research Article

Al Thabiani Aziz*

A study on the larvicidal and adulticidal potential of *Cladostepus spongiosus* macroalgae and green-fabricated silver nanoparticles against mosquito vectors

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Abstract: Mosquito vectors in the present universe cause a major problem due to the transmission of pathogens with high morbidity. The present research aimed to explore the larvicidal and adulticidal toxicity of the *Cladostepus spongiosus* extract and its fabricated AgNPs on key mosquito vectors. The synthesized AgNPs were confirmed by UV-Vis spectroscopy, transmission electron microscopy, energy-dispersive X-ray spectroscopy, Fourier transform infrared spectrophotometry, and X-ray diffraction analysis. In the mosquitocidal assay, the *C. spongiosus* extract has shown good larvicidal mortality against *Aedes aegypti* (88.9%), *Anopheles stephensi* (84.1%), and *Culex. quinquefasciatus* (81.6%). Further, adulticidal mortality percentages were 78.8%, 75.4%, and 67.6% against *An. stephensi, Ae. Aegypti*, and *Cx. quinquefasciatus* at 1,000 ppm. AgNPs revealed larvicidal mortality percentages of 94.8% against *An. stephensi*, 92.8% against *Ae. Aegypti*, and 90.6% against *Cx. quinquefasciatus*; the adulticidal potential was also revealed to be higher against *An. stephensi* (89.4%) followed by *Ae. aegypti* (86.8%) and *Cx. quinquefasciatus* (83.2%). Comparing the results achieved from the *C. spongiosus* extract and its derived AgNPs, promising activity was attained against key mosquito vectors at a minimal dose of 70 ppm of AgNPs. Thus, *C. spongiosus*-mediated AgNPs can be an alternative tool in controlling key mosquito vectors.

Keywords: nano-biotechnology, macroalgae, bio-toxicity, mosquito-borne diseases, dengue

*** Corresponding author: Al Thabiani Aziz,** Biology Department, Faculty of Science, University of Tabuk, P.O. Box. 741, Tabuk 71491, Saudi Arabia, e-mail: aalthbyani@ut.edu.sa, tel: +966-536811051

1 Introduction

Mosquitoes are the arthropod vectors for diseases like malaria, dengue, and filariasis, which cause significant global mortality and morbidity with increased insecticide resistance [1,2]. World Health Organization reports showed 247 million cases of malaria in 2021 and 619,000 deaths [3]; it is a primordial disease caused by *Plasmodium* parasites and spread through the bites of infected female *Anopheles* mosquitoes [4,5]. Dengue is a viral infection mainly transmitted by *Aedes aegypti* and *Ae. albopictus* [6], and it has been estimated that 100–400 million infections occur per year [7]. Also, the *Aedes* mosquito vector carries the pathogens, which cause yellow fever, dengue, zika fever, and encephalitis [8]. The most common clinical manifestations observed are fever, rash, eye pain, arthralgias, myalgias, and haemorrhage [9]. The *Culex* species are the vector of encephalitis and West Nile fever in tropical and subtropical climatic conditions [10]. It is opportunistic and feeds on humans and animals [11]. The life stages of the mosquito are usually targeted using diverse conventional and microbial agents [12]. However, these agents have adverse effects on human health and the environment [13,14]. Therefore, recently green sources have been implemented to augment the control of mosquito vectors, with special reference to botanical mosquitocidal. Botanical is endowed with good photochemical potential, which has promising activities against insect vectors [15] and non-target effects against other beneficial organisms [12,16–18]. Besides terrestrial, oceans are known to have magnificent diversities of living organisms covering 70% of the total surface area of the Earth. Algae are photosynthetic organisms and include a wide range of species with the extreme habitat of microalgae to seaweeds [19]. Algae are rich in antioxidants due to the presence of ascorbic acid, reduced glutathione, phenols, and flavonoids [20–22]; they have been reported as insecticidal, repellent, and oviposition deterrence on insects [21,23,24].

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Cladostephus spongiosus (Hudson) is a marine brown alga, ranging 2–3 mm in width, and appears narrow, tough, and wiry in nature. Alves et al. [25] highlighted that the algae exhibit a number of biological activities including anti-microbial, anti-cancer, anti-inflammatory, and anti-protozoal. Insecticides synthesized from natural products, such as metal and metal oxide nanomaterials of herbal origin, could have a prominent role in the existing situation. Nanomaterials of varied sizes have gained broad applications based on definite particulate dispersions with a size range of 1–100 nm [26,27]. Silver nanoparticles (AgNPs) possess potential microbial, anticancer, and mosquitocidal properties [28–30].

Nanoparticles are smaller in their structure with definite shape and are more specific in their action; hence, they have gained significance in the research fraternity and many other diverse fields [26,27]. The AgNPs synthesized have more potential against microbes, cancer, mosquitoes, and anticancer [28,31]. Green synthesized nanoparticles from plants are gaining significance owing to their simplicity and the rapid rate of synthesis of nanoparticles of diverse morphologies and eco-friendliness [32]. The present study aimed to explore the bio-fabrication of macro-algae-mediated AgNPs and the produced nanomaterials were categorized through various biophysical techniques. Further, the larvicidal and adulticidal potentials of the *C. spongiosus* extract and *C. spongiosus*-synthesized AgNPs against key mosquito vectors, *An. stephensi, A. aegypti*, and *Cu. quenifascienses*, are studied.

2 Materials and methods

2.1 Collection of plant materials and extraction

The alga *Cladostepus spongiosus* ((Hudson) C. Agardh, 1817) was collected from Haql (29°20025.400 N, 34°56053.5100 E), in the Tabuk region, Saudi Arabia, during morning hours and identified by a plant taxonomist at the Department of Biology, Tabuk University, Saudi Arabia. The algae harvested were washed in running tap water twice or thrice and air-dried in shade at room temperature (25 ± 2°C). The dried algae were chopped into fine pieces using an electric blender (Torrington, CT, USA) and used for further extraction and experimental procedures.

C. spongiosus was extracted using Soxhlet apparatus with ethanol for 48 h at 76–78°C. About 200 g of fine-powdered algae was dissolved in 750 mL of ethanol, and the concentrated crude extract obtained was further evaporated in a rotary evaporator at 40–60°C to remove excess solvent and stored at 2–8°C for further activities.

2.2 Preparation of the standard solution

The standard solution was prepared by adding 1 mL of the extract to 98.5 mL of deionized water and 0.5 mL of Triton-×100 as an emulsifying agent. This gives a concentration of 1,000 ppm, which can be diluted for further experiments.

2.3 Synthesis of *C. spongiosus*-based AgNPs and characterization

About 1 mL of the *C. spongiosus* ethanol extract was added to 1 mL of silver nitrate ($AgNO_3$) 10^{-3}, 97.5 mL of distilled water, and 0.5 mL of Triton-X100. The above solution was further incubated at room temperature until the colour changed to grey, which indicated the synthesis of nanoparticles [33]. The nanoparticles synthesized were subjected to Fourier transform infrared spectrophotometry (FTIR), energy-dispersive X-ray (EDX) spectroscopy, and transmission electron microscopy (TEM).

2.4 Mosquito culture

Mosquitoes of Indian strains *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* were cultured and maintained in the entomology laboratory, at Tabuk University, Saudi Arabia, for more than 6 years without exposure to insecticides. Cultures were maintained at 27 ± 2°C and 75–85% relative humidity under a 14:10 L/D photoperiod. Larvae were fed with Brewer's yeast and dog biscuits, and algae were collected at 3:1:1. The pupae that emerged were transferred to a cup of tap water and replaced in a cage (dimension: 50 cm × 50 cm × 50 cm) for adult emergence. Adults were fed with 10% sucrose solution, and further larvae emerging from the eggs were used for further experimental purposes.

2.5 Larvicidal bioassays

Bioassays were performed for 24 instars of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* at varied concentrations: 300, 450, 600, and 750 ppm of the crude extract of *C. spongiosus* and 10, 25, 40, 55, and 70 ppm of *C. spongiosus*-fabricated AgNPs. No food was provided throughout our experiments and the mortality rate was observed after 24 h. The experiments were repeated five times and each concentration had a control. The mean mortality was determined using Abbott's [34] formula:

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100. \quad (1)$$

2.6 Adulticidal bioassay

Different concentrations of the ethanolic algal extract at 200, 400, 600, 800, and 1,000 ppm and 10, 25, 40, 55, and 70 ppm of *C. spongiosus* green-fabricated nanoparticles were tested for adulticidal activity. Different concentrations of extracts were impregnated to Whatman no. 1 filter paper (12 cm × 15 cm); ethanol was used as a control. The impregnated paper was air-dried for 5 min and kept in an exposure tube. Twenty adult mosquitoes, 2–5 days old, starved for blood meal were introduced into the holding tube for 1 h. The tested mosquitoes were blown back to the holding tube after exposure. The tubes were accomplished with a 10% glucose solution, which was soaked in cotton and was used as feed for the surviving mosquitoes. At the end of 24 h, the number of dead mosquitoes was recorded to determine their mortality.

2.7 Statistical analysis

The observed mortality from our experiment was exposed to the analysis of variance (ANOVA of arcsin, logarithmic, and square root transformed percentages). The significant differences obtained between treated and control groups were evaluated by Tukey's multiple range test (significance at $P < 0.05$) using the Minitab®17 program.

3 Results

The nanofabricated AgNPs synthesized from *C. spongiosus* were characterized using FTIR, spectrophotometry, and they showed significant peaks. The peaks observed were 461.33, 711.49, 780.48, 873.74, 1,034.74, 1,423.08, 2,521.27, 2,930.71, and 3,409.34 cm^{-1} and indicated the presence of S═S disulfide asym., ═CH out of plane, C–H out of plane, C–H out of plane, P–OR ester, S═O sulphate ester, S–H (sharp), and CH_2, ArO–H bond, respectively. The results obtained are shown in Figure 1 and Table 1.

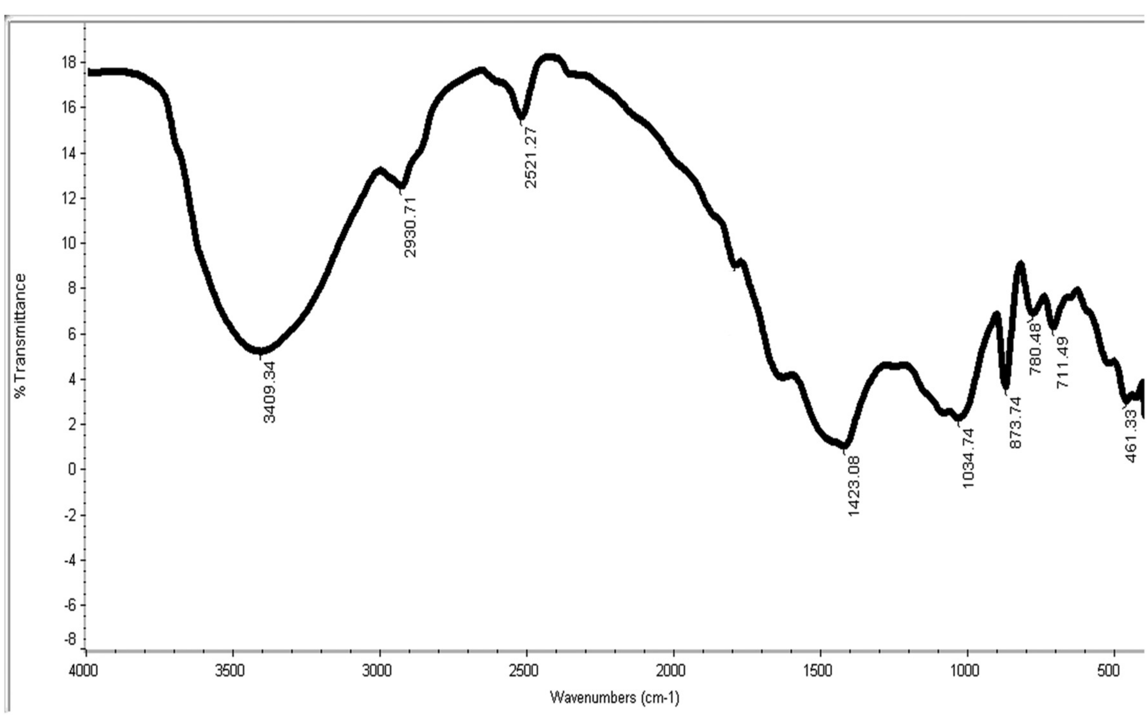

Figure 1: FTIR analysis of green-fabricated *C. spongiosus*.

Table 1: FTIR analysis of green-fabricated *C. spongiosus*

Peak	Class	Structure	Assignments
461.33	Misc	S═S disulphide	S═S disulphide asym.
711.49	Alkenes	*Trans* RCH = CHR	═CH out of plane
780.48	Aromatics	1,2,3-trisub	C–H out of plane
873.74	Aromatics	1,2,3,4,5-pentasub	C–H out of plane
1034.74	Misc	P-OR ester	P–OR ester
1423.08	Misc	S═O sulphate	S═O sulphate ester
2521.27	Misc	S═H thiols	S–H (sharp)
2930.71	Alkanes	CH_2	CH_2
3409.34	Phenols	ArO–H bonded	ArO–H bonded

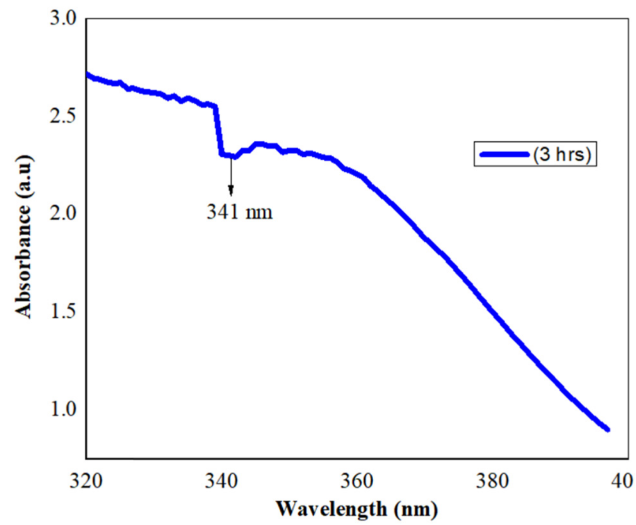

Figure 3: UV-Vis spectra of *C. spongiosus* green-fabricated AgNPs.

EDX analysis of *C. spongiosus*-mediated green-fabricated AgNPs showed strong signals of silver atoms at 3 keV. This implies the synthesis of AgNPs, as shown in Figure 2. The signals apart from silver, such as carbon and copper, are due to the TEM grid.

UV-visible spectrophotometric analysis of synthesized nanoparticles showed an absorption peak at 341 nm, which indicated the synthesis of AgNPs. The results obtained are shown in Figure 3.

The TEM results also implied the synthesis of nanoparticles. The shape and size of the nanoparticles synthesized were 20 and 50 nm, as shown in Figure 4.

X-ray diffraction (XRD) analysis of the synthesized nanoparticles showed the corresponding peaks of (111), (200), (220), and (311) planes, confirming that the material synthesized was nanoparticles. The results obtained are shown in Figure 5.

The larvicidal activity of the ethanolic crude extract of *C. spongiosus* against treated mosquito vectors such as *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* showed promising activity, which was evident by its mortality percentage. Larvae treated at concentrations of 150, 300, 450, 600, and 750 ppm showed good mortality in a dose-dependent manner. The mortality percentage was higher at higher concentrations and least at lower concentrations. At 150 ppm, the mortality percentages were 24.2% against *Cx. quinquefasciatus*, 26.7% against *Ae. Aegypti*, and 29.5% against *An. stephensi.* At a higher concentration of 750 ppm, the mortality percentages were 81.6% in *Cx. quinquefasciatus*, 84.1% in *Ae. aegypti*, and 88.9% in *An. stephensi.* This implies that the extract has exceeded its potential to control mosquito larvae vectors. The results obtained are shown in Figure 6.

The larvicidal activity of green-fabricated *C. spongiosus*-mediated AgNPs against mosquito vectors showed good activity. Larvae treated at different concentrations of 10, 25, 40, 55, and 70 ppm showed good potential to control

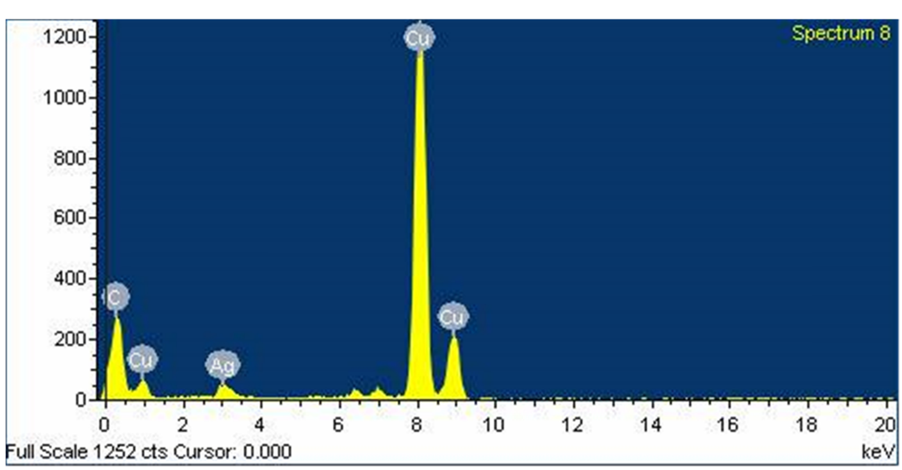

Figure 2: EDX spectrum of *C. spongiosus*-mediated AgNPs showing distribution of elements.

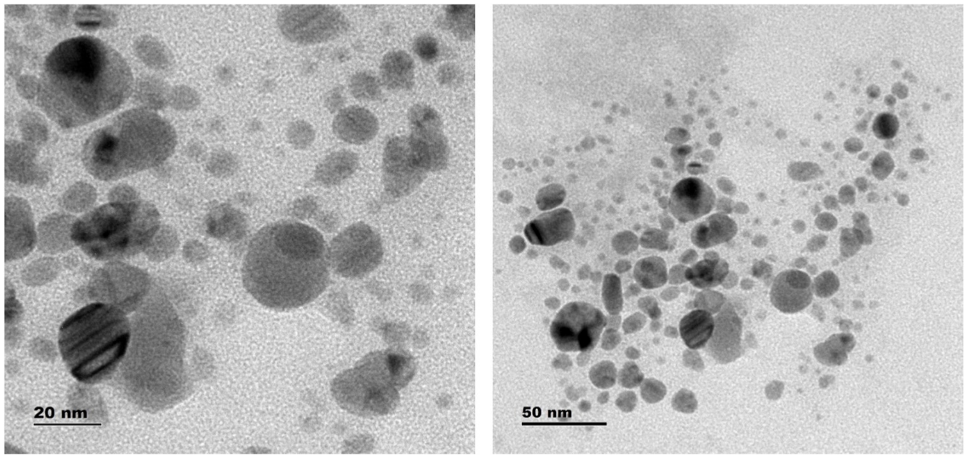

Figure 4: TEM image of the synthesized AgNPs at 20 and 50 nm.

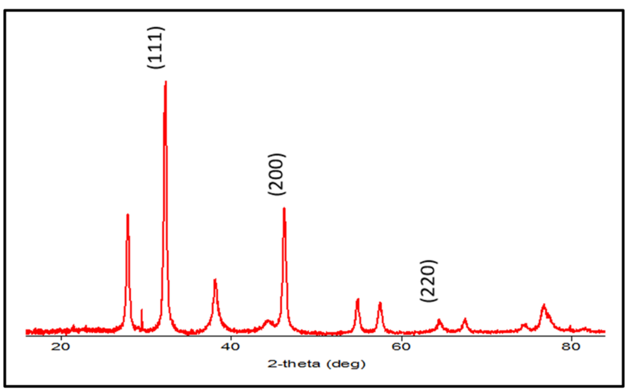

Figure 5: XRD spectrum of AgNPs *C. spongiosus*-mediated green-fabricated AgNPs.

mosquito populations in their larval stage. The results obtained at 10 ppm concentration showed mortality of 35.2% against *Cx. quinquefasciatus*, 37.4% against *Ae. aegypti*, and 40.6% against *An. stephensi*. The nanosynthesized *C. spongiosus*-mediated AgNPs showed mortality percentages of 90.6%, 92.8%, and 94.8% against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi,* respectively. The mortality percentages obtained from green-fabricated *C. spongiosus*-mediated AgNPs against mosquito vectors are shown in Figure 7.

The adulticidal activity of the ethanolic extract of *C. spongiosus* showed good activity against the treated vectors. The adulticidal potential was implied by its mortality

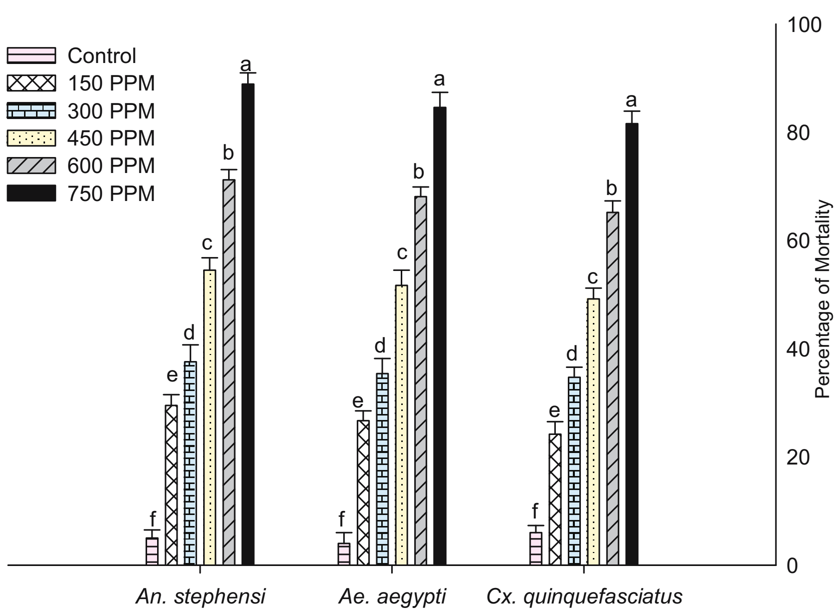

Figure 6: Larvicidal activity of the ethanolic extract of *C. spongiosus* against mosquito vectors.

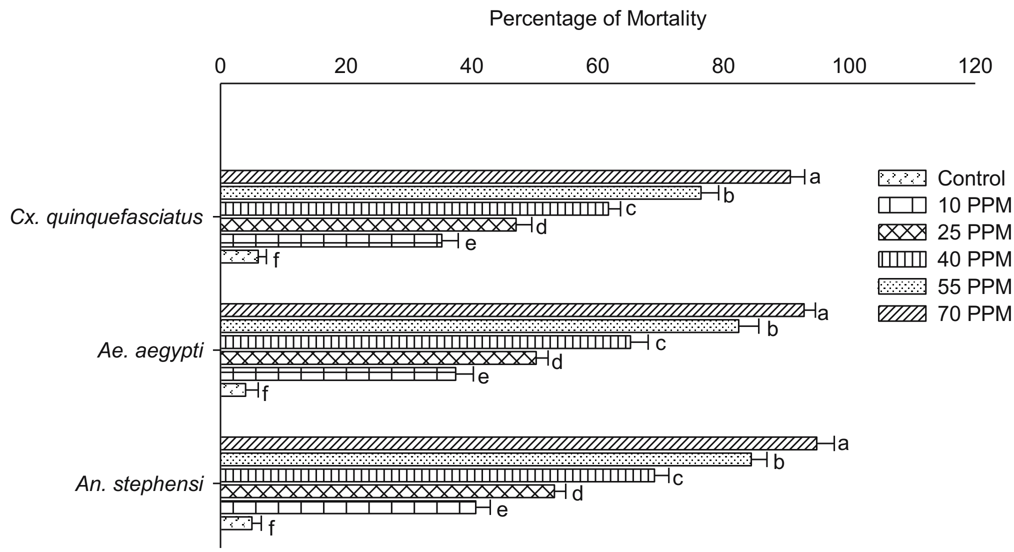

Figure 7: Larvicidal activity of green-fabricated *C. spongiosus*-mediated AgNPs against mosquito vectors.

percentage obtained. Adults treated with 200, 400, 600, 800, and 1,000 ppm of the *C. spongiosus* extract showed the mortality percentage in a dose-dependent manner. The mortality percentages were 24.0%, 28.8%, and 30.4% at a concentration of 200 ppm against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*, respectively. At a concentration of 1,000 ppm, the mortality percentages were 67.6% against *Cx. quinquefasciatus*, 75.4% against *Ae. aegypti*, and 78.8% against *An. stephensi.* The results obtained are displayed in Figure 8.

Green-fabricated *C. spongiosus*-mediated AgNPs against adult mosquito vectors showed good results at lower concentrations. The adults treated at 10, 25, 40, 55, and 70 ppm showed significant mortality. At a lower concentration of 10 ppm, the mortality percentages were 49.6%, 51.2%, and 55.2% against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*, respectively. At a higher concentration of 70 ppm, the mortality percentages were higher, 83.2%, 86.8%, and 89.4% against *Cx. quinquefasciatus*,

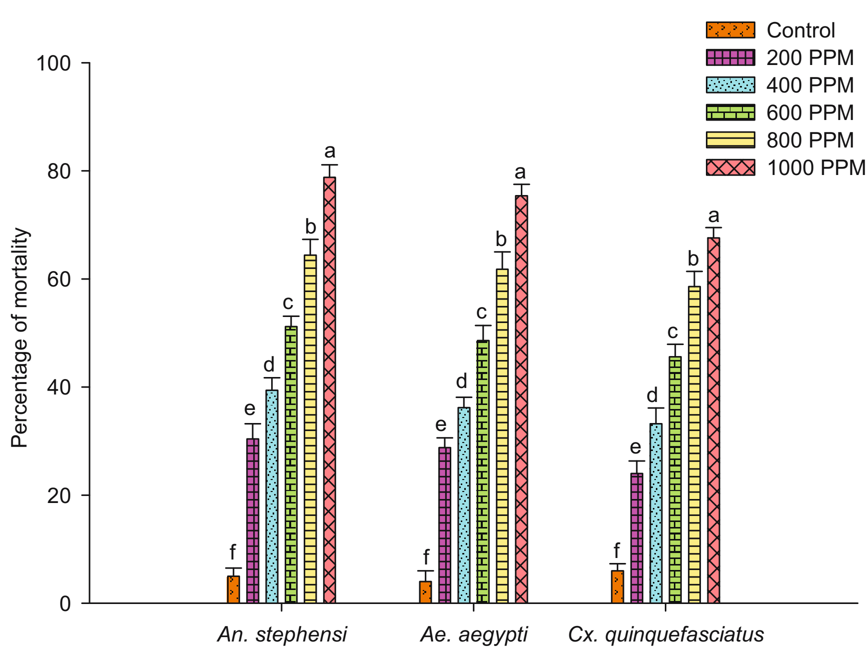

Figure 8: Adulticidal activity of the ethanolic extract of *C. spongiosus* against mosquito vectors.

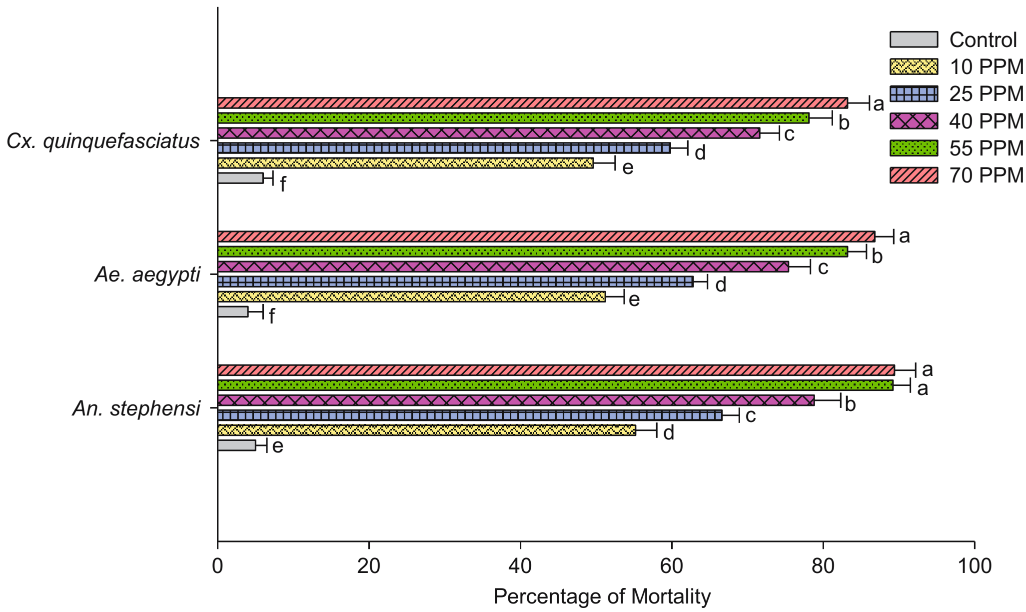

Figure 9: Adulticidal activity of green-fabricated *C. spongiosus*-mediated AgNPs against mosquito vectors.

Ae. aegypti, and *An. stephensi*. The results obtained are shown in Figure 9.

4 Discussion

The mosquito control program is challenging in the present environment due to its resistance against most of the insecticides. The continuous use of chemical pesticides to control vector-based disease spread has become a global problem against food chain and environmental pollution [35]. Insecticides developed from the natural environment or plant-based origin have good potential with no toxicity against the environment. Several studies are in progress to develop potential insecticides at low cost and with high mortality [35–37].

Seaweeds are widely distributed in the universe and can be obtained at lower costs from the seas and oceans [24]. Thus, it can be an alternative source for the present synthetic insecticides [27]. *C. spongiosus* extracted with ethanol showed promising activities against mosquito vectors. Similarly, earlier research reports reveal that the algae have good larvicidal potential owing to the presence of bioactive compounds [38].

Nanosynthesized green-fabricated nanoparticles have more advantages than crude extracts due to their rapid action at the lowest concentration. Green-fabricated *C. spongiosus*-mediated AgNPs synthesized via ethanol extraction showed colour changes. Cittararasu et al. [39] revealed that changes in the colour indicated the initial confirmation of nanoparticles, which was compared with the Ag nanoparticles synthesized biologically from *Barleria longiflora.* The FT-IR spectra of the synthesized nanoparticles showed the presence of S═S disulfide asym., ═CH out of plane, C–H out of plane, C–H out of plane, P–OR ester, S═O sulphate ester, S–H (sharp), and CH_2, ArO–H. Similarly, *Sargassum myriocystum*-mediated AgNPs showed the presence of N–H stretch, C═C stretch, 1 C–C (O)–C stretch with esters, and C–C stretch with alkyl halides [40].

EDX analysis revealed the crystalline nature of the particles synthesized. Balaraman et al. [40] revealed the phase formation and crystalline nature of the nanoparticles synthesized from *S. myriocystum*. The nanoparticles synthesized from *C. spongiosus* showed Ag ions in the range of 2.7–3 keV. A similar study was reported in which Ag NPs were synthesized from *S. siliquosum* that shows Ag ions at ~3 keV [41].

UV-Vis spectrophotometric analysis of the synthesized nanoparticles showed an absorption peak at 341 nm, which indicated the synthesis of AgNPs. Similar to our report, AgNPs prepared on montmorillonite clay in *n*-hexanol exhibited fluorescence peaks at 341 nm [42].

TEM analysis showed that the synthesized nanoparticles were of size 20 and 50 nm. Reports from earlier findings reveal that particle sizes in the range of 1–100 nm and solid particles in the range of 10–1,000 nm are considered to be nanoparticles [43,44].

The XRD analysis of the synthesized nanoparticles showed corresponding peaks of (111), (200), (220), and

(311) planes, confirming that the material synthesized is nanoparticle. Our reports agree with the findings of other researchers, who found similar findings when analysed for marine fabricated AgNPs [43,45,46].

The ethanolic extract of *C. spongiosus* algae showed promising activities against *Cx. quinquefasciatus, Ae. aegypti*, and *An. stephensi* larvae. Similarly, the methanolic extract of brown, green, and red algae tested against *Ae. albopictus* and *Ae. aegypti* showed strong larvicidal activity [28]. The ethanolic extract of *C. spongiosus* treated at concentrations of 150, 300, 600, and 750 ppm showed good mortality against *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus.* This implies that the bioactive compounds available in the extract exhibit their potential by binding to their target site [47]. Similarly, the leaf extract of *Annona muricata* treated against mosquito vectors *An. stephensi, Cu. quinquefasciatus*, and *Ae. aegypti* showed good activity when treated at concentrations of 100, 200, 300, 400, and 500 ppm [48].

The adulticidal potential of the ethanolic *C. spongiosus* extract showed significant activity against all vectors showing maximum activity at 1,000 ppm. The adults showed a maximum of 78.8% followed by 75.4% and 67.6% against *An. stephensi, Ae. aegypti*, and *Cx. Quinquefasciatus,* respectively. Similarly, the ethanolic extract of *Sargassum polycystum,* which was tested against adulticidal potential, showed good activity; when treated at 100, 150, 200, 250, and 300 ppm, it showed significant activity on *Ae. aegypti* and *Cx. quinquefasciatus* [49].

The synthesized green-fabricated *C. spongiosus*-mediated AgNPs which were tested against larvae of *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus* showed their potential by the mortality percentage. The mortality percentages obtained against the vectors are higher even at lower concentrations. The larvae treated at the lowest concentration of 10 ppm exhibited mortality percentages of 40.6%, 37.4%, and 35.2% against *An. stephensi, Ae. aegypti*, and *Cx. Quinquefasciatus,* respectively. Similarly, plant-fabricated nanoparticles show higher mortality against mosquito larvae at lower concentrations [46].

The maximum larvicidal activity was obtained at a concentration of 70 ppm against *An. stephensi* (94.8%), followed by 83.8% and 90.6% against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Similarly, *R. mucronate*-mediated AgNPs showed maximum activity at a concentration of 10 $mg{\cdot}L^{-1}$, which showed 100% mortality against *Ae. aegypti* and *Cx. quinquefasciatus* [50].

The adulticidal potential of *C. spongiosus*-mediated AgNPs was good in governing mosquito adults. The treated adults showed maximum percentages of mortality at a concentration of 70 ppm: 89.4% against *An. stephensi* adults, 86.4% against *Ae. Aegypti,* and 83.2% against *Cx. quinquefasciatus.* Likewise, AgNPs fabricated using the *Acacia caesia* showed promising potential against treated adults at a lower concentration of 45 $\mu g{\cdot}mL^{-1}$, with a mortality percentage of 100% against *An. subpictus,* 98.2% against *Ae. albopictus*, and 95.6% against *Cu. tritaeniorhynchus* [51].

The percentage of mortality against larvae and adults of *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus* via *C. spongiosus* may change from place to place within the same species. This may directly depend on the availability of bioactive compounds in the algae with respect to environmental factors or stress. A similar result was stated by earlier researchers: species of the same division change due to ecological and geographical factors, etc. [52].

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Conflict of interest: The author states no conflict of interest.

Data availability statement: The author confirms that the data supporting the findings of this study are available in the article.

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