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ORIGINAL PAPER

Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*

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Abstract Nearly 1.4 billion people in 73 countries worldwide are threatened by lymphatic filariasis, a parasitic infection that leads to a disease commonly known as elephantiasis. Filariasis is vectored by mosquitoes, with special reference to the genus *Culex*. The main control tool against mosquito larvae is represented by treatments with organophosphates and insect growth regulators, with negative effects on human health and the environment. Recently, green-synthesized nanoparticles have been proposed as highly effective larvicidals against mosquito vectors. In this research, we attempted a reply to the following question: do green-synthesized nanoparticles affect predation rates of copepods against mosquito larvae? We proposed a novel method of seaweed-mediated synthesis of silver nanoparticles using the frond extract of *Caulerpa scalpelliformis*. The toxicity of the seaweed extract and silver nanoparticles was assessed against the filarial vector *Culex quinquefasciatus*. Then, we evaluated the predatory efficiency of the cyclopoid crustacean *Mesocyclops longisetus* against larval instars of *C. quinquefasciatus* in a nanoparticle-contaminated water environment. Green-synthesized silver nanoparticles were characterized by UV–vis spectrum, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). In mosquitocidal assays, the LC_{50} values of the *C. scalpelliformis* extract against *C. quinquefasciatus* were 31.38 ppm (I), 46.49 ppm (II), 75.79 ppm (III), 102.26 ppm (IV), and 138.89 ppm (pupa), while LC_{50} of silver nanoparticles were 3.08 ppm, (I), 3.49 ppm (II), 4.64 ppm (III), 5.86 ppm (IV), and 7.33 ppm (pupa). The predatory efficiency of the copepod *M. longisetus* in the control treatment was 78 and 59 % against I and II instar larvae of *C. quinquefasciatus*. In a nanoparticle-contaminated environment, predation efficiency was 84 and 63 %, respectively. Predation was higher against first instar larvae over other instars. Overall, our study showed that seaweed-synthesized silver nanoparticles can be proposed in synergy with biological control agents against *Culex* larvae, since their use leads to little detrimental effects against aquatic predators, such as copepods.

Keywords Biocontrol · Biopesticides · Copepods · Culicidae · Integrated pest management · Mosquitoes · Nanobiotechnologies · Predaceous arthropods

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Introduction

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. More than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema (WHO 2014). Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty. Lymphatic filariasis is caused by Filariodidea nematodes, namely *Wuchereria bancrofti*, which is responsible for 90 % of cases, *Brugia malayi*, and *Brugia timori*. Microfilariae are transmitted to humans by different mosquitoes. *Culex* species, with special reference to *Culex quinquefasciatus*, are the most common vectors across urban and semi-urban areas of Asia (Chadee et al. 2002; WHO 2014).

Currently, the main control tool against *Culex* larvae is represented by treatments with organophosphates and insect growth regulators, with negative effects on human health, the environment, and non-target aquatic organisms (Brown 1986; see also Conti et al. 2014). In this scenario, botanical products constitute a valuable alternative, due to their reduced toxicity towards vertebrates and high biodegradability. Plant-borne chemicals have been used by human communities in different parts of the world as mosquitocidals, adult repellents, and oviposition deterrents, against a wide number mosquito species (e.g., Amer and Mehlhorn 2006a, b; Coelho et al. 2009; Freitas et al. 2010; Govindarajan 2010; Hafeez et al. 2011; Ravikumar et al. 2011; Benelli et al. 2013a, b, 2015a, b, c).

Nanotechnologies open new perspectives for unraveling a huge array of applications in the field of catalysis, sensors, optoelectronics, magnetic devices, drug delivery, antimicrobials, and parasitology (Shirkhanzadeh et al. 1995; Chan and Nie 1998; El-Sayed et al. 2005; Vaseashta and Dimova-Malinovska 2005; Aurel et al. 2007; Kim et al. 2007; Magana et al. 2008; Rai et al. 2009). The plant-mediated biosynthesis of nanoparticles is advantageous over chemical and physical methods, since it is cheap and eco-friendly, does not require high pressure, energy, temperature, and the use of highly toxic chemicals (Goodsell 2004). A growing number of plants and fungi have proposed for efficient and rapid extracellular synthesis of silver and gold nanoparticles (Shankar et al. 2003, 2004; Chandran et al. 2006; Song et al. 2009), which showed excellent mosquitocidal properties, also in field conditions (Soni and Prakash 2012; Dinesh et al. 2015; Suresh et al. 2015).

Concerning eco-friendly control of mosquito vectors, a further way to tackle the problem is the employment of aquatic organisms that predate Culicidae larvae in biological control programs. Good examples are odonate young instars, water bugs, tadpoles, fishes, crabs, and copepods (Bowatte et al. 2013; Kalimuthu et al. 2014 and references therein). Copepods are small aquatic crustaceans; most of them are omnivorous and can prey on immature mosquitoes, especially first-instar larvae, but rarely on later stages (Hurlbut 1938; Riviere and Thirel 1981; Marten et al. 1989; Williamson 1999). In particular, several species of copepods, such as *Mesocyclops aspericornis*, *Mesocyclops guangxiensis*, *Mesocyclops longisetus*, and *Mesocyclops thermocyclopoides*, have been reported as potential biological control agents against mosquitoes (Rawlins et al. 1997; Jekow et al. 1998; Manrique-Saide et al. 1998; Schaper 1999; Locantoni et al. 2006). Operationally, the use of *M. longisetus* against mosquitoes in urban and semi-urban habitats is not expensive and requires little labor for colony maintenance (Soumare and Cilek 2011; Chitra et al. 2013).

To the best of our knowledge, few studies investigated the non-target effects of nanoparticles against predatory copepods (Oberdorster et al. 2006; Jarvis et al. 2013; Park et al. 2014, see Fabrega et al. 2011, and Baun et al. 2008 for reviews), and no evidences are available about the toxicity of green-synthesized nanoparticles against these predaceous aquatic organisms. No efforts have been carried out to integrate classic biological control programs and nanobiotechnological tools for eco-friendly control of mosquito vectors.

Do green-synthesized nanoparticles affect predation rates of copepods against mosquito larvae? In this research, we proposed a new method of seaweed-mediated synthesis of silver nanoparticles using the frond extract of the seaweed *Caulerpa scalpelliformis* (Caulerpaceae). Green-synthesized silver nanoparticles were characterized by UV–vis spectrum, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The toxicity of the seaweed extract and silver nanoparticles was assessed against the filarial vector *C. quinquefasciatus*. Then, we evaluated the predatory efficiency of the cyclopoid crustacean *M. longisetus* against larval instars of *C. quinquefasciatus* in a nanoparticle-contaminated water environment. Our research highlighted that the seaweed-synthesized silver nanoparticles can be employed in synergy with biological control agents against mosquito vectors, leading to little detrimental effects against aquatic predators, such as copepods.

Materials and methods

Seaweed study species

The marine environment is an outstanding reservoir of bioactive natural products, which have many therapeutic

applications, such as antiviral, antibacterial, antifungal, antifertility, and anticancer activities (Ireland et al. 1988; Bazes et al. 2009; Ravikumar et al. 2009, 2010; Chen and Wilson 2010; Kamaraj et al. 2011; Tennyson et al. 2012). The frond extract of the seaweed *C. scalpelliformis* is toxic against different arthropod pests, including mosquitoes. Raniello et al. (2007) reported the phytotoxic property of caulerpenyne. Other phytochemicals include saponins, tannins, terpenoids, alkaloids, steroids, and flavonoids (Engel et al. 2006).

C. scalpelliformis collection and extraction

C. scalpelliformis was collected from coastal areas of Rameshwaram (Tamil Nadu, Southern India). *C. scalpelliformis* fronds were washed with tap water and shade-dried at room temperature. The dried plant material was powdered using an electrical blender. Then, 500 g of the powdered plant material were extracted using 1.5 L of ethanol for 72 h. The crude plant extract was concentrated at reduced temperature using a rotary evaporator, and stored at 22 °C. One gram of the plant residue was dissolved in 100 mL of acetone (fixative agent to separate the aqueous impurities altering the chemical composition of plant crude extract) and considered as 1 % stock solution. From this stock solution, experimental concentrations were prepared.

C. quinquefasciatus rearing

Eggs of *C. quinquefasciatus* were collected from local breeding habitats in Coimbatore (India) using an "O" type brush (Dinesh et al. 2015). Eggs were transferred to laboratory conditions [27±2 °C, 75–85 % R.H., 14:10 (L:D) photoperiod] and placed in 18×13×4 cm plastic containers containing 500 mL of tap water, waiting for larval hatching. Larvae were reared in the plastic containers described above, and fed daily with a mixture of crushed dog biscuits and hydrolyzed yeast at 3:1 ratio (*w*:*w*). Water was renewed each 2 days. The breeding medium was checked daily and dead individuals were removed. Breeding containers were kept closed with muslin cloth to prevent contamination by foreign mosquitoes. Larvae and pupae for experiments were collected daily from culture containers and transferred to glass beakers containing 500 mL of water.

M. longisetus rearing

Copepods were collected from a pond (Muthanamkulam, Coimbatore, India) using a mesh net. All collected samples were identified as *M. longisetus* by Dr. Y. Ranga Reddy (Department of Zoology, Acharya Nagarjuna University, India). *M. longisetus* was reared following the method reported by Kosiyachinda et al. (2003). Isofemale lines were established from gravid females and maintained at Department of Zoology, Bharathiar University (Coimbatore, India). Gravid females from different isofemale lines were pooled and mass reared in dechlorinated water (pH 7) in fish tanks (15 L) at 27 ±2 °C and natural photoperiod. Food was *Paramecium* spp. prepared from boiled rice straw water extract and commercial powdered fish food.

Seaweed-mediated synthesis and characterization of silver nanoparticles

The *C. scalpelliformis* aqueous extract was prepared adding 10 g of washed and finely cut leaves in a 300-mL Erlenmeyer flask filled with 100 mL of sterilized double distilled water and then boiling the mixture for 5 min, before finally decanting it. The extract was filtered using Whatman filter paper No. 1, stored at −15 °C and tested within 5 days. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature (Dinesh et al. 2015). A brown-yellow solution indicated the formation of silver nanoparticles, since aqueous silver ions were reduced by the plant extract generating stable silver nanoparticles in water. Silver nitrate was purchased from the Precision Scientific Co. (Coimbatore, India).

The presence of green-synthesized silver nanoparticles was confirmed 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 a UV-3600 Shimadzu spectrophotometer at 1-nm resolution. The reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min, and the resulting pellet was dissolved in de-ionized water and filtered through a Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) analyses, and energy dispersive X-ray (EDX) spectroscopy (Dinesh et al. 2015; Suresh et al. 2015).

FTIR spectra were recorded and analyzed using a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}. The structure and composition of freeze-dried purified silver particles was analyzed by using a 10 kV ultra-high-resolution scanning electron microscope with 25 μL of sample was sputter coated on copper stub and the images of nanoparticles were studied using a FEI QUANTA-200 SEM. SEM-EDX was conducted using a JEOL-MODEL 6390. The XRD pattern was phase-matched using match software version 1.10c Inc. Standard values are obtained from the International Centre for

Diffraction Data ICDD. Hkl indices for the observed peaks were determined to be 111, 200, and 311 according to the Bragg's reflection for face-centered cubic structure.

Toxicity of *C. scalpelliformis* extract and silver nanoparticles against *C. quinquefasciatus*

Following the methods reported in Suresh et al. (2015), 25 *C. quinquefasciatus* larvae (I, II, III or IV instar) or pupae were placed in a 500-mL glass beaker filled with 249 mL of dechlorinated water and 1 mL of the desired concentration of *C. scalpelliformis* extract or green-synthesized silver nanoparticles was added. Larval food (0.5 mg) was provided for each tested concentration. Each concentration was replicated five times against all instars. In control treatments, 25 larvae or pupae were transferred in 249 mL of dechlorinated water plus 1 mL of acetone (seaweed extract control), or 250 mL dechlorinated water (silver nanoparticle control). Percentage mortality was calculated as follows:

$$\text{Percentage mortality} = (\text{number of dead individuals}/\text{number of treated individuals}) \times 100$$

Predation of *M. longisetus* against *C. quinquefasciatus*

In this experiment, the predation efficiency of *M. longisetus* adults was assessed against *C. quinquefasciatus* larvae. For each instar, 100 mosquitoes were introduced, with 10 copepods, in a 500-mL glass beaker containing 250 mL of dechlorinated water. Mosquito larvae were replaced daily with new ones. For each mosquito instar, four replicates were conducted. Control was 250 mL of dechlorinated water without copepods. All beakers were checked after 1, 2, 3, 4, and 5 days and the number of prey consumed by copepods was recorded. Predatory efficiency was calculated using the following formula:

$$\text{Predatory efficiency} = [(\text{number of consumed mosquitoes}/\text{number of predators})/\text{total number of mosquitoes}] \times 100$$

Predatory efficiency of *M. longisetus* after treatment with silver nanoparticles

In this experiment, the predation efficiency of *M. longisetus* adults was assessed against *C. quinquefasciatus* larvae, after a mosquitocidal treatment with silver nanoparticles. For each instar, 100 mosquitoes were introduced with 10 copepods in a 500-mL glass beaker filled with 249 mL of dechlorinated water and 1 mL of the desired concentration of *C. scalpelliformis*-synthesized silver nanoparticles (1 ppm, i.e., 1/3 of the LC_{50} calculated against first instar larvae of *C. quinquefasciatus*). Mosquito larvae were replaced daily with new ones. For each mosquito instar, four replicates were conducted. Control was 249 mL of dechlorinated water with 1 mL of acetone, without copepods. All beakers were checked after 1, 2, 3, 4, and 5 days and the number of prey consumed by copepods was recorded. Predatory efficiency was calculated using the above-mentioned formula.

Data analysis

Toxicity data were subjected to two-way ANOVA with two factors (i.e., the targeted mosquito instar and the tested dosage). Means were separated using Duncan's multiple range tests by Alder and Rossler (1977). The average mosquito mortality data were subjected to probit analysis. LC_{50} and LC_{90}, were calculated using the method by Finney (1971). Data were analyzed using the SPSS Statistical Software Package version 17.0. A probability level of $P<0.05$ was used for the significance of differences between values.

Copepod predation data were analyzed by JMP 7 using a weighted general linear model with one fixed factor: $y = X\beta+\varepsilon$ where y is the vector of the observations (the number of consumed preys), X is the incidence matrix, β is the vector of fixed effect (the targeted mosquito instar), and ε is the vector of the random residual effect. A probability level of $P<0.05$ was used for the significance of differences between values.

Results and discussion

Toxicity of *C. scalpelliformis* extract against *C. quinquefasciatus*

In laboratory assays, the frond ethanol extract of *C. scalpelliformis* was highly toxic against larval instars (I–V) and pupae of *C. quinquefasciatus*. Larvicidal activity was proportional to the concentration of *C. scalpelliformis* extract (Table 1). LC_{50} values were 31.38 ppm (I instar), 46.49 ppm (II), 75.79 ppm (III), 102.26 ppm (IV), and 138.89 ppm (pupa) (Table 1). To the best of our knowledge, this is the first report

Table 1 Larval and pupal toxicity of *Caulerpa scalpelliformis* extract against the filariasis vector *Culex quinquefasciatus*

Targeted instar	Mortality (%)					LC_{50} (LC_{90})	95 % Confidential limit LC_{50} (LC_{90})		χ^2
	10 ppm	20 ppm	40 ppm	80 ppm	160 ppm		LCL	UCL	
I	33.20±1.92^{d}	48.20±2.30^{e}	59.00±2.00^{d}	71.00±1.58^{d}	93.00±3.16^{e}	31.38 (139.44)	19.36 (120.34)	41.54 (168.36)	3.41 n.s.
II	29.40±3.04^{cd}	41.80±1.30^{d}	53.80±2.28^{c}	66.00±2.12^{c}	81.00±2.44^{d}	46.49 (192.85)	3.95 (137.26)	78.73 (389.00)	5.79 n.s.
III	23.00±2.12^{bc}	31.60±3.04^{c}	42.00±2.54^{b}	57.20±1.64^{b}	73.00±2.34^{c}	75.79 (226.83)	62.30 (191.02)	91.34 (285.73)	4.23 n.s.
IV	19.20±2.16^{ab}	23.80±1.92^{ab}	37.40±2.30^{b}	44.20±3.11^{a}	66.00±2.54^{b}	102.26 (263.04)	86.59 (219.27)	123.72 (337.24)	3.15 n.s.
Pupa	13.20±2.58^{a}	18.60±2.70^{a}	24.20±3.19^{a}	39.40±2.96^{a}	53.00±2.91^{a}	138.89 (311.25)	117.57 (255.34)	172.78 (410.73)	3.27 n.s.

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. LC_{50} lethal concentration that kills 50 % of the exposed organisms, LC_{90} lethal concentration that kills 90 % of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit, χ^2 chi-square. Within each column means followed by the same letter(s) are not significantly different ($P<0.05$); *n.s.* not significant

of mosquitocidal properties of *C. scalpelliformis*. However, several seaweed species have been studied for their toxic properties against mosquitoes. In agreement with our results, Kalimuthu et al. (2014) observed high mortality rates in larvae of *Aedes aegypti* exposed to methanol extract of the seaweed *Gracilaria firma*. Kumar et al. (2012) studied the larvicidal and pupicidal activity of the brown seaweed *Sargassum wightii* towards *Anopheles sundaicus*, with LC_{50} ranging from 0.88 mg/L (I instar) to 1.171 mg/L (pupae). Lastly, insoluble bound phenolic acids and soluble conjugated phenolic acid fractions of *Chaetomorpha antennina* had a larvicidal effect against *A. aegypti,* with LC_{50} of 23.4 and 44.6 μg/mL, respectively (Vimaladevi et al. 2014).

UV–vis spectrum of seaweed-synthesized silver nanoparticles

UV–vis spectrum showed maximum absorbance at 350 nm, which increased over time during the incubation of silver nitrate with the seaweed extract (Fig. 1). The fronds extract without $AgNO_3$ did not show any change in color over time. *C. scalpelliformis*-mediated reduction of silver ions to silver nanoparticles was linked with changes in the UV–vis spectra. The appearance of the yellowish-brown color was an indication of formation of colloidal silver nanoparticles in the medium. The dark color may be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Ahmad et al. 2003; Krishnaraj et al. 2010). Our results are in agreement with previous research on color variations in fresh suspension of *Vitex negundo* and silver nitrate solution (Zargar et al. 2011).

Fourier transform infrared spectroscopy of seaweed-synthesized silver nanoparticles

FTIR spectra of aqueous silver nanoparticles prepared from the *C. scalpelliformis* frond extract exhibited prominent peaks

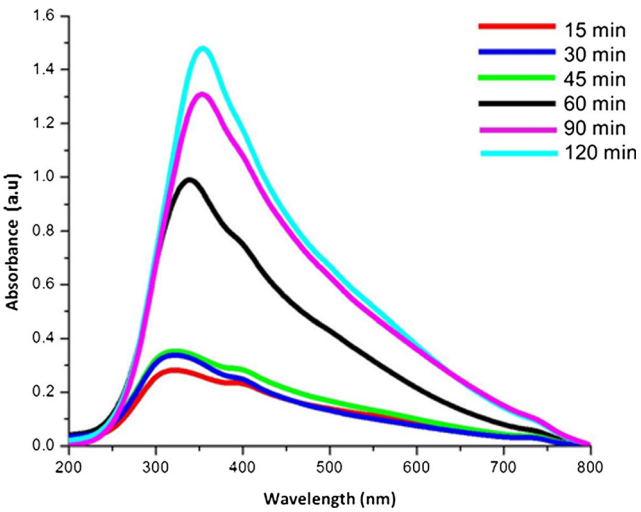

Fig. 1 UV-visualization at different time intervals of the absorption spectra of silver nanoparticles synthesized using *Caulerpa scalpelliformis* extract plus an aqueous solution $AgNO_3$ (1 mM)

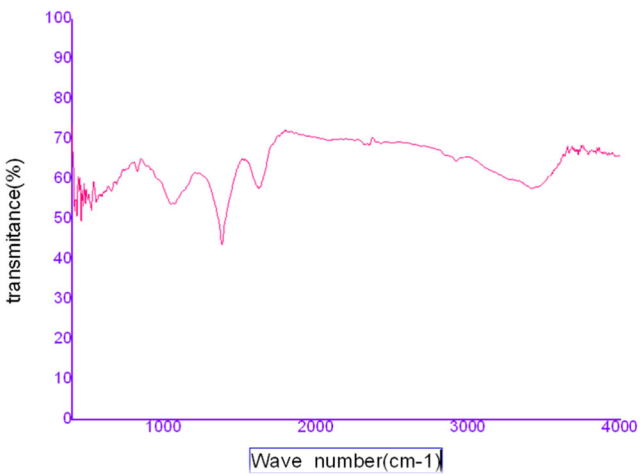

Fig. 2 Fourier transform infrared (FTIR) spectra of vacuum-dried powder of silver nanoparticles synthesized using the extract of the seaweed *Caulerpa scalpelliformis*

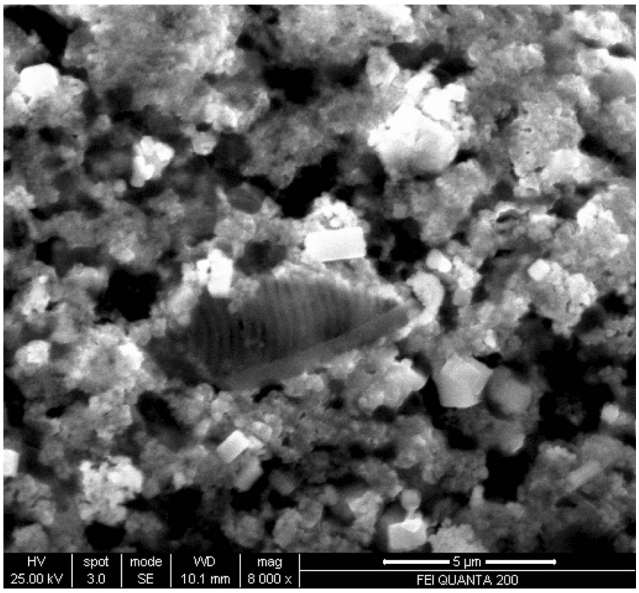

Fig. 3 Scanning electron microscopy (SEM) micrograph showing the morphological characteristics of silver nanoparticles synthesized using the extract of the seaweed *Caulerpa scalpelliformis*

at 3459.67, 2345.02, 1605.45, 1382.71, 1081.87, 634.466, 562.148, 519.722, 482.117, 437.762, 425.227, and 412.692 cm^{-1} (Fig. 2). The observed peaks were considered as major functional groups in different chemical classes such as flavonoids, triterpenoids, and polyphenols (Asmathunisha et al. 2010). A broad intense band at 3402 cm^{-1} in both spectra was assigned to the N–H stretching frequency arising from the peptide linkages present in the proteins of the extract (Mukherjee et al. 2008). FTIR analysis reveals that the carbonyl group from amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer that cover the metal nanoparticles (i.e., capping of silver nanoparticles), preventing agglomeration and thereby stabilizing the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Sathyavathi et al. 2010; Song et al. 2009). FTIR spectra of aqueous silver nanoparticles prepared from the *C. scalpelliformis* frond extract indicated that carboxyl (−C═O), hydroxyl (−OH) and amine (−NH) groups are involved in fabrication of silver nanoparticles (see also Dinesh et al. 2015; Suresh et al. 2015).

Scanning electron microscopy, energy dispersive X-ray spectroscopy, and X-ray diffraction analysis of seaweed-synthesized silver nanoparticles

Figure 3 showed a SEM micrograph of the reaction mixtures containing 50 mg of *C. scalpelliformis* fronds extract powder and 1.0 mM of silver nitrate incubated for 6 h magnified

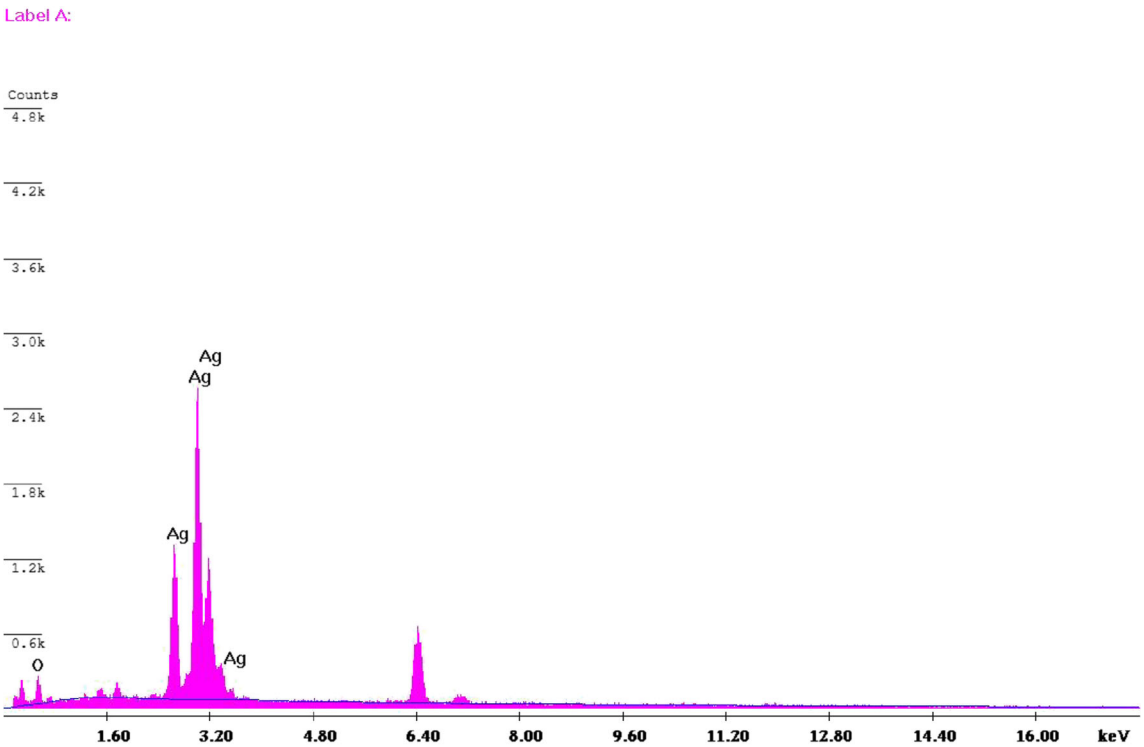

Fig. 4 Energy dispersive X-ray (EDX) profile of silver nanoparticles synthesized using the extract of the seaweed *Caulerpa scalpelliformis*

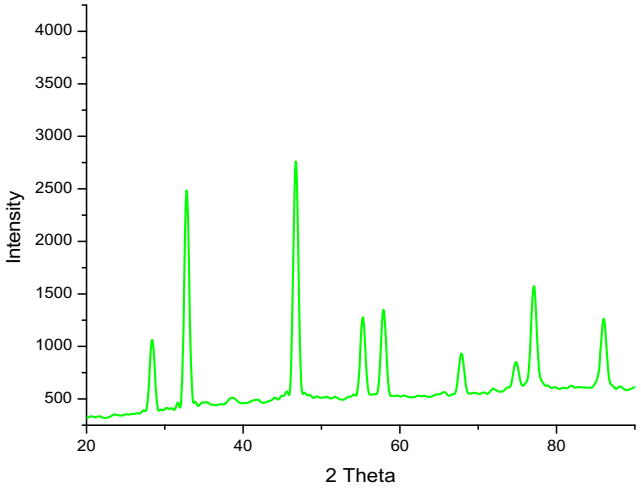

Fig. 5 X-ray diffraction (XRD) pattern of silver nanoparticles synthesized using the extract of the seaweed *Caulerpa scalpelliformis*

×8000. Seaweed-synthesized nanoparticles were monodispersed with spherical and cubic structures with mean sizes of 20–35 nm. Similar morphological features of silver nanoparticles have been obtained via green-synthesis with aqueous extracts from different terrestrial plants (Chandran et al. 2006; Huang et al. 2007; Dinesh et al. 2015; Suresh et al. 2015). Recent SEM studies also showed that "capped" silver particles were stable in solution for more than 8 weeks (Suganya et al. 2013). In the spot EDX profile, strong signals from elemental silver atoms in the nanoparticles were observed (Fig. 4). Our finding is in agreement with a previous study on silver nanoparticle synthesis using the fungus *Trichoderma viridae* (Fayaz et al. 2010); metallic silver nanoparticles generally show typical absorption peak approximately at 3 KeV due to SPR (Magudapathy et al. 2001).

The XRD pattern of seaweed-synthesized silver nanoparticles showed a number of Bragg reflections at 2θ=32.4, 46.4 and 28.0 (Fig. 5). They matched the face centered cubic structure of the bulk silver with the broad peaks at 32.4, 46.4, and 28.0. These corresponded to (111), (200), (311) planes, respectively. The line broadening of the peaks was primarily due to small particle size (see also Shameli et al. 2011a, b). The XRD pattern observed in this study was consistent with previous reports (Bar et al. 2009). Also, Dubey et al. (2009) reported the size of silver nanocrystals as estimated from the full width at half-maximum of (111) peak of silver using the Scherrer's formula was 20–60 nm.

Toxicity of seaweed-synthesized silver nanoparticles against *C. quinquefasciatus*

C. scalpelliformis-synthesized silver nanoparticles were highly toxic against larvae and pupae of the filarial vector *C. quinquefasciatus* (Table 2). LC_{50} values were 3.08 ppm (I instar), 3.49 ppm (II), 4.64 ppm (III), 5.86 ppm (IV), and 7.33 ppm (pupa). Results of larvicidal and pupicidal assays indicated that the percentage of mortality was directly proportional to concentration of the silver nanoparticles. No mortality was observed in control treatment. To the best of our knowledge, few studies have been conducted to evaluate the toxic activity of seaweed-synthesized nanoparticles against insects and bacteria. Recently, Vinayaga Moorthi et al. (2015) reported the insecticidal activity of silver nanoparticles synthesized using *Sargassum muticum* against the common castor butterfly *Ariadne merione*, outlining changes in the protein profile of hemolymph, morphology of hemocytes, and deteriorated midgut inclusions. Furthermore, *Sargassum longifolium*-synthesized silver nanoparticles have been found toxic against the pathogenic fungi *Aspergillus fumigatus*, *Candida albicans*, and *Fusarium* sp., even at low dosages (Rajeshkumar et al. 2014).

Predation of *M. longisetus* against *C. quinquefasciatus* before and after treatment with seaweed-synthesized silver nanoparticles

M. longisetus adults actively predate *C. quinquefasciatus* young larval instars. The predatory efficiency per copepod

Table 2 Larval and pupal toxicity of *Caulerpa scalpelliformis*-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus*

Targeted instar	Larval and pupal mortality (%)					LC_{50} (LC_{90})	95 % Confidential limit LC_{50} (LC_{90})		χ^2
	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm		LCL	UCL	
I	40.00 ± 2.54^{c}	61.60 ± 3.78^{d}	73.20 ± 2.58^{d}	94.40 ± 1.14^{e}	99.00 ± 1.22^{d}	3.08 (7.46)	2.44 (6.85)	3.59 (8.29)	4.65 n.s.
II	36.60 ± 2.30^{c}	57.20 ± 1.48^{d}	67.60 ± 1.81^{cd}	86.20 ± 2.58^{d}	95.00 ± 1.73^{cd}	3.49 (8.89)	2.77 (8.11)	4.07 (9.99)	1.63 n.s.
III	30.80 ± 2.38^{b}	42.40 ± 2.50^{c}	61.20 ± 3.42^{c}	72.20 ± 3.11^{c}	92.20 ± 2.58^{c}	4.64 (10.43)	4.01 (9.48)	5.19 (11.79)	3.48 n.s.
IV	22.40 ± 2.60^{a}	36.80 ± 1.64^{b}	51.00 ± 2.64^{b}	63.40 ± 2.96^{b}	81.00 ± 3.16^{b}	5.86 (12.38)	5.25 (11.11)	6.46 (14.30)	0.62 n.s.
Pupa	18.60 ± 2.07^{a}	28.40 ± 2.30^{a}	43.80 ± 1.78^{a}	54.20 ± 2.58^{a}	66.00 ± 2.91^{a}	7.33 (15.15)	6.62 (13.22)	8.20 (18.33)	0.33 n.s.

Mortality rates are means±SD of five replicates. No mortality was observed in the control. *LC_{50}* lethal concentration that kills 50 % of the exposed organisms, *LC_{90}* lethal concentration that kills 90 % of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit, χ^2 chi-square. Within each column means followed by the same letter(s) are not significantly different ($P<0.05$); *n.s.* not significant

Table 3 Predation of the copepod *Mesocyclops longisetus* against larvae of *Culex quinquefasciatus*

Targeted instar	Number of consumed preys						Total predation (n)	Consumed preys per copepod per day (*n*)
	Control	1 day	2 days	3 days	4 days	5 days		
I	0	83±2.1	81±6.7	75±9.6	78±8.0	73±8.8	390	7.8[a]
II	0	73±8.9	69±8.1	58±5.9	50±4.6	45±6.0	295	5.9[b]
III	0	22±4.6	18±4.1	15±3.7	11±3.0	6±1.9	72	1.4[c]
IV	0	12±3.6	10±3.1	8±2.8	5±0.9	2±0.7	35	0.7[d]

Predation rates are means±SD of four replicates (10 copepods vs. 100 mosquitoes per replicate). Control was water without copepods. Within each column means followed by the same letter(s) are not significantly different ($P<0.05$)

per day was 7.8, 5.9, 1.4, and 0.7 larvae (I, II, III, and IV, respectively) (Table 3). Our results are in agreement with previous evidences on other species. Indeed, adult copepods have been found very effective to control young larval instars of different mosquitoes (e.g., *Aedes albopictus* and *A. aegypti*), while little predation rates have been observed against late-instar larvae (Kay et al. 1992; Schreiber et al. 1993; Marten et al. 1994; Murugan et al. 2011, 2013).

Post-treatment with seaweed-synthesized silver nanoparticles, the predatory efficiency of a single *M. longisetus* per day was 8.0, 6.3, 0.8, and 0.2 larvae (I, II, III, and IV, respectively) (Table 4). Also in this experiment, copepods were effective predators of first and second instars of mosquitoes, while they are not active control agents against late larval instars. Our results highlighted that a combined approach using green-synthesized silver nanoparticles and predaceous aquatic organisms is effective against the filarial vector *C. quinquefasciatus*. The higher predation rates of *M. longisetus* against *C. quinquefasciatus* young larvae may be due to the impact of nanoparticles treatment on the prey organism, since they can affect the physiological and metabolic activities, thus motility. This has been hypothesized also by Murugan et al. (2011), reporting higher predation rates of the copepod *M. aspericornis* against *A. aegypti*, after the treatment with neem seed kernel extract. Similarly, the predatory efficiency of a single adult copepod of *M. thermocyclopoides* was 6.5, 4.6, 0.76, and 0.14 *C. quinquefasciatus* larvae per day (I, II, III, and IV instar, respectively), while it was 8.7, 5.9, 1.2, and 0.36 larvae day (I, II, III, and IV instar, respectively), after treatment with *Solanum xanthocarpum* fruit extract (Mahesh Kumar et al. 2012).

Conclusions

This research highlighted that seaweed-borne compounds are highly effective against larval populations of the filarial vector *C. quinquefasciatus*, and can be used as effective reducing agent for the synthesis of mosquitocidal silver nanoparticles. The novel method we proposed is simple and cheap. Furthermore, we consider the predatory copepod *M. longisetus* as a reliable biological control agents against young larvae of *C. quinquefasciatus*. Interestingly, in a nanoparticle-contaminated environment, the predation efficiency of this crustacean is still high. Overall, our study firstly showed that seaweed-synthesized silver nanoparticles can be proposed in synergy with biological control agents against *Culex* larvae, since their use leads to little detrimental effects on predation efficacy of aquatic predators, such as copepods. However, further research is needed to shed light on long-term toxicity and sub-lethal effects of nanoparticles against copepods (Baun et al. 2008; Fabrega et al. 2011).

Table 4 Predation efficiency of the copepod *Mesocyclops longisetus* against larvae of *Culex quinquefasciatus* after a treatment with seaweed-synthesized silver nanoparticles (1 ppm)

Targeted instar	Number of consumed preys post-treatment with silver nanoparticles						Total predation (*n*)	Consumed preys per copepod per day (*n*)
	Control	1 day	2 days	3 days	4 days	5 days		
I	0	90±7.6	83±7.1	79±4.2	75±6.09	71±9.1	398	8.0[a]
II	0	53±6.8	48±8.3	45±8.0	41±5.7	38±7.0	315	6.3[b]
III	0	9±3.5	9±3.5	8±4.4	7±4.06	7±2.2	40	0.8[c]
IV	0	3±0.9	3±0.7	2±0.7	2±1.8	1±1.2	11	0.2[d]

Predation rates are means±SD of four replicates (10 copepods vs. 100 mosquitoes per replicate). Control was water without copepods. Within each column, means followed by the same letter(s) are not significantly different ($P<0.05$)

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