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Research Article

MULTI-POTENT ACTIVITY OF *TURBINARIA ORNATA* MEDIATED NANOPARTICLE AGAINST MOSQUITOES AND MICROBES

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ABSTRACT

Mosquito vectors are spread deadly diseases such as malaria, dengue, chikungunya and lymphatic filariasis now a day nanotechnology used for eco-friendly approach to control of mosquitoes and platforms in the area microbial biology have attracted remarkable attention. This research proposed a novel method of biosynthesis silver nanoparticles using *seaweed, Turbinaria ornata.* Nanoparticles *were characterized by* UV–vis spectroscopy, FTIR, SEM, EDX and XRD. In laboratory assays, the *T. ornata*-synthesized silver nanoparticles against *A. stephensi* the LC_{50} values ranging from 9.150 ppm (I) to 24.977 ppm (pupae); LC_{50} values for *Cx. quinquefasciatus* ranging from 11.963 ppm (I) to 27.643 ppm (pupae) and LC_{50} values for *A. aegypi* ranging from 10.214 ppm (I) to 23.630 ppm (pupae). In addition, the antibacterial activity of *T. ornata* -synthesized AgNp was evoked inhibition zone wider. Overall results of this study demonstrate that the green synthesized nanoparticles potential to be used as a perfect eco-friendly method for the control of mosquitoes and microbes.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are blood-thirsty insects and worst enemy in human societies, while they act as vectors for devastating parasites and pathogens, including malaria, yellow fever, dengue fever, zika virus and filariasis (Benelli *et al*., 2016). To complicate matters, there are more than 3,500 species of mosquitoes existing globally, among those, the members of three genera of mosquito, Anopheles, Aedes and Culex are the leading causes of mortality and morbidity in humans (Perng, 2015). Over one million people worldwide die from mosquito-borne diseases annually addressed in WHO guidelines WHO (2015).

Dengue fever, Chikungunya and Zika virus spreads to people primarily through the bite of an infected Aedes species mosquito (*Ae. aegypti*). People can also get Zika through sexual contact and spread the virus unborn child through pregnant mother. There are no vaccines to prevent infection with dengue virus (Benelli *et al*., 2016). Lymphatic filariasis is an abandoned tropical disease. Infection occurs when filarial parasites are transmitted to humans through Culex mosquito species (WHO, 2017).

The emergence of insecticide resistance in targeted vectors (Hemingway and Ranson 2000), as well as their damaging on non-target organisms and environment, increase an urgent search of new and better mosquito control methods that are economical and efficient as well as safe for non-target organisms and the environment (see also Govindarajan and Benelli 2016a, b; Pavela and Benelli, 2016; Suresh *et al*., 2017; Benelli *et al*., 2017).

Newly, the nanoparticle playing significant role on mosquito control there are various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots (CdS, ZnS), among these, Silver nanoparticles to possess anti-bacterial, anti-viral, anti-fungal activity (Murugan *et al*., 2016a; Akl *et al*., 2012; Umesh *et al*., 2013; Nethra Devi *et al*., 2012; Geoprincy *et al*., 2011; Rogers *et al*., 2008; Panaceka *et al*., 2009). Synthesis of nanoparticles using plants or microorganisms can effectively eliminate this problem by making the nanoparticles more bio-compatible. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for low-cost, energy-efficient, and nontoxic production of metallic nanoparticles (Thakkar *et al*., 2010).

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Marine halophytes are the specialized group of plants adopted for high saline conditions which include seaweeds, mangrove and seagrass. The biodiversity of marine ecosystem provides important sources of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal, antifertility and anticancer activities (Ravikumar *et al*., 2009; Ravikumar *et al*., 2010a; 2010b; 2010c). Several studies have been proven to have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to nematicidal and insecticidal in lower form of animals (Chen *et al*., 2010; Bazes *et al*., 2009; Kamaraj *et al*., 2011; Tennyson *et al*., 2012).

Recently, several seaweeds *Ulva lactuca*, *Centroceras clavulatum*, *Codium tomentosum*, *Gracilaria edulis*, *Sargassum muticum*, were used for synthesized silver nanoparticles (Murugan *et al*., 2015a, 2015b; 2016a; Madhiyazhagan *et al*., 2015; 2016). Peoples are often infected by microorganisms such as bacteria, molds, yeasts, and viruses present in their living environments. Because of the emergence and increase in the number of multiple antibiotic-resistant microorganisms and the continuing emphasis on health-care costs, many scientists have researched methods to develop new effective antimicrobial agents that overcome the resistances of these microorganisms and are also cost-effective. Such problems and needs have led to resurgence in the use of silver-based antiseptics that may be linked to a broad-spectrum activity and considerably lower propensity to induce microbial resistance compared with those of antibiotics (Jones *et al*., 2004; Pinto *et al*., 2009; Shahverdi *et al*., 2007; Silva Paula *et al*., 2009). In particular, silver ions have long been known to exert strong inhibitory and bactericidal effects as well as to possess a broad spectrum of antimicrobial activities (Berger *et al*., 1996).

Based on the scientific information in this study, investigate synthesized AgNP were characterized by UV–vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction analysis (XRD) and to investigate larvicidal and pupicidal properties of the *Turbinaria ornata* -synthesized AgNP against the dengue vector, *Ae. Aegypti*, malarial vector, *A. stephensi* and filarial vector, *Cx. quinquefasciatus*. In addition, the silver nanoparticles (AgNPs) were evaluated for their applicability in increasing antibacterial activities against *Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Seaweed collection and preparation of the extract

Turbinaria ornata (figure 1a) was collected in the Gulf of Mannar (Tamil Nadu, India; latitude: from 8° 47 to 9° 15 N; longitude: from 78° 12 to 79° 14 E). The seaweed was washed with distilled water and shade-dried for 2 days at 28 °C. Ten grams of washed and finely cut fronds was stored in a 300-mL Erlenmeyer flask filled with 100 mL of sterile distilled water. Following the method by Murugan *et al*. (2015c), the mixture was boiled for 5 min and then decanted. The aqueous extract was stored at 4 °C and tested within 5 days.

Synthesis and characterization of silver nanoparticles

To reduce Ag+ ions to Ag0, 10 mL seaweed aqueous extract was added to 190 mL aqueous $AgNO_3$ (1 mM). The effect of reaction time on synthesis rate and size of AgNP was studied by carrying out the reaction in a water bath at 95 °C with reflux (elapsed time: from 10 min to 4 h). AgNP were subjected to repeated centrifugation at 15,000 rpm for 20 min followed by re-dispersion of the pellet in de-ionized water. UV–vis spectra were recorded as a function of reaction time on a UV-3600 Shimadzu spectrophotometer operated at a resolution of 1 nm. After freeze-drying of the purified AgNP, the structure and composition were analyzed by 10 kV Ultra High Resolution SEM (FEI QUANTA-200 SEM) and EDX. The surface groups of the nanoparticles were qualitatively confirmed using FTIR spectroscopy. FTIR spectra were recorded on a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. XRD using Cukα radiation (PAN analytical X'pert Pro MPD diffractometer) was used to determine the crystalline structure of AgNP. In addition, X-ray analysis on dried AgNP was carried out using a Philips Model PW1050/37 diffractometer, operating at 40 kVand 30 mA, with a step size of 0.02° (2θ).

Tested concentrations

Two milliliter of *T. ornata*-synthesized AgNP was diluted in 100 mL distilled water for the preparation of 2 % (v/v) stock solution. Then, the experimental concentrations (i.e., 10, 20, 30, 40, and 50 ppm) were prepared by subsequent dilution of stock solution in distilled water. All stocks and dilutions were kept refrigerated at −4 °C and tested within 8 weeks.

Mosquito rearing

Eggs of *A. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* Larvicidal toxicity on mosquitoes were collected from different water reservoirs in Coimbatore (Tamil Nadu, India) using an "O"-type brush. Batches of 100–110 eggs were transferred to 18x13x4 cm^3 enamel trays containing 500mL of water, where eggs were allowed to hatch in laboratory conditions (27±2 °C and 75–85% RH;14:10 (L/D) photoperiod). These three larval species were fed daily with 5g of ground dog biscuits (Pedigree, USA) and hydrolyzed yeast (Sigma-Aldrich, Germany) in a 3:1 ratio. Newly emerged larvae and pupae were collected and used in the experiments (Anitha *et al*., 2016).

Larvicidal toxicity on mosquitoes

Larva and pupae of *A. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were reared in laboratory (27±2 °C and 75–85 % R.H.; 14:10 (L:D) photoperiod) as described by Murugan *et al*. (2015d). Following Suresh *et al.* (2015), 25 *A. stephensi Ae. aegypti* and *Cx. quinquefasciatus* larvae (I, II, III or IV instar) or pupae were stored in a glass beaker and exposed to 250 ml of dechlorinated water plus the desired concentration of green-synthesized *Turbinaria ornata* nanoparticles (10-50 ppm). Each concentration was replicated 5 times against all larval instars and pupae. In control, 25 larvae or pupae were transferred in 250 ml of dechlorinated water. Mortality was assessed after 24 h.

Bacterial Strains

Microorganisms used in this study were originally purchased from Microbial Type Culture Collection and Gene Bank (MTTC), Institute of Microbial Technology, Sector 39-A,

Chandigarh-160036. Selected bacterial strains were *Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae.*

Preparation of Suspension of Test Bacteria

18 -24 hrs old culture were used for preparation of the test bacteria. Each of the three bacterial strains was inoculated into nutrient broth and incubated overnight (37 °C). After 24 hours the young culture attained 0.5 OD or 2 × 10^{-6} cfu/ml, which was further used for Antibacterial assay.

Nutrient Broth Composition

Ingredients	Gms/liter
Peptone	5.00
Yeast extract	1.50
Beef extract	1.50
Sodium chloride	5.00
Ph	7.4

- 13gm of Nutrient broth was suspended into 100 ml of distilled water.
- 25ml of Nutrient broth was transferred into each of 4 conical flask
- The test tubes were autoclaved at 121^0c for 15 min at 15psi.
- After autoclaving the test tubes appropriate organism were inoculated into the tubes.
- Then, incubate at 37°C for 24hrs.

Assessment of Antibacterial Activity by Disk Diffusion Method

AgNPs synthesized seaweed *Turbinaria ornata* was selected for the anti-bacterial activity. The agar disk diffusion method was employed for the determination of the antimicrobial activity of the AgNPs synthesized seaweed *Turbinaria ornata* against the three pathogenic bacteria such as *Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae.* The test bacteria were swabbed on the Muller Hinton agar medium plates. The three disks are inserted in each plate with three different concentrations (50mg/L, 100mg/L and 150mg/L) of sample. Then the plates were incubated at 37ºC for 24 hours. After the incubation, the zones of inhibition were measured.

Data analysis

Larvicidal and pupicidal data were transformed into arcsine√proportion values and analyzed using ANOVA with three factors (i.e., tested dose, mosquito species, and instar). Means were separated using Turkey's HSD test. The average mosquito mortality data were subjected to probit analysis. LC_{50} and LC_{90} were calculated using the method by Finney (1971). All data were analyzed using the SPSS 22.0 software (SPSS Inc., USA). A probability level of $P < 0.05$ was used for the significance of differences between values.

RESULTS

Characterization of T. ornata mediate silver nanoparticles

The aqueous silver nitrate was reduced to silver nanoparticles when added to natural seaweed extract of *T. ornata.* It is well known that the silver nanoparticles shows yellowish brown colour in water, after heating at different time interval it was change into light to dark brown gradually *(figure 1b)*. It was observed the vibration peak at 426nm at 120 mins and the absorption peak ranging between 341 - 426nm shown in figure 1c.

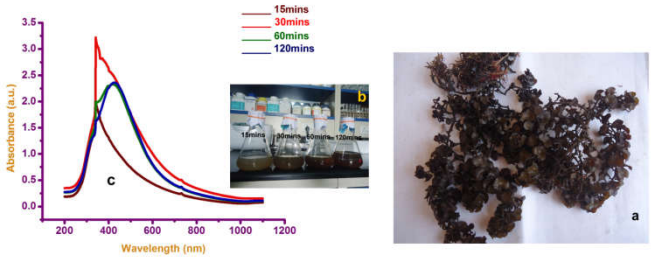

Figure 1 UV–vis spectra of aqueous silver nitrate with *Turbinaria ornata* aqueous extract at different time intervals

FTIR spectroscopy was carried out to identify the biomolecules responsible of the reduction of Ag^+ ions to AgN as well as of capping of the bio-reduced AgN synthesized by using *T. ornata*. Figure 2 showed that the AgN synthesized using the *T. ornata* extract had absorption peaks at 3415.74 (Amine N-H stretch), 1639.40 (Alkene C=C stretch), 1095.50 (Alkyl Halide C-F stretch) and 603.68 (Alkyl Halide C-Cl Stretch) cm^{-1}).

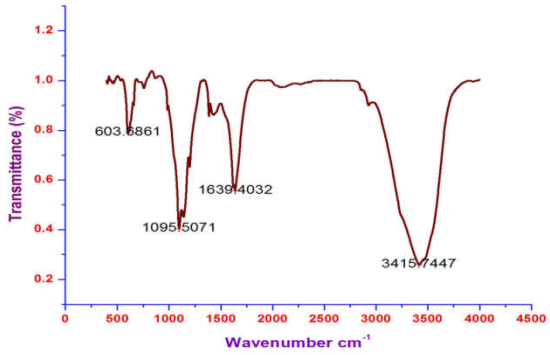

Figure 2 FTIR spectra of vacuum-dried powder of synthesized AgNPs using *Turbinaria ornata* extract

The formation of silver nanoparticles as well as their morphological dimensions in the FESEM study demonstrated that the average size of *Turbinaria ornata* was *spherical in shape with the size measured at 68–96 nm (figure 3)*.

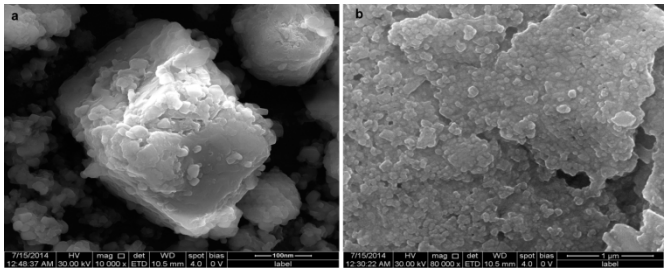

Figure 3 SEM micrograph showing the morphological characteristics of silver nanoparticles synthesized using *Turbinaria ornata* seaweed extract. a, Lower magnification (300nm), b, Higher magnification (1µm)

EDX spectra recorded from the silver nanoparticles are shown in Figure 4 from EDX spectrum, it is clear that silver nanoparticles reduced by *Turbinaria ornata* have recorded 62.82% weight of silver nanoparticle along with 37. 18 weight of oxygen molecules. EDS profile shows strong silver signal along with strong oxygen peak, which may originate from the biomolecules that are bound to the surface of the silver nanoparticles.

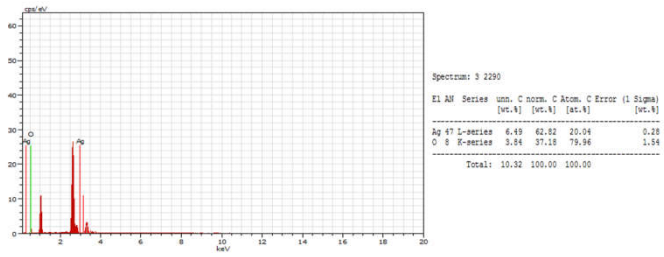

Figure 4 EDX spectrum of biosynthesized silver nanoparticles using seaweed of *Turbinaria ornata*

The biosynthesized silver nanoparticles using 5% *Turbinaria ornata* leaf broth at 95°C was investigated by X-ray diffraction technique (Figure 5). The diffractometer was operating at 40 kV and 30 mA, with a step size of 0.02° (2θ). The scanning was done in the region of 35˚to 85˚ for 2θ. The XRD pattern of the Ag NPs synthesized *Turbinaria ornata* extract are shown in Figures 4 the crystalline nature of Ag nanoparticles was confirmed from the X-ray diffraction analysis. The intense diffraction peaks due to AgNPs at 17.8, 37.00, 51.64, 64.24, and 76.78 corresponding to the (002), (101), (104), (110) and (200) facets of the face centered hexagonal crystal structure (JCPDS File number 87- 0598).

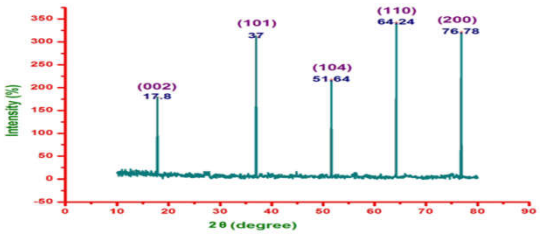

Figure 5 XRD pattern of bio-synthesized AgNPs using *Turbinaria ornata* aqueous seaweed extract

In laboratory condition, a dose-dependent effect of *T. ornata*-synthesized AgNP was highly toxic against larval instars (I-IV) and pupae of three threatening vectors. LC_{50} values for *A. stephensi* were 9.150 (I), 10.701 (II), 12.967 (III), 13.886 (IV) and 24.977 ppm (pupae) (Table 1). LC_{50} values for *Cx. quinquefasciatus* were 11.963 (I), 13.939 (II), 15.717 (III), 17.934 (IV) and 27.643 ppm (pupae) (Table 2) and LC_{50} values for *A. aegypi* were 10.214 (I), 11.280 (II), 13.217 (III), 15.251 (IV) and 23.630 ppm (pupae) (Table 3).

The larval mortality is expressed as mean±SD of five replicates. Nil mortality was observed in the control. Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range tests. *LFL* – Lower Fiducidal Limit; *UFL* - Upper Fiducidal Limit. χ^2, Chi-Square value. *Significant at $P<0.05$ level.

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Silver nitrate has long been considered as a powerful and natural antibiotic and antibacterial agent. The antibacterial activity of the synthesized silver particles has been investigated against *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumonia* **(Table 4; Figure 6)**. Silver nanoparticles synthesized using *Turbinaria ornata* also showed very strong inhibitory action against *Bacillus subtilis* were 6.4mm at 50mg/L; 8.2mm at 100mg/L; 11.2mm at 150mg/L zone of inhibition followed by *Salmonella typhi* were 5.8mm at 50mg/L; 8.2mm at 100mg/L; 10.8mm at 150mg/L zone of inhibition and *Klebsiella pneumonia* were 7.2mm at 50mg/L; 9.4mm at 100mg/L; 12.6mm at 150mg/L zone of inhibition, respectively.

Table 1 Larvicidal and pupicidal efficacy of AgNPs synthesized by using *Turbinaria ornata against An. Stephensi*

Larval instars	Larval mortality±SD (%) Concentrations (ppm)					LC_{50} (LC_{90})	95% Confidence limit		Chi-Square value (χ^2)
	10	20	30	40	50		LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
I	54±0.3	68±0.9	77±0.1	87±0.7	99±0.5	9.150 (39.896)	2.879 (35.785)	13.381 (45.926)	5.857*
II	52±0.5	63±0.6	74±0.4	82±0.1	97±0.2	10.701 (44.968)	4.187 (40.090)	15.073 (52.457)	4.858*
III	49±0.8	59±0.8	68±0.5	78±0.4	93±0.3	12.967 (51.584)	6.251 (45.484)	17.448 (61.487)	3.338*
IV	48±0.1	57±0.6	65±0.8	74±0.3	89±0.2	13.886 (58.169)	6.271 (50.387)	18.776 (71.778)	2.498*
Pupae	39±0.2	45±0.5	53±0.3	62±0.6	71±0.4	24.977 (86.216)	18.025 (69.924)	30.326 (121.867)	0.213*

Table 2 Larvicidal and pupicidal efficacy of AgNPs synthesized by using *Turbinaria ornata against Cx. Quinquefasciatus*

Larval instars	Larval mortality±SD (%) Concentrations (ppm)					LC_{50} (LC_{90})	95% Confidence limit		Chi-Square value (χ^2)
	10	20	30	40	50		LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
I	51±0.5	60±0.2	72±0.3	83±0.8	96±0.1	11.963 (45.809)	5.927 (40.912)	16.106 (53.287)	3.663*
II	47±0.7	58±0.4	66±0.8	78±0.2	90±0.6	13.939 (54.269)	7.179 (47.618)	18.452 (65.291)	1.517*
III	45±0.6	55±0.5	64±0.3	75±0.4	87±0.5	15.717 (58.597)	8.997 (50.988)	20.232 (71.621)	0.960*
IV	43±0.2	52±0.4	61±0.6	71±0.3	83±0.1	17.934 (64.924)	11.117 (55.670)	22.543 (81.629)	0.675*
Pupae	34±0.3	41±0.5	52±0.7	63±0.4	71±0.8	27.643 (79.030)	22.567 (66.360)	32.219 (10.323)	0.171*

Table 3 Larvicidal and pupicidal efficacy of AgNPs synthesized by using *Turbinaria ornata against Ae. Aegypti*

Larval instars	Larval mortality±SD (%) Concentrations (ppm)					LC_{50} (LC_{90})	95% Confidence limit		Chi-Square value (χ^2)
	10	20	30	40	50		LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
I	53±0.3	65±0.7	75±0.6	87±0.8	98±0.4	10.214 (41.398)	41.938 (37.148)	14.328 (47.675)	3.909*
II	51±0.5	62±0.3	72±0.7	85±0.1	95±0.6	11.280 (45.506)	49.677 (40.598)	15.559 (53.022)	2.048*
III	48±0.7	59±0.9	68±0.3	81±0.4	92±0.9	13.217 (50.620)	68.339 (44.807)	17.545 (59.889)	1.605*
IV	45±0.2	56±0.4	65±0.8	78±0.6	88±0.1	15.251 (55.713)	88.629 (48.870)	19.605 (67.051)	0.726*
Pupae	37±0.4	46±0.8	57±0.5	64±0.3	76±0.2	23.630 (74.367)	17.808 (62.745)	28.119 (96.372)	0.300*

Table 4 Zone of inhibition of *Turbinaria ornata* -synthesized silver nanoparticles against bacteria *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Salmonella typhi*

Treatment	Inhibition Zone (mm)		
	Bacillus subtilis	*Salmonella typhi*	*Klebsiella pneumonia*
Control	0.2±0.2[a]	0.2±0.1[a]	0.2±0.2[a]
50mg/L	6.4±0.8[b]	5.8±0.4[b]	7.2±0.8[b]
100 mg/L	8.2±0.4[c]	8.2±0.2[c]	9.4±0.6[c]
150 mg/L	11.2±0.2[d]	10.8±0.6[d]	12.6±0.2[d]

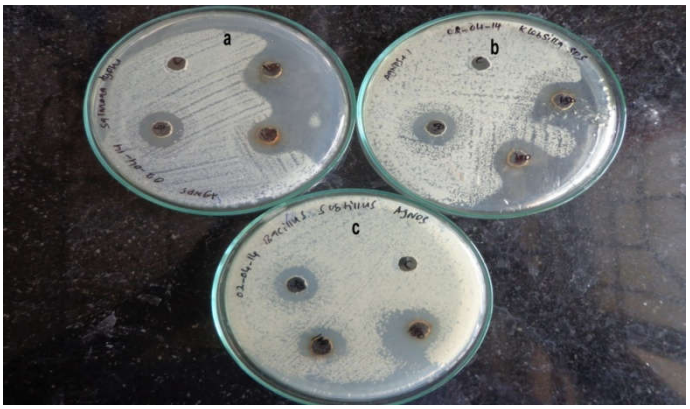

Figure 6 Inhibition zone induced by *Sargassum muticum* synthesized silver nanoparticles against different bacteria species: a) *Salmonella typhi*, b) *Klebsiella pneumoniae*, and c) *Bacillus subtilis*

DISCUSSION

UV-vis spectroscopy analysis depends on the arising of color in the reaction due to the excitation of surface Plasmon resonance band in a reaction mixture and was recorded as different functional time, there is no peak was formed at the initial stage indicate that there is no synthesis of silver nanoparticles was observed. Further, Madhiyazhagan *et al*. (2015) noticed that the absorption spectra of *Sargassum muticum*-synthesized AgNP showed an intense peak at 438 nm. Recently, a peak with maximum absorption at 374 nm characterized the biosynthesis of ZnONP synthesized using *Limonia acidissima* (Patil and Taranath, 2016). The peak at 3479 cm^{-1} shows the bonds due to O-H stretching of phenolic compound (Rajathi *et al*., 2013) and 3851 cm-1 corresponds to O-H stretching of hydroxyl groups (Jannathul Firdhouse *et al*., 2013). Peak at 1640 cm-1 was a strong absorption peak which indicates the characteristics IR absorption of polysaccharides shows the bonds due to C=O stretching, amines (Rastogi *et al*., 2011). For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons (Jores *et al*., 2004). Previous studies proved that spherical shape of nanoparticle size between 20nm to 120nm (Rajathi, 2012; Murugan *et al*., 2016b).

The presence and crystalline nature of silver nanoparticles in the material were observed by EDX analysis. It is well know that silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface Plasmon resonance (Magudapatty *et al*., 2001). (Ganesan *et al*., 2013) believed that the aminoacids present in the biomass of sea weed may be the reducing agent which reduces the silver ions to silver nanoparticles. The EDS study shows a strong silver signal along with weak oxygen and carbon peaks, which may have originated from the biomolecules bound to the surface of the silver nanoparticles. Similarly, (Shankar *et al*., 2004; Song and Kim, 2009) Carbon and copper peaks may be due to the same being present in the grids. It has been reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth and are, thus, stable in solution up to 4 weeks after synthesis. The presence of structural peaks in XRD patterns clearly illustrate that the AgNPs synthesized by our green method were nanocrystalline in nature. In addition to the Bragg peaks representative of facets of the face centered silver nanocrystals. In addition unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occurs on the surface of the silver nanoparticles.

However, in the present study higher mortality was observed in all the larval and pupal stages of *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. stephensi*. The larval mortality slowly decreased when the larval ages increased. Recently, for instance an increasing number of green-synthesised nano particles showed comparable larvicidal and pupicidal toxicity against different mosquito vectors (Udaiyan Suresh *et al* 2017; Sabah and Rajesh, 2017; Benelli *et al*., 2017). Safaepour *et al*. (2009) clearly state that the mortality effect of silver nanoparticles on mosquito larvae may be enabled by the small size of the particles, which allows passage through the insect cuticle and into individual cells where they interfere with molting and other physiological processes. This study agreed with (Jha *et al*., 2009; Kasthuri *et al*., 2009; Singh *et al*., 2010) another reason behind the mortality AgNPs synthesis were also reported from combination of reducing agents and secondary metabolites of the plant. Early studies envisaged that the Indian

marine algae extracts possessed potential larvicidal activity (Rao *et al*., 1995).

It is well known that Ag ions and Ag-based compounds have strong antimicrobial effects and many investigators are interested in using nanoparticles as antibacterial agents (Crabtree *et al*., 2003; Furno *et al*., 2004; Hamouda *et al*., 2000). The nanoparticles synthesized using seaweeds in the present study have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. But the nanoparticle targets the microbes by creating free radicals which leads to immediate death of the cells. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenols which derive radical to stabilize and delocalize the unpaired electron (chain-breaking function) (Chanda *et al*., 2009).

CONCLUSION

This evaluated study belongs to the seaweed mediated nanoparticle as a reducing and stabilizing agent and AgNP were clustered, crystalline in nature, *spherical in shape* with a mean size of *68–96 nm*. The green synthesized silver nanoparticle may be employed at low dosages to reduce larval and pupal populations. Furthermore, the AgNP highly pathogenic against *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumonia* using disk diffusion method. The incubation zone was dose dependent. Therefore, the overall study conclude that the seaweed based insecticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable.

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Conflicts of Interest

The Authors declare no conflicts of interest.

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.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

References

Akl, M., and Awwad Nidà, M. 2012. Green Synthesis of Silver Nanoparticles by Mulberry Leaves Extract. Nanoscience and Nanotechnology, 2(4), 125-128.

Anitha, J., Murugan, K., Panneerselvam, C., Madhiyazhagan, P., *et al*. Earthworm-mediated synthesis of silver nano-particles: a potent tool against hepatocellular carcinoma, pathogenic bacteria, Plasmodium parasites and malaria mosquitoes. Parasitol. Int, 2016; 65, 276–284.

Bazes, A., Silkina, A., Douzenel, P., Fay, F., Kervarec, N., and Morin D. 2009. Investigation of the antifouling constituents from the brown alga Sargassum sp. (Yendo) Fensholt. J. Appl. Phycol, 21, 395-403.

Benelli, G. 2016. Spread of Zika virus: the key role of mosquito vector control. Asia Pacif. J. Trop. Biomed, 6, 468–471.

Benelli, G., Caselli, A., and Canale, A. 2017. Nanoparticles for mosquito control: Challenges and constraints. J. King Saud Univ.Sci, 29: 424–435.

Benelli, G., Pavela, R., Maggi, F., Petrelli, R., and Nicoletti, M. 2017. Commentary: Making green pesticides greener? The potential of plant products for nanosynthesis and pest control. J. Clust. Sci, doi: 10.1007/s10876-016-1131-7.

Berger, T.J., Spadaro, J.A., Chapin, S.E., and Becker, R.O. 1996. Electrically generated silver ions: quantitative effects on bacterial and mammalian cells, Antimicrob. Agents Ch, 9:357-358.

Chanda, S., Dave, R. 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview, Afr. J. Microbiol. Res, 3(13): 981-996.

Chen, L.H., and Wilson, M.E. 2010. Dengue and chikungunya infections in travelers, Curr. Opin. Infect. Dis, 23(5): 438-444.

Crabtree, J.H., Burchette, R.J., Siddiqi, R.A., Huen, I.T., Handott, L.L., and Fishman, A. 2003. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections, Perit. Dial. Int, 23(4): 368-74.

Finney, D.J. 1971. Probit analysis. Cambridge University, London, pp 68–78.

Furno, F., Morley, K.S., Wong, B., Sharp, B.L., Arnold, P.L., and Howdle, SM. 2004. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection, J. Antimicrob. Chemother, 54: 1019–1024.

Ganesan, V., Aruna Devi, J., Astalakshmi, A., Nima, P., and Thangaraja, A. 2013. Eco- Friendly Synthesis of Silver Nanoparticles using a Sea Weed, *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva, Int. J. Eng. Adv. Tech, 2(5): 559-563.

Geoprincy, G., Saravanan, P., NagendraGandhi, N., and Renganathan, S. 2011. A novel approach for studying the combined antimicrobial effects of silver nano particles and antibiotics through agar over layer method and disk diffusion method, Digest. J. Nanomat. Biostruct, 6(4): 1557-1565.

Govindarajan, M., and Benelli, G. 2016a. *Artemisia absinthium*-borne compounds as novel larvicides: effectiveness against six mosquito vectors and acute toxicity on non-target aquatic organisms, Parasitol. Res, 115(12): 4649-4661.

Govindarajan, M., and Benelli, G. 2016b. α-humulene and β-elemene from *Syzygium zeylanicum* (Myrtaceae) essential oil: highly effective and eco-friendly larvicides against *Anopheles subpictus, Aedes albopictus* and

Culex tritaeniorhynchus (Diptera: Culicidae), Parasitol. Res, 115; 2771–2778.

Hamouda, T., Myc, A., Donovan, B., Shih, A., Reuter, J.D., and Baker, J.R. 2000. A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, envelop. vir. fungi. Microbiol. Res, 156: 1-7.

Hemingway, J., and Ranson, H. 2000. Insecticide resistance in insect vectors of human disease, Annu. Rev. Entomol, 45: 371–391.

Jannathul Firdhouse, M., and Lalitha P. 2013. Green Synthesis of Silver Nanoparticles using the aqueous extract of *Portulaca Oleracea* (L.), Asian J. Pharma. Clinic. Res, 6(1): 92-93.

Jha, A.K., Prasad, K., Kulkarni, A.R. 2009. Plant system: nature's nanofactory, Colloids. Surf. B. Biointerf, 73: 219-223.

Jones, S.A., Bowler, P.G., Walker, M., and Parsons, D. 2004. Controlling wound bioburden with a novel silver-containing Hydrofiber dressing, Wound Repair Regen, 12: 288-294.

Jores, K., Mehnert, W., Drecusler, M., Bunyes, H., Johan, C., and MAder, K. 2004. Investigation on the stricter of solid lipid nanopartuicles and oil-loaded solid nanoparticles by photon correlation spectroscopy, field-flow fractionasition and transmission electron microscopy, J Control Release, 17:217-227.

Kamaraj, C., Bagavan, A., Elango, G., Zahir, A.A., Rajakumar, G., Marimuthu, S. 2011. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchu*, Indian J. Med. Res, 134: 101-106.

Kasthuri, J., Kathiravan, K., Rajendiran, N. 2009. Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: A novel biological approach, J. Nanopart. Res, 11: 1075-1085.

Madhiyazhagan, P., Murugan, K., Naresh Kumar, A., Nataraj T, *et al*. 2016. One pot synthesis of silver nanocrystals using the seaweed *Gracilaria edulis*: biophysical characterization and potential against the filariasis vector *Culex quinquefasciatus* and the midge *Chironomus circumdatus*, J. Appl. Phycol, DOI 10.1007/s10811-016-0953-x.

Madhiyazhagan, P., Murugan, K., Naresh Kumar, A., Nataraj, T, *et al*. 2015. *Sargassum muticum*-synthesized silver nanoparticles: an effective control tool against mosquito vectors and bacterial pathogens, Parasitol. Res, DOI 10.1007/s00436-015-4671-0.

Magudapatty, P., Gangopadhyayrans, P., Panigrahi, B.K., Nair, K.G.M., Dhara, S. 2001. Electrical transport studies of Ag nanoparticles embedded in glass matrix, Physica. B, 299: 142-146.

Murugan, K., Christina Mary, S., Panneerselvam, C., Higuchi, A, *et al*. 2015a. Seaweed-synthesized silver nanoparticles: an eco-friendly tool in the fight against *Plasmodium falciparum* and its vector *Anopheles stephensi*?, Parasitol. Res, 114: 4087–4097.

Murugan, K., Aruna, P., Panneerselvam, C., Madhiyazhagan, P., *et al*. 2015b. Fighting arboviral diseases: low toxicity on mammalian cells, dengue growth inhibition (in vitro), and mosquitocidal activity of *Centroceras clavulatum*-synthesized silver nanoparticles, Parasitol. Res, DOI 10.1007/s00436-015-4783-6.

Murugan, K., Benelli, G., Ayyappan, S., Dinesh, D., *et al*. 2015c. Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*, Parasitol. Res, 114: 2243–2253.

Murugan, K., Aarthi, N., Kovendan, K., Panneerselvam, C., *et al*. 2015d. Mosquitocidal and antiplasmodial activity of *Senna occidentalis* (Cassiae) and *Ocimum basilicum* (Lamiaceae) from Maruthamalai hills against *Anopheles stephensi* and *Plasmodium falciparum*, Parasitol. Res, doi: 10. 1007/s 00436-015-4593-x.

Murugan, K., Panneerselvam, C., Subramaniam, J., Madhiyazhagan, P., *et al*. 2016. Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. Environ, Sci. Pollut. Res, DOI 10.1007/s11356-016-6832-9.

Murugan, K., Nataraj, D., Jaganathan, A., Dinesh, D., *et al*. 2016a. Nanofabrication of Graphene Quantum Dots with High Toxicity Against Malaria Mosquitoes*, Plasmodium falciparum* and MCF-7 Cancer Cells: Impact on Predation of Non-target Tadpoles, Odonate Nymphs and Mosquito Fishes, J. Clust. Sci, DOI 10.1007/s10876-016-1107-7.

Murugan, K., Panneerselvam, C., Aziz, A.T., Subramaniam, J., Madhiyazhagan, P., *et al*., 2016b. Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods, Environ. Sci. Poll. Res, 23: 16671–16685.

Nethra Devi, C., Sivakumar, P., and Renganathan, S., 2012. Green synthesis of silver nanoparticles using *Datura metel* flower extract and evaluation of their antimicrobial activity, Inter. J. Nanomat. Biostruct, 2(2), 16–21.

Panaceka, A., Milan, K., Renata, V., Robert, P., Jana, S., Vladimır, K., Petr, H., Radek, Z., and Libor, K., 2009. Antifungal activity of silver nanoparticles against Candida spp, Biomat, 30, 6333–6340.

Pavela, R., and Benelli, G., 2016. Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors – a review, Exp. Parasitol, 167C, 103-108.

Perng G.C., 2015. Threat of Mosquito-Borne Human Viral Diseases, J. Bioter. Biosaf, 1 (1), 1-2.

Pinto, R.J.B., Marques, P.A.A.P., Neto, C.P., Trindade, T., Daina, S., and Sadocco. P., 2009. Antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers, Acta. Biomater, 5, 2279-2289.

Rajathi, F.A.A., Parthiban, C., and Ganesh Kumar, V., 2012. Biosynthesis of antibacterial gold nanoparticles using brown alga, *Stoechos permummarginatum* (kutzing), Spectrochim. Acta. Part A Mol. Biomol. Spectrosc, 99, 166-173.

Rajathi, K., and Sridhar, S., 2013. Green Synthesized Silver Nanoparticles from the Medicinal Plant *Wrightia*

tinctoria and Its Antimicrobial Potential. Inter, J. Chem. Tech. Res, 5 (4), 1707-1713.

Rao, D.R., Mani, T.R., Rajendran, R., Joseph, A.S., Gjanana, A., and Reuben, R. 1995. Development of high level resistance to Bacillus sphericus in a field population of *C. quinquefasciatus* fro, Kochi, India, J. Am. Mosq. Contr. Assoc, 11, 1–5.

Rastogi, L., and Arunachalam, J., 2011. Sunlight based irradiation strategy for rapid green synthesis of highly stable silver nanoparticles using aqueous garlic (*Allium sativum*) extract and their antibacterial potential, Mat. Chem. Phy, 129, 558– 563.

Ravikumar, S., Ramanathan, G., Inbaneson, S.J., and Ramu, A. 2010a. Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*, Parasitol. Res, 108, 107-113.

Ravikumar, S., Abideen, S., Ali, M.S., and Selvam, M.B. 2010b. In vitro human sperm immobilizing activity of marine halophytes, J. Pharm. Res, 4 (4), 1291-1293.

Ravikumar, S., Inbaneson, S.J., Suganthi, P., and Gnanadesigan, M. 2010c. In vitro antiplasmodial activity of ethanolic extracts of mangrove plants from south east coast of India against chloroquine-sensitive *Plasmodium falciparum*, Parasitol. Res, 108, 873-878.

Rogers, J.V., Parkinson, C.V., Choi, Y.W., Speshock, J.L., and Hussain, S.M. 2008. A preliminary assessment of silver nanoparticle inhibition of monkey pox virus plaque formation, Nanoscal. Res. Lett, 3, 129–133.

Sabah, S., and Rajesh P. 2017. Biosynthesis of Silver Nanoparticles for Mosquito Control, British J. Med. Health Res, 4(4), 48-55.

Safaepour, M., Shahverdi, A.R., Shahverdi, H.R., Khorramizadeh, M.R., and Gohari, A.R. 2009. Green synthesis of small silver nanoparticles using geraniol and its cytotoxicity against *Fibrosarcoma-wehi*, Avicenna J. Med. Biotechnol, 1, 111–115.

Shaalan, M., Saleh, M., El-Mahdy, M., and El-Matbouli, M. 2016. Recent progress in applications of nanoparticles in fish medicine: A review Nanomedicine, Nanotech. Biol. and Med, 12, 701–710.

Shahverdi, A.R., Fakhimi, A., Shahverdi, H.R., and Minaian, S. 2007. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*, Nanomed. Nanotechnol, 3, 168-171.

Shankar, S.S., Rai, A., Ahmad, A., and Sastry, M. 2004 Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth, J. Colloid Interface Sci, 275, 496–502.

Silva Paula, M.M.d., Franco, C.V., Cesar, B.M., Rodrigues, L., *et al*., 2009. Synthesis, characterization and antibacterial activity studies of poly-{styrene-acrylic acid} with silver nanoparticles, Mater. Sci. Eng, 29, 647-650.

Singh, A.K., Talat, M., Singh, D.P., and Srivastav, A. 2010. Biosynthesis of gold and silver nanoparticles by natural precursor clove and their fictionalization with amine group, J. Nanopart. Res, 12, 1667-1675.

Song, J.Y., and Kim, B.S., 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extract, Bioproc. Biosyst. Eng, 32, 79–84.

Suresh, U., Murugan, K., Benelli, G., and Nicoletti, M. 2015. Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae), Parasitol. Res, 114, 1551–1562.

Suresh, U., Murugan, K., Panneerselvam, C., Rajaganesh, R., et al., 2017. Suaeda maritima-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector Aedes aegypti and the tobacco cutworm Spodoptera litura, Physiol. and Mol. Plant Pathol, http://dx.doi.org/10.1016/j.pmpp.2017.01.002 0885-5765/©.

Suresh, U., Murugan, K., Panneerselvam, C., Rajaganesh, R., Roni, M., *et al*., 2017. *Suaeda maritima*-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector *Aedes aegypti* and the tobacco cutworm *Spodoptera litura*, Physiol. Mol. Plant Pathol, doi: 10.1016/j.pmpp.2017.01.002.

Taranath, T.C and Patil, B.N., 2016. *Limonia acidissima* L. leaf mediated synthesis of zinc oxide nanoparticles: A potent tool against *Mycobacterium tuberculosis*, , 5 (2), 197-204. doi: 10.1016/j.ijmyco.2016.03.004.

Tennyson, S., Ravindran, K.J. and Arivoli, S., 2012. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera : Culicidae), Asian Pac. J. Trop. Biomed, 2, S1130-S1134.

Thakkar, K.N, Mhatre, S.S., and Parikh, R.Y., 2010. Biological synthesis of metallic nanoparticles, Nanomed, 6, 257-262.

Umesh, B., Jagtap Vishwas, A., and Bapat. 2013. Green synthesis of silver nanoparticles using *Artocarpus heterophyllus* Lam. Seed extract and its antibacterial activity, Indus. Crops and Prod, 46, 132–137.

WHO. 2015. World malaria report, WHO Global Malaria Programme World Health Organization, Geneva, Switzerland.

WHO. 2017. http://www.who.int/mediacentre/factsheets/fs102/en/

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