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Larvicidal Activities of N-(2-Hydroxyl) Propyl-3-Trimethyl Ammonium Chitosan Chloride (HTCC) and Silver Nanoparticles against Two Mosquito Species, *Aedes* and *Culex*: A Comparative Study

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Research Article

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ABSTRACT

This paper mainly focuses on using biopolymer as a potential larvicide. This work initially starts with extracting chitosan from the shrimp waste by a chemical method and it was characterized further. Chitosan is a natural carbohydrate biopolymer derived by deacetylation (DA) of chitin that is nontoxic, biodegradable, and biocompatible. But usually chitosan will not completely dissolved in water at normal conditions. Therefore, to increase the solubility and reaction rate, quaternized derivative of chitosan, N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC), is obtained and characterized. It highly dissolves in water and has more positive charge (quaternary ammonium groups) compared with chitosan. Next the larvicidal activity of the obtained HTCC against two mosquito species, *Aedes* and *Culex*, is evaluated. Next part of the work continues with synthesizing silver nanoparticles from the plant *Euphorbia Antiquorum* and characterized by UV Spec, FTIR, and SEM; then the larvicidal activity is examined similar to HTCC and the results obtained are compared with each other; Though both, silver nanoparticles and HTCC, show similar larvicidal activity, this study suggests HTCC is a better larvicidal agent having in mind the drastic effects of silver nanoparticles on aquatic ecosystem; Nanoparticles in aquatic systems are responsible for agglomeration and aggregation, dissolution, redox reactions and transformation into new solid phases, whereas chitosan and chitosan derivatives are completely degradable, biocompatible, and nontoxic to plants, animals, or human.

INTRODUCTION

World health organization has declared mosquitoes as a public health pest throughout the world as they are responsible for the transmission of various dreadful disease-causing pathogens [1,2]. Though over 3,500 species of mosquitoes exist [3], only few of them are found to be awful, which act as vectors for a number of infectious diseases, belong to the genera *Culex*, *Aedes* and *Anopheles*.

Therefore, control over mosquito population is vital. It is more easier to control mosquitoes when they are in larval stages than controlling the dispersed adults [4] All the three stages of mosquito, i.e., egg, larvae, and pupa, need water for their growth and survival. Since a brood of larval mosquito can be killed while they concentrate in a pool of water. Using chemical pesticides such as DDT, organochlorines, organophosphates, methoprene, etc, were one of the methods followed in killing mosquitoes. Despite high cost, food safety concerns, and environmental hazardous [5,6], mosquitoes also develop genetic resistance [7,8] to these chemicals. Therefore, alternative vector control strategies, especially effective, environmental safe, biodegradable, low

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cost, and indigenous methods are extremely imperative [9-12]. Thereof, investigations and searching of natural and environmental friendly insecticidal substances are ongoing worldwide [13-15].

Biopolymers as Larvicidal Agent

Though silver nanoparticles are Eco synthesized, one of the effective methods widely used, they are researched to be responsible for biomagnification and heavy metal toxicity. Therefore, to overcome the issues of silver nanoparticle, biopolymer materials are used. One among the most applicable biopolymer is chitosan, extracted from the crustacean wastes, that has well-known antibacterial and antifungal activities. More than that, after treatment they break down into monomers and at particular time completely disintegrates. Therefore, this work attempts to examine the larvicidal activity of N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC), synthesized from the chitosan extracted from shrimp shell wastes which is highly positively charged and easily soluble in water against two mosquito species *Culex* and *Aedes*. The obtained results are compared with the activity of silver nanoparticles synthesized from the plant *Euphorbia antiquorum.*

MATERIALS AND METHODS

Synthesis of Silver Nanoparticles

Ten grams of fresh plant was washed and chopped in to pieces, later it was boiled in 100 ml deionized water for 10 minutes. The extract was then cooled and filtered using Whatman No 1 filter paper. 10 ml of this plant extract was added 90 mL of 1Mm silver nitrate solution. The solution was allowed to react at room temperature, after 24 h color change was observed from pale yellow to brick red. The reduction mechanism of silver ions in to nanoparticles was confirmed through the UV-visible spectrophotometer analysis at 420 nm. It is centrifuged at 13000 rpm and the pellet was air dried and collected in an air-tight eppendorf tube. Thus-obtained silver nanoparticles were characterized using UV-Vis spectrometer and SEM.

Extraction and Characterization of Chitosan and HTCC from Shrimp Waste

Ten grams of shrimp shell powder is mixed with 4% NaOH for 21 h and washed several times until neutral pH and dried at 60˚C for 3 hours. It was followed by demineralization using 2% HCl for 2 h at 70˚C. It was washed several times and the left out thus obtained is chitin. Thus-obtained chitin is DE acetylated using 60% NaOH for 72 hours. After deacetylation samples are cooled at room temperature and washed several times using distilled water until neutral pH, the remaining is the chitosan that is oven-dried completely for further use. HTCC was synthesized according to a known method [16].

Selection and Culturing of Mosquito Species

Mosquito species *Aedes* aegypti and *Culex* were selected for this study. Entire analyses were carried out against laboratory-reared vector mosquitoes which are free of insecticidal and pathogenic exposure.

Larvicidal Bioassay of Silver Nanoparticles

Larvicidal activity of silver nanoparticles was performed using a standard protocol of WHO. Synthesized nanoparticles were diluted to 5.0, 4.0, 2.0, 1.0, and 0.5 mg/l using double distilled water. Ten larvae of each species is added to each test solution with beakers containing 200 ml of water, and control is a beaker with distilled water. The mortality was recorded after 24 and 48 h and the experiment was repeated for four times for average value. Percentage mortality of both experiments was recorded using Abott's formula.

$$\%\text{Motality} = \frac{\%\text{test molarity} - \%\text{control molarity}}{100 - \%\text{control molarity}}$$

Larvicidal Bioassay of HTCC

Larvicidal bioassay of HTCC was carried out according to WHO standard procedures with slight modifications. Different concentrations ranging from 200 to 1000 ppm of HTCC were redissolved in 200 ml of tap water of 250 ml beakers and 10 larvae per concentration were used for all the experiments. A control was 200 ml of water. The number of dead larvae at the end of 24 and 48 h was recorded.

RESULTS

Characterization of Silver Nanoparticles

Color conformation: Change of the solution color from pale yellow to brick color after 48 hours initially confirms the formation of silver nanoparticles, as shown in **Figure 1**.

UV-VIS Spectrometer

Figure 2 shows the absorption spectra of AgNPs formed in the reaction media that have absorption maxima at 450 nm due to surface plasmon resonance of AgNPs confirmed the formation of silver nanoparticles.

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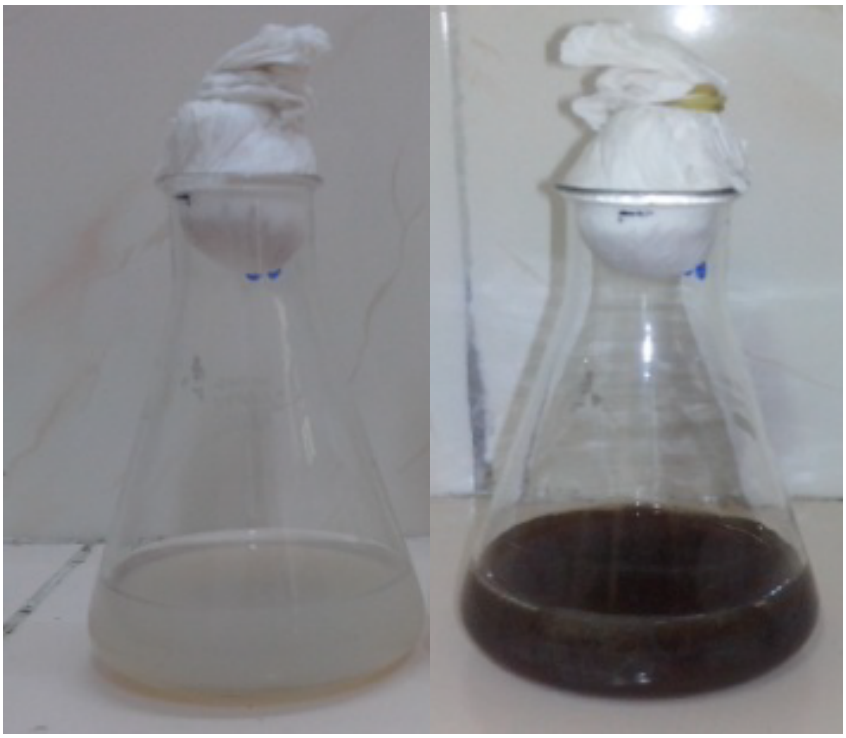

Figure 1. Formation of silver nanoparticles by color changing from pale yellow to brick red after 48 h.

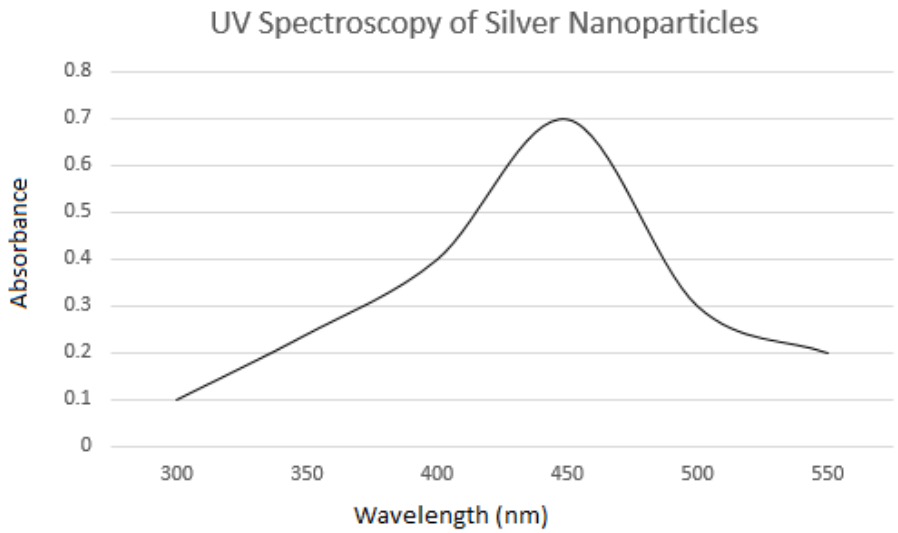

Figure 2. UV Spectroscopy of silver nanoparticles synthetized from *Euphorbia antiquorum*.

SEM

Figure 3 shows the SEM of silver nanoparticles synthesized from Euphorbia Antiquorum. The silver nanoparticles were spherical in shape with particle size range from 5 to 40 nm and they are aggregated to each other.

Characterization of Chitosan and HTCC

Table 1 shows color, yield percentage, ash content, molecular weight, degree of deacetylation, solubility, water and oil absorbing percentage, pH and viscosity of the extracted and obtained chitosan and HTCC.

FTIR of Chitosan and HTCC

Figure 4 shows FTIR peak of chitosan. There is a broad band at 3438 cm^{-1} that is because of $-NH^2$ groups, -OH groups, and intermolecular hydrogen bonds overlap each other. The peak at 2991 cm^{-1} represents the CH stretch [17]. Peak at 1639 cm^{-1} shows the presence of carbonyl group, which is due to incomplete deacetylation of chitin to chitosan. A significant peak is also observed at 1560 cm^{-1} indicates N-H bending of the primary amine.

Figure 5 shows FTIR of HTCC with a new peak at 1470 cm^{-1} represents the C-H bending of trimethylammonium group of HTCC. It should also be noted that the peak at 1560 cm^{-1} in chitosan is disappeared in HTCC due to the change of primary amine

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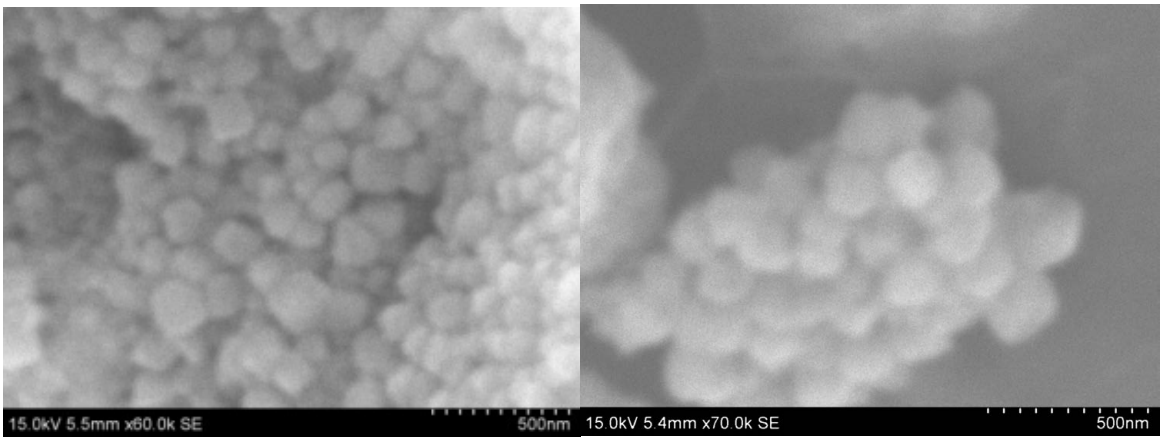

Figure 3. SEM Image of Silver nanoparticles synthetized from Euphorbia antiquorum.

Table 1. Physical Properties of Chitosan and HTCC extracted from shrimp shell.

S No	Properties	Chitosan	HTCC
1	Color	Creamy White	Creamy White
2	Yield	21	–
3	Ash content	0.8%	–
4	Molecular weight	10,197.49	–
5	Degree of Deacetylation	76%	–
6	Solubility	35%	98%
7	WBC%	498.7%	864.8%
8	OBC%	356%	665.9%
9	Ph	8.9	7.2
10	Viscosity	298cps	

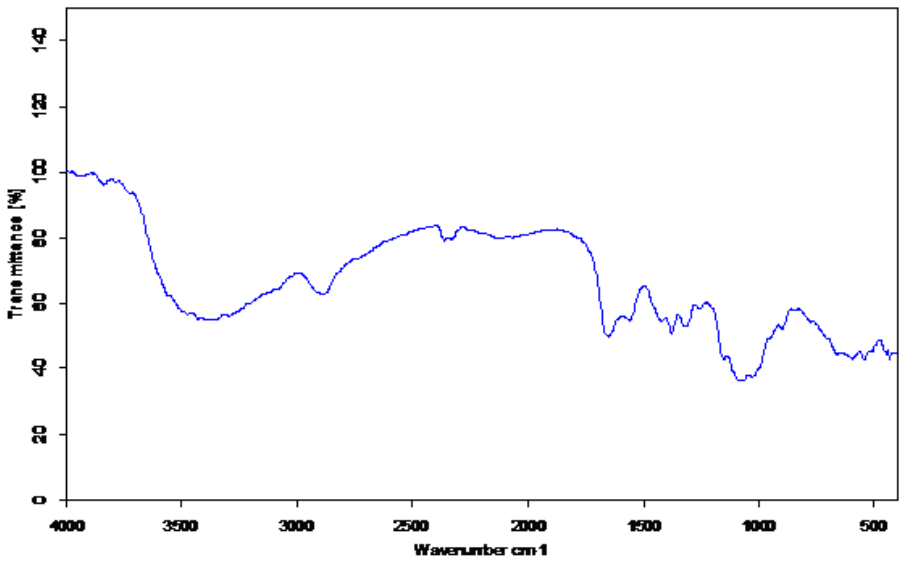

Figure 4. FTIR peak of Chitosan Synthesized from Shrimp waste.

in chitosan to secondary amine in HTCC. The peak at 3430 cm^{-1} confirms the N-H stretching of a secondary amine and this peak proves the synthesis of HTCC by forming N-H group.

From the experiments carried out, *Aedes* was found to be resistive than *Culex* to both silver nanoparticles and HTCC and the rate of mortality was dose and time dependent.

Table 2 shows the larvicidal activity of silver nanoparticles on *Aedes* and *Culex* at 24 and 48 h, respectively. It can be seen that percentage of mortality increases with increasing concentration. In *Culex*, at least concentration, 0.5 ppm, the mortality was 10%; whereas at highest concentration, 5 ppm, mortality was 100%. In case of *Aedes*, it was 6% and 100% at 0.5 and 5pmm, respectively. Therefore, at all concentrations, *culex* is more susceptible than *Aedes*.

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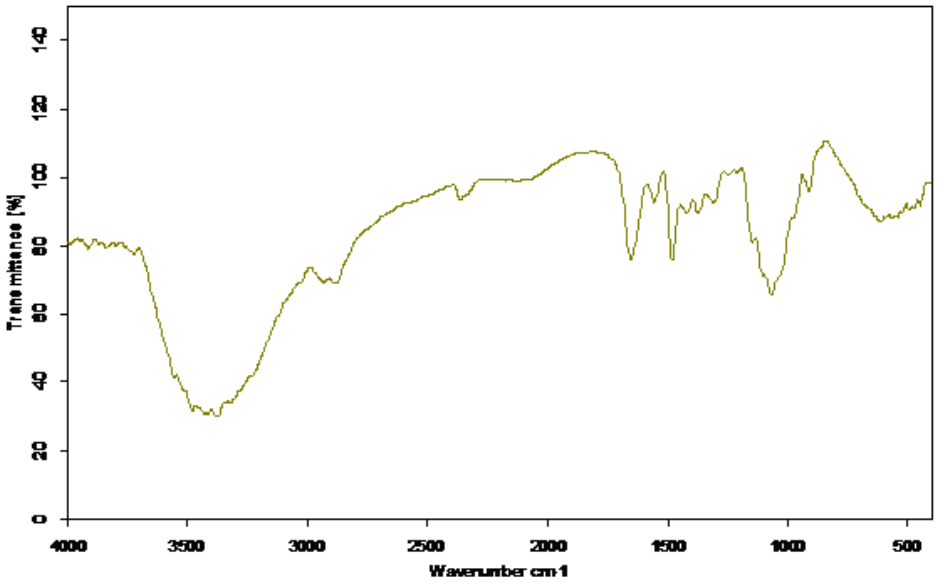

Figure 5. FTIR peak of HTCC synthesized from Chitosan.

Table 2. Percentage Mortality of Silver Nanoparticles on Culex and Aedes at 24 h.

Concentration	Culex	Aedes
	24 h	**24 h**
5.0	100%	100%
4.0	82	78%
2.0	60	58%
1.0	33	28
0.5	10	6

Table 3 shows the percentage mortality of HTCC against *Aedes* and *Culex*. As before, *Culex* is more susceptible than *Aedes* and mortality increased with increasing concentration. *Culex* showed 12% mortality at low concentration and 100% at highest concentration. *Aedes* showed 10% mortality at low concentration and 100% at highest concentration, respectively. Both Silver nanoparticles and HTCC showed 50% mortality at 2 ppm and 600 ppm, respectively.

Table 3. Percentage Mortality of HTCC on Culex and Aedes at 24 h.

Aedes	Culex	
Concentration	**24 h**	**24 h**
5.0	100%	100%
4.0	82	70%
2.0	54	50%
1.0	28	20
0.5	10	10

Table 4 shows lethal concentration (LC50 and LC90) values of the silver nanoparticles and HTCC on *Culex quinquefasciatus* and *Aedes aegypti.* The LC50 values of silver nanoparticles and HTCC on *Culex quinquefasciatus* were 1.179 and 2.212, respectively; 2.392 and 2.097, respectively, for *Aedes aegypti*. The LC90 values are 4.555 and 4.521 for *Culex* and 5.129 and 4.428 for Ades treated with Silver nanoparticles and HTCC, respectively, and the chi-square values are significant at p.

DISCUSSION

Larvicidal activity of silver nanoparticles was already reported by literature [12-16] Besides the larvicidal activity, negative impact of silver nanoparticles on the aquatic life after treatment is more concerned. The most important processes affecting the fate of nanoparticles in aquatic systems are agglomeration and aggregation, dissolution, redox reactions and transformation into new solid phases [18-22] though used in very less quantity. The use of nanomaterials and their potential environmental and human health risks [23] are of increasing concern and social debate [16,24] and have been the subject of many government reports.

There are several reports evidenced the drastic effects of nanoparticles on aquatic environment. Julia Fabrega, et al. reported that concentrations of Ag NPs, as low as, just a few ng/L, can affect prokaryotes, invertebrates, and fish and also they also studied the mechanisms of toxicity. researched Ag ion toxicity in vivo in freshwater fish species with LC10 values as low as 0.8 µg/ L had effect on certain freshwater fish species [25].

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Table 4. Lethal concentration (LC_{50} and LC_{90}) values of the silver nanoparticles and HTCC on *Culex quinquefasciatus* and *Aedes aegypti.*

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit	LC_{90} (ppm)	95% confidence limit	Intercept ± SE	Slope Pt ± SE	χ^2		
			LL	UL		LL	UL			
Culex quinquefasciatus	Silver nanoparticles	1.179	0.837	1.456	4.555	3.812	6.001	3.6 ± 0.26	3.95 ± 0.56	6.9*
	HTCC	2.212	1.882	2.532	4.521	3.810	5.880	3.57 ± 0.27	4.12 ± 0.58	5.1*
Aedes aegypti	Silver nanoparticles	2.392	2.034	2.749	5.129	4.243	6.954	3.53 ± 0.27	3.86 ± 0.56	7.3*
	HTCC	2.097	1.766	2.414	4.428	3.711	5.811	3.73 ± 0.25	3.94 ± 0.56	5.4*

Therefore, completely nontoxic biocomponent is indeed for treating mosquito larvae which reside in water bodies. Chitosan is a polysaccharide which forms the basis of the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs, and lobster [26]. There are several studies evidenced the antimicrobial activity of chitosan [27,28] and also reported that chitosan has effective antifungal activity which inhibits spore germination, germ tube elongation, and radial growth. Few works also evidenced antiprotozoal activity of chitosan [15,29]. The killing mechanism is due to the interaction mediated by the electrostatic forces between the protonated NH^{+3} groups and the negative residues [30], presumably by competing with Ca^{2+} for electronegative sites on the membrane surface of the microbes.

To improve further the solubility of chitosan in water, various derivates of chitosan were prepared. Among the various chitosan derivatives, the derivatives with quaternary ammonium groups have shown higher efficient activity against microbes compared to those of chitosan [31,32]. This may be due to enhanced positive charged quaternary amine group, known to targeted on the negatively charged cytoplasmic membrane of microbes, altering membrane properties and impeding nutrients entering the cells [33,34]. Therefore, same mechanism may occur in killing mechanism of mosquito larvae [35-40].

From the results it can be concluded that HTCC has equivalent and better larvicidal activity when compared with silver nanoparticles [41-46], which shows 100% mortality 1000 ppm in both *Aedes* and *Culex*. And the activity can also further enha nced by synthetizing HTCC nanoparticles [47-50].

CONCLUSION

This study compares the larvicidal activity of three silver nanoparticles and HTCC. Despite equal efficacy, this study suggests using HTCC as a larvicidal agent is advisable and more beneficial; because chitosan is completely degradable, biocompatible, and nontoxic to plants, animals, or human. This study can be further extended in future by synthesizing HTCC nanoparticles and examining its larvicidal activity. This work also aims to explore the parasitic activity of HTCC in future studies.

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