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Green synthesis of silver nanoparticles using chitosan extracted from *Penaeus indicus* and its potential activity as aquatic larvicidal agent of *Culex pipens*

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ABSTRACT

In the present study, silver nanoparticles (AgNPs) were greenly synthesized using two different reducing/capping agents, chitosan (Cs) and Molar Hinton Broth (MHB) agar. With the use of Fourier transform infrared spectroscopy (FTIR), the potential functional groups responsible for reducing/capping silver ions were determined, as well as the characterization and computation of the degree of deacetylation of the produced chitosan employed in AgNPs synthesis. The synthesized silver nanoparticles were validated and described using ultra-violet (UV–Vis) spectrophotometric analysis for surface plasmon resonance and a transmission electron microscope (TEM). Also, the AgNPs solutions were evaluated to combat the aquatic larvae of *Culex pipens*. The results revealed that silver ions can be reduced by both chitosan and MHB agar to generate well stable silver nanoparticles solution although chitosan has weaker reduction properties. UV–vis spectrum for both AgNPs solutions showed a single absorption peak of 400 nm range indicating that the nanoparticles exist and are spherical in shape. TEM analysis showed both AgNPs are round in shape with an average size of 5 to 15 for MHB-AgNPs and from 10 to 50 for chitosan silver nanoparticles Cs-AgNPs. The findings of combat of aquatic larvae of mosquito showed the superiority of CS-AgNPs over MBH-AgNPs in killing mosquito larvae with LC_{50} (279.33, 321.33, and 367.37 µM) and LC_{90} (468.85, 505.84, and 568.07 µM) while (MBH-AgNPs) showed less effectiveness with LC_{90} (483.87, 543.12, and 611.4 µM) and LC_{90} (870.23, 811.97, and 967.91 µM), respectively. In conclusion, the greenly synthesized silver nanoparticles have the potential to be used as an alternative eco-friendly larvicide for mosquito control in its larval aquatic stages.

INTRODUCTION

The development of well-established process for the green synthesis of nanoparticles is an important aspect of current nanotechnology research (**Hasabllah *et al.*, 2021a**) since greenly synthesized silver nanoparticles (AgNPs) are highly effective and eco-friendly alternative to chemical and physical methods (**Parveen *et al.*, 2016**). Generally, AgNPs have distinctive physio-chemical properties, including a high electrical

and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity, and nonlinear optical behavior; besides, AgNPs exhibit broad spectrum antibacterial and antifungal activities that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, and pastes. Nano silver can also be utilized either in the textile industry or employed in filtration membranes of water purification systems (**Tran and Le, 2013**).

The biological and chemical diversity of marine environments are among the richest and most complex ecosystems (**Mona *et al*., 2019; Shaban *et al*., 2020, Darweesh *et al*., 2021 a,b; El-Nagaar *et al*., 2022a**). Many of marine organisms have huge nutritionally important substances, and they use an arsenal of chemical defenses to protect themselves (**Ibrahim *et al*., 2017; Metwally *et al*., 2020**). Bioactive substances isolated from marine organisms possess several biological activities such as anti-microbial, anti-inflammatory, anti-fungal, anti-cancer, and other activities (**Cui *et al*., 2020; EL-Naggar *et al*., 2022b**). Despite of the efforts have been exerted by the scientific communities to isolate and characterizethe biologically active natural products from marine sources, nowadays there are still many compounds that have yet to be discovered that can be help solving various health problems and environmental in the future (**El-Damhougy *et al*., 2017; Hasaballah *et al*., 2021b**).

Chitosan is a polysaccharide is commercially syntheses via deacetylation of chitin, that the structural component of crustaceans' exoskeleton. Its use in medical and pharmaceutical sector has grown rapidly due to its interesting properties such as strong antibacterial, biocompatibility, biodegradability, non-toxicity and high humidity absorption. Furthermore, other biological properties such as analgesic, antitumor, haemostatic, hypocholesterolaemic and antioxidant properties have also been reported in recent studies on chitosan (**Victor *et al*., 2020**).

Many researchers have investigated of chitosan as a potential accelerator for wound healing (**Muzzarelli *et al*., 1988**). Chitosan can be used in dentistry for treating oral mucosal sores. Also, it is been discovered that chewing chitosan-containing gum inhibits the growth of cryogenic bacteria (**Hayashi *et al*., 2007**). Chitosan that derived from shrimp shells is a better broad spectrum antibacterial agent (**Teli *et al*., 2012**). Chitosan silver nanoparticles (Cs-AgNPs) are intriguing due to their unique characteristics, which can be used in antimicrobial and antifungal (**Ashrafi *et al*., 2020**). Moreover, Cs-AgNPs have a good effect as an antifungal against *Neoscytalidium dimidiatum* (**Ngoc and Nguyen, 2018**) and used for cancer therapy (**Manjusha *et al*., 2010**). Recently, **Alebouyeh *et al*. (2020)** stated that the chitosan nanoparticles extracted from the shirmp (*Penaeus semisulcatus*) is highly inhibiting the growth of Listeria monocytogenes and Salmonella typhi. It also can be used as a flocculant to water purify (drinking and pool water) and metals removal from water (**Mohanasrinivasan *et al*., 2014**). Also, chitosan derivatives have promise applications in food sciences (**Luo and Wang, 2013**). It forms protective, fungistatic, and antibacterial coating for vegetables and

fruits (**Jianglian and Shaoying, 2013**). Chitosan derivatives have huge nanotechnology application. Chitosan has high solubility, low toxicity, and versatile routes make it an idealistic matter for nanotechnology.

Mosquito is serious insect not only as discomfort biter, but also as a vector of significant diseases including dengue fever, filariasis, and malaria. *Culex* mosquito species are excellent vector of West Nile virus (**Fonseca *et al*., 2004; Hasaballah and El-Naggar, 2017; El-Mehdawy *et al*., 2021**), Zika virus infections in Latin America and the Caribbean and lymphatic filariasis parasitic worm (**El-Naggar and Hasaballah, 2018**) circulating diseases among human and animals causing massive damage in both economy and human health. Presently, more than 1.4 billion human beings in 73 countries are living in areas where lymphatic filariasis is endemic and facing the risk of being infected (**WHO, 2015; Attia *et al*., 2021**). The control of mosquito is the only way to management and prevents these diseases, according to the World Health Organization.

The life cycle of mosquito contain four stages: egg, larva, pupa, and adult. The first three stages are aquatic giving high opportunity forth success of combat it in its natural aquatic habitat. Since a long time, insecticides have been widely utilized to control of mosquitos. The hunt for eco-friendly insecticide alternatives has become increasingly urgent due to their negative health and environmental effects. Culex mosquito had developed some kind of resistance over years against traditional larvicides along with its toxicity problems. In the matter of facts, resistance strains of *Culex pipiens* larvae to organophosphates insecticides was recorded in Egypt (**Zayed *et al*., 2006; Hasaballah *et al*., 2021b**), so there is need to explore new larvicides have more effectiveness and less harmful environmental impact.

Therefore, the aim of this research is greenly synthesis of silver nanoparticles using two different reducing/capping agents (MHB) agar and chitosan and investigates their larvicidal activities against the aquatic larvae of *Culex Pipens*.

MATERIALS AND METHODS

2.1. Raw materials of chitosan preparation

Raw materials of chitosan preparation were obtained by collect the crusts of Indian white prawn *Penaeus indicus* H. Milne Edwards, 1837. The Indian white prawn was brought from prawn farm at Muthallath El-Deeba region at Damietta on the North East coast of the Mediterranean, Egypt. The current work was carried out during 2020.

2.2. Preparation of chitosan.

Two major steps involved in preparation of chitosan from shrimp shells

1. Extraction of chitin

- The shells of shrimps were scraped free of loose tissue, washed, and dried for three days under sunlight. The dried shells were blended by electric blender to create shrimp shells powder that passes from 300 m mesh size sieves.

- The prepared powder of shrimp shells was weighted then deproteinizationed using NaOH (2N) with ratio of 1:12 (w/v) at 70°C. This treatment lasted for four hours. The residue was collected using filter paper, rinsed with distilled water, and dried to a consistent weight in a 50°C oven.
- The weighted deproteinized product was immediately demineralized with a diluted HCl solution (10 %) in a 2.5:10 (w/v) ratio for 24 hours at room temperature. With distilled water, the demineralized product was washed. The residue was collected by filter paper and dried in oven at 50°C to a constant weight.
- The product from demineralization process was weighted and decolorized by placing it in acetone in room temperature for 24 hrs. Herein, the end product is the shrimp shell chitin.

2. De-acetylation of chitin for formation of chitosan.

To obtain chitosan powder, the final product from decolorization (chitin) was exposed to N-deacetylated using NaOH (12.5N) with ratio of 1:20 (w/v) at different temperatures for different times. The residue was washed with distilled water and collected by filter paper and dried in oven at 50°C to a constant weight. The final product from this process is chitosan.

2.3. Characterization of the prepared chitosan.

Chitosan was characterized using Fourier Transform-Infrared (FTIR) analysis, which was conducted using a JASCO-FT-IR-4600 with a scanning range of 4000 to 400 cm^{-1} to analyse the presence of functional groups in the chitosan.

2.4. Synthesis of silver nanoparticles using Molar Hinton broth (MHB) agar.

To synthesis of silver nanoparticles using MHB agar, 1.0 gm of Molar Hinton Broth (MHB) agar was dissolved in 50 ml deionized water to make soluble media and then the media was sterilized in an autoclave at 120ºC for 15 min. The pH of this media was adjusted to 10 by added NaOH (2M) and measured by pH meter. A stock of 0.1 M silver nitrate aqueous solution was prepared by carefully dissolved of $AgNO_3$ (0.16987 gm.) in deionized water (10 ml) in a dark conical flask. Silver nitrate solution was added to the previously prepared MHB media for reach the final concentrations of silver ion to 1×10^{-3} mM. The solution was then heated at 60°C with constant stirring until it turned brown.

2.5. Synthesis of silver nanoparticles using chitosan.

For synthesis of silver nanoparticles using chitosan, 0.25 g of already synthesized chitosan was dissolved in 50 ml of 1% (v:v) ascorbic acid under magnetic stirring for 90 minutes at room temperature to gain 0.5% (w:v) chitosan solution. A stock of 0.1 M silver nitrate aqueous solution was prepared by carefully dissolved of $AgNO_3$ in deionized water in a dark conical flask. To reach the ultimate concentrations of silver ions of 1×10^{-3} mM, the silver nitrate solution was added to the previously produced chitosan. After that, 3 ml of NaOH (2 M) was added to the solution drop by drop with constant stirring until a brown gel was obtained. The brown gel was dissolved in ascorbic acid

(1% v/v) and agitated until completely dissolved to create silver/chitosan nanocomposite colloidal, and pH was readjusted to 5 by using 1.0 ml acetic acid.

2.6. Characterization of the Prepared Nanomaterials

The localized surface plasmon resonance (LSPR) phenomenon of synthesized silver solutions was examined to assure the generation and structure of silver nanoparticles (AgNPs) by using UV-Vis spectrophotometer BioTek power wave XS2 Ultraviolet-visible (UV-Vis) spectroscopy at 300 - 600 nm wavelength. The JEM–2100 electron microscope was used to conduct the transmission electron microscopy (TEM) analysis. Finally, FTIR measurements were performed using a JASCO-FT-IR-4600 with a scanning range of 4000 to 400 cm^{-1} to determine the existence of different functional groups involved in Ag^{+} bio-reduction and silver nanoparticle capping.

2.7. Control of aquatic larvae of mosquito by chitosan- AgNPs

2.7.1. Collection and culture of *Culex pipiens* mosquito

Immature stages (larvae and pupae) of *Culex pipiens* mosquito were collected from the fish farm in St. Makarios monastery, Beni Salama, Al-Natron Valley, El-Beheira, Egypt (30º17'29.9" N, 30º28'32.3' 'E). The larvae were identified according to the keys described by (**Harbach, 1985**) and reared for six generations in Mosquito Insectary Lab., Animal House, Department of Zoology, Faculty of Science, Al-Azhar University under controlled conditions as temperature (27±2ºC), relative humidity (70±10%), and photoperiods (12:12) using a standard procedure described by **Hassanain *et al*. (2019)**.

2.7.2. Larvicidal bioassay.

Larvicidal bioassay was carried out according to the procedure of WORLD HEALTH ORGANIZATION (**WHO, 1996**). Different concentrations of each tested nanomaterials (AgNPs) were prepared in volume of 200ml using dechlorinated tape water and placed in 300 ml beakers. Subsequently, twenty of 3^{th} instar larvae of *C. pipiens* were immediately placed into each beaker. The larvae were deprived of food during their AgNPs exposure to ensure the effect was only happened by body penetration. Three replicates were usually used for each tested concentration. All beakers were incubated under the controlled conditions of the mosquitos' colony. Survived larvae were recorded subsequently after 24, 48, and 72 hr. Control larvae survived in 200ml dechlorinated tap water alongside.

2.8. Required chemicals.

Some chemicals were required to accomplish the experiment such as sodium hydroxide (NaOH) with molecular weight 40, hydrochloride acid (HCl) with molecular weight 36.46, Molar Hinton Broth agar, Silver nitrate ($AgNO_3$) with molecular weight 169.87, ascorbic acid (molecular weight 176.12) and glacial acetic acid (molecular weight 60). All using chemicals were provided from Sigma-Aldrich co., Cairo, Egypt.

2.9. Statistical analysis.

Probity analysis were applied to average survived larvae values for calculating LC50 and LC90 at 95% lower and upper confidence limits. All statistical analyses were done using Statistical Package Social Science (SPSS) software version 11.5.

RESULTS AND DISCUSSION

3.1. Characterization of chitosan.

The FTIR analysis performed to assess the functional groups present in the chitosan (Figure 1). The band in the 3437 cm^{-1} region correlates to N-H and O-H stretching, in addition to the intra-molecular hydrogen bonds. C-H symmetric and asymmetric stretching could be responsible for the absorption bands about 2922 and 2876 cm^{-1}. These bands are characteristics of polysaccharide spectra such as glucans (**Wolkers *et al*, 2004**) and xylan (**Melo-Silveira *et al*, 2012**). The presence of residual N-acetyl groups (amide I) was confirmed by the band around 1655 cm^{-1}. The N-H bending of the primary amine is represented by the band at 1589 cm^{-1}. The appearance of bands near 1429 corroborated the CH2 bending and CH3 symmetrical deformations. The asymmetrical stretching of the C-O-C bridges could explain the absorption band at 1152 cm^{-1}. C-O stretching is represented by the band at 1078 cm^{-1}. All previous bands are found in samples spectra of chitosan reported by **Song *et al*. (2013)** and **Fernandes *et al*. (2015)**. In spite of the investigated chitosan was prepared from animal origin, however it didn't contaminated by glycosaminoglycans (GAGs), GAGs are sulphate, and the existence of sulphate groups covalently bound to the polysaccharide can be verified by the appearance of a very significant band about 1270 cm^{-1} in the infrared spectra. The signal at 1270 cm^{-1} in the spectrum obtained from produced chitosan is very modest and so does not correlate to sulphate groups, ruling out chitosan contamination by GAGs. This band at 1270 cm^{-1} was assigned as the bending vibrations of hydroxyls present in chitosan. The signal around 874 cm^{-1} corresponds to the CH bending out of the plane of the ring of monosaccharides (**Fernandes *et al*, 2015**)

3.2. Degree of deacetylation (DDA)

Degree of deacetylation (DDA) of the chitosan means how much chitin is N-deacylated to chitosan. The produced chitosan's FTIR spectrum can be used to calculate the average Degree of Deacetylation (DDA) using several equations such as:

1. $DDA\ \% = A_{1655}/A_{3430} \times 100/1.33 = 92/86 \times 100/1.33 = 80.4\ \%$ (1)
2. $DDA\ \% = A_{1655}/A_{2870} \times 100/1.33 = 92/93{,}5 \times 100/1.33 = 73.9\%$ (2)

Where A_{1655} is the absorbance at 1655 cm^{-1} of the amide I band as a measure of the N-acetyl group content while A_{3430} is the absorbance at 3430 cm^{-1} due to hydroxyl group and A_{2870} is the absorbance at 2870 cm^{-1} referred to C-H symmetric and asymmetric stretching as an internal standard. The value 1.33 represents the ratio of this absorbance for a fully acetylated compound (**Czechowska-Biskup *et al*., 2012**).

3. $DDA\ \% = 100 - (A_{1320}/A_{1420} - 0.3822) \times 1/0.0313$

= 100- (95/91-0.3822) × 1/0.0313 = 78.9% (3)

Where band at 1320 is characteristic of the acetylated amine and the other band 1420 $cm^{-1}$is used as the reference band. (**Fatima, 2020**).

DDA = Average of (1), (2), (3) = 77.73%

3.3. Characterization of silver nanoparticles

3.3.1. Ultra-violet (UV) - Visible.

To confirm the formation and shape of AgNPs, the phenomenon of LSPR was investigated. LSPR was performed for both Molar Hinton Broth agar capped-silver (MHB-AgNPs) and Cs-AgNPs nanoparticles solutions. The results showed single absorption peak of UV-Vis spectra in 400 nm ranges for both solutions (410 nm for MHB-AgNPs, 430nm for Cs-AgNPs) as shown in Figure (2). The particles are existed and spherical in shape (**Kathiresan *et al*., 2009; Susilowati, 2019**).

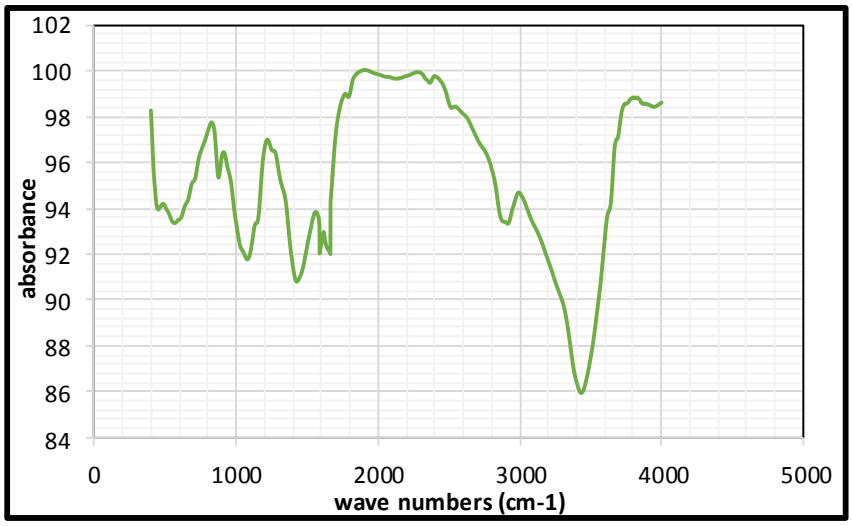

Fig 1. diagram shows FTIR, analysis of chitosan

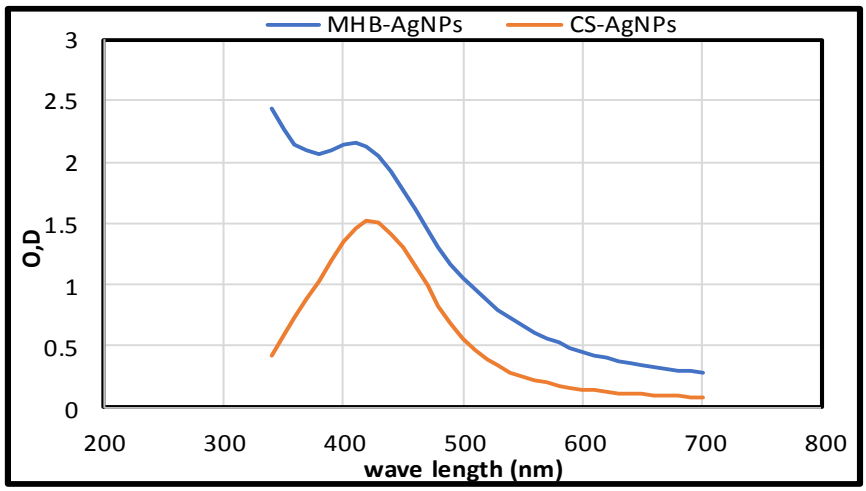

Fig 2. UV-Vis spectra in 400 nm range for MHB-AgNPs and Cs-AgNPs).

3.3.2. Transmission electron microscope (TEM)

It is clear from the Figures (3-6) that TEM pictures showed both AgNPs are round in shape with average size of 5 to 15 for MHB-AgNPs and from 10 to 50 for Cs-AgNPs. Despite both silver nanoparticle's solutions are prepared greenly, the average size of Cs-AgNPs is relatively big due to the weakness of chitosan as reducing agent (**López-Carballo *et al*., 2013; Susilowati and Maryani, 2018**).

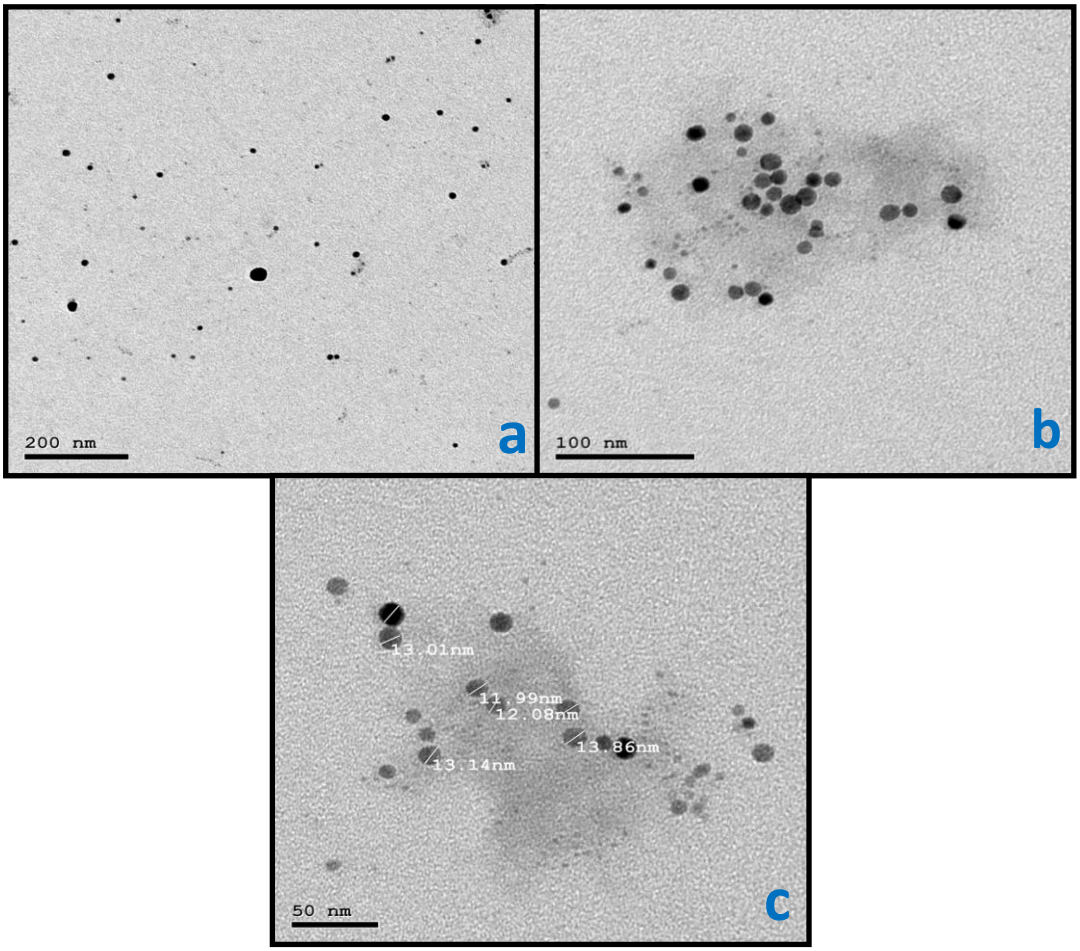

Fig 3. TEM images of MHB-AgNPs with scale bars of a) 200 nm, b) 100 nm and c) 50 nm

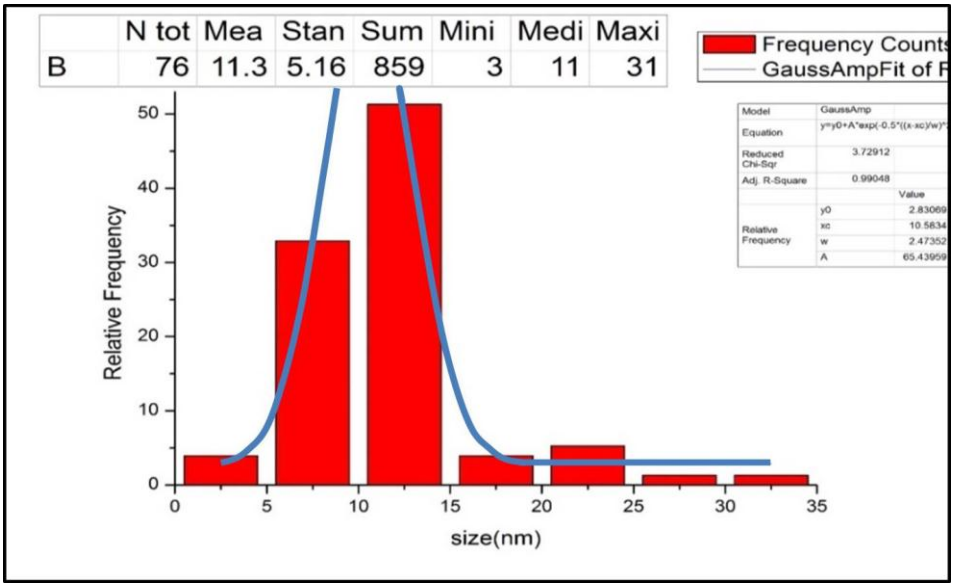

Fig 4. The average size of MHB-AgNPs.

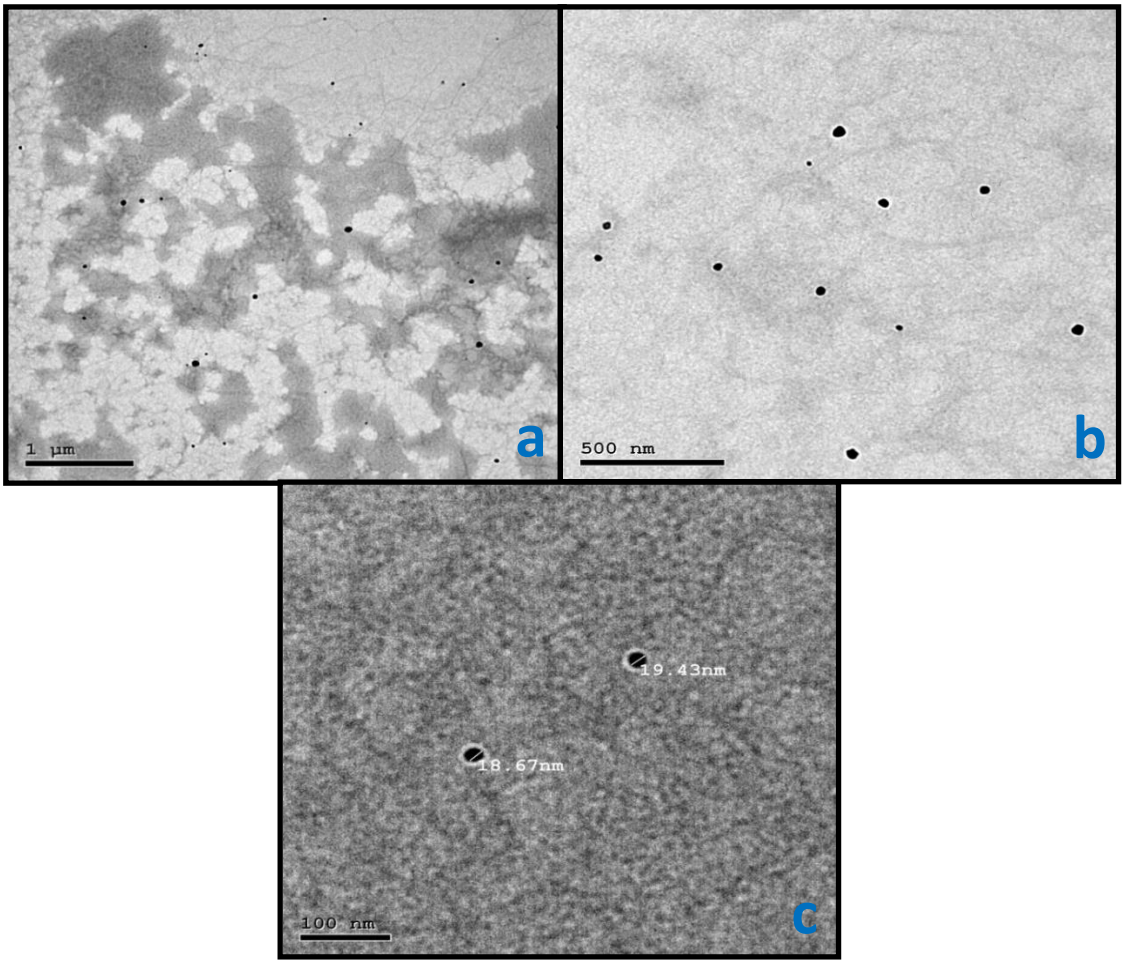

Fig 5. TEM images of CS-AgNPs with scale bars of a) 1.0 µm, b) 500 nm and c) 1000 nm

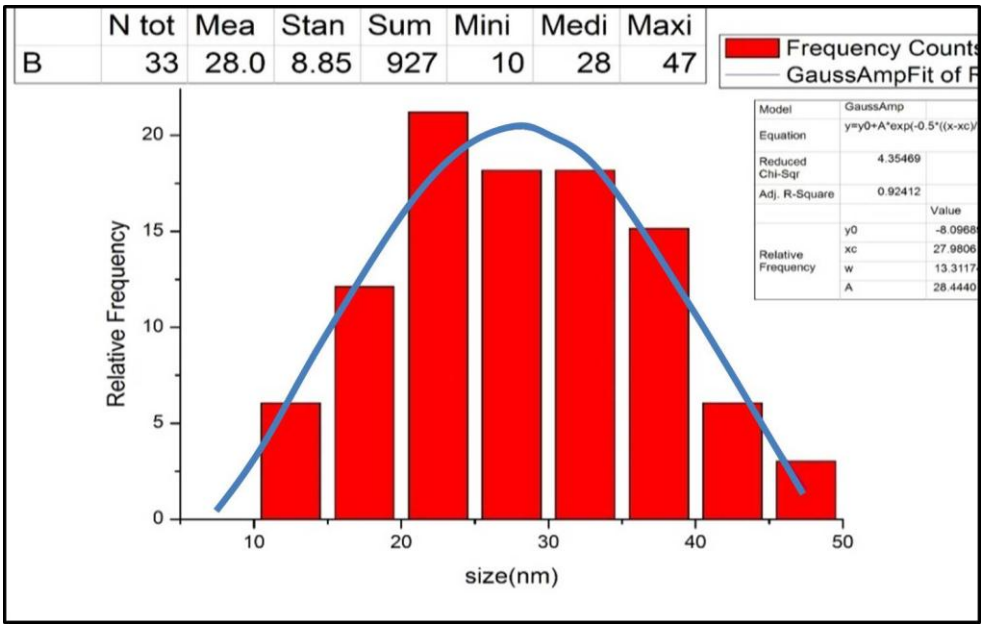

Fig 6. The average size of CS-AgNPs.

3.3.3. Fourier transform-infrared (FTIR).

FTIR analysis carried out to identify the presence of various functional groups responsible for the bio reduction of Ag^{+} and capping/stabilization of silver nanoparticles. As shown in Figure (7), both silver nanoparticles solutions spectra showed distinctive bands that are responsible for reducing/capping of silver ions. MHB-AgNPs spectrum has bands at 3934, 3811, 3436, 2918, 2851, 1632, 1460, and 1042 cm^{-1} while Cs-AgNPs spectrum exhibits bands at 3442, 1640, 1542, 1414, 1346, 1053, and 650 cm^{-1}. **Thomas *et al*. (2014)** observed that the bands around 3934, 3811, 3442, and 3436 cm^{-1} correspond to -OH stretching of the hydroxyl group of alcohol and phenolic-compounds. While, **Jyoti *et al*. (2016)** noticed that the bands observed around 2918 and 2851 cm^{-1} region arising from C–H and C–H stretching vibration of aromatic compound. Whereas, **Santhoshkumar *et al*. (2011)** found that the bands around 1632 cm^{-1} indicate the presence of proteins since they correlate to (NH) C=O stretching vibrations. In addition, the bands at 1640 cm^{-1} and 1542 cm^{-1} correlate to the binding vibrations of proteins' amide I and amide II, respectively **(Shankar *et al*., 2003).** Also, **Jyoti *et al*. (2016)** discovered that the bands at 1460, 1057, and 1042 cm^{-1} were allocated to N–H and C–N stretch vibrations of proteins (amines), respectively, while **Ahmad *et al*. (2011)** discovered that the band at 1346 cm^{-1} was allocated to C–H bending vibrations. As a result, the band at 1414 cm^{-1} correspond to aromatic amine C–C stretching vibrations, but the band at 650 cm^{-1} correspond to stabilized silver nanoparticle C–H bending vibrations, showing that chitosan is engaged in the stabilisation process (**Momin *et al*., 2019; Vijayakumar *et al*., 2020**).

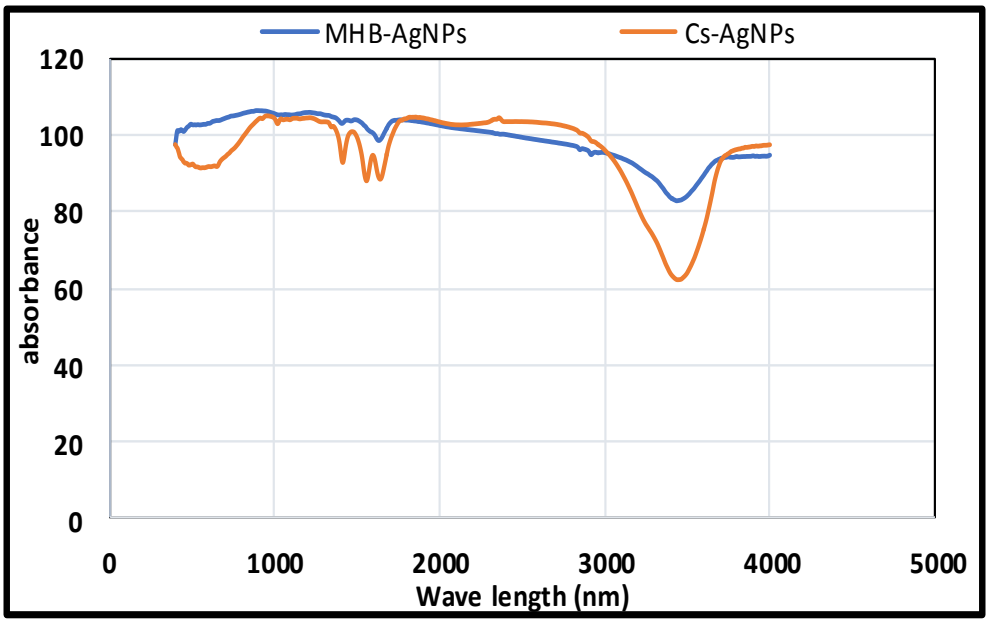

Fig. (7): FTIR analysis for both CS-AgNPs and MBH-AgNPs

3.4. Larvicidal bioassay

As indicated in the Tables (1 and 2), CS-AgNPs were shown to be superior to MBH-AgNPs in killing mosquito larvae. The CS-AgNPs exhibited LC_{50} values (279.33, 321.33, and 367.37 µM) and LC_{90} values (468.85, 505.84, and 568.07 µM) higher than MBH-AgNPs that showed less effectiveness LC_{50} values (483.87, 543.12, and 611.4 µM) and LC_{90} values (870.23, 811.97, and 967.91 µM), as shown in Table (3).

Table 1. The toxicity of different concentrations of silver-agar nanoparticles on 3th instar larval of *C. pipiens*.

Concentration (µM)	Survived larvae percentage (%)		
	24 h.	48 h.	72 h.
Control	100.0±0.0	100.0±0.0	100.0±0.0
100	100.0±0.0	97.5±3.54	92.5±3.54
200	97.5±3.54	90.0±0.0	82.5±3.54
300	85.0±0.0	80.0±0.0	72.5±3.54
400	75.0±0.0	70.0±0.0	65.0±0.0
500	67.5±3.54	62.5±3.54	52.5±3.54
600	52.5±3.54	45.0±0.0	37.5±3.54
700	42.5±3.54	32.5±3.54	22.5±3.54
800	30.0±0.0	20.0±0.0	12.5±3.54
900	12.5±3.54	0.0	0.0

Table 2. The toxicity of different concentrations of silver- chitosan nanoparticles on 3th instar larval of *C. pipiens*.

Concentration (µM)	Survived larvae percentage (%)		
	24 h.	48 h.	72 h.
Control	100.0±0.0	100.0±0.0	100.0±0.0
77	100.0±0.0	95.0±0.0	92.5±3.54
154	95.0±0.0	87.5±3.54	77.5±3.54
231	82.5±3.54	77.5±3.54	62.5±3.54
308	62.5±3.54	52.5±3.54	40.0±7.07
385	47.0±3.54	35.0±7.07	25.0±7.07
462	32.5±3.54	22.5±3.54	10.0±0.0
539	12.5±3.54	0.0	0.0

Table 3. Propit values (µM) of silver nanoparticles synthesized using agar and chitosan against 3th instar larvae of *C. pipiens*.

Nanomaterial	Time	LC_{50}	95% Confidence limits (LCL-UCL)	LC_{90}	95% Confidence limits (LCL-UCL)
CS-AgNPs	24 h.	367.37	(234.40-500.33)	568.07	(370.17-765.96)
	48 h.	321.33	(272.09-370.56)	505.84	(353.74-657.94)
	72 h.	279.33	(278.06-280.60)	468.85	(334.29-603.41)
MHB-AgNPs	24 h.	611.4	(588.28-634.52)	967.91	(964.10-971.72)
	48 h.	543.12	(525.36-560.87)	870.23	(803.84-936.61)
	72 h.	483.87	(391.43-576.30)	811.97	(789.86-834.08)

In agreement with the current revealed findings, the greenly synthesized silver nanoparticles showed great efficacy in controlling larvae of *Culex pipens* (**Fouad *et al*., 2017**) as well as *Aedes aegypti*, *Anopheleus stephensi*, *Anopheles subpictus*, *Aedes albopictus*, *Culex tritaeniorhynchus*, and *Culex quinquefasciatus* (**Balakrishnan *et al*., 2016; Bhuvaneswari *et al*., 2016; Kumar *et al*., 2016; Morejón *et al*., 2018; Kumar, *et al*., 2018**).

The prepared nanoparticles using chitosan as coating/reducing polymer exhibit biodegradable character, good antimicrobial activity, and prolonged action of silver on the affected cells (**Sanpui *et al*., 2008**). Chitosan increases the attachment time at the absorption site, this attributed to its mucoadhesive properties as a result of possibility of hydrogen bond formation and also, due to the availability of bonding groups such as carboxylic and hydroxyl groups (**Huo *et al*., 2010**). The electrostatic interaction between the positive charge on $R\text{-}NH3^{+}$ group and the negative charge on the mucosal surfaces, the interaction of amine groups with the cell membrane results in an irreversible structural reorganization in the protein-associated tight junctions, which is followed by opening of these tight junctions, that increases the time of attachment at the absorption site (**Sinha *et al*., 2004**). In addition to the mucoadhesive nature, chitosan possesses superior properties such as biocompatibility, low toxicity, biodegradability, ease of

fabrication of polymeric nanoparticles without using hazardous solvents, and ability to control the release of the nanoparticles makes chitosan the polymer of choice for developing the polymeric nanoparticle (**Ahmed and Aljaeid, 2016**).

CONCLUSION

Greenly synthesized silver nanoparticles are easy to produce, stable over time, and can be used with minimum concentrations to effectively reduce mosquito vectors populations' superior to traditional larvicides. This study concluded that AgNPs are considered as an alternative eco-friendly larvicide for the mosquito control in its larval aquatic stages.

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