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RESEARCH ARTICLE

Larvicidal Activity of Green Synthesized Silver Nanoparticles and Chitosan Nanoparticles Encapsulated Aloe vera Gel Extract Against *Musca domestica* (Diptera: Muscidae)

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Abstract: ***Background*:** There is a worldwide interest in metal nanoparticles synthesized by various chemical reactions for use in biomedical applications. These processes exhibit a broad range of toxicity in non-target organisms. To avoid chemical toxicity, green synthesis of metal nanoparticles is proposed as a cost-effective and eco-friendly alternative. Aloe vera (A. vera) leaf extract is a medicinal agent with multiple properties, including antibacterial effects. Its constituents include lignin, pectin and hemicellulose, which can be used in the reduction of silver ions to produce silver nanoparticles *(*AgNPs).

***Objective*:** The study aimed at the use of naturally occurring compounds as a reducing and stabilizing agent for the biosynthesis of nanoparticles and investigation of the insecticidal activity of these compounds against *Musca domestica* (*M. domestica*) larvae.

***Methods*:** Phytochemical analysis of A. vera gel extract was done and the phytochemical components were identified by Gas Chromatography–Mass Spectrometry (GC-MS) analysis. AgNPs and encapsulated Chitosan Nanoparticles (CsNPs) were prepared by an eco-friendly method using A. vera gel extract as a reducing and stabilizing agent. A. vera-AgNPs and A. vera encapsulated CsNPs were characterized using Ultraviolet-visible spectrophotometer (UV–vis spectrum), Transmission Electron Microscopy (TEM), Fourier-Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). Then the insecticidal activity of these compounds was investigated against late second instar larvae of the house fly.

***Results*:** The most active ingredients identified by GC-MS analysis were Terpene and Sesquiterpene hydrocarbons. The synthesized AgNPs were spherical with an average size of about 12-75 nm, as revealed by TEM. While encapsulated CsNPs ranged between 34-75 nm and the shape seemed spherical with dark parts confirming the encapsulated plant extract. FTIR and XRD results confirmed the successful encapsulation of the gel extract within the chitosan nanoparticles**.** Results proved the insecticidal potential of the tested compounds against *M. domestica* larvae, and the relative potency of encapsulated CsNPs was nearly 148.51 times more potent than A. vera crude extract whereas AgNPs was nearly 40.65 times more potent than A. vera crude extract. Furthermore, a prolongation of larval duration and reduction in the percentage pupation and adult emergence were observed.

***Conclusion*:** Overall, green-synthesized silver and chitosan nanoparticles have the potential for application as a biopesticide for house fly population control through the use of a safer and cost-effective approach.

Keywords: *Musca domestica*, Aloe vera, gel extract, silver nanoparticles, encapsulated chitosan, nanoparticles green synthesis.

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1. INTRODUCTION

The house fly, Musca domestica, is the most common fly species found in houses and has spread all over the world. It transmits many diseases such as cholera, diarrhea, typhoid, bacillary dysentery and parasitic worms [1, 2]. The excessive use of chemical insecticides increases the deterioration of the environment and leads to the development of resistance in insects [3]**.** In search of ecofriendly-effective pesticides, plant extracts are recommended [4-6]. *Aloe vera* contains many active compounds that make it more specific than chemical insecticides [7-11]. Nanotechnology which uses plant extracts has emerged as a promising area for developing and utilizing nanosized particles with a wide range of uses, including pesticides applications [12, 13]. Silver nanoparticles have been used in many medical, agricultural and pharmaceutical applications because they are non-toxic to humans and possess a wide array of biological activities such as insecticidal and antimicrobial activities [14]. Chitosan is a versatile polymer that has been used in drug delivery systems as nanoparticles because of their biodegradable and biocompatible nature, and low toxicity [15]. Plant extract is currently used for the green synthesis of nanoparticles which is a rapid, low cost, ecofriendly and a single-step method for the biosynthesis process [16, 17]. Therefore, the present study dealt with the activity of *Aloe vera* gel extract and its synthesized silver and chitosan nanoparticles against *Musca domestica*.

2. MATERIALS AND METHODS

2.1. Rearing Technique

The house fly colony used in the present study was reared at the Entomology Department [18].

2.2. Plant Material and Isolation of Components

2.2.1. Plant Collection / Preparation of Gel Extract

Fresh leaves of *Aloe vera* were collected from the graves of El-Ramla Village, Qalyubia Governorate, Egypt. Leaves were properly cut, *i.e.* their hard epidermis was peeled off by sharp knife and the inner gel was scooped out by a spoon. Gel was kept in a glass jar for further experimental analysis. The gel was lyophilized and ground. Lyophilized gel powder was packed into soxhlet apparatus and extracted with 90% methanol for 4 h at 90°C. The methanol containing the extract was filtered and concentrated using a rotary evaporator and stored at 4°C [19].

2.3. Gas Chromatography/ Mass Spectrometry (GC-MS) Analysis of *A. vera*

GC-MS analysis was carried out for the methanolic gel extract of A. vera using a Shimadzu GC-MS-QP 2015 plus instrument (Kyoto, Japan). The analysis was performed by injecting 0.5 µl of the examined gel extract into Hewlett Packard chromatograph model 5970, equipped with a flame ionization detector (FID) and a 50 meter HP capillary column (0.2 mm I.D.). The oven temperature was programmed at 3 minutes from 60°C to 200°C, then isothermally at 200°C for 25 minutes. Detector and injector temperatures were set as 250°C and 200°C, respectively. The carrier gas was helium and the gas flow rate was 1ml/minute. Diluted samples (1% v/v) were injected with a split ratio of 15:1, and the injected volume was 1 µl. The MS operative parameters were as follows: interface temperature: 280°C, ion source temperature: 200 °C, EI mode: 70 eV, scan range: 35-500 amu. In order to identify the peak obtained, the retention time of each peak was compared with that of the standard. The quantization of the components was done by comparing the area of the resulting peaks with data from the WILEY, NIST and Tutor libraries [20, 21].

2.4. Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were prepared by mixing 10 ml filtrate of A. vera gel extract with 90 ml of 1 mmole aqueous solution of silver nitrate ($AgNO_3$) along with continuous stirring at 60°C for one hour. The change of solution color to dark brown indicated the formation of silver nanoparticles, which was confirmed and portrayed by UV-Vis Spectrophotometer and Transmission Electron Microscopy (TEM) [22].

2.5. Synthesis of Chitosan Nanoparticles Encapsulated Plant Extract

Low molecular weight chitosan (0.5 g) was dissolved in 90 ml (2%) aqueous acetic acid solution under stirring until complete dissolution. 10

ml filtrate of A. vera gel extract was added to the above solution with continuous stirring for about 1 h and sonicated for another 1h. Tripolyphosphate (TPP) (0.3 g) was dissolved in 50 ml distilled water and added drop by drop to the chitosan mixture solution with continuous stirring at room temperature. The solution turned into milky suspension, indicating chitosan nanoparticles formation [23]. The plant extract encapsulated CsNPs were centrifuged and freeze-dried to obtain encapsulated CsNPs powder. Encapsulated CsNPs were confirmed and portrayed by UV-Vis Spectroscopy, Fourier Transform Infrared (FTIR), Transmission Electron Microscopy (TEM) and X-ray Diffraction (XRD).

2.6. Larvicidal Bioassay

The larvicidal activities of methanol gel extract, biosynthesized silver and chitosan nanoparticles of A. vera were evaluated by the standard methods reported [24]. Twenty late second instar larvae of *M. domestica* were exposed to food contaminated with different concentrations of the used compounds (10, 20, 40, 80,160 ppm for gel extract and 0.25, 0.5, 1, 2, 4, and 8 ppm for AgNPs and CsNPs). The control group reared on media was only supplied with water. Three replicates were used, and all experiments were carried out in laboratory conditions (27±2°C and 70±5% relative humidity). The larval mortality was recorded at 24, 48 and 72 h post-treatment, and the lethal concentrations (LC_{50} and LC_{90}) were calculated.

For biological measurements, second instar larvae of *M. domestica* were fed on bait treated with different concentrations of each tested compound. Twenty larvae were transferred to a 250 ml beaker containing 3 g treated bait with three replicates for each treatment. Control experiments were carried out without any treatment. All experiments were conducted at laboratory conditions and daily examined to estimate the time required for larvae to develop into pupae, pupation %, pupal mortality and adult emergence.

2.6.1. Calculations

Pupation percentage = (A / B) × 100.

Where: A; number of pupae, B; number of tested larvae

Adult emergence % = (A / B) × 100.

Where: A; number of emerged adults, B; number of tested larvae.

Relative potency= LC_{50} of the lowest toxic compound/LC_{50} of tested compounds x 100.

2.7. Statistical Analysis

Data obtained were analyzed using one-way Analysis of Variance (ANOVA) followed by LSD post-hoc multiple comparisons with SPSS software package version 20 program. When ANOVA statistics were found to be significant ($P< 0.05$), the means were compared by Duncan's multiple range test. The values of percentage mortality and their Standard Error (SE) were calculated. Also, the obtained data were analyzed by statistics package (LDP-line) for goodness of fit (Chi-square test) and to detect LC_{50} & LC_{90} values with corresponding 95% Confidence Limits (C.L.), slope and correlation coefficient.

3. RESULTS

The phytochemical components were identified in A. vera gel methanolic extract using GC-MS analysis. The identification of phytochemical compounds was done based on the peak area, molecular weight and molecular formula. As indicated in Table **1**, twelve bioactive phytochemical compounds have been found in the gel extract of *A. vera*. The main compounds included terpenes with an average rate of 56.62% and sesquiterpenes hydrocarbons with an average rate of 11.47%. So, terpenes exhibited the highest average rate. These results have been found to be in agreement with the findings reported earlier [25-27].

3.1. Characterization of *A. vera*-AgNPs

The fresh gel extract of A. vera was characterized by its green color. However, after the addition of the gel extract to the $AgNO_3$ solution, the solution turned to dark brown color, as shown in Fig. (**1**), indicating the formation of nanoparticles. The formation of AgNPs has been easily monitored by UV-Vis spectroscopy. UV-Vis absorption spectrum of silver nanoparticles is shown in Fig. (**2**). The absorption spectra showed an absorption peak at wavelength of around 430 nm, related to surface plasmon resonance of silver nanoparticles, suggesting that the nanoparticles have

Table 1. Phytocomponents identified in A. vera gel methanolic extract using GC-MS analysis.

Peak	RT	Area% (Average Rate)	Name	Molecular Formula	Molecular Weight (gm/mol)	Compound Nature	Activity
1	4.68	2.37	Debocane, 4 methyl	C_9H_9NO	135.16	Heterocyclic compound	Insecticide, larvicide
2	6.60	5.59	Trocosane	$C_{24}H_{50}$	338.65	Sesquiterpenes hydrocarbon	Antifeedant
3	13.25	2.61	6-Hydroxy hexan3-3-1	C_7H_{16}	100	Sesquiterpenes hydrocarbon	Antifeedant
4	13.50	3.05	1-Dodecamol	$C_{12}H_{26}O$	186.34	Terpene	Insecticide
5	17.19	5.20	1-Octadecanol	$C_{18}H_{38}O$	270.49	Terpene	Insecticide
6	21.61	22.22	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	Terpene	Insecticide
7	24.72	16.2	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.47	Terpene	Insecticide
8	25.16	4.99	Octadecanoic acid	$C_{18}H_{34}O_2$	282.47	Terpene	Insecticide
9	26.91	3.09	1-Phenyl thioxomethyl piperidine	$C_7H_{16}N_2$	219.35	Heterocyclic cpd	Insecticide, larvicide
10	29.56	3.27	Docosane	$C_{22}H_{40}$	310.60	Sesquiterpenes hydrocarbon	Antifeedant
11	38.78	2.89	Sitosterol	$C_{29}H_{50}O$	414.17	Terpene	Insecticide
12	36.10	2.1	Stigmasterol	$C_{29}H_{48}O$	412.69	Terpene	Insecticide

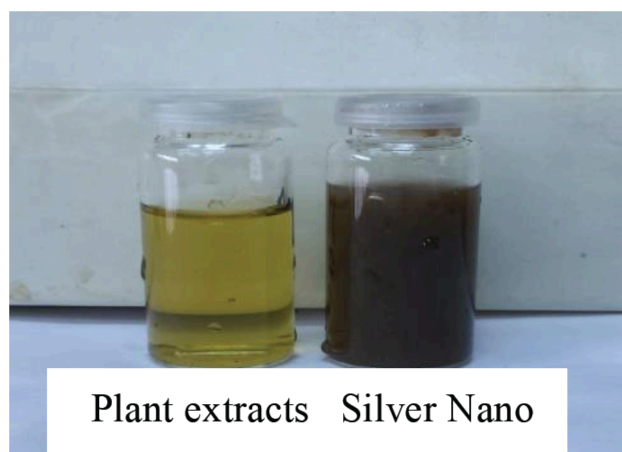

Fig. (1). Preparation of *A.vera*-AgNPs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

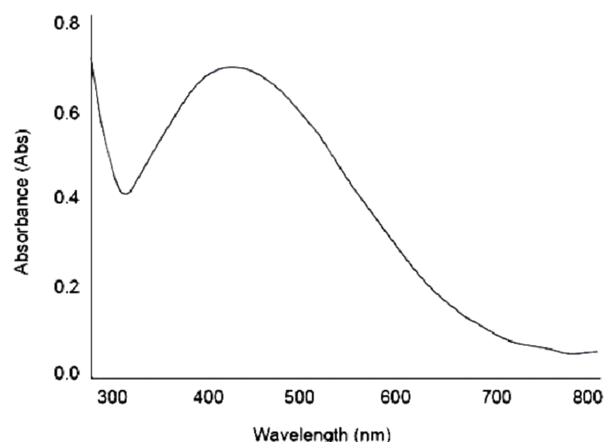

Fig. (2). UV-Vis spectroscopy of AgNPs.

been formed successfully. The formed nanoparticles have been dispersed in the aqueous solution with no appearance of any aggregation, which may be due to stabilization by the functional organic compound present in the plant extract [28, 29]. The silver colloidal nanoparticles characterization has also been confirmed by TEM. The synthesized silver nanoparticles obtained using this green method have shown spherical shape morphology with an average size of about 12-75 nm. The average particles size was measured using Image J program, and it showed the size of the majority of the particles to be around 12 nm, as clearly shown in Fig. (**3**) [27, 30, 31].

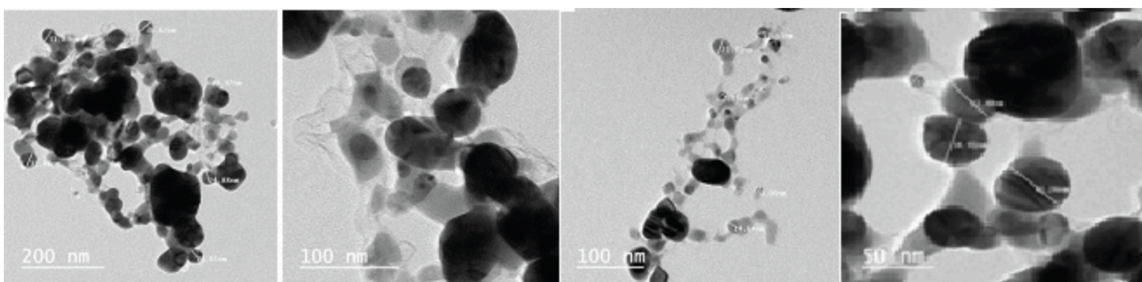

Fig. (3). TEM image of biosynthesized AgNPs using *A. vera*. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

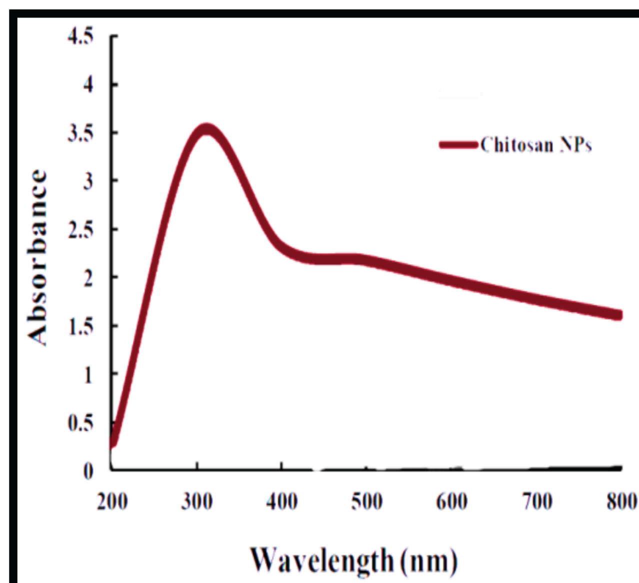

Fig. (4). UV-Vis spectra of CsNPs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. Characterization of A. vera Extract Encapsulated CsNPs

3.2.1. UV-Vis Spectra

The formation of CsNPs can easily be monitored by the naked eye, that is the formation of turbid suspension during the addition of TPP to chitosan solution. The CsNPs can also be explored using UV-Vis spectroscopy, and the absorption spectrum is shown in Fig. (**4**). Chitosan-NPs colloidal solution showed an absorption peak at a wavelength of 330 nm, suggesting that the nanoparticles have been formed and have been dispersed in the aqueous solution with no aggregation formation [32, 33].

3.2.2. FTIR Spectroscopy

FTIR spectra are shown in Fig. (**5**). In general, chitosan powder showed characteristic peaks at 3433 (-OH and $-NH_2$ stretching), 2920 (-CH stretching), 1647 (amide I), 1088 (C-O-C stretching) and 591 cm^{-1} (pyranoside ring stretching vibration) [34]. For chitosan nanoparticles (Fig. **5-S**), the peak of amide I ($-NH_2$ bending) shifted from 1647 to 1685 cm^{-1}, and new peaks appeared at 1338 (C-O-C stretch) and 1560 cm^{-1} (amide II), implying the complex formation *via* electrostatic interaction between NH_3^+ groups of chitosan and phosphoric groups of TPP within the nanoparticles [35]. Moreover, in comparison with the FTIR spectrum of chitosan nanoparticles, the addition of A. vera water extract resulted in a marked decrease in the intensity of the ($-NH_2$) and (-CH) stretching peaks at 3449 and 2922 cm^{-1}, indicating an increase in the hydrogen bonds formed between the amino group of the nano chitosan and the hydroxylic groups from the plant extracts. The results indicate that plant extract was encapsulated into the chitosan nanoparticles.

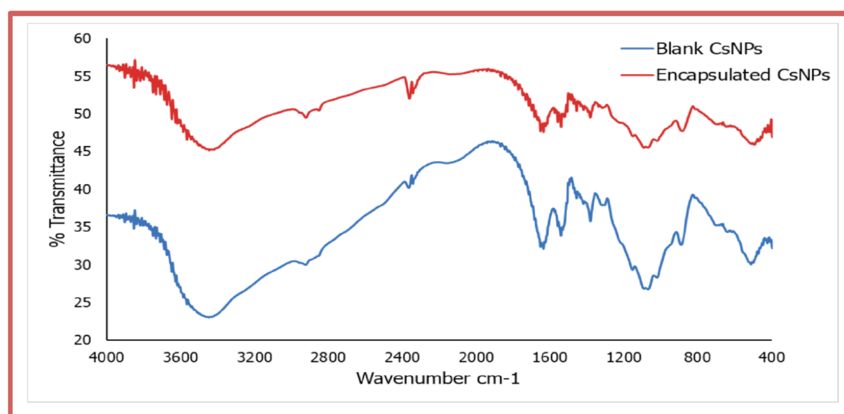

Fig. (5). FTIR spectra, S; refer to CsNPs, A; refer to *A.vera*-CsNPs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

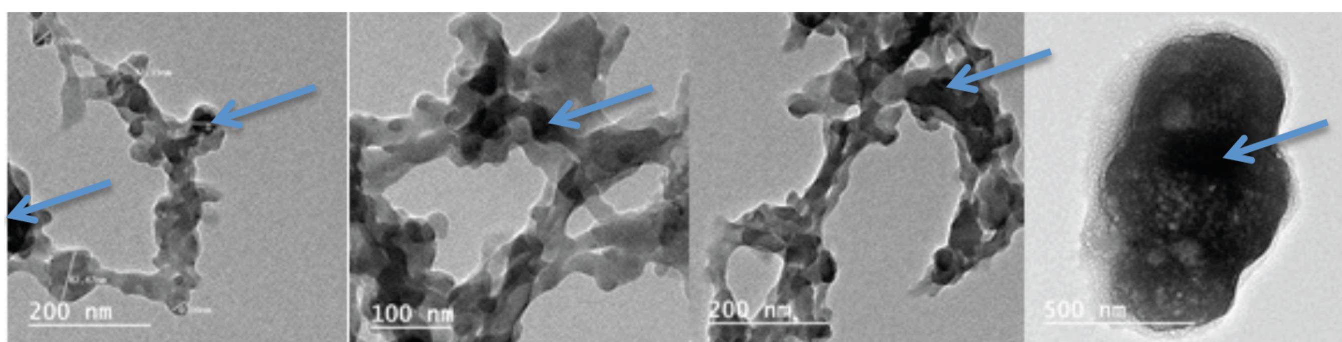

Fig. (6). TEM image of biosynthesized CsNPs (Arrows refer to A. vera extract). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2.3. Transmission Electron Microscopy (TEM)

The colloidal Chitosan-NPs characterization has also been confirmed by TEM, as shown in Fig. (**6**). The figure displays that the chitosan nanoparticles had a relatively spherical shape with an average diameter of about 34-75 nm. The encapsulated plant extract can be confirmed by the appearance of dark parts within the chitosan nanoparticles. The average particles size was measured using the ImageJ program, which showed that the size of the majority of the particles was around 34, as clearly shown in Fig. (**6**).

3.2.4. X-Ray Diffraction

The XRD study of chitosan nanoparticles with and without the plant extraxt is shown in Fig. (**7a**). It is well known that pure chitosan has a high degree of crystallinity with well-characterized peaks at (2θ) of 20 and 10 degrees associated with crystallographic planes (110) and (020), respectively, related to the non-deacetylated part of chitosan (chitin) [36]. The crystallinity largely decreases for the nano chitosan due to the crosslinking with TPP during formation of chitosan nanoparticles, which indicates the amorphous nature of chitosan nanoparticles; this in agreement with a previously reported work [37]. For chitosan encapsulated A. vera extract, the figure displayes new peaks at 2θ: 24.50° and 44.40°, which may be referred to the effect of the encapsulated *A.vera* extract**.** The XRD study demonstrated the nearness of trademark diffraction peaks of Chitosan-NPs (Fig. **7a**). Chitosan-NPs demonstrated a broad diffraction peak at 2h estimations of 18.59°, which are the mill fingerprints of Chitosan-NPs [38, 39]. The lower force displayed by the diffraction peaks of Chitosan-NPs uncovered that they are indistinct in nature. The non-appearance of some other diffraction peaks corresponding to impurities was found in the XRD examples of Chitosan-NPs, showing their immaculateness. The ionic cooperation among TPP and – NH_3^+ of chitosan particles has brought about the development of Chitosan-NPs [35]; the force of diffraction tops was expanded as an outcome of changing indistinct chitosan into

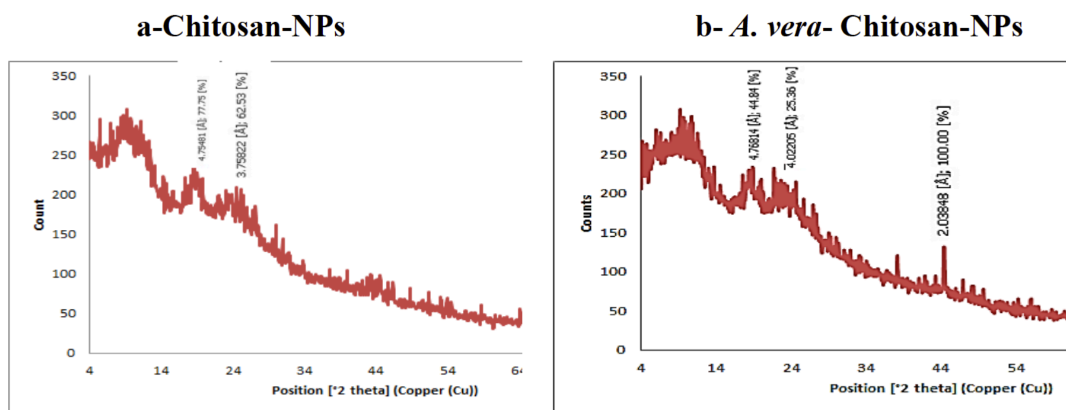

Fig. (7). X-ray diffraction (XRD) pattern of chitosan nanoparticles (**a**) and chitosan nanoparticles with *Aloe Vera* (**b**) recorded in the 2h range of 20 θ –80 θ. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 2. Larvicidal activity of *Aloe vera* gel extract against *Musca domestica.*

Concentrations ppm	% Mortality ± SE		
	24h	48h	72h
10	6.66±0.33[a]	10.00±0.58[a]	11.66±0.33[a]
20	15.00±0.58[ac]	16.66±0.33[a]	25.00±0.00[ac]
40	25.00±0.10[a]	26.66±0.33[ac]	28.33±0.33[ad]
80	40.00±1.53[ad]	43.33±1.20[ad]	48.33±0.67[ae]
160	58.33±5.36[bcd]	60.00±5.51[bcd]	61.66±5.17[bcde]
Control	0.000	0.000	0.000
F	2.681	2.56	2.874
P	0.094	0.104	0.080

[*]Means within a column and followed by the same letter are not significantly different; SE, standard error.

the solidified structure after response with TPP [38]. The diffraction pinnacle of unadulterated chitosan has somewhat moved to a lower stem (18.59°) in the present examination, and this can be credited to the response of Chitosan-NPs to TPP and the solidified structure of Chitosan-NPs, which was found to be in great accordance with the previous reports [32, 40]. On the other hand, XRD studied showed the presence of characteristic diffraction peak of *A. vera*-CsNPs (Fig. **7b**). It showed a main broad diffraction peak at 2h estimations of 18.59° for the chitosan-NPs, and other new peaks at 2h = 20.00°, 24.50° & 44.40° referring to the effect of the encapsulated *A.vera* extract [23, 41, 42].

3.3. Larvicidal Activity of Aloe vera Gel Extract Against *Musca domestica*

The insecticidal activity of A. vera gel extract was evaluated against the late 2nd instar larvae of *M. domestica* at 24, 48 and 72 h post-treatment; the results are given in Table **2**. A. vera gel extract was found to be quite effective against 2nd instar larvae of *M. domestica* where larval mortality was observed to be 61.66% at the highest concentration (160 ppm) after 72h exposure. Non-significant (P>0.05) increase was observed in the larval mortality with the time, and the larval mortality was found ot be directly proportional to the concentration.

3.4. Larvicidal Activity of Aloe vera-AgNPs against *Musca domestica*

In this study, the biosynthesized AgNPs using A. vera gel extract were tested at different concentrations for their larvicidal activity against *M. domestica*; the results are presented in Table **3**. Considerable mortality was observed in the treated larvae after 24h; the larval mortalities were 8.33, 18.33, 26.66, 36.66, 50.00 and 70.00% at concen-

Table 3. Larvicidal activity of *Aloe vera*-AgNPs against *Musca domestica*.

Concentrations ppm	% Mortality ± SE		
	24h	48h	72h
0.25	8.33±0.08[a]	11.66±0.88[a]	13.33±0.58[a]
0.5	18.33±0.33[a]	21.66±0.65[a]	23.33±0.03[a]
1	26.66±0.88[a]	30.00±0.58[a]	31.66±0.33[a]
2	36.66±0.33[b]	41.66±0.67[b]	43.33±0.33[b]
4	50.00±1.00[c]	53.33±0.88[c]	55.00±1.58[c]
8	70.00±2.00[d]	75.00±4.00[d]	76.66±1.33[d]
Control	0.000	0.000	0.000
F	41.44	37.45	41.40
P	0.000*	0.000*	0.000*

Means within a column and followed by the same letter are not significantly different; SE, standard error.

Table 4. Larvicidal activity of *Aloe vera* encapsulated CsNPs against *Musca domestica*.

Concentrations ppm	% Mortality ± SE		
	24h	48h	72h
0.25	25.00±0.58[a]	28.33±0.65[a]	31.66±0.65[a]
0.5	38.33±1.23[b]	41.66±0.45[b]	45.00±1.65[b]
1	58.33±0.33[d]	63.33±0.88[bf]	65.00±0.58[bf]
2	68.33±0.33[e]	71.66±0.88[cfg]	75.00±1.53[cfgi]
4	71.66±1.20[f]	76.66±3.67[dgh]	80.00±1.00[dgh]
8	78.33±3.33[g]	83.33±2.33[eh]	86.66±3.33[ehi]
Control	0.000	0.000	0.000
F	91.19	23.33	13.93
P	0.000*	0.000*	0.000*

Means within a column and followed by the same letter are not significantly different; SE, standard error.

trations 0.25, 0.5, 1, 2, 4 and 8 ppm, respectively. The percentage of larval mortality was found to be significantly increased ($P<0.05$) by increasing the concentrations to reach the maximum (76.66±1.33) at 8 ppm concentration after 72h.

3.5. Larvicidal Activity of *Aloe vera* Encapsulated CsNPs against *Musca domestica*

The larvicidal activity of A. vera encapsulated CsNPs against *M. domestica* was evaluated in the present study, and the results are tabulated in Table **4**. The larvicidal activity of chitosan nanopaticles was found to be statistically significant against *M. domestica* at different time intervals, and the response of house fly larvae was found to be time and concentration dependent. 78.33, 83.33 and 86.66% mortalities were achieved after 24, 48 and 72h, respectively, at 8 ppm. The control showed no mortality in all the assays.

The Probit analysis (Table, **5**) revealed that the LC_{50} values after 72 hours post- exposure were 95.94 ppm for A. vera crude extract, 2.36 ppm for AgNPs and 0.646 ppm for CsNPs. Based on the LC_{50} value of A. vera crude extract, data showed that the toxicity index was 0.54, 21.9 and 80 for crude extract, AgNPs and CsNPS, respectively. The relative potency of CsNPs was nearly 148.51 times more potent than A. vera crude extract, whereas that of AgNPs was nearly 40.65 times more potent than A. vera crude extract.

3.6. Biological Activity of A. vera Gel Extract, *A. vera*-AgNPs and *A. vera*-CNPs against *Musca domestica*

The biological effects of the tested materials on *M. domestica* are presented in Table **6**. Results revealed that the mean larval duration of the control larvae was 8.6±0.23 days. A significant

Table 5. Relative potency of the tested compounds against 2nd instar larvae of *M. domestica*.

Compound	LC50 (ppm)	95% Confidence Limit LFL - UFL	Slope	x^2	Relative Potency
Gel extract	95.94	78.290 - 118.31	1.146±0.091	2.936	1
AgNPs	2.36	1.943 - 2.887	1.201±0.093	3.329	40.65
CsNPs	0.646	0.5197 - 0.7915	1.158±0.092	4.422	148.51

Table 6. Biological effects of A. vera gel extract, *A. vera*-AgNPs and *A. vera*-CsNPs against *Musca domestica*.

Compound	Conc. ppm	Larval Duration (days) ± SD	Pupation %	Pupal Mortality %	Adult Emergence %
Gel extract	10	$9.0±0.23^a$	88.35	2.33	86.02
	20	$9.1±0.15^a$	75.00	3.67	71.33
	40	$9.2±0.15^b$	71.65	3.67	67.98
	80	$9.9±0.12^c$	51.65	2.33	49.32
	160	$10.6±0.12^d$	38.35	1.33	37.02
A. vera -AgNPs	0.25	$10.0±0.05^a$	86.67	0.67	86.00
	0.5	$10.0±0.10^a$	76.67	1.67	75.00
	1	$10.5±0.06^b$	68.35	1.67	66.68
	2	$11.3±0.12^c$	56.65	1.67	54.98
	4	$11.2±0.20^{bc}$	45.00	1.33	43.67
	8	$11.9±0.12^d$	23.35	1.67	21.68
A. vera-CsNPs	0.25	$10.6±0.21^a$	68.34	3.00	65.34
	0.5	$11.1±0.12^a$	66.67	3.67	63.00
	1	$11.8±0.20^b$	35.00	3.60	31.40
	2	$12.5±0.06^c$	25.00	3.67	21.31
	4	$13.0±0.06^d$	13.33	2.67	10.66
	8	$14.0±0.14^e$	3.33	3.33	0.00
	Control	8.6±0.23	100	0.00	100

Means within a column for each compound and followed by the same letter are not significantly different.

prolongation in mean larval duration was observed in the larvae treated with A. vera gel extract. In addition, a highly significant prolongation in the larval duration was observed in larvae treated with *A. vera*-AgNPs and *A. vera*-CsNPs. Significant decreases in the pupation percent were observed in treatments with *A. vera*-AgNPs and *A. vera*-CsNPs. The percent pupation was found to be inversely proportional to the concentration of the tested materials. The highest pupal mortality was induced by *A. vera*-CsNPs followed by gel extract. Regarding adult emergence, all the tested materials induced a reduction in the percent of adults emerged from treated larvae. There were significant differences observed between the tested compounds: *A. vera*-CsNPs were the most effective compound in inhibiting adult emergence; it induced complete inhibition of adult emergence at 8 ppm.

4. DISCUSSION

4.1. Larvicidal Activity of *Aloe vera* Gel Extract against *Musca domestica*

Results of this study showed that *Aloe vera* gel extract was quite effective against 2nd instar larvae of *M. domestica*, and the larval mortality was directly proportional to the concentration. A. vera leaves contain a range of biologically active compounds. As GC-MS analysis indicated, twelve compounds were identified with two main compounds, terpenes and sesquiterpenes. The biologi-

cal activity of A. vera extract might be due to these compounds [43]. These findings corroborate with earlier findings [44] which reported that the treatment of *M. domestica* larvae with different concentrations of A. vera extract exhibited relatively lower percent mortality after a shorter duration (24hrs) than after a longer duration (48hrs). The toxicity of A. vera gel extract against other insects was investigated, and results showed that the mortality varied with the exposure time [45, 46].

4.2. Larvicidal Activity of *A. vera*-AgNPs against *Musca domestica*

Considerable mortality was observed after 24h in the larvae treated with biosynthesized AgNPs using A. vera gel extract. The percentage of larval mortality was significantly increased by increasing the concentrations to reach the maximum at the highest concentration after 72h. Similar results were obtained [30] when the mosquitocidal activity of *Aloe vera* leaf extract and silver nanoparticles synthesized using A. vera extract was investigated against the malaria vector *Anopheles stephensi*.

4.3. Larvicidal Activity of A. vera Encapsulated CsNPs against *Musca domestica*

Up to now, there are no reports on the larvicidal effect of A. vera encapsulated CsNPs against *M. domestica*. Therefore, the larvicidal activity of A. vera encapsulated CsNPs against *M. domestica* was evaluated in this study and the activity was found to be statistically significant at different time intervals. Our results have been found to be in agreement with the earlier reports [47] which have reported chitosan nanoparticles to be toxic against *Anopheles sundaicus* larvae even at low concentrations, while chitosan nanoparticles were found to be highly toxic.

Based on LC_{50} values, the relative potency of CsNPs was found to be nearly 148.51 times more than A. vera crude extract, whereas the relative potency of AgNPs was nearly 40.65 times more than A. vera crude extract. The high larvicidal efficacy of synthesized nanoparticles using plant extracts may be due to their ability for exoskeleton penetration into insect cells, where they restrict macromolecules like DNA and proteins, changing their structure and their function, thereby leading to the loss of cellular function and cell death [12,13]. Similar observations have been reported earlier [48], indicating chitosan NPs to be highly toxic than silver nanoparticles against *Culex pipens*.

4.4. Biological Activity of A. vera Gel Extract, *A. vera*-AgNPs and *A. vera*-CNPs against *Musca domestica*

All the tested materials induced significant prolongation in the mean larval duration as well as reduction in the pupation rate, and the percent of adults emerging from treated larvae. The disturbing effects on insect development, including prolongation of larval duration and inhibition of molting, may be due to the interference of the active metabolites of the plant with bio-formation of ecdysone hormone, which affects cytochrome-P450 involved in the control of molting process in insects [6]. Prolongation of the larval duration with the tested plants was found to be similar to that reported in *M. domestica* [18, 49, 50]. Similar observations have also been made regarding the reduction in pupation percentage and adult emergence in *M. domestica* [3, 6, 51].

CONCLUSION

In this study, we synthesized silver nanoparticles for the first time using *Aloe vera* gel extract as a bio-reducing agent and chitosan nanoparticles encapsulated same extract for larvicidal activity against *M. domestica*. Biosynthesized Ag NPs were spherical in shape, with the mean size ranging between 12 and 75 nm. While encapsulated CsNPs ranged between 34-75nm, the biosynthesized nanoparticles showed significant larvicidal and biological effects against *M. domestica* even at low concentrations, which established their potency as a promising alternative for the control of *M. domestica.* Further studies are needed to clarify the mechanism of action of Ag and Cs nanoparticles against *M. domestica*.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

RESEARCH INVOLVING PLANTS

Fresh leaves of Aloe vera were collected from the graves of El-Ramla Village, Qalyubia Governorate, Egypt.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data used to support the findings of this study have been included in the article.

FUNDING

None.

CONFLICTS OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Chantawee A, Soonwera M. Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly *Musca domestica*. Asian Pac J Trop Med 2018; 11: 621-9. http://dx.doi.org/10.4103/1995-7645.246338

[2] Habibi H, Firouzi S, Rohollahzadeh H. The flies' as a mechanical vector of avian viral pathogens. Int J Agric Environ Bioresearch 2018; 3: 221-7.

[3] Abd El-Hamid MM, Helal EM, Mohamadeen FT. Laboratory evaluation of the toxicity of silver nanoparticles against housefly, *Musca domestica* (Diptera: *Muscidae*). Alexandria Sci Exch J 2018; 39: 511-20. http://dx.doi.org/10.21608/asejaiqjsae.2018.16744

[4] El-Sayed AA, Amr A, Kamel OMHM, El-Saidi MMT, Abdelhamid AE. Eco-friendly fabric modification based on AgNPs@ *Moringa* for mosquito repellent applications. Cellulose 2020; 27: 8429-42. http://dx.doi.org/10.1007/s10570-020-03355-8

[5] Farag SM, El-Sayed AA, Abdel-Haleem DR. Larvicidal efficacy of *Nigella sativa* seeds oil *Culex pipiens* and *Musca domestica*. Egyptian Society of Parasitology 2020; 50: 215-20.

[6] Abdel Razik MAA. Toxicological and developmental effects of selected insecticides, plant volatile oils and plant extracts on house fly, *Musca domestica* L. Am J Biochem Mol Biol 2017; 7: 127-37. http://dx.doi.org/10.3923/ajbmb.2017.127.137

[7] Zareen S, Khan SN, Adnan M, *et al*. Mixture of olive oil and *Aloe vera* gel: a natural mosquito repellent and a skin moisturizer. Int J Mosq Res 2016; 3: 48-9.

[8] Kumari BN, Sharmila M. *Aloe vera* its medicinal uses: a review. Int J Pharm Pharm Sci 2015; 2: 16-21.

[9] Riat AK, Kocher D. Study of histoarchitectural changes in *Anopheles stephensi* larvae following exposure to *Eucalyptus globulus* and *Aloe vera* oils. Turk J Zool 2017; 41: 763-73. http://dx.doi.org/10.3906/zoo-1701-4

[10] Noor A, Gunasekaran S, Manickam AS, Vijayalakshmi MA. Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin-induced diabetic rats. Curr Sci 2018; 94: 1070-6.

[11] Raksha B, Pooja S, Babu S. Bioactive compounds and medicinal properties of *Aloe vera* L. J Plant Sci 2014; 2: 102-7.

[12] Sharma A, Thomas S, Mathew AM, Agarwal AK. Synthesis of silver nano-particles from natural products and its applications. Mapana J Sci 2019; 18: 37-44.

[13] Benelli G. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res 2016; 115(1): 23-34. http://dx.doi.org/10.1007/s00436-015-4800-9 PMID: 26541154

[14] Shehata AZI, Mahmoud AM. Efficacy of leaves aqueous extract and synthesized silver nanoparticles using *Lagenaria siceraria* against *Culx pipiens* liston and anopheles pharoensis theobald (Diptera: *Culicidae*). Egyptian Society of Parasitology 2019; 49: 381-7.

[15] Lee E, Park SJ, Lee JH, Kim MS, Kim CH. Preparation of chitosan-TPP nanoparticles and their physical and biological properties. Asian J Pharm Sci 2016; 11: 166-7. http://dx.doi.org/10.1016/j.ajps.2015.11.065

[16] Athanassiou CG, Kavallieratos NG, Benelli G, Losic D, Usha Rani P, Desneux N. Nanoparticles for pest control: current status and future perspectives. J Pest Sci 2018; 91: 1-15. http://dx.doi.org/10.1007/s10340-017-0898-0

[17] El-Sayed AA, Khalil AM, El-Shahat M, Khaireldin NY, Rabie ST. Antimicrobial activity of PVC-pyrazolone-silver nanocomposites. J Macromol Sci Part A 2016; 53: 346-53. http://dx.doi.org/10.1080/10601325.2016.1166000

[18] Elkattan NAI, Ahmed KS, Elbermawy SM, Abdel-Gawad RM. Effect of some botanical materials on certain biological aspects of the house fly, *Musca domestica* L. Egypt J Hosp Med 2011; 42: 33-48.

[19] Noor A, Gunasekaran S, Amirtham SM, Vijayalakshmi MA. Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin-induced diabetic rats on JSTOR. Curr Sci 2008; 94: 1070-6.

[20] Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. AmazonCom: Books. 4th ed. TX UDA: Texensis Publishing Gruver 2017.

[21] Beckley L, Gorder K, Dettenmaier E, Rivera-Duarte I, McHugh T. On-site gas chromatography/mass spectrometry (GC/MS) analysis to streamline vapor intrusion investigations. Environ Forensics 2014; 15: 234-43. http://dx.doi.org/10.1080/15275922.2014.930941

[22] Jafer FS, Mohammed R, Annon M. Larvicidal effect of pure and green-synthesized silver nanoparticles against *Tribolium castaneum* (Herb.) and *Callosobruchus maculatus* (Fab.). J Glob Pharma Technol 2018; 10: 448-54.

[23] Othman N, Masarudin MJ, Kuen CY, Dasuan NA, Abdullah LC, Md Jamil SNA. Synthesis and optimization of chitosan nanoparticles loaded with l-ascorbic acid and thymoquinone. Nanomaterials 2018; 8(11): E920. http://dx.doi.org/10.3390/nano8110920 PMID: 30405074

[24] Wright JW. The WHO programme for the evaluation and testing of new insecticides. Bull World Health Organ 1971; 44(1-3): 11-22.
PMID: 5315342

[25] Thu K, Mon Y, Khaing TA, Tun OM. Study on phytochemical properties, antibacterial activity and cytotoxicity of *Aloe vera* L. Int J Biotechnol Bioeng 2013; 7(5): 1-5.

[26] Das P, Srivastav AK. Phytochemical extraction and characterization of the leaves of *Aloe vera* barbadensis for its anti-bacterial and anti-oxidant activity. Int J Sci Res ISSN 2015; 4: 658-61.

[27] Parthasarathy G, Saroja M, Venkatachalam M. Biosynthesized Nano-formulation of zinc oxide- *Aloe vera* and to study their characterization and antibacterial activities against multiple pathogens. Int J Pharm Sci Res 2017; 8: 900-7.

[28] Karthikeyen TRS. Synthesis of the silver nanoparticles from *Aloe* barbadensis extract and its application against the urinogenial tract infection. Int Res J Eng Technol 2017; 4: 364-7.

[29] Pal S, Mondal S, Maity J. Green Synthesis of silver nanoparticles using *Moringa Oleifera* leaf extract: investigation of photocatalytic activity. J Nanosci Nanoeng Appl 2018; 8: 60-8.

[30] Dinesh D, Murugan K, Madhiyazhagan P, *et al*. Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* extracts: towards an effective tool against the malaria vector Anopheles stephensi? Parasitol Res 2015; 114(4): 1519-29.
http://dx.doi.org/10.1007/s00436-015-4336-z PMID: 25653031

[31] Tippayawat P, Phromviyo N, Boueroy P, Chompoosor A. Green synthesis of silver nanoparticles in *Aloe vera* plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. PeerJ 2016; 4: e2589.
http://dx.doi.org/10.7717/peerj.2589 PMID: 27781173

[32] Ghadi A, Mahjoub S, Tabandeh F, Talebnia F. Synthesis and optimization of chitosan nanoparticles: potential applications in nanomedicine and biomedical engineering. Caspian J Intern Med 2014; 5(3): 156-61.
PMID: 25202443

[33] Divya K, Jisha MS. Chitosan nanoparticles preparation and applications. Environ Chem Lett 2018; 16: 101-12.
http://dx.doi.org/10.1007/s10311-017-0670-y

[34] Zvezdova D. Synthesis and characterization of chitosan from marine sources in Black Sea. Assoc Adv Automot Med 2010; 49: 65-9.

[35] Yoksan R, Jirawutthiwongchai J, Arpo K. Encapsulation of ascorbyl palmitate in chitosan nanoparticles by oil-in-water emulsion and ionic gelation processes. Colloids Surf B Biointerfaces 2010; 76(1): 292-7.
http://dx.doi.org/10.1016/j.colsurfb.2009.11.007 PMID: 20004558

[36] Zidan TA, Abdelhamid AE, Zaki EG. N-Aminorhodanine modified chitosan hydrogel for antibacterial and copper ions removal from aqueous solutions. Int J Biol Macromol 2020; 158: 32-42.
http://dx.doi.org/10.1016/j.ijbiomac.2020.04.180 PMID: 32353502

[37] Ali MEA, Aboelfadl MMS, Selim AM, Khalil HF, Elkady GM. Chitosan nanoparticles extracted from shrimp shells, application for removal of Fe(II) and Mn(II) from aqueous phases. Sep Sci Technol 2018; 53: 2870-81.
http://dx.doi.org/10.1080/01496395.2018.1489845

[38] Jonassen H, Kjøniksen AL, Hiorth M. Stability of chitosan nanoparticles cross-linked with tripolyphosphate. Biomacromolecules 2012; 13(11): 3747-56.
http://dx.doi.org/10.1021/bm301207a PMID: 23046433

[39] Zhao LM, Shi LE, Zhang ZL, *et al*. Preparation and application of chitosan nanoparticles and nanofibers. Braz J Chem Eng 2011; 28: 353-62.
http://dx.doi.org/10.1590/S0104-66322011000300001

[40] Anand M, Sathyapriya P, Maruthupandy M, Hameedha Beevi A. Synthesis of chitosan nanoparticles by TPP and their potential mosquito larvicidal application. Front Lab Med 2018; 2: 72-8.
http://dx.doi.org/10.1016/j.flm.2018.07.003

[41] Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F. Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in vitro* release study. Carbohydr Polym 2013; 95(1): 50-6.
http://dx.doi.org/10.1016/j.carbpol.2013.02.031 PMID: 23618238

[42] Asiri SM, Khan FA, Bozkurt A. Synthesis of chitosan nanoparticles, chitosan-bulk, chitosan nanoparticles conjugated with glutaraldehyde with strong anti-cancer proliferative capabilities. Artif Cells Nanomed Biotechnol 2018; 46(sup3): S1152-61.
http://dx.doi.org/10.1080/21691401.2018.1533846 PMID: 30704296

[43] Zhang Y, Zhang Q, Luo J, Ding W. Acaricidal active fractions from acetone extract of *Aloe vera* L. against Tetranychus cinnabarinus and Panonychus citri. Acta Physiol Plant 2017; 39: 1-7.
http://dx.doi.org/10.1007/s11738-017-2496-4

[44] Jesikha M. Evaluation of larvicidal efficacy of *Aloe vera* extract against *Musca domestica*. Toxicol Food Technol (IOSR-JESTFT) 2012; 2: 1-03.

[45] Mallavadhani UV, Rajendra PB, Lakshmi SP, Babu RM, Ratanlal M. *Aloe vera* (L.) Burm. f.: A highly useful Indian traditional plant for the management of maize storage pest, *Sitophilus oryzae* L. (Coleoptera: *Curculionidae*). J Biopesticides 2016; 9: 157-66.

[46] Lubis R, Ilyas S, Panggabean M. The effectivity test of *Aloe vera* leaf extract to larvae *Aedes* sp. Asian J Pharm Clin Res 2018; 11: 262-6.
http://dx.doi.org/10.22159/ajpcr.2018.v11i7.24463

[47] Murugan K, Anitha J, Suresh U, *et al*. Chitosan-fabricated Ag nanoparticles and larvivorous fishes: a novel route to control the coastal malaria vector Anopheles sundaicus. Hydrobiologia 2017; 797: 335-50.
http://dx.doi.org/10.1007/s10750-017-3196-1

[48] Rajesh P, Mahadik N. Chitosan mediated nanoparticles from *Saccharomyces cerevisiae* and its mosquito larvicidal activity. J Appl Adv Res 2017; 2: 179-83.

[49] Shakir MM, Ahmed S, Khan AS, Nisar MS. Effect of aqueous extracts of different plants on life cycle and population build up parameters of house fly, *Musca domestica* L. (Diptera: *Muscidae*). Res Rev J Agric Sci Technol 2018; 4: 1-6.

[50] Attaullah A, Zahoor MK, Zahoor MA, *et al*. Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: *Muscidae*) to some indigenous weed plant extracts. Saudi J Biol Sci 2020; 27(1): 106-16.
http://dx.doi.org/10.1016/j.sjbs.2019.05.009 PMID: 31889824

[51] Kamel AA, Mohamed MB, El-Dakhly KM. Larvicidal activity and bio-efficacy of some products against larvae of

the housefly, *Musca domestica* (L) (Diptera: *Muscidae*). J Appl Sci 2019; 19: 427-33. http://dx.doi.org/10.3923/jas.2019.427.433