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Larvicidal activity of aqueous *Mimusops elengi* seeds-synthesized silver nanoparticles against *Aedes aegypti* and *Qulex quinquefasciatus*

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Abstract
To investigate the synthesized silver nanoparticles using aqueous *Mimusops elengi* (*M. elengi*) seeds extract against two important mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*. The *M. elengi*-synthesized silver nanoparticles (Ag NPs) were characterized by UV–vis spectroscopy, Fourier-transform infra-red (FT-IR) and further monitored by TEM and SEM. The *M. elengi*-synthesized Ag NPs to be effective against *A. aegypti* (LC_{50}= 16.59 µg/ml; LC_{90}= 30.46 µg/ml) and *C. quinquefasciatus* (LC_{50}= 18.75 µg/ml; LC_{90}= 33.60 µg/ml). The control showed nil mortality in the concurrent assay. The UV-visible spectroscopy showed a maximum absorbance peak at 470 nm which corresponds to the Ag NPs. The FT-IR spectrum showed prominent peaks (3433.29, 3387.00, 2924.09, 2765.92, 2364.73, 2092.77, 1774.51, 1639.49, 1508.33, 1384.89, 825.53 and 462.92 cm^{-1}) in the region of 4000–400 cm^{-1}. The TEM image showed the NPs with an average size of 27.44 nm. The SEM image of *M. elengi*-synthesized Ag NPs revealed their shape spherical, pentagonal and triangular. To conclude *M. elengi*-synthesized Ag NPs have a potential to be used as a drug for the control of *A. aegypti* and *C. quinquefasciatus* through eco-friendly and cost-effective approach.

Keywords: Mosquito larvicidal, silver nanoparticles, *Mimusops elengi,* TEM, SEM

1. Introduction

Mosquitoes are serious vectors of a few diseases such as Zika, Dengue, Malaria and Japanese encephalitis. These diseases are causing millions of death worldwide. Among vector- borne diseases, dengue and filariasis are of major health concern in the urban and semi-urban areas. Dengue is a viral infection, transmitted through *Aedes aegypti*. About 390 million of dengue infected cases were reported each year, and over 96 million people were at risk due to favorable environmental conditions for the vector [1]. Filariasis is caused by *Wuchereria bancrofti*, transmitted through *Culex quinquefasciatus*. Approximately 120 million people from 83 countries were affected each year [2].

Due to the rapid changes in environmental conditions, water supply and sanitation facilities, the incidence of vector-borne diseases dramatically increased worldwide. The development of vaccines against viral diseases often turns ineffective due to the high mutation rate of the parasites, cost, the long trial process required, and developing quick resistance towards the vaccine. Vector management, therefore, is the only effective measure to prevent and control such diseases [3]. Larviciding is one of the best way of sinking mosquito densities in their propagation places earlier than they appear into adults [4].

Chemical insecticides such as methoprene, pyrethroids, carbamates, temephos, and organophosphates were largely being used in pest management programs. Currently, such chemical insecticides are not listed in integrated vector management programs due to their harm to non-target organisms, emerging avoidance and resistance behavior of mosquitoes towards insecticides, high-risk environmental protection from insecticides and sustainable development [5-7]. Recently, nanoparticles have proved significant effective in controlling insects [8]. The combined use of plant extract and nanoparticles may be an alternative strategy for controlling mosquito vectors since they are non-toxic, cheap, and biodegradable [9].

Plant-mediated synthesis of silver nanoparticles has received an extraordinary attention over other biological organisms like bacteria, algae, and fungi as they are easily available, safe to handle, non-toxic, and only a few steps are required in downstream processing and have the faster rate of Ag NPs synthesis [10, 11]. Till date, only a few reports have been published related to synthesis of silver nanoparticles using plant extracts and their larvicidal activity against various mosquito species. Silver nanoparticles synthesized using *Eclipta prostrata* [12], *Pergularia daemia* [13], *Cadaba indica* [14], *Heliotropium indicum* [15], *Achyranthes aspera* [16] and *Polianthus tuberose* [2] have shown very strong larvicidal activity against different mosquito vectors. Besides, Ag NPs had no side effects against non-targeted organisms as compared to conventional insecticides and are environmentally sustainable as reported by various authors [8-16]. Incidentally, so far, there is no published report on the larvicidal activity of synthesized Ag NPs using seed extract of *Mimusops elengi.*

Mimusops elengi Linn (Family: Sapotaceae) commonly known as Bakul and is found all over the different parts of India, Pakistan, Bangladesh [17]. It has a long history of being used in Indian traditional medicine [18]. Several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this plant [19]. The various extracts of the plant (bark, fruit, leaves, seed and flowers) have been reported to be cardiotonic, alexipharmic, stomachic, hypotensive, antibacterial, anthelmintic, anti-gastric ulcers, teeth cleaner and antimalarial activities [20]. The present study was aimed to investigate the *M. elengi*-synthesized silver nanoparticles against two important mosquito species, *A. aegypti* and *C. quinquefasciatus*.

2. Materials and Methods

2.1. Collection and identification of plants

M. elengi seeds were collected from different places of Vellore District, Tamilnadu, India (Fig. 1a). The collected plant seeds were taxonomically identified by Dr. V. Chelladurai, Retired Research Officer-Botany and Central Council for Research in Ayurvedha and Sida, Tirunelveli District, Government of India.

Fig 1a: *Mimusops elengi* seeds

2.2. Preparation of *M. elengi* seed extract

M. elengi seeds were dried in shade and ground to fine powder in an electric grinder (Fig. 1b). Aqueous extract was prepared by mixing 50 g of dried seed powder with 500 ml of boiled and cooled distilled water with constant stirring on a magnetic stirrer. The suspension of dried seed powder in water was left for 3 h and filtered through Whatman no. 1 filter paper and the filtrate was stored in an airtight bottle at 10°C for further use.

Fig 1b: *Mimusops elengi* seeds powder

2.3. Synthesis of silver nanoparticles

The fresh *M. elengi* seed extract was prepared by taking 10 g of thoroughly washed and finely cut seeds in a 300-ml flask along with 100 ml of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1 and stored at −15°C. Aqueous 1mM silver nitrate (21.2 mg of silver nitrate powder in 125 ml distilled water) solution was prepared in flask and incubated at room temperature. 88 ml of an aqueous solution of 1 mM silver nitrate was reduced using 12 ml of *M. elengi* seed extract at room temperature for 10 min, resulting in a brown–yellow solution indicating the formation of Ag NPs. The *M. elengi*- synthesized Ag NPs were characterized by UV, FT-IR, TEM, SEM and EDX analysis.

2.4. Insect rearing

Mosquito *A. aegypti* larvae were collected from water tank, broken bottles, small water courses and *C. quinquefasciatus* larvae were from drainage, septic tank, cock pits, polluted water area of our campus surrounding areas. To start the colony, the larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory [21]. The collected larvae of *A. aegypti* and *C. quinquefasciatus* were identified by Zonal Entomological Team, Velapadi, Vellore-1, Tamilnadu, India.

2.5. Larvicidal bioassay

The larvicidal activity of aqueous *M. elengi* seeds extract (60, 120, 180, 240 and 300 µg/ml) and *M. elengi*-synthesized Ag NPs (7, 14, 21, 28 and 35 µg/ml) were evaluated according to the WHO protocol [22]. Based on the wide range and narrow range tests, plant extract was tested. Twenty numbers of third instar larvae were introduced into 500 ml disposable cups containing 199 ml of dechlorinated water and 1 ml of desired concentrations of the extracts was added. For each concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. The control experiments (distilled water with one ml of acetone) were also run parallel with each replicate. The mortality of mosquito larvae was recorded according to the following criteria: moribund larvae, which were incapable of rising to the surface or did not show the characteristic diving reaction when water was disturbed, had discoloration, an unnatural position or rigor.

2.6. Data analysis

Data were subjected to probit analysis in order to estimate the LC_{50} and LC_{90} values. Lethal concentration that killed 50% and 90% of the population (LC_{50}–LC_{90}), fiducial limit (FL) with a 95% confidence limit (CL) and the chi-square value were calculated. *p* value less than 0.05 were considered to indicate statistical significance [23].

3. Results and Discussion

Phytochemicals may act as suitable alternatives to chemical insecticides in the future as these are non-toxic, inexpensive, bio-degradable and easily available in many areas of the world. Different parts of plants contain a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites, which act as mosquitocidal agents.

3.1. Larvicidal activity

The results of larvicidal activity of aqueous *M. elengi* seeds extract and *M. elengi*-synthesized Ag NPs against the third instar of *A. aegypti* and *C. quinquefasciatus* were presented in Table 1 and 2. Considerable mortality was noted in both the treated groups. The LC_{50} and LC_{90} values of aqueous *M. elengi* seeds extract appeared to be effective against *A. aegypti* (LC_{50} 151.08 µg/ml and LC_{90} 271.15 µg/ml), followed by *C. quinquefasciatus* (LC_{50} 165.75 µg/ml and LC_{90} 291.89 µg/ml). Most considerable mortality was evident after the treatment of silver nanoparticles. *M. elengi*-synthesized Ag NPs against the vector *A. aegypti* had LC_{50} and LC_{90} values of 16.59 and 30.46 µg/ml and *C. quinquefasciatus* had LC_{50} and LC_{90} values of 18.75 and 33.60 µg/ml. The crude aqueous *M. elengi* seed extract was less toxic than *M. elengi*-synthesized Ag NPs in both mosquito species. The control showed nil mortality in the concurrent assay. χ^2 value was significant at $p \leq 0.05$ level.

In the present investigation larvicidal activity of *M. elengi*-synthesized Ag NPs showed significantly more activity against *A. aegypti* than *C. quinquefasciatus*. Vector management is one of the major issues due to the capacity of resistance against the usual insecticides. Therefore, an urgent need has emerged to develop the new insecticides. Hence, the invention of nanometals using *M. elengi* as represented here could be provided a new product to control the mosquitoes by replacing the synthetic larvicidal products and this route would make available for larvicides to prevent several dreadful diseases. It is interesting to mention that the rate of mortality was significantly enhanced with increasing concentration. This result is also comparable to earlier reports of Morejon *et al.* [24] who observed that the *Ambrosia arborescens*-mediated Ag NPs were more toxic than the plant extract against the larvae of *A. aegypti*.

Table 1: Larvicidal activity of aqueous *M. elengi* seed extract against *A. aegypti* and *C. quinquefasciatus*

Mosquito species	Concentration (µg/ml)	24 h mortality (%) ± SD[a]	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
A. aegypti	Control	00 ± 00	151.08 (118.13-183.88)	271.15 (229.41-349.59)	14.496
	60	24.2 ± 1.2			
	120	40.3 ± 1.6			
	180	61.5 ± 0.8			
	240	76.3 ± 1.3			
	300	96.8 ± 1.5			
C. quinquefasciatus	Control	00 ± 00	165.75 (137.14-195.65)	291.89 (251.48-363.07)	10.980
	60	20.1 ± 1.4			
	120	36.6 ± 0.8			
	180	53.7 ± 1.5			
	240	72.2 ± 0.7			
	300	93.4 ± 1.4			

Table 2: Larvicidal activity of *M. elengi*-synthesized Ag NPs against *A. aegypti* and *C. quinquefasciatus*

Mosquito species	Concentration (µg/ml)	24 h mortality (%) ± SD[a]	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
A. aegypti	Control	00 ± 00	16.59 (11.93-21.07)	30.46 (25.09-41.80)	19.641
	7	28.2 ± 0.6			
	14	45.3 ± 1.4			
	21	62.1 ± 1.2			
	28	78.9 ± 1.5			
	35	98.6 ± 1.6			
C. quinquefasciatus	Control	00 ± 00	18.75 (14.91-22.72)	33.60 (28.98-43.42)	13.829
	7	22.6 ± 0.2			
	14	38.4 ± 1.6			
	21	56.3 ± 1.8			
	28	72.1 ± 1.2			
	35	94.5 ± 1.4			

No mortality was observed in the control. SD- standard deviation, LC50 -lethal concentration that kills 50% of the exposed organisms, LC90 -lethal concentration that kills 90% of the exposed organisms, UCL 95% -upper confidence limit, LCL 95% -lower confidence limit, χ2 -chi square, a -Values are mean ± SD of five replicates.

3.2. Biosynthesis and characterization of silver nanoparticles

3.2.1. UV–Vis spectra analysis

The aqueous silver ions were reduced to silver nanoparticles when added to the aqueous *M. elengi* seeds extract. It was observed that the colour of the solution turned from light dark to dark brown in colour after 30 min from the reaction, which indicated the formation of Ag NPs (Fig. 2). This is due to the Surface Plasmon Resonance (SPR) vibrations of *M. elengi*-synthesized Ag NPs in the reaction medium. The formation of SPR peak was influenced by various factors such as size, shape and particles formed. The UV–Vis absorption spectrometric analysis showed absorbance peak at around 470 nm, suggesting bioreduction of silver nitrate into Ag NPs (Fig. 3). Ag NPs stability could be attributed to capping or stabilizing agents, which contained proteins and other biomolecules presenting the extract into the reaction. In agreement with our results, a peak with maximum absorption at 463.50 nm also characterized *Artemisia nilagirica*-fabricated Ag NPs [25]. Furthermore, Pilaquinga *et al*. [26] have reported that the absorption spectrum of *Solanum mammosum*-fabricated Ag NPs showed symmetric single absorption bands with a peak at 411.5 nm.

Fig 2: *M.elengi*-synthesized silver nanoparticles

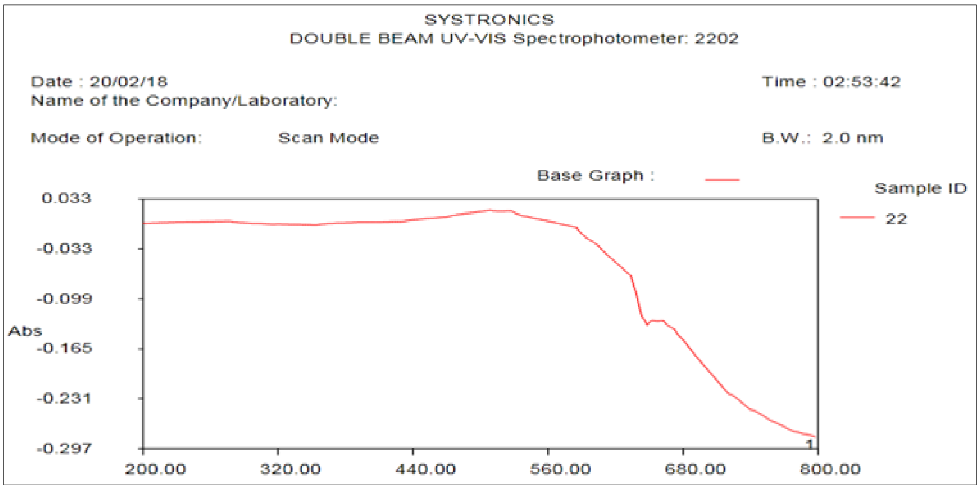

Fig 3: UV visualization of the absorption spectrum of *M. elengi*-synthesized Ag NPs

3.2.2. FT-IR analysis

The FT-IR spectra were carried out to identify the functional groups found on the *M. elengi*-synthesized silver nanoparticles. In the spectrum, transmittance peaks were at 3433.29, 3387.00, 2924.09, 2765.92, 2364.73, 2092.77, 1774.51, 1639.49, 1508.33, 1384.89, 825.53, 462.92 cm^{-1} (Fig. 4). The peaks corresponding to presence of primary amines, alcohol, phenol, ammonium ions, aldehydes, ammonium ions, aldehyde, ketones, aromatic, nitro compounds, vinyl and iodo alkanes. The spectra absorption band assigned to the stretching vibration of N-H, O-H, N-H, C-H, N-H, C-C, C=O, C-N, C-C, N-O, C-H and C-X group respectively (Table 3). They were denoted as possible biomolecules responsible for stabilizing, capping and reducing agents of the Ag NPs. Results suggested that alcohols, alkanes, amines and amides were involved in the bioreduction and capping of silver ions. Similarly, the role of alcohols, aldehydes and amines in bioreduction, capping and stabilization of silver ions during nanoparticle formation was also described earlier [25, 27, 28].

Table 3: FT-IR analysis of *M. elengi*-synthesized Ag NPs

Absorption peak cm-1(nm)	Bond	Type of bonds	Specific type of bond	Appearance
3433.29	N-H	Primary amines	Any	Strong
3387.00	O-H	Alcohol, phenol	High concentration	Broad
2924.09	N-H	Ammonium ions	Any	Multiple broad peaks
2765.92	C-H	Aldehydes	Any	Medium

2364.73	N-H	Ammonium ions	Any	Multiple broad peaks
2092.77	C-C	C=C	Terminal alkanes	Very weak
1774.51	C=O	Aldehyde, ketones	Cyclic 4 membered	Often indistinguishable
1639.49	C-N	C=N	Any	Similar conjugation effects
1508.33	C-C	Aromatic C=C	Any	Weak to strong
1384.89	N-O	Nitro compounds	Alphatic	Weaker
825.53	C-H	Vinyl	Trisubstituted alkenes	Strong to medium
462.92	C-X	Iodo alkanes	Any	Medium to strong

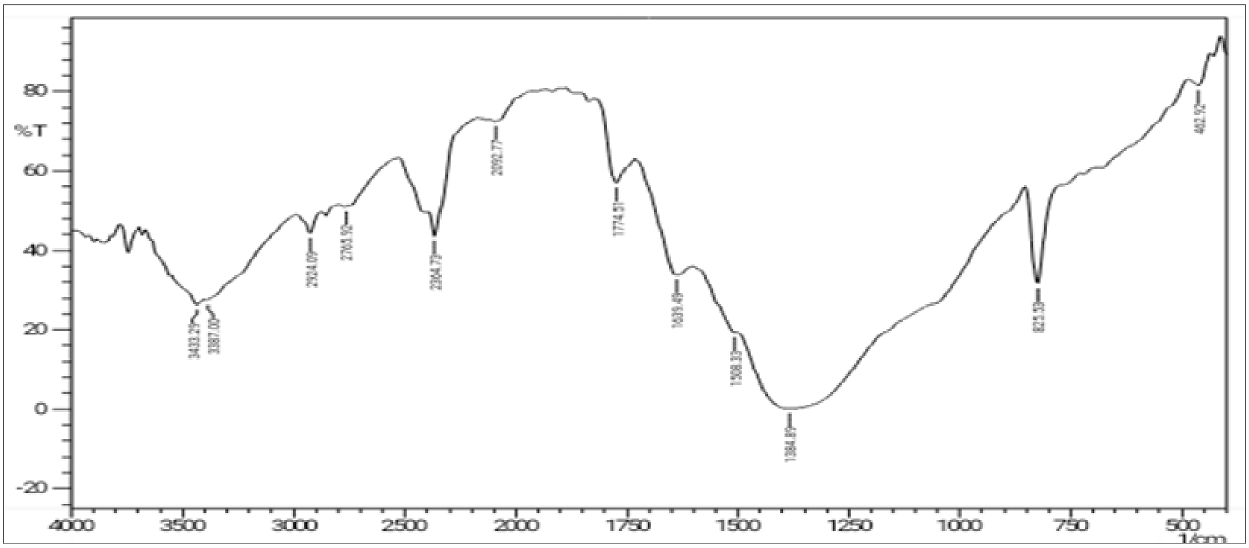

Fig 4: FT-IR spectrum of *M. elengi*-synthesized Ag NPs

3.2.3. TEM analysis

TEM micrograph shows that *M. elengi*-synthesized Ag NPs was spherical in structure. The particle size varies from 18.15 to 38.50 nm and the average size of the Ag NPs was 27.44 nm (Fig. 5a, 5b and Table 4). This is in agreement with recent results by Rawani [2], who reported that *Polianthus tuberose*-fabricated Ag NPs were spherical in shape with a size range of 50 ± 2 nm.

Table 4: Selected area electron diffraction (SAED) pattern of TEM

Spot#	d-Spacing (nm)	Rec. Pos.(1/nm)	Degrees to Spot 1	Degrees to x-axis	Amplitude
1	0.3312	3.020	0.00	156.10	973.16
2	0.2861	3.496	19.39	136.71	387.55
3	0.2394	4.177	11.22	167.32	281.92
4	0.2018	4.955	39.00	117.10	395.56
5	0.2000	5.001	14.95	141.15	2372.55
6	0.1639	6.100	0.91	157.01	668.80
7	0.1423	7.026	8.54	164.64	267.97
8	0.1160	8.618	7.57	163.67	298.03

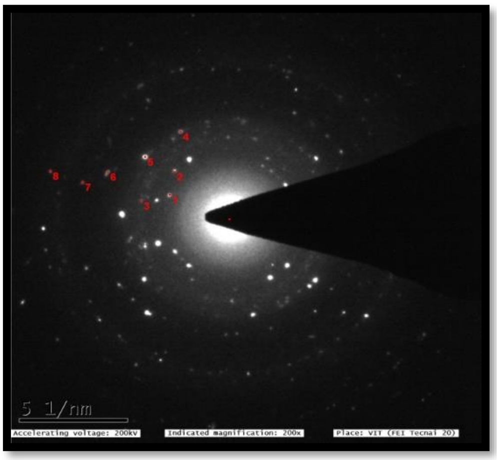

Fig 5a: Transmission electron micrograph of *M. elengi*-synthesized Ag NPs

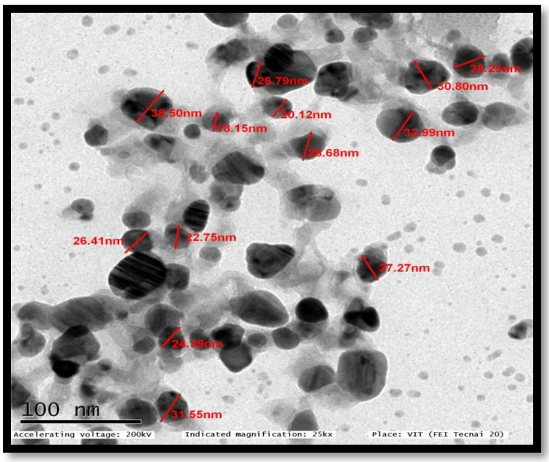

Fig 5b: SAED pattern of TEM

3.2.4. SEM analysis

SEM micrograph of *M. elengi*-synthesized Ag NPs was found to be spherical, pentagonal and triangular in structures. It was magnified at ×4000 and measured at 20 µm (Fig. 6). Most recently, Jinu *et al.* [29] reported spherical and hexagonal shaped silver and copper nanoparticles using *Prosopis cineraria* leaf extracts.

3.2.5. Energy dispersive X-ray (EDX) analysis

M. elengi-synthesized Ag NPs were allowed to identify the presence of metallic elemental signals by EDX spectroscopy analysis. The EDX spectrum indicates a strong silver ion signal along with other weak signals, including carbon, nitrogen, oxygen and sodium, which may have arisen from the bioactive molecules bound to the surface of the biosynthesized nanoparticles (Table 5). EDX results exhibited the silver elemental peak at 2.5 KeV (Fig. 7). Jeeva *et al.* [30] has described the formation of triangular shape silver nanoparticles at 2.4 keV using *Caesalpinia coriaria* leaf extracts. The weak elemental signals might have occurred from the bioactive compounds capped on the surface of the engineered silver nanoparticles. It is most likely; the signals might have originated because of the X-ray emission from macromolecules such as carbohydrates/proteins/enzymes appeared in the cell wall [31].

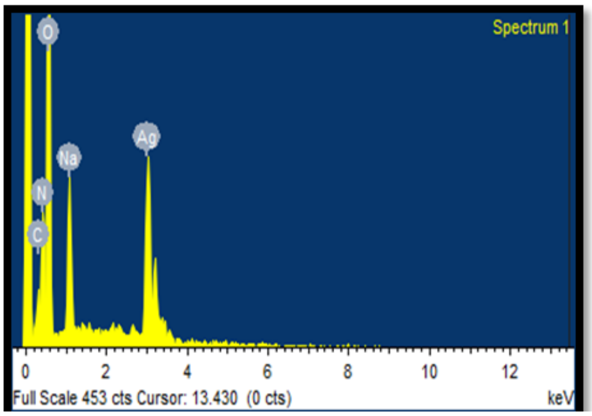

Fig 6: SEM of *M. elengi*-synthesized Ag NPs

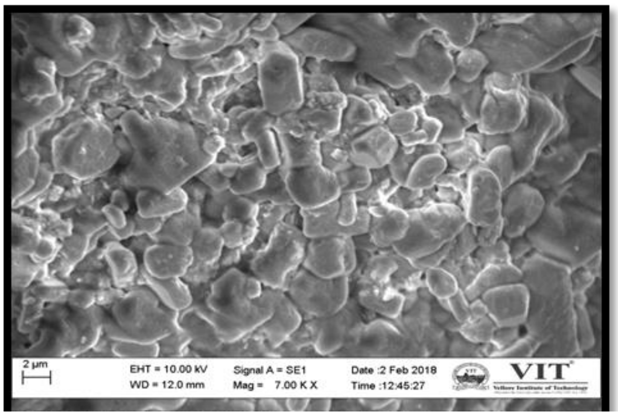

Fig7: EDX of *M. elengi*-synthesized Ag NPs

Table 5: Energy dispersive X-ray (EDX) spectroscopy present complete chemical composition of *M. elengi*-synthesized Ag NPs

Element	Weight%	Atomic%
C K	1.84	3.39
N K	20.02	31.61
O K	38.22	52.83
Na K	5.28	5.08
Ag L	34.64	7.10
Total	100.00	100.00

4. Conclusions

The biosynthesized Ag NPs using aqueous *M. elengi* seeds extract as reducing and stabilizing agent. Ag NPs were mostly spherical, triangular and pentagonal in shape, crystalline in nature, with face-centred cubic geometry, and their mean size was 27.44 nm. Our study highlighted the concrete potential of *M. elengi*-synthesised Ag NPs in mosquito control, since they are cheap, easy to produce and could be employed to strongly reduce larval populations of dengue and filarial vector. Overall, the chance to *M. elengi*-synthesized Ag NPs for control of mosquito vectors seems promising, since they are effective at low doses and may constitute an advantageous alternative to build newer and safer control tools.

5. Competing Interests

The authors declare that they have no competing interests.

6. Acknowledgement

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