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BIOEFFICACY OF *SESBANIA GRANDIFLORA* LEAVES SILVER NANOPARTICLES AGAINST *AEDES AEGYPTI* LARVAE

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ABSTRACT

The present study evaluated the green synthesis of silver nanoparticles using the leaf extract *Sesbania grandiflora* and charecterized by the UV-Vis Spectrophotometry, Particle size analyzer and Fourier Transform Infra-Red Spectroscopy (FTIR) and also evaluated its killing effect against larval and pupal stages of *Aedes aegypti*. The production of silver nanoparticles was observed by the colour changes from yellow to dark brown and further confirmed by UV-Vis spectroscopy showed an absorption band at 340.8nm; the size of the nanoparticles is around 723 nanometer according to particle size analysis. FTIR analysis showed that functional group of synthesized silver nanoparticles belongs to free amino or carboxyl group. FTIR analysis showed the functional group of synthesized silver nanoparticles and the absorption bands were seen at 518.82cm^{-1}, 730.01cm^{-1}, 873.69cm^{-1}, 1093.56cm^{-1}, 1139.85cm^{-1}, 1640.35cm^{-1}, 2088.76cm^{-1}, 3446.56cm$^{-1.}$ The effect of leaf extract of *S. grandiflora* on the survival and development of fourth instar larvae of *Ae. Aegypti* was sudied. LC_{50} values of aqueous leaf extract of *S. grandiflora* against *Ae. aegypti* and silver nanoparticles synthesized from aqueous leaf extract of *S. grandiflora* were 293.87 ppm and 5.548ppm respectively. The killing effect increased along with the increasing concentration of aqueous and silver nanoparticles synthesized from leaf extract of *S. grandiflora*. The synthesized silver nanoparticles from the leaf extract *S. grandiflora* were more efficient than the aqueous leaf extract. Thus the present study also suggested that the *S. grandiflora* leaf mediated silver nanoparticles could be used as an effective larvicidal and pupicidal agents.

Keywords: FTIR, Green synthesis, Particle size analyser, *Sesbania grandiflora,* UV-Vis spectroscopy.

1. INTRODUCTION

Mosquito belongs to the phylum arthropod and is important vector for many vector-borne diseases. It causes millions of death among the population worldwide. It holds life-threatening parasitic pathogens on their thorax region, causes illness by transferring pathogens to humans through the mode of biting [1]. The important genera *Aedes, Anopheles* and *Culex* cause several deadliest diseases such as Zika, Chikungunya, Dengue fever, Yellow fever, Malaria, West Nile, Rift valley fever, Murray valley encephalitis, Japanese encephalitis and Western equine encephalitis and Dog heart worm [2]. Mosquito-borne diseases are endemic to India due to favorable ecological conditions for the vectors, their close contact with humans and their reproductive biology. In rubber plantations, the rich organic content, stagnant water, low light levels and protected conditions in the coconut shells usd in rubber production, favors intense breeding [3]. *Ae. aegypti* (Diptera: culicidae) is an exasperating creature to the human being and cause more diseases than any other organisms such as Dengue, Chikungunya and yellow fever [4]. Another example of *Ae. aegypti* disease is Zika fever, which has re-infected various parts of the world and became a serious problem as a human pathogen [5]. *Ae. aegypti* originally native to Africa is a strongly anthropophilic and synanthropic species which, due to its capacity to flourish in human settlements, has been able to expand its distribution all along the tropical and subtropical regions of the globe [6]. Female *Ae. Aegypti* become arbovirus vectors after ingesting an contagious blood meal. Once the pathogen starts being secreted with the insect's saliva, the mosquito becomes infective and will transmit the pathogen every time it blood feeds on a vulnerable host, for the rest of its life [7]. The use of plants for synthesis of nanoparticles express low cost, eco-friendly and safe for human therapeutic use [8]. One of the most important methods to control the

insect vector is to prevent mosquitoes breeding by using insecticides which can affect different stages and involved using classical chemical insecticides such as DDT, chlordane, benzene hexachloride and hexamethyl tetraphosphate. Though, with due respect to their effective control in the elimination of mosquitoes population, these classical chemical cause serious undesirable effects on human health and to the environment [29]. In current context, green nanotechnology opens a new horizon in the field of bio-control of mosquitoes [10]. Nanotechnology concerns with the development of tentative processes for the synthesis of nanoparticles of different sizes, shapes and controlled disparity [11]. Silver is the one of the most commercialized nano-material with five hundred tons of silver nanoparticles manufacture per year [12] and is estimated to increase in next few years. A number of techniques are available for the synthesis of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc., [13]. *S. grandiflora* (also known as agati, syn. *Aeschynomene grandiflora*) belongs to the family Fabaceae is one of the most popular green vegetables and also traditional medicinal plants of India. *S. grandiflora* has been known to have antibacterial, antifungal, anti-bdiabetic, antioxidant and anti-tumorigenic activities [14]. The plant has been widely used in traditional medicine for the treatment of a broadspectrum of diseases including leprosy, gout, rheumatism, tumour and liver disorders. Leaf extract believed to have antibiotic, anthelmintic, antitumor and contraceptive properties [15].In the present study, the evaluation of larvicidal activity was carried out by applying Silver Nanoparticle synthesized by the leaf extracts of *S. grandiflora*.

2. MATERIAL AND METHODS

In the present study, the larvae of the mosquito, *Aedes aedypti* and the aqueous extract and silver nanoparticle synthesized extract of *S. grandiflora* were used.

2.1. Preparation of Plant extract

The freshly harvested plant leaves were washed thoroughly in tap water, pat dried with paper towel, and shade-dried at room temperature (35±12°C). The Dried leaves were ground to fine powder using a blender. Ten grams of leaf powder was added into each three Erlenmeyer flasks containing 100 ml of double distilled water. The suspension was mixed well and left for 5 hours without disturbance, and the extracts obtained were filtered through Whatmann No. 1 filter paper. The filtrate was used to find out the larvicidal and pupicidal activity against the target vector.

2.2. Silver nitrate preparation

Silver nitrate was used as precursor for the synthesis of silver nanoparticles. Analytical grade, silver nitrate ($AgNO_3$) was used. For preparation of solution, 16.96 mg of silver nitrate was carefully weighed and dissolved in 90 ml of Milli-Q-water. This aqueous Silver nitrate solution was always prepared fresh.

2.3. Collection and maintenance of target vector

Different larval instars and pupae of *Ae.aegypti* were collected from the Indian Council for Medical Research, Madurai and were brought to the laboratory safely without disturbance. These larvae and pupae were maintained in enamel trays containing deionized water and allowed to feed on brewer's yeast, dog biscuits and sucrose in a 3: 1:1 ratio in the laboratory at room temperature for 24 hours, before start of the experiment.

2.4. Synthesis of silver nanoparticles from leaf extract

Aqueous leaf extract of *S. grandiflora* was prepared by placing 10 g of chopped fresh leaves in a 250 ml Erlenmeyar flask and boiled with 100 ml of sterile double distilled water up to 60 min at 60°C in a water bath. The crude extract was passed through Whatmann filter paper (no.1), and the filtrates (aqueous leaf extract) were stored at 4°C and used within 3 days. Ten ml of aqueous leaf extract was treated with 90 ml of prepared 1mM aqueous $AgNO_3$ Solution in an Erlenmeyer flask and incubated in dark at room temperature. The aqueous solution of 1mM of Ag NO_3 was leading to change of pale yellow to dark brown resulting in synthesis of Ag NPs [16].

2.5. Characterization of synthesized silver nanoparticles

2.5.1. UV-Visible spectral analysis

Initial characterization of silver nanoparticles was carried out using UV-Visible spectroscopy. The bio-reduction of silver ions to silver was monitored by measuring the UV-Vis spectrum of the reaction mixture (silver nitrate+aqueous leaf extract). The reaction mixture (1ml) was drawn at different time interval (min), and the absorption measurements were carried out on UV-Visible spectrophotometer at a resolution of

1nm between 200-800 nm. Distilled water was used as blank. The spectra recorded were then plotted [17].

2.5.2. Particle size analysis

The particle size of the synthesized silver nanoparticles was characterized by Laser Diffraction Particle Size Analyzer (Shimadzu model and Model No: 2300). The particle size was determined using the scattered light intensity pattern.

2.5.3. Fourier Transform Infra Red Spectroscopy

A dry nanoparticle powder was obtained in the following manner. Silver nanoparticles synthesized after 5 hours of reaction of 1mM $AgNO_3$ solution with *Capsicum annuum* extract centrifuged at 10,000 rpm for 15 minutes at room temperature, after which the pellet was redispersed in sterile distilled water. The process of centrifugation and redispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the nanoparticels. The purified pellet was then dried and subjected to Fourier Transform Infrared (FTIR, IR Affinity-1, Shimadzu Corporation, Tokyo, Japan) spectroscopy measurement using the potassium bromide (KBr) pellet technique in diffuse reflection mode at a resolution of 4 cm^{-1}. The nanoparticle powder was mixed with KBr and exposed to an infrared source of 500-4000 cm^{-1} [18].

2.6. Larvicidal and pupicidal activity

The larvicidal and pupicidal activity was evaluated using WHO method (1996) with slight modifications. Different test concentrations of aqueous leaf extract and AgNPs in 200 ml de-ionized water were prepared in 250 ml capacity autoclaved glass bottles. Bio-efficacy test was conducted against the larvae and pupa of target vector at ten different concentrations of aqueous leaf extract and synthesized AgNPs, 10 larvae were exposed to each test at different concentrations. Similarly, each test included a set of control group (Tap water) with three replicates for each individual concentration. Mortality rate was recorded after 24 h of exposure period. The dead larvae in ten replicates were combined expressed as a percentage of larval and pupal mortality for each concentration.

2.7. Statistical analysis

The results obtained were subjected to statistical analysis to ascertain their credibility. Standard deviation and mean separation statistical tools were employed for analysis of larval and pupal mortality obtained in the present investigation using computer software. The dose response mortality data were concerned to probit analysis for finding the $LC_{50,}$ upper and lower confidence limit at 95 % confidence, and values determined using the software SPSS, 2007.

3. RESULTS AND DISCUSSION

In the present research work, green synthesis of silver nanoparticles was carried out using the leaf extract of *S. grandiflora* (Table 1) and it was found to be a conquering biological agent for the control of *Ae. aegypti* larvae.

Table 1: Preparation of different concentration of the test solutions of the aqueous leaf extract of *S. grandiflora* and AgNPs synthesized from leaf extract of *Sesbania grandiflora*

S. No	Required volume of stock solution in ml		Required volume of water in ml		Concentration in ppm	
	Aqueous leaf extract	AgNPs synthesized from leaf extract	Aqueous leaf extract	AgNPs synthesized from leaf extract	Aqueous leaf extract	AgNPs synthesized from leaf extract
1	50	0.1	950	99.9	50	1.0
2	100	0.2	900	99.8	100	2.0
3	150	0.3	850	99.7	150	3.0
4	200	0.4	800	99.6	200	4.0
5	250	0.5	750	99.5	250	5.0
6	300	0.6	700	99.4	300	6.0
7	350	0.7	650	99.3	350	7.0
8	400	0.8	600	99.2	400	8.0
9	450	0.9	650	99.1	450	9.0
10	500	1.0	500	99.0	500	10

Recently, biosynthesis of nanoparticles has received significant attention due to the urgent need to develop environmentally benign technologies in material sciences. For illustration, a great deal of effort has been

put into the biosynthesis of nanoparticles, especially metal nanoparticles using plants [19]. The synthesis of metallic nanoparticles, such as silver, gold and platinum have been generally used in the control of insect vectors and pharmaceutics products [20].

3.1. Visible observation of silver nanoparticle synthesis

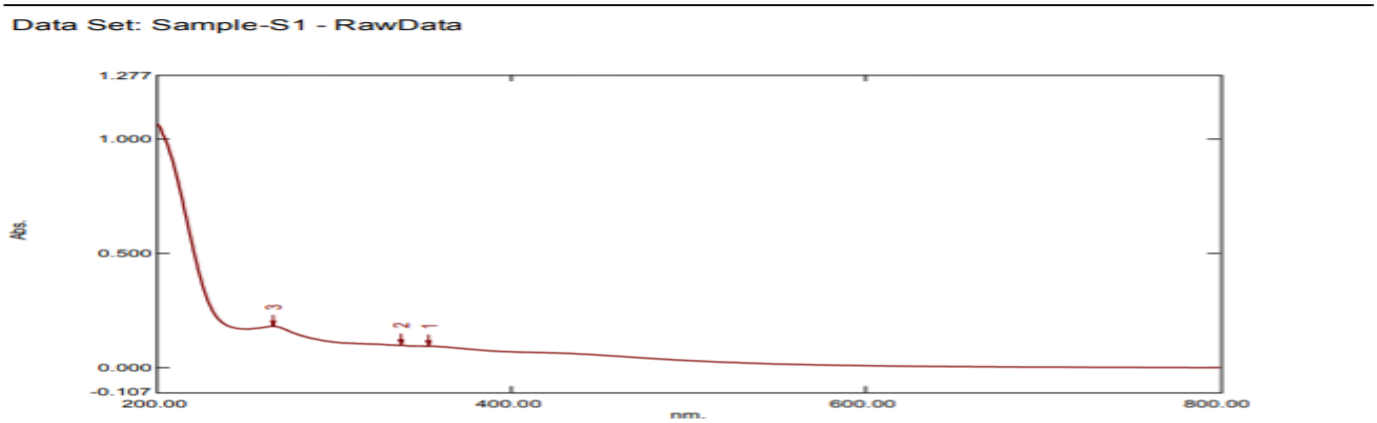
Green synthesis of silver nanoparticles was carried out by means of aqueous leaf extract of *S. grandiflora* with the help of 1mM $AgNO_3$. The green synthesis of silver nanoparticles was at first confirmed by the visible observation of colour change. The fresh suspension of *S. grandiflora* was yellowish-green in colour and after addition of $AgNO_3$ the yellowish-green colour was transformed to dark brown within 5 hours incubation at room temperature. The appearance of brown colour is due to the excitation of surface Plasmon vibration. The present study provides the evidence that the aqueous leaf extract of *S. grandiflora* have the prospective to convert silver nitrate to silver nanoparticles by the reduction of silver ions (Ag^+ into Ag°) and also has the larvicidal and pupicidal effect on mosquito. The colour of the reduction mixure changed from transparent to brown in present experimental liquid. Scientists [21] reported the similar study in *Polyalthia longifolia* samples which changes colour from almost colourless to brown. Similar results were observed [22] in *Punica granatum* samples that showed changes in colour from almost yellowish to dark brownish the concentration of the colour was increased during the period of incubation.

3.2. UV-Visible spectroscopy analysis

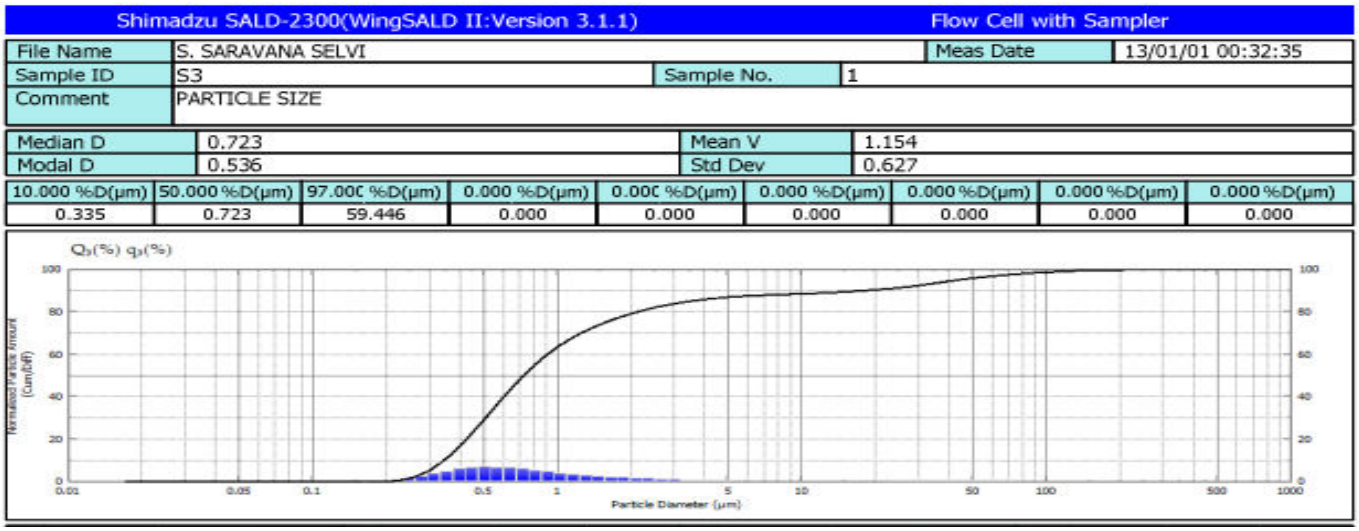
The UV-Visible spectroscopy is used to characterize the excitation spectra of the $AgNP_3$ samples, which is useful to prove the presence of nanoparticles. The UV-Visible spectra gives an absorption band at 340.8 nm (Fig.1(a)) which keep up a correspondence to the absorbance of silver nanoparticles. This absorption is exclusive property of metal nanoparticles called SPR (Surface Plasmon Resonance) that happen due to conduction of electrons on surface of silver nanoparticles (Fig.1(a)). UV-visible spectroscopy is very useful to identify the formation of metal nanoparticles in reaction mixture [23]. In the present study, UV-visible spectra shows an absorption band at 340.8nm which communicates to the absorbance of silver nanoparticles. This absorption is unique property of metal nanoparticles called SPR (Surface Plasmon Resonance) arises due to conduction of electrons on surface of silver nanoparticles. Similar results were observed in *Solanum torvum* [24] which shows characteristic surface Plasmon resonance (SPR) absorption band at 420nm for silver nanoparticles. Similar results were reported in *Pedalium murex* leaf extract [25].

3.3. The Particle size analyzer

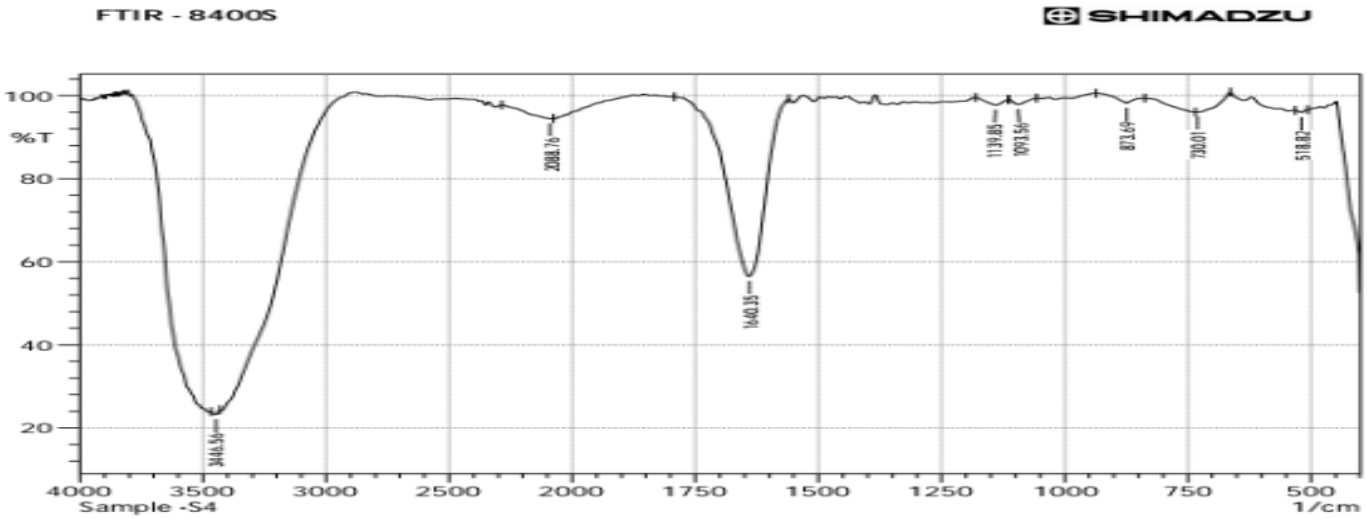
The particle size of the synthesized silver nanoparticles was characterized by laser diffraction Particle Size Analyzer (Shimadzu model and model no: 2300). The particle size of the synthesized nanoparticle is shown in the Graph (Fig.1 (b)). The light intensity pattern thus obviously shows that the silver nanoparticles are crystalline in nature due to the reduction of Ag^+ ion by *S. grandiflora* leaf extract. The size of the silver nanoparticles synthesized from *S. grandiflora* was predicted as 723 nm (Fig.1 (b)). The particle size of the synthesized silver nanoparticles was characterized by Laser diffraction Particle size Analyzer. Further, analysis of the silver nanoparticles by scattered light intensity pattern was done. The size of the silver nanoparticles synthesized from *S. grandiflora* was predicted as 723nm. Similar results were reported in *Azhadirachta indica* [26].



(a)



(b)



(c)

Fig. 1: Characterization of silver nanopartiicles synthesized from leaf extract of *Sesbania grandiflora*.(a) UV-Visible absorption spectrum, (b)Particle size analyzer Patterns, (c) FTIR spectrum

3.4. FTIR Analysis

Fourier Transform Infrared Spectroscopy is used to find out the functional group. The FTIR technique is used to compute the absorption of various infrared radiations by the target materials. It produces an IR spectrum that can be used to recognize functional group and molecular structure of the sample. The FTIR spectrum of produced silver nanoparticles had many absorption bands and the absorption bands seen at (Fig.1(c)) 518.82cm^{-1}, 730.01cm^{-1}, 873.69cm^{-1}, 1093.56cm^{-1}, 1139.85cm^{-1}, 1640.35cm^{-1}, 2088.76cm^{-1}, 3446.56cm^{-1} were assigned to the C-Br stretching of alkyl halides, C-H bends of aromatic compounds, C-H bends of aromatic compounds, C-O stretching of alcohols, C-O stretching of alcohols, N-H bends of amides, C-H stretching of alkanes, O-H stretching of alcohols. This points out that the silver nanoparticles synthesized using aqueous leaf extract of *S. grandiflora* was proved to have all these functional group in it (Table 2; Fig.1(c)). These results were supported by the study [27] that FTIR spectra of silver nanoparticles exhibited outstanding peaks at 2927cm^{-1}, 1631cm^{-1} and 1383cm^{-1}. The spectra showed sharp and strong band at 1631cm^{-1} assigned to the stretching vibration of (NH) C=O group. The band 1383cm^{-1} developed for C-C and C-N stretching. Similar results were observed in Green synthesis of silver nanoparticles using *Abies webbiana* leaves extract for the antibacterial studies [28]. Similar results were reported by scientists [21], the spectrum exhibits the bands at 1418cm^{-1} equivalent to aromatic group.

Proteins present in the extract can bind to silver nanoparticle through either free amino or carboxyl groups in the proteins.

3.5. Killing Effect

3.5.1. LC_{50} values

LC_{50} values for the aqueous leaf extract of *S. grandiflora* and synthesized silver nanoparticles were calculated against the fourth instar larvae of *Ae. aegypti* using probit analysis. The $LC_{50}$of the aqueous leaf extract of *S. grandiflora* against *Ae. aegypti* was 293.87ppm and LC_{50} of the synthesized silver nanoparticles against *Ae. aegypti* was 5.548 ppm (Table 3). In the present study LC_{50} values for the aqueous leaf extract of *S. grandiflora* and synthesized silver nanoparticles were calculated against the IV instar larvae of *Ae. Aegypti* using probit analysis. The LC_{50} value of the aqueous leaf extract of *Sesbania grandiflora* against *Ae. aegypti* was 293.87 ppm and LC_{50} value of the synthesized silver nanoparticles against *Ae. aegypti* was 5.548 ppm. Comparable observations were resulted by study [29] in silver nanoparticles synthesized from the leaf extract of *A.marina* against the larvae of *Ae. aegypti*. Scientists [30] accounted that the LC_{50} of the ethereal extracts of *Heliopposiiti folia* and *Jaegeria hirta* are 41ppm and 24ppm.

Table 2: Functional groups present in silver nanoparticles synthesized leaf extract revealed by FT-IR

S. No	Absorption (cm^{-1})	Class of compounds	Bond
1	518.82	Alkyl halide	C-Br stretch
2	730.01	Aromatic compound	C-H bend
3	873.69	Aromatic compound	C-H bend
4	1093.56	Alcohol	C-O stretch
5	1139.85	Alcohol	C-O stretch
6	1640.35	Amide	N-H bend
7	2088.76	Alkanes	C-H stretch
8	3444.56	Alcohol	O-H stretch

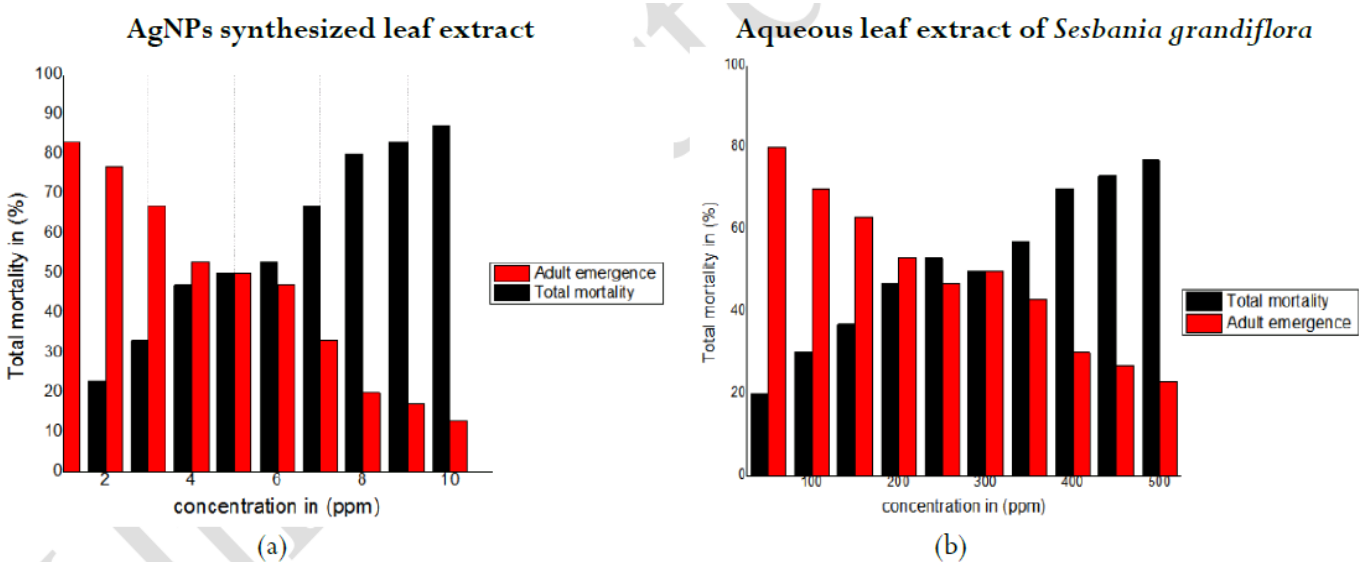


Fig. 2: Effect of different concentrations of aqueous (a) and AgNPs synthesized(b) leaf extract of *S. grandiflora* on the larval mortality and adult emergence of *Ae. Aegypti*

Table 3: LC_{50} value of the test solutions for Aqueous leaf extract and AgNPs synthesized leaf extract of *S. grandiflora* against IV instar larvae of *Ae. Aegypti*

Types of leaf extract	Test solution	LC_{50} values in ppm
Leaf extract of *Sesbania grandiflora*	Aqueous extract of *Sesbania grandiflora*	293.87ppm
Silver nanoparticles of *Sesbania grandiflora*	AgNPs synthesized from leaf extract of *S. grandiflora* leaves	5.5488ppm

3.5.2. Larval Killing Effect

The larval Killing effect of aqueous extract of *S. grandiflora* and silver nanoparticles synthesized from aqueous leaf extract of *S. grandiflora* against the IVth instar larvae of *Ae. aegypti* was recorded (Table 4, 5). The larval killing effect of aqueous extract of *S. grandiflora* increased from 1-10 larvae with the increasing concentration from 50-500ppm (Table 4; Fig 2(a)). The larval killing effect increased from 1-10 larvae with the increasing concentration from 1.0-10.0 ppm by using silver nanoparticles synthesized aqueous leaf extract of *S. grandiflora* (Table5; Fig 2(b)). In the present study, the larval killing effect of aqueous extract and silver nanoparticles synthesized from *S. grandiflora* extract against the fourth instar larvae of *Ae. aegypti* was recorded. The larval killing effect of aqueous extract increased from 1-10 larvae with the concentration from 50-500ppm. Parallel results were observed by [31] in the aqueous plant extract of *Daemia extensa* against the fourth instar larvae *Ae. aegypti*. High larval killing effect (80-100%) was noticed in mixture treatment, *V.negudo, Z.officinalis* and *O.santum* which may be due to the chemical constituents present in leaf and seed extracts that arrest the metabolic activities of larvae.

3.5.3. Pupal Killing Effect

The pupal killing effect was recorded in the aqueous extract of *Sesbania grandiflora*. Three pupae were killed in 200ppm and two pupae were killed in 100, 150ppm and one pupa was killed in 50, 250 ppm. There is no pupal mortality in control (Table 4). In the silver nanoparticles synthesized from aqueous leaf extract of *Sesbania grandiflora* there is no pupal killing effect. Three pupae were killed in 4.0 ppm and two pupae were dead in 2.0, 3.0ppm and one pupa were killed dead in 1.0, 5.0ppm concentration (Table 5).

Table 4: Effect of *S. grandiflora* (aqueous leaf extract) on various stages of *Ae. Aegypti*

S.No	Factors Observed	Control	Dosage of *S. grandiflora* (aqueous) in ppm									
			50	100	150	200	250	300	350	400	450	500
1	Larval stage (days)	4	4	4	4	4	4	4	4	4	4	4
2	Pupal stage (days)	2	2	2	2	2	2	2	2	2	2	2
3	Larvaekilled	0	5	7	9	11	15	15	17	21	22	23
4	Pupae killed	0	1	2	2	3	1	0	0	0	0	0
5	Adults killed	0	0	0	0	0	0	0	0	0	0	0
6	Total killing effect (%)	0	20	30	37	47	53	50	57	70	73	77
7	Adult Emergence %)	100	80	70	63	53	47	50	43	30	27	23

Table 5: Effect of *S. grandiflora* (AgNPs) on various stages of *Ae. aegypti*

S.No	Factors Observed	Control	Dosage of *S. grandiflora* (AgNPs) in ppm									
			1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
1	Larval stage (days)	4	4	4	4	4	4	4	4	4	4	4
2	Pupal stage (days)	2	2	2	2	2	2	2	2	2	2	2
3	Larvae killed	0	4	5	8	11	14	16	20	24	25	26
4	Pupae killed	0	1	2	2	3	1	0	0	0	0	0
5	Adults killed	0	0	0	0	0	0	0	0	0	0	0
6	Total killing effect (%)	0	17	23	33	47	50	53	67	80	83	87
7	Adult Emergenc(%)	100	83	77	67	53	50	47	33	20	17	13

3.5.4. Adult Killing Effect

In aqueous extract and silver nanoparticles synthesized aqueous leaf extract, adult killing effect was not found at all different concentrations (Table 4 and 5). In the present study, adult killing effect was not found in aqueous leaf extract of *S. grandiflora* and silver nanoprticles synthesized aqueous extract of *S. grandiflora* at various concentrations 50-500ppm. Similar results also observed for the acetone and aqueous extract of *Ptaeroxy lonobliquum* and *Pittosporum viridiflorum* against the malarial vector *Anopheles arabiensis* [32].

3.5.5. Percentage of total killing effect and adult emergence

The total killing effect increased from 10-100% with the increasing concentrations of aqueous extract of *S. grandiflora* from 50-500ppm (Table 4). The total killing effect increased from 10-100% with increasing

concentration of silver nanoparticles synthesized leaf extract of *S. grandiflora* from 1.0-10.0ppm (Table 5). India, being rich in herbal plants diversity, can make use of its herbs for such reason, plants not only being pesticides and insecticides, they can also act as an effective antifungal, anti-malrial, antimicrobial and antiparasitic agents. Thus, it has been affluently oppressed for these properties; here in the present study, we have used the plant source *S. grandiflora* to synthesis AgNPs as larvicidal and pupicidal agent against *Ae. aegypti*.

4. CONCLUSION

The present study confirmed that the silver nanoparticles synthesized from the leaf extract of *S. grandiflora* have larvicidal, pupicidal and adulticidal activity against the mosquitoes *Ae. aegypti*. Thus the present study concluded that the leaf extract of *S. grandiflora* has the potential to produce silver nanoparticles. Further the silver nanoparticles of *S. grandiflora* can be used as larvicidal, pupicidal and adulticidal tool against *Ae. aegypti*. Hence, the AgNPs synthesized from leaves of *S. grandiflora* extract can be used as an ecofriendly larvicidal, pupicidal and adulticidal agent in the vector management programme.

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Conflict of interest

None declared

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