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Green Synthesis of Silver Nanoparticles from *Eclipta Alba* (L.) Collected from Kolli Hills and their Larvicidal Activity Against *Aedes Aegypti* (L.).

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Abstract*—Green synthesis of nanoparticles helps to develop a clean, nontoxic, and eco-friendly methods. They also find place in the insecticides like nonmaterial's based insecticides. They provide green and efficient alternatives for pest management without harming the nature. Synthesis of silver nanoparticles has the potent to be an eco-friendly, approach for the control of mosquito population. *Aedes aegypti* and *Aedes albopictus* are the primary and secondary worldwide vectors; they breed in per domestic man-made water containers, and their control is the most effective way to reduce the viral transmission. In the present study, synthesized silver nanoparticles of *Eclipta alba* leaf collected from Kolli Hills were used as larvicides against *Aedes aegypti* mosquito larvae. The synthesized AgNPs were confirmed by UV-visible absorption spectrum, X-Ray diffraction (XRD), Fourier Transform Infrared (FTIR) and Scanning Electron Microscopy (SEM) analysis. *Aedes aegypti* larvae were exposed to different doses at two of phases 12 and 24 hours duration. The UV spectral absorption at 357nm corresponds to Surface Plasmon Resonance (SPR). SEM analyses shows the spherical and cluster shaped structures of 2µm in size. Mortality observed after 12 and 24 hours of duration was high at 10ppm. The third instar larvae mortality rate was relatively high than the other larval instars. Larvicidal activity after 12 hours of observation of the third instar larvae was (LC_{50}=3.48ppm and LC_{90} =50.18ppm) and after 24 hours duration (LC_{50}= 2.50 ppm and LC_{90}=29.79 ppm) respectively. Bioactive compounds present in synthesized AgNPs of *Eclipta alba* leaf extract exhibited mortality in the aquatic habitat of *Aedes aegypti*, since the reduced silver ions can be suspended in liquids and cause a controlling factor for mosquito menace.

***Index Terms*—*Aedes aegypti*, *Eclipta alba*, Green Synthesis, Larvicidal Activity, Leaf extract, Silver nanoparticles, Reduced Silver ions.**

I. Introduction

Dengue is an arbovirus transmitted by species of Aedes mosquitoes. Aedes aegypti and Aedes albopictus are the primary and secondary worldwide vectors; they breed in peri domestic man-made water containers, and their control is the most effective way to reduce the viral transmission [1]. Plants produce compounds to protect themselves from insects, and it can affect insect development in many ways. Hundreds of plant species have been tested for their effects against mosquitoes [2]. The metal nanoparticles are emerging as one of the fastest growing materials due to their unique physical, chemical and biological properties [3]. Much of the research against Aedes aegypti mosquitoes has been focused on by-products of plants already utilized for economic gain, or on already recognized medicinal plants. Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. Biological methods of nanoparticles synthesis using microorganism [4, 6], enzyme [5], and plant or plant extract [7] have been suggested as possible ecofriendly alternatives for chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [7]. It can also be suitably scaled up for large-scale synthesis of nanoparticles. [7] Reported on the synthesis of pure metallic nanoparticles of silver and gold by the reduction of Ag+ and Au3+ ions using Neem Azadirachta indicia leaf broth. Ecofriendly alternative integrated control programs have emerged as promising alternatives [8] and essential oil based nanoemulsions have been recognized as valuable products for mosquito control [9]. Reports are available on larvicidal activity of AgNPs against various mosquitoes [10], [11]. In this study, the investigation is reported that Eclipta alba leaf extracts of silver nanoparticles exhibited considerable mortality against III instar larva of Aedes aegypti. The mortality rate was observed after 12 and 24 hours. The synthesized silver nanoparticles were confirmed by UV-visible spectroscopy and SEM images. The XRD and FTIR analysis prove the presence of various bio-molecules present in the sample. The peculiar properties of silver nanoparticles are small in size, with high surface area, easy to suspend in liquid medium and have a deep access to cells and organelles that can easily strike on the mosquito larvae in the aquatic phase of its life cycle.

II. Materials and Methods

A. Collection of Plant Material

The medicinal plant *Eclipta alba* widely grown in Kolli hill was collected from Solakkadu region.

B. Preparation of Leaf Extract

The leaves of *Eclipta alba* were washed with running tap water and distilled water. The leaves were shade dried for 20days, the dried leaves was powdered mechanically using electrical stainless steel blender Fig.1. The leaf powder of 10 g were weighed and boiled for 10-15 minutes with 100 mL double distilled water at 60°C and the extracts were filtered through Whatman filter paper No.1. The filtered extract was stored in refrigerator at 4°C for further use in the synthesis of silver nanoparticles.

C. Synthesis of Silver Nanoparticles

100mL (10^{-3}M) aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Then 10 ml of leaf extract and 90ml of double distilled water were added to 60 µl of aqueous silver nitrate solution kept in a separate conical flask of 250ml at room temperature. The conical flask was covered and kept in dark chamber until the solution colour changes from pale yellow to dark yellow. After 30 minutes, the solution turns from pale yellow to dark brown indicating the formation of silver nanoparticles Fig.2. The bio reduction of silver ions was monitored by sampling using UV Spectrophotometer, Fig.3.

D. Separation of silver Nanoparticles

The synthesized silver nanoparticles were separated by centrifugation using a REMI centrifuge at 10,000rpm for 15min. The supernatant liquid was re-suspended in the sterile double distilled water. The process was carried out thrice to get rid of any uncoordinated bio molecules. After, the desired reaction period, the supernatant liquid was discarded and the pellets were collected and stored at 4°C for further use.

E. Lyophilization

The pellet obtained was then lyophilized by using freeze dryer to enhance the stability of silver nanoparticles.

F. Characterization of Silver Nanoparticles

A colour change from pale yellow to dark brown upon incubation due to Surface Plasma Resonance (SPR) vibration was observed indicating the formation of nanoparticles. The optical absorbance between 300 nm and 800 nm with a Shimadzu UV-Visible spectrophotometer (UV-1800, Japan) were performed to investigate the reduction of silver ions by leaf extract. For Scanning electron microscopic studies, the particle size and surface morphology was confirmed using the images of nanoparticles were studied using Scanning Electron Microscopy (SEM; JEOL, ModelJFC-1600) and measurements were operated at an accelerating voltage of 120 kV. FTIR spectra of the samples were measured using Perkin-Elmer Spectrum instrument in KBr pellets is used to obtain the infrared spectra of absorption and emission of the formed silver nanoparticles. FTIR spectra were recorded from wave number 600-4000 cm^{-1}. For XRD studies, the spectra were recorded by using Phillips PW 1830 instrument operating at a voltage of 40 kV with CuKα1 radiation.

G. Larvicidal Bioassay

The larvicidal activity was carried out at room temperature. The larvicidal activity was assessed by the standard procedure of WHO [12] with little modifications. Twenty five in numbers of 1^{st},2^{nd},3^{rd} and 4^{th} instar larvae of *Aedes aegypti* were transferred separately from culture being maintained in the laboratory to the 250 ml disposable cups containing the 100 ml of desired concentration of AgNPs synthesized leaf extract of 1.00, 2.50, 5.00, 7.50 and 10ppm respectively. The control was set up with dechlorinated tap water. The moribund larvae were countedafter12 hrs and 24 hours of exposure and the percentage mortality was recorded for the average of five replicates, Fig.7.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae treated}} \times 100 \quad (1)$$

The values of LC_{50}, LC_{90} and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL) and Chi-square values were calculated by using Probit analysis [13].

III. Statistical Analysis

The average larval mortality data were subjected to Probit analysis, for calculating LC_{50} and LC_{90}, values were calculated by using the Finney (1971) method. Results with $P < 0.05$ were considered to be statistically significant from Table-1.

Fig. 1. *Eclipta alba* leaf powder

Fig. 2. Synthesis of AgNPs

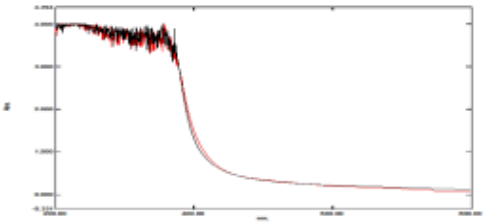

Fig. 3. UV-visible spectroscopy

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TABLE I
LARVICIDAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES FROM LEAVES OF *ECLIPTA ALBA* AGAINST *AEDES AEGYPTI* LARVAL STAGES I, II, III AND IV AFTER THE EXPOSURE PERIOD OF 12 HOURS

Larval instars	Dose in Con.(ppm)	%Mortality±S.D	LC_{50}ppm (LCL-UCL)	LC_{90}ppm (LCL-UCL)	$\chi 2$ chi-square
I Instar	Control	0±0	3.44(2.73-4.33)	59.67(26.94-132.16)	0.1751
	1.00	31.20±21.35			
	2.50	44.00±29.34			
	5.00	57.60±37.19			
	7.50	64.80±40.51			
	10.00	69.60±42.14			
II Instar	Control	0±0	3.35(2.67-4.21)	55.91(26.19-119.34)	3.364
	1.00	32.80±22.48			
	2.50	44.80±29.91			
	5.00	52.00±33.23			
	7.50	65.60±41.08			
	10.00	73.60±44.97			
III Instar	Control	0±0	3.48(2.81-4.31)	50.18(25.11-100.27)	4.1735
	1.00	31.20±21.35			
	2.50	43.20±28.77			
	5.00	55.20±35.49			
	7.50	60.80±37.68			
	10.00	76.00±46.66			
IV Instar	Control	0±0	2.27(1.82-2.83)	22.86(14.48-36.08)	3.3391
	1.00	36.80±25.31			
	2.50	50.40±33.87			
	5.00	62.40±40.58			
	7.50	77.60±49.56			
	10.00	82.40±51.19			

Control—Nil mortality. Significant at P<0.05 level
LC_{50} lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, $\chi 2$ chi-square.

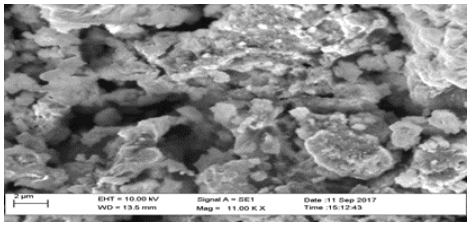

Fig. 4. SEM image

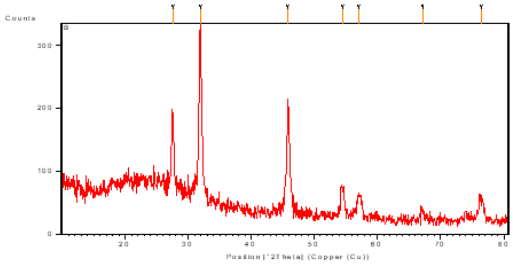

Fig. 5. XRD reading

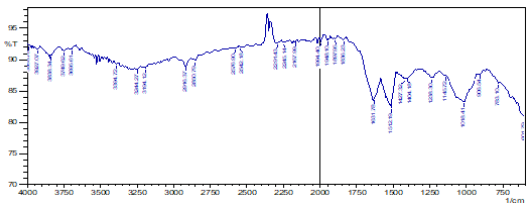

Fig. 6. FTIR analysis

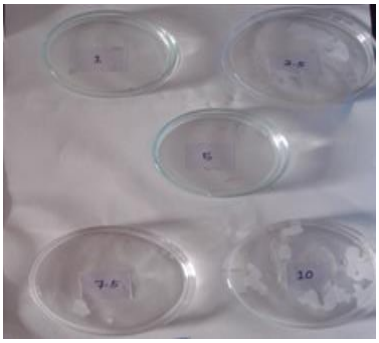
Fig. 7. Larvicidal bioassay

IV. RESULTS AND DISCUSSION

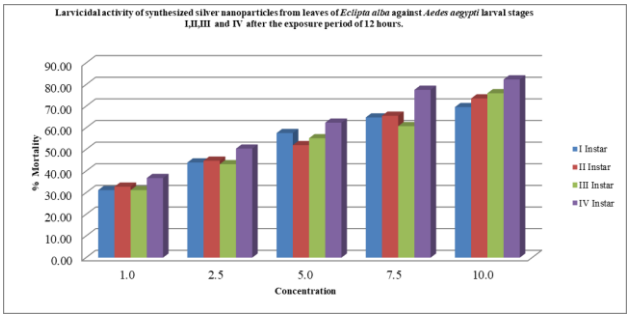

Fig. 8. Larvicidal activity of synthesized silver nanoparticles from leaves of *Eclipta alba* against *Aedes aegypti* larval stages I, II, III and IV after the exposure period of 12 hours

TABLE II
LARVICIDAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES FROM LEAVES OF *ECLIPTA ALBA* AGAINST *AEDES AEGYPTI* LARVAL STAGES I, II, III AND IV AFTER THE EXPOSURE PERIOD OF 24 HOURS

Larval instars	Dose in Con.(ppm)	%Mortality±S.D	LC_{50}ppm (LCL-UCL)	LC_{90}ppm (LCL-UCL)	$\chi 2$ chi-square
I Instar	Control	0±0	2.79(2.26-3.43)	15.76(10.72-23.18)	2.3811
	1.00	33.60±23.05			
	2.50	46.40±31.04			
	5.00	59.20±38.32			
	7.50	70.40±44.47			
	10.00	79.20 ±48.93			
II Instar	Control	0±0	2.39(1.91-2.98)	26.19(15.94-43.03)	4.8214
	1.00	36.80±25.31			
	2.50	49.60±33.30			
	5.00	60.00±38.89			
	7.50	73.60±46.73			
	10.00	83.20±51.76			
III Instar	Control	0±0	2.50(2.00-3.13)	29.79(17.42-50.94)	8.8923
	1.00	38.40±26.44			
	2.50	45.60±30.47			
	5.00	58.40±37.75			
	7.50	71.20±45.04			
	10.00	84.00±52.32			
IV Instar	Control	0±0	2.10(1.65-2.68)	24.74(15.01-40.78)	1.0298
	1.00	38.40±26.44			
	2.50	52.00±35.00			
	5.00	66.40±43.41			
	7.50	75.20±47.87			
	10.00	81.60±50.62			

Control—Nil mortality. Significant at P<0.05 level
LC_{50} lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, $\chi 2$ chi-square.

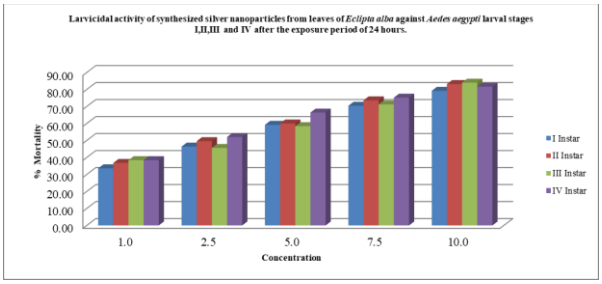

Fig. 9. Larvicidal activity of synthesized silver nanoparticles from leaves of *Eclipta alba* against *Aedes aegypti* larval stages I,II,III and IV after the exposure period of 24 hours

Vector management is one of the major issues due to the capacity of resistance against the usual insecticides. Therefore, an urgent need has been emerged to develop the new insecticides [14]. Identification of chemical constituents and larvicidal activity of essential oil from *Murraya exotica* L. (Rutaceae) against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). The larvicidal effects of aqueous extracts from leaves of *Ricinus communis* (*R. communis*) showed the LC_{50} values of 1091.44, 1364.58 and 1445.44 ppm against 2nd , 3rd and 4thlarval instars of *Culex quinquefasciatus*(15).The highest larval mortality was found in leaf ethyl acetate of *A. marmelos* and *Eclipta prostrata*, hexane, and methanol of *Andrograph is paniculata* and *C. hirsutus* showing LC_{50} values of 167.00, 78.28, 67.24, and 142.83 ppm and LC90 of 588.31, 360.75, 371.91, and 830.01 ppm, respectively [16]. The larvicidal activity of synthesized AgNPs was studied against the 1st, 2nd, 3rd, 4th instar larva at 12 and 24 h of exposure time. LC_{50} value observed after 12 hrs were 3.44, 3.35, 3.48, 2.27ppm and LC_{90} values were 59.67, 55.91, 50.18 and 22.86ppm respectively. After 24 hrs of exposure the observed LC_{50} value were 2.79, 2.39, 2.50, 2.10ppm and LC_{90} values were15.76, 26.19, 29.79, 24.74ppm respectively. In the present study, the larvicidal activity of AgNPs showed mortality rate at the 3rd instar was relatively higher against *Aedes aegypti*. During synthesis and characterization of silver nanoparticles, it is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The UV-visible spectra was observed at the maximum absorbance occurs at 357 nm Fig. 3. The silver nanoparticles were characterized by UV-Visible spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles [17], indicating the presence of spherical Ag nanoparticles. The excitation of Surface Plasmon Resonance (SPR) band at 410 to 430 nm confirmed the synthesis of AgNPs from plant products [18]. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the organic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating

stabilization of the nanoparticles by a capping agent [19]. SEM analysis shows high-density of AgNPs synthesized, wich shown that they are relatively spherical and uniform AgNPs formed with diameter of 2µm Fig: 4. The larger silver particles may be due to the aggregation of the smaller ones, in the SEM measurements. [20] reported the unidentified crystalline peaks (27.89°, 32.30°, 46.26°, 54.79°) are also apparent in many works in which the XRD pattern includes the relevant 2θ range. These peaks are due to the organic compounds which are present in the extract. Fig. 5, shows the XRD pattern of AgNPs and peak values at 2θ degrees of 27.6°, 31.9°, 45.7°,54.4°, 57.1°,67.0°, 76.4° corresponding to (311), (100), (111), (022), (020), (222), (131). Whereas XRD examination produces a diffraction pattern that is subsequently compared with data contained in a standard crystallographic database to determine structural information. Analysis of the XRD data identifies crystalline structure, preferred crystal orientation, and phases present in samples [21], [22]. FT-IR spectroscopy can be used to investigate surface chemistry and identify surface residues such as functional groups like carbonyls and hydroxyls moieties that attach to the surface during nanoparticle synthesis [23]. FTIR spectrum indicated the clear peaks with (1018.41, 1512.19, 1631.78, 2916.37, 3838.34 cm^{-1}) different values in the above peak values they corresponded to functional groups like, (C-N stretching amine group1018.41 cm^{-1}), (N=O nitroso group 1512.19 cm^{-1}), (C=N amine group 1631.78 cm^{-1}), (C-H alkane group 2916.37 cm^{-1}), (O-H alcohol group 3838.34 cm^{-1}) Fig.6. FTIR spectrum the most intense band at 1620cm^{-1} and 1636 cm^{-1} represent carbonyl groups from polyphenols; the results suggest that molecules attached with AgNPs have free and bound amide groups. These amide groups may also be in the aromatic rings. This concludes that the compounds attached with the AgNPs could be polyphenols with an aromatic ring and bound amide region (24). The functional groups such as alcohol, amines, amides, alkanes, methyl, aliphatic and halides confirmed their presence in AgNPs and these are the main classes in most of the functional groups. They were denoted as possible bio molecules responsible for stabilizing, capping and reducing agents of the AgNPs [25]-[27]. Hence the invention of new synthesized nanoparticles from leaf extract of *Eclipta alba* can thus replace the synthetic larvicidal products.

V. Conclusion

Natural products have been recognized as the valuable sources of insecticidal agents. On this context, many researchers have focused to evaluate these substances as promising tools for integrative control programs. Moreover, many natural products have poor water solubility. This fact should be considered, for example, if an effective larvicidal product is desired for *Aedes aegypti* control, the main vector of dengue, a public health problem in many developing countries. Due to the fact that application of adulticides may cause temporary diminish in the adult population, therefore more efficient and liable approach in mosquito control program is to target out the larval stages in their breeding sites as larvicides. Therefore, in this present study an attempt is made to control the larval population of *Aedes aegypti*, where significant larvicidal activity was seen using synthesized silver nanoparticles of *Eclipta alba* leaf extract.

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