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Insecticidalactivity of Green Synthesized silver Nanoparticles using *Coleus aromaticus* and *Wrightia tinctoria* Leaf Extracts against *Culex quinquefasciatus*

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Abstract

Background: Mosquitoes transmit dreadful diseases, causing millions of deaths every year. The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. The plant extracts seems to be a better alternative to control mosquitoes due to the presence of many bioactive compounds. In addition the green synthesized silver nano-particles have vital application in biological research. The present study addresses the green synthesized silver nano-particles (AgNPs) of *Coleus aromatics* and *Wrightia tinctoria* leaf extract and their larvicidal and pupicidal activity against *Culex quinquefasciatus*.

Methods: The synthesized AgNPs were characterized by UV-Vis spectrum, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy analysis (FTIR) and XRD measurements. The II, III, IV instar and pupa of *C. quinquefasciatus* were exposed to different concentration of silver nano-particles (5 ppm to 50 ppm) for 24 hours.

Results: LC_{50} values of II, III, IV instars and pupa of *C. quinquefasciatus* were 13.37 ppm and 16.26 ppm, 36.07 ppm and 41.47 pp. LC_{90} values are 35.47 ppm, 53.57 ppm and 67.60 ppm, 76.68 ppm against *Coleus aromatics*. Likewise *Wrightia tinctoria* LC_{50} values were 14.56 ppm, 18.77 ppm, 42.76 ppm and 51.06 ppm and LC_{90} values were 46.03 ppm, 62.17 ppm, 79.89 ppm and 93.16 ppm.

Conclusion: Bio synthesis of silver nano-particles using leaf aqueous extract of *Coleus aromaticus, Wrightia tinctoria* provides potential source for the larvicidal and pupicidal activity against *Cx. quinquefasciatus*. Between the two plants studied, the LC_{50} and LC_{90} values indicate that *Coleus aromatics* synthesized silver nano-particles were more effective than *Wrightia tinctoria*.

Keywords

Larvicidal activity; *Culexquin quefasciatus*; Green synthesized silver nano-particles

Introduction

Mosquitoes are important group of insects in terms of public health. Malaria, Filaria, Japanese Encephalitis, Dengue Fever, Heamorragic Fever, Yellow Fever, Chikungunya and Zika fever are the most dreadful diseases transmitted by the mosquitoes that lead to millions of deaths every year [1,2]. *C. quinquefasciatus* is one such mosquito species that spread disease in an estimated population of 120 million, among these 44 million people have chronic manifestation [3,4]. Repeated use of synthetic insecticides in controlling the mosquitoes have disrupted the natural biological control systems and led to resurgences of mosquito population [5-7]. This has also resulted in the development of resistance, undesirable effects on non-target organisms [8-10]. Bio-pesticides are an alternate for chemical insecticides as it is long lasting, low cost, eco-friendly and harmless to non-target organisms [11,12]. In the present study to enhance the activity of plants derived bioactive compound using modern technique adapted by synthesis of nano-particles using silver, copper, gold and zinc due to their unique physical, chemical and biological properties [13]. In this regard, an attempt was made to find out the differences in the larvicidal properties of green synthesized silver nanoparticles of plants such as *Coleus aromatics* and *Wrightia tinctoria* leaves against filarial vector *C. Quinquefasciatus.*

Materials and Methods

Collection of plant materials

The leaves of *Coleus aromatics* (Lamiaceae) and *Wrightia tinctoria* (Apocynaceae) leaves collected from Karambayam village (10.49°N, 79.30°E), Thanjavur district, Tamilnadu, India.

Preparation of plants extract

Fresh leaves were thoroughly washed with distilled water. 25 g leaves were taken and crushed with 100 ml sterile distilled water. The extract was filtered through Whatman No.1 filter paper (Pore size 25 µm). The filtrate was further filtered using a 0.6 µm sized filters [14].

Synthesis of silver nanoparticles

2.0 mM aqueous solution of silver nitrate ($AgNO_3$) used for the synthesis of silver nanoparticles. 10 ml of leaf extract was added to 90 ml of aqueous solution of 2 mM silver nitrate for reduction of Ag^+ ions and kept at room temperature for a period 5 hours. As a result, a brown solution is formed which indicates the formation of silver nanoparticles. The sample was centrifuged at 5000 rpm for 20 min and the resulting suspension was redispersed in 10 ml sterile distilled water. Centrifugation and redispersion process was repeated three times. The purified suspension was freeze dried to obtain dried powder. The dried nanoparticles were used for further analysis [15, 16].

Characterization of silver nanoparticles

UV-Vis Spectra Analysis**:** UV-Vis spectra analysis is an important technique to preview the morphology and stability of nanoparticles. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum (UV-3024) of the reaction medium after 5 hours diluting a small aliquot of the sample into distilled water.

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SEM Analysis of Silver Nanoparticles**:** Scanning Electron Microscopic (SEM) analysis was done using Vega 3 Tescan SEM machine. Thin film of the sample was made on carbon coated copper grid by just dropping a small amount of the sample, extra solution was removed by using a blotting paper and the film formed on the SEM grid was allowed to dry by keeping under a mercury lamp for 5 min [17].

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis was carried out at the central instrumentation facility of St. Joesph's College, Tiruchirappalli, Tamilnadu. Two milligram of silver nanoparticle was prepared by mixing with 200 mg of spectroscopic grade KBr. FTIR spectra were recorded using a Nicolet 520 P spectrometer with detector at 4000-400 cm^{-1} resolution and 20 scans per sample [18].

XRD-measurements

Green synthesized silver nanoparticles were determined by an X-pert pro x-ray diffractometer (Rigaku Ultima III XRD) operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in a θ-2θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform stains, using the Debye Scherrer's formula.

$D=0.94\lambda/\beta \, Cos \, \theta$

Where D is the average crystalline domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle [18].

Preparation of stock solution and test concentration

50 mg of green synthesized silver nano-particles first dissolved in 10 ml of distilled water this solution used for experiment and the make the test concentration 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm respectively.

Culture of test animal

Filarial vector, *C. quinquefasciatus* egg rafts were collected from stagnant sewage water of Thanjavur. The hatched larvae were cultured and maintained in the laboratory at room temperature (27°C ± 2°C) and 75%–85% relative humidity. The larvae were fed with dog biscuits and yeast powder in the ratio 3:1. Adults emerged from these larvae were reared in mosquito cage and the females were allowed to feed on avian (pigeon) blood and the males were provided with 10% glucose solution socked in cotton. Sample from this parent population was used to confirm the species by The District Entomologist of Malarial Control Program, Thanjavur. Eggs laid by these adults were cultured in a separate container and larvae developed from these eggs were used for bioassay studies [19,20].

Larvicidal and pupicidal bioassay

Mosquito larvicidal bioassay was carried out according to WHO [21] Standard procedure with slight modification. 200 ml of was it dechlorinated water was taken in a series of 250 ml beakers. The concentration starting 5 ppm to 50 ppm of silver nanoparticles of *Coleus aromatics* and *Wrightia tinctoria* were used for the experiment. A control was also maintained separately by adding now the total volume become 202 ml. was it the case with experimental insects also. 10 larvae per concentration were used for all the experiments. The number of dead larvae at the end of 24 h was recorded and the percent corrected mortality was calculated by using Abbott's formula [22]. The mortality rates of *C. quinquefasciatus* pupae were estimated in the same manner.

Statistical analysis

LC_{50} and LC_{90} data for the mortality and the relationship between concentration and mortality (dose dependent mortality) were subjected to regression analysis and a 95% confidential limited was calculated by probit analysis [23]. ANOVA was performed to find out the relationship between the green synthesized silver nanoparticles and larval mortality. SPSS 16 [24] version was used for doing the statistical analysis (Tables 1 and 2).

Results

Leaves extracts of *C. aromatics* and *W. tinctoria* were filtered and treated with 2 m M $AgNO_3$ solution and incubated at room temperature for 5 hours. The colour of the solution was changed into dark brown that this indicates confirmed that formation of nano-particles. Further the synthesis of silver nano-particles is confirmed

Table 1: The LC_{50} and LC_{90} values of *Coleus aromaticus* and *Wrightia tinctoria* synthesised silver nano-particle against the II, III, IV instar and pupa of *Culex quinquefasciatus* after 24 h of exposure.

Larval stages	LC_{50} (ppm) (UCL-LCL)	LC_{90} (ppm) (UCL-LCL)	x^2	Regression equation
Coleus aromatics				
II instar	13.37 (17.70–11.65)	35.47 (39.80–3.75)	3.250	Y=1.78+0.18X
III instar	16.26 (19.91–14.95)	53.47 (39.80 –33.75)	2.381	Y=1.42+0.16X
IV instar	36.07 (42.31–34.49)	67.60 (77.08–4.86)	.530	Y=-1.24+0.16X
Pupa	41.47 (51.49–39.21)	76.68 (82.92–5.10)	.258	Y=-1.31+0.12X
Wrightia tinctoria				
II instar	14.56 (45.28–13.17)	46.03 (49.75–44.64)	1.018	Y=1.62+0.17X
III instar	18.77 (22.41–17.49)	62.17 (65.81-60.97)	1.257	Y=0.99+0.16X
IV instar	42.76 (50.65–41.22)	79.89 (87.78–78.35)	.031	Y=-1.46+0.14X
Pupa	51.06 (59.26–50.04)	93.16 (101.95–92.14)	.376	Y=-1.256+0.10X

by UV-Visible absorption by spectroscopy. The peak was seen at 441 nm for *C. aromatics* whereas for *W. tinctoria* it was obtained at 418 nm (Figures 1 and 2). FTIR of synthesized silver nanoparticles was observed at 3336, 2922, 2278, 1612, 1389, 1016, 759, 525 cm^{-1} for *C. aromatics* and 2914, 2843, 2430, 2309, 2228, 2129, 1607, 1375, 1051,822, 771, 603, 442 cm^{-1} for *W. tinctoria* (Figures 3 and 4). In X-ray diffraction the characteristic peaks as exhibited between 20 and 80, where a distinct diffraction peak was obtained at 27°, 32°, 46°, 57° at 2θ values indexed to 220, 122, 220, 241 respectively for *C. aromatics* whereas *W. tinctoria* the peaks were obtained at 27°, 32°, 38°, 46° and 57° to a corresponding sets of lattice planes to 220, 122, 111, 200, and 241 respectively (Figures 5 and 6). Scanning Electron Microscopic (SEM) image analysis showed a clear shape and size of the AgNPs for *C. aromaticus* (79.46 nm, 89.39 nm) and *W. tinctoria* (99.34 nm,129.149 nm) (Figures 7 and 8). XRD analysis reveals the nature of the green synthesized silver nanoparticles (Tables 3 and 4).

Results of larvicidal bioassay of *C. aromaticus* and *W. tinctoria* synthesized AgNPs against II, III, IV instars and pupa of *C.*

Table 2: Two way ANOVA to test the validity of relationship in mortality (LC_{50}) as a function of two plants green silver nano-particles and larval and pupa.

Source of variation	SS	df	F	P-Value	F-crit
Green synthesized silver nanoparticles	49.60	1	6.69	0.081	10.12
Larval stages	1527.13	3	68.72	0.002	9.27
Error	22.21	3	2.29		
Total	1598.95	7			

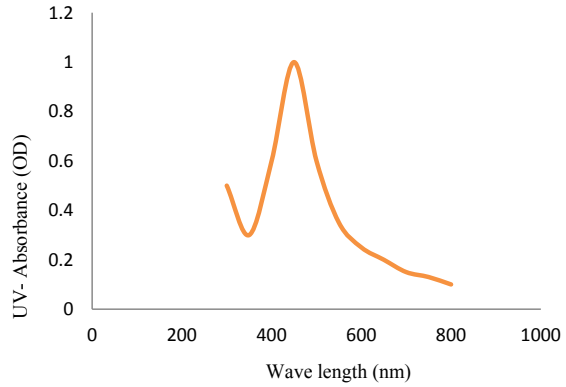

Figure 1: UV-Vis absorption spectra of silver nanoparticles synthesized by *Coleus aromaticus* leaf extract.

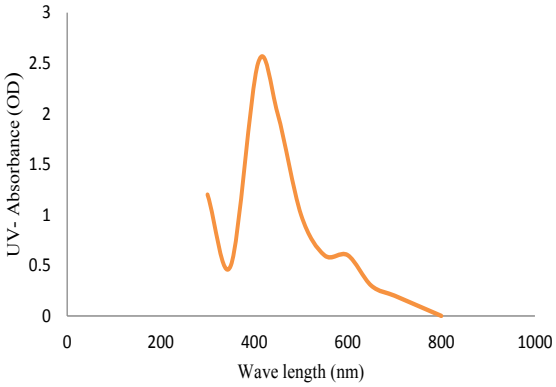

Figure 2: UV-Vis absorption spectra of silver nanoparticles synthesized by *Wrightia tinctoria* leaf extract.

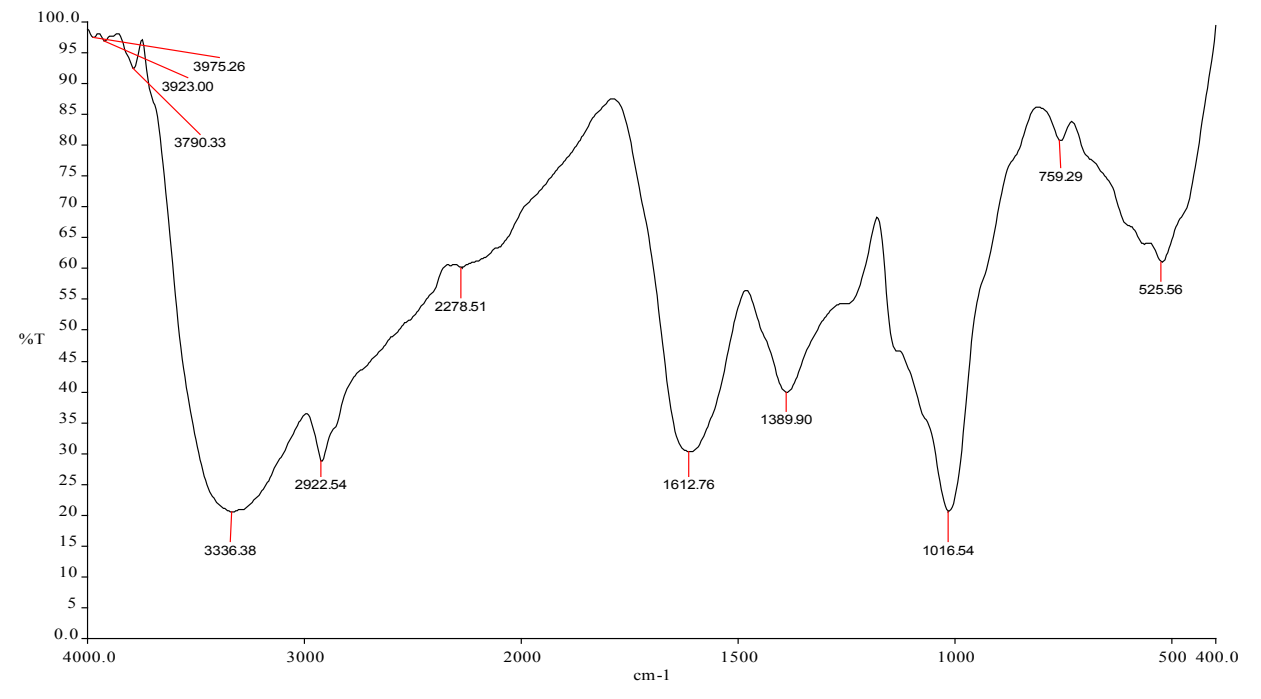

Figure 3: FTIR spectrum of silver nanoparticles synthesized by *Coleus aromaticus* leaf extract.

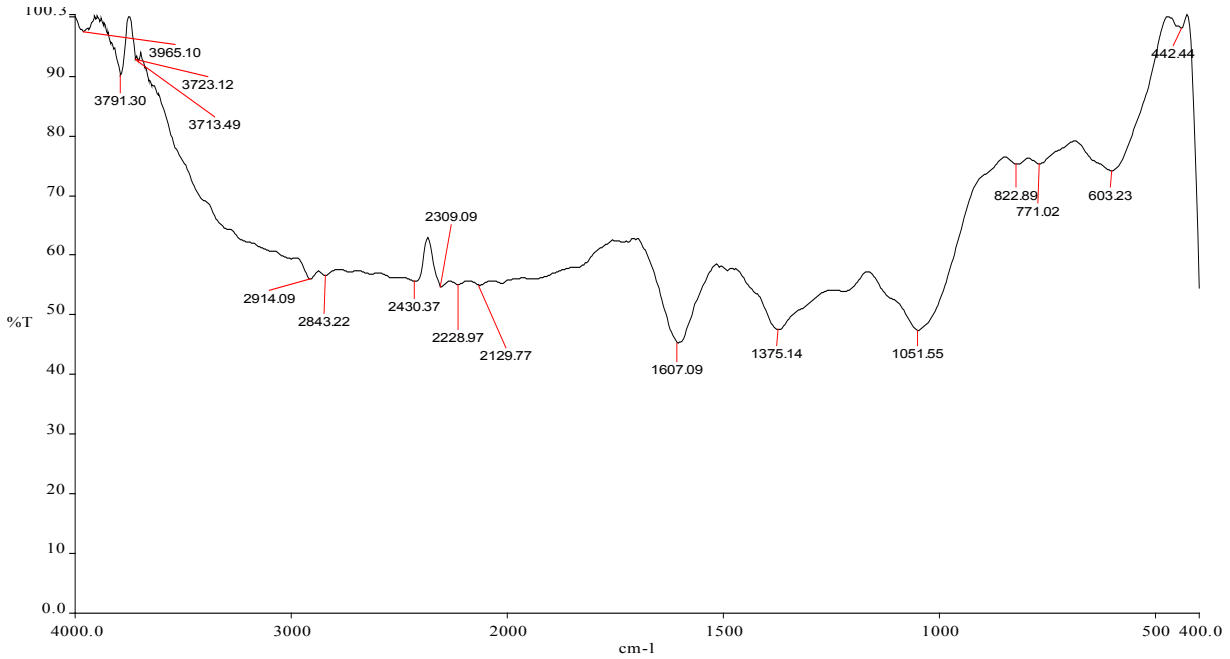

Figure 4: FTIR spectrum of silver nanoparticle synthesized by *Wrightia tinctoria* leaf extract.

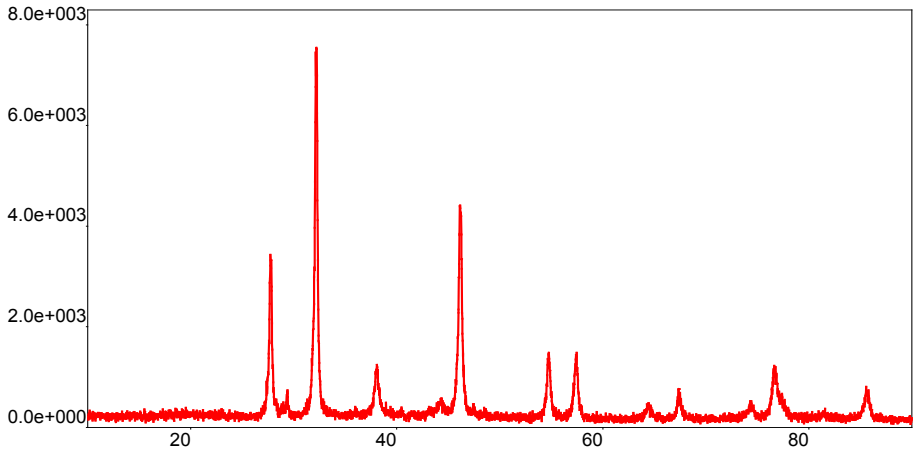

Figure 5: XRD analysis of silver nanoparticles synthesized by *Coleus aromaticus* leaf extract.

quinquefasciatus are given in Table 1. LC_{50} values were13.37 ppm and 16.26 ppm, 36.07 ppm and 41.47 ppm and the LC_{90} values are 5.47 ppm, 53.57 ppm and 67.60 ppm, 76.68 ppm for *C. aromatics*. Likewise, *W. tinctoria* synthesized silver nano-particles LC_{50} values were 14.56 ppm and 18.77 ppm, 42.76 ppm and 51.06 ppm and LC_{90} values are 46.03 ppm, 62.17 ppm, 79.89 ppm and 93.16 ppm against Cx. *quinquefasciatus*.

From the present study it is found that II instar of *C. quinquefasciatus* has more susceptible to green synthesized silver nanoparticles ($P<0.05$) than other larval stages for two plants. *C. aromaticus* was more effective than *W. tinctoria* nano-particles used. A two way analysis of variance was performed to find out the difference among the two plant species and larval stages on mortality and the analysis indicate that there is a significant difference ($P<0.05$) (Table 2) existing between the larval mortality as a function of larval stages and green synthesized silver nano-particles.

Discussion

The effect of green synthesized silver nanoparticles on the adult emergence was also studied. Though there was a reduction in the rate of adult emergence as a function of concentration, there was no significant difference among the green synthesized silver nanoparticles. Green synthesized nanoparticles are much popular as larvicide in controlling mosquitoes and most of these studies prefer silver nano-particles [25-27] while other metals like Copper [28], Zinc [29], Titanium [30], Magnesium [31] and Gold [32] are also in use.

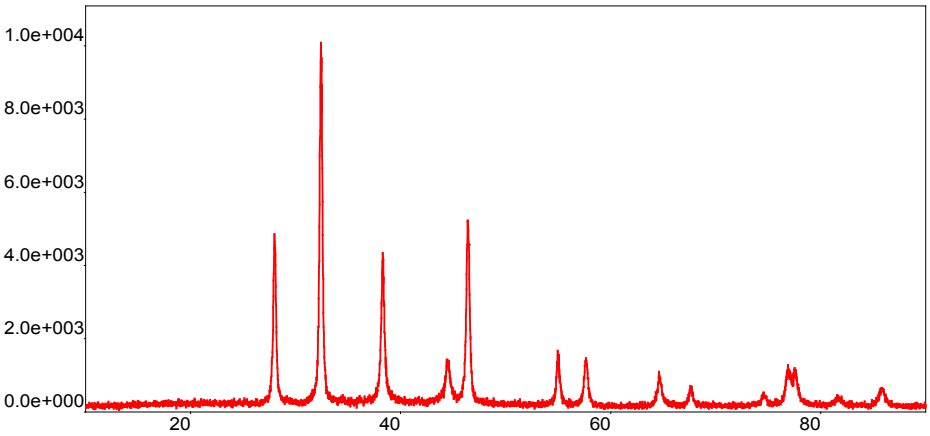

Figure 6: XRD analysis of silver nanoparticles synthesized by *Wrightia tinctoria* leaf extract.

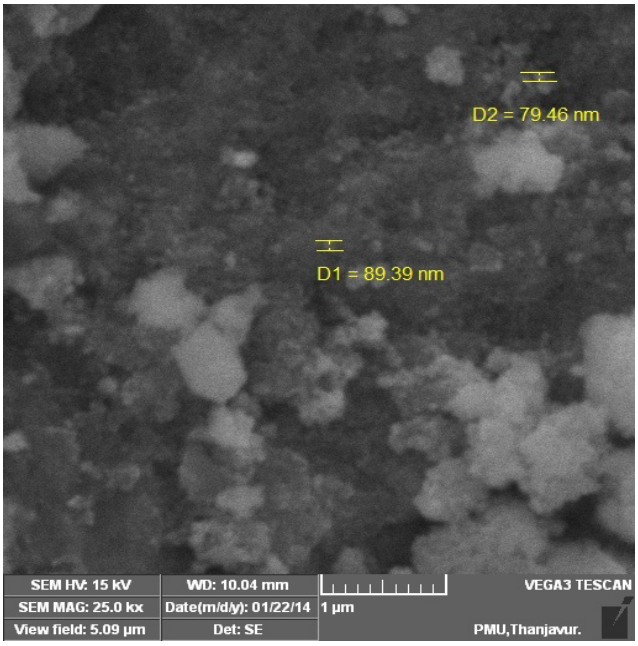

Figure 7: SEM image of silver nanoparticles synthesized by *Coleus aromaticus* leaf extract.

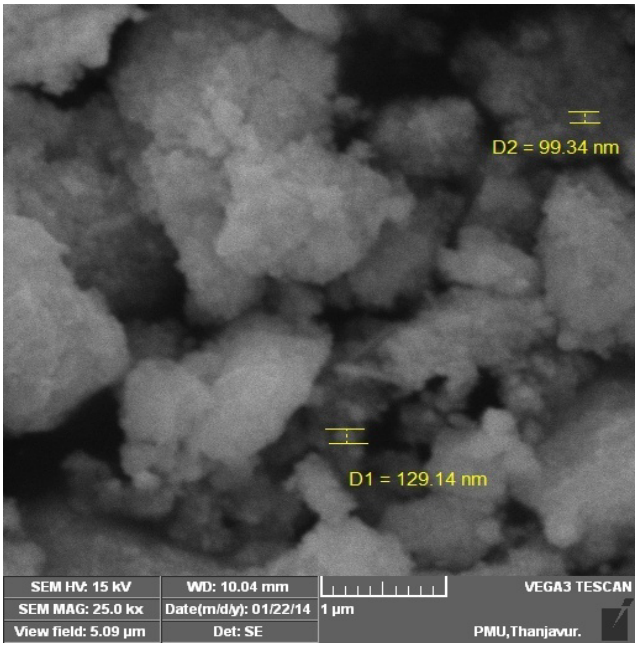

Figure 8: SEM image of silver nanoparticles synthesized by *Wrightia tinctoria* leaf extract.

Table 3: XRD patterns of *Coleus aromaticus* synthesized silver nano-particles.

No.	2-theta (deg)	D (ang.)	Height (cps)	FWHM (deg)	Int. I (cpsdeg)	Int. W (deg)	Asym. factor
1	27.747(7)	3.2125	2904	0.278	1326	0.46	0.63
2	32.165(4)	2.7806	6550	0.283	3038	0.46	0.64
3	46.144(9)	1.9656	3624	0.335	1910	0.53	0.68
5	57.39(3)	1.6043	861	0.45	521	0.61	0.9

Table 4: XRD patterns of *Wrightia tinctoria* synthesized silver nano-particles.

No.	2-theta (deg)	D (ang.)	Height (cps)	FWHM (deg)	Int. I (cps deg)	Int. W (deg)	Asym. factor
1	27.983(9)	3.1860	3140	0.294	1296	0.41	1.10
2	32.400(6)	2.7610	6849	0.288	2663	0.389	1.14
3	38.275(14)	2.3496	2812	0.305	1339	0.48	1.1
5	46.349(10)	1.9574	3472	0.339	1512	0.44	0.74
7	57.58(2)	1.5994	881	0.391	433	0.49	0.77

A comparative study on the larvicidal activity of *Nelumbo nucifera* leaf crude extract and silver nanoparticles was performed against the malarial and filarial vectors. LC_{50} values obtained for crude methanol, aqueous, and synthesized silver nanoparticles against the larvae of *A. subpictus* are 8.89, 11.82, and 0.69 ppm respectively while the LC_{90} values are 28.65, 36.06, and 2.15 ppm. In the case of *C. quinquefasciatus,* the LC_{50} values are 9.51, 13.65, and 1.10 ppm and the LC_{90} values are 28.13, 35.83, and 3.59 ppm for methanol, aqueous and silver nanoparticles respectively. From this it is known that green synthesized silver nanoparticles more toxic to malarial and filarial vectors than other extracts [33].

Similarly, Gnanadesigan [26] reported that the control *Ae. aegypti* and *C. quinquefasciatus* larvae using *Rhizophora mucronata* F. In this study also green synthesized nanoparticles are toxic to *C. quinquefasciatus* (LC_{50}=0.585 mg/l) than *Ae. aegypti* ($LC_{50=}$0.891 mg/l). Nano-particles synthesized using fungi and bacteria such as *Afaricus bisporus, Penicilium* spp, *E. coli* and *Vibrio* sp were also reported and the results indicate that *A. bisporus* synthesized nano-particles is promising in controlling *C. quinquefasciatus* [34]. To support this work, Borase [17] reported bio-synthesized nanoparticles of *Jatropha gossypifolia, Euphorbia tirucalli, Padilanthus tithymaloides* and *Alseuosmia macrophylla* against IV instar of *Ae. aegypti* and *An. stephensi*. Of these nanoparticles, nanoparticles synthesized using *J. gossypifolia* was effective against *Ae. aegypti* (LC_{50}=4.44 ppm) and *An. stephensi* (LC_{50}=4.90 ppm).

Green synthesized silver nanoparticle using *Adhatoda vasica* was tested against *C. quinquefasciatus* with a LC_{50} value of 450.03 ppm for III instar [35]. Velayutham and Ramanibai [36] studied the larvicidal activity of green synthesized silver nanoparticles of *Annonasquamosa* leaves against the larvae of *C. quinquefasciatus*. The LC_{50} values obtained for the first to fourth instar larvae are 2.50, 2.78, 3.02, 3.05 µg/ml respectively. Likewise Adesuji [17] studied the effect of biosynthesized silver nanoparticles (AgNPs) of *Cassia hirsute* aqueous leaf extracts against *C. quinquefasciatus* where the LC_{50} value is 4.43 ppm for fourth instar larvae. Larvicidal activity of silver nanoparticles synthesized by using the leaf extracts of *Azadirachta indica* was tested against *C. quinquefasciatus*. The results of this study indicates its highest toxicity is LC_{50}=500 ppm [35].

The relationship between concentration and adult emergence is also studied and the results indicate that irrespective of the plant species, when the concentration is increased, there is reduction in adult emergence. Though there is a reduction in the rate of adult emergence as a function of concentration, there is no significant difference among the two plants silver nanoparticles studied. Such studies indicate the enhanced activity of leaf extracts by applying nanotechnology which is reviewed recently by Mondal [27] and Benelli [37]; Elemike [38] and Khader [39].

Conclusion

In the present study, we have synthesized green silver nanoparticles using *C. aromaticus* and *W. tinctoria* leaf extract. These green synthesized AgNPs were characterized using UV-Vis, XRD, FTIR and SEM and evaluated for their larvicidal activity against *C. quinquefasciatus* larvae and pupa. Of the two plant extracts used, *C. aromaticus* has more effective than *W. tinctoria*. Hence these green synthesized plant extracts has potential to be used as larvicidal agent in mosquito bio-control program.

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