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Larvicidal and pupicidal activity of green synthesized silver nanoparticles using selected plants extract against *Culex quinquefasciatus*

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Abstract
Control of mosquitoes by using chemical insecticides creates several problems including development of resistance. This leads to find out alternative methods via plant products. Viewing this mind the present study addresses the green synthesis of silver nano-particles of *Aegle marmelos* and *Colocasia esculenta* leaf extract and it is larvicidal activity against II, III, IV instar and pupa of *Cx. quinquefasciatus.* 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 *Cx. quinquefasciatus* were exposed to different concentration Silver nanoparticles (5 ppm to 50 ppm) for 24 hr. LC_{50} values obtained for a period of 24 hours for *Aegle marmelos* leaf nanoparticle was 13.14ppm for II instar, 16.52ppm for III instar, 33.40 ppm for IV instar and 43.21ppm for pupa respectively. Likewise for *Colocasia esculenta* leaf nanoparticles the LC_{50} value is 9.64ppm for II instar, 13.88ppm for III instar, 32.69 for IV instar and 44.52ppm for pupa. Bio synthesis of silver nanoparticles using leaf aqueous extract of *Aegle marmelos, Colocasia esculenta* provides potential source for the larvicidal activity against *Cx. quinquefasciatus*. Among the two plants studied the LC_{50} values indicate that *Aegle marmelos* nanoparticles is effective than *Colocasia esculenta.*

Keywords: Larvicidal activity, pupicidal activity, *Culex quinquefasciatus*, green synthesized silver nanoparticles

1. Introduction
Mosquito borne diseases are the most health hazardous problems in tropic and sub- tropics and several mosquito species belonging to genera *Anopheles, Culex* and *Aedes* act as vectors of malaria, filariasis, Japanese encephalitis, dengue fever, hemorrhagic fever, yellow fever, chickungunya and zika fever etc [1,2]. Attempts were made to control mosquito at larval as well as adult stage [3]. Synthetic chemical pesticides are much in use to control mosquitoes which create pesticidal pollution and have an adverse effect on non target organism; these are also non-degradable [4]. In this contest bio-pesticides are developed which are plant based have promising results [5]. To enhance the activity, these plant based product nanotechnology is applied in mosquito larval control. Biosynthesized nanoparticles are easily degradable, nontoxic to non target organisms and environmentally safe [6].
A. marmelos is commonly known as Bael and belong to the family Rutaceae. Medicinal value of the leaf, root, bark, seeds and fruits [7]. The leaves of Bael are astringent, a laxative, and an expectorant and are useful in the treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation and asthmatic complications [8].
C.aromaticus a member of family Lamiaceae is an Indian traditional herb with several medicinal properties. The plant is traditionally used externally for burns and insect bites, while internally it is used as a carminative and to control asthma. C. aromaticus is reported to also possess few other medicinal properties as antiepileptic, antimutagenic, antitumourogenic and antigenotoxic effects, antiinflammatory and antitumor, diuretic, antioxidant and antimicrobial activities [9]. In the present study to enhance the activity of plants derived bioactive compound using modern technique adapted by synthesis of nano-particles using silver nanoparticles using *Aegle marmelos* and *Colocasia esculenta* leaf extract and tested these extract against for *Cx. quinquefasciatus* larvae and pupa.

2. Materials and Methods

2.1 Collection of plant materials

The plant leaves of *Aegle marmelos* (Rutaceae) and *Colocasia esculenta* (Asteraceae) were collected from Karambayam village (10.49^0N, 79.30^0E), Thanjavur district Tamilnadu, India.

2.2 Preparation of plants extract

Fresh leaves were collected and thoroughly washed with distilled water of these 25g leaves were taken and crushed with 100ml sterile distilled water. The extract was filtered through Whatman No.1 filter paper (Pore size 25µm). The filtrate was further filtered using a 0.6 µm sized filters [10].

2.3 Synthesis of silver nanoparticles

2.0 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of leaf extract was added to 90 ml of aqueous solution of 2mM silver nitrate for reduction into 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 to further analysis [11].

2.4 Characterization of silver nanoparticles

1.0mg of NPs was dissolved in 1.0ml of distilled water and particles size distributions were measured at a light scattering angle of 90º. The intensity-weighted mean valued was recorded as the average of three measurements.

2.5 UV-Vis spectra analysis

UV-Vis spectra analysis to preview the morphology and stability of nanoparticles. The reduction of pure Ag+ ions was monitored by measuring the UV- Vis spectrum of the reaction medium after 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral was analysis done by using UV-Vis spectrometer UV-3024.

2.6 SEM analysis of silver nanoparticles

Scanning Electron microscopic (SEM) analysis was also done using Vega 3 Tescan SEM machine. Thin film of the sample was made a 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

2.7 Fourier transforms 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 were prepared mixing with 200 mg of spectroscopic grade KBr. FTIR spectra were recorded using a Nicolet 520P spectrometer with detector at 4000-400 cm-1 resolution and 20 scans per sample [12].

2.8 XRD measurements

Silver nanoparticles were determination by an Xpert pro X-ray diffract meter (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.

2.9 Preparation of stock solution and test concentration

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

2.10 Culture of test animal

The egg rafts were collected from stagnant water body. The hatched larvae were cultured and maintained in the laboratory at 27 ± 2°C and 85% relative humidity. The larval forms were fed with dog biscuit and yeast power in the ratio 3:1[13, 14].

2.11 Larvicidal bioassay

Mosquito larvicidal bioassays were carried out according to WHO [15] standard procedure with slight modification. 200 ml of tap water was taken in a series of 250 ml beakers. The test concentration was made 5 ppm to 50 ppm of silver nano-particle of *Aegle marmelos* and *Colocasia esculenta.* A control was also maintained separately by adding 2 ml of distilled water to 200 ml of water. 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 mortality rate (%) was calculated by using Abbott's formula [16].

2.12 Pupicidal bioassay

Pupicidal activity of green synthesized silver nanoparticles of *A. marmelos* and *C. esculenta* plants was evaluated against *Cx. quinquefasciatus* pupae. The LC_{50} and LC_{90} values of pupae against *A. marmelos* are 43.21ppm and 72.47 ppm. Likewise *C. esculenta* the LC_{50} value are 44.52 ppm and LC_{90} value is 78.80 ppm. It is known that AgNPs of *A. marmelos* was effective against the pupa of *Cx. quinquefasciatus* than the *C. esculenta* nanoparticles used.

2.13 Statistical analysis

SPSS 16 (SPSS 2007) [17] version package was used for determination of LC_{50} and LC_{90} data for the mortality and the relationship between concentration mortality was subjected to regression analysis and 95% Confidential limited was calculated by Probit analysis[18] and ANOVA was performed to find out the relationship between the green synthesized silver nanoparticles and larval and pupa mortality.

3. Results

The aqueous extract of *Aegle marmelos*, *Colocasia esculenta* leaf extract was filtered and treated with 2mM AgNPs solution and incubated at room temperature for 5 hours. The colour of the solution was changed into dark brown. Which indicates the synthesis of nano-particles. Further the synthesis of silver nano-particles is confirmed by UV-Visible

absorption spectroscopy. The peak is seen at 434nm for *Aegle marmelos* and at 456nm for *Colocasia esculenta* [Figure 1, Figure 2]. FTIR of synthesized Ag nano-particles was observed at 3967, 3889, 3790, 3241, 2921, 1603, 1390, 1235, 1149, 1075, 1021, 833, 758, 570, 529 cm-1 for *Aegle marmelos* and 3911, 3962, 3776, 3260, 2923, 2285, 1593, 1383, 1032, 772, 615, 535 cm-1 for *Colocasia esculenta* [Figure 3, Figure 4]. In X-ray diffraction the characteristic the peak was exhibited between 20-80, where distinct diffraction peak at was obtained 27^0,32^0, 46^0, 54^0, 57^0, 67^0 and 76^0 corresponding sets of lattice planes to (220),(122),(200),(331),(220) and (220) for *Aegle marmelos* and *Colocasia esculenta* peak at 27^0, 29^0, 32^0, 46^0, 54^0, 57^0, 64^0 and 76^0, at 2θ values indexed to (220),(111),(122), (200),(331), (241), (220) and (220) based silver nano particles (Figure 5, Figure 6) Scanning Electron Microscopic (SEM) image analysis showed a clear shape and size of the nano-particles of AgNPs for *Aegle marmelos* (101.38nm, 92.17nm) and *Colocasia esculenta* (92.17nm, 82.95nm) [Figure 7, Figure 8].

The result of larvicidal bioassay of synthesized AgNPs against II, III, IV instars and pupa of *Culex quinquefasciatus* LC_{50} and LC_{90} given (Table. 1). The LC_{50} and LC_{90} of *Aegle marmelos* green synthesis of silver nanoparticles against *Culex quinquefasciatus* II, III, IV instars and pupa are 13.14ppm and 16.52ppm, 33.40ppm and 43.21ppm and 36.18ppm, 54.54ppm and 72.47ppm,71.98 respectively. For like likewise 9.64ppm and 13.88ppm, 32.69ppm, 44.52 and 28.89ppm, 48.44ppm and 77.22ppm, 78.80ppm for *Colocasia esculenta*. Control was no mortality.

From the present study it is inferred that II instar larvae are more susceptible to green synthesized silver nanoparticles (P<0.05). among the two plants, *C. esculenta* was effective than other green synthesized silver nanoparticles used. A two way analysis of variance was performed to find out the difference among the plant species and larval stages on mortality and the analysis indicate that there is a significant difference (P<0.05) existing between the larval mortality as a function of larval stages and green synthesized silver nanoparticles (Table.2).

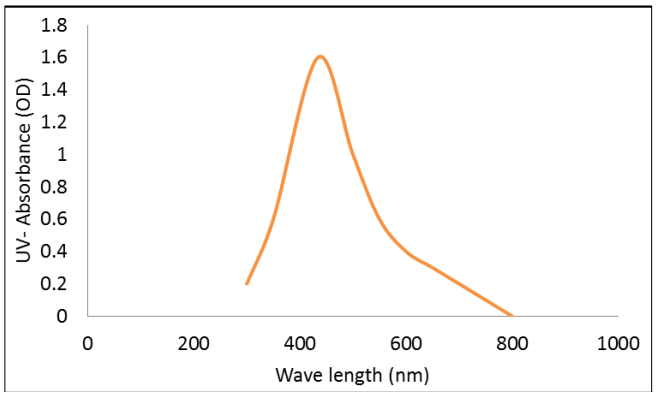

Fig 1: UV-Vis absorption spectra of silver nano-particles synthesized by *Aegle marmelos* leaf extract

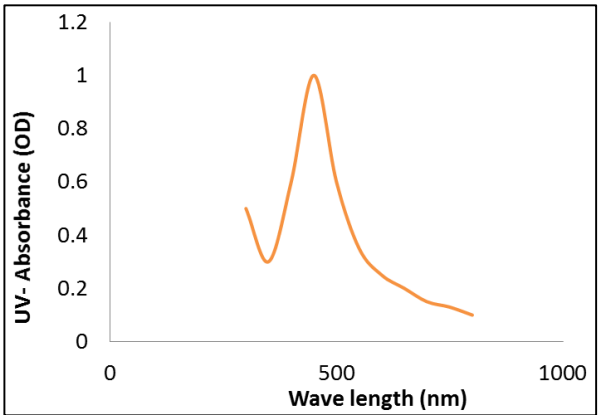

Fig 2: UV-Vis absorption spectra of silver nano-particles synthesized by *Colocasia esculenta* leaf extract

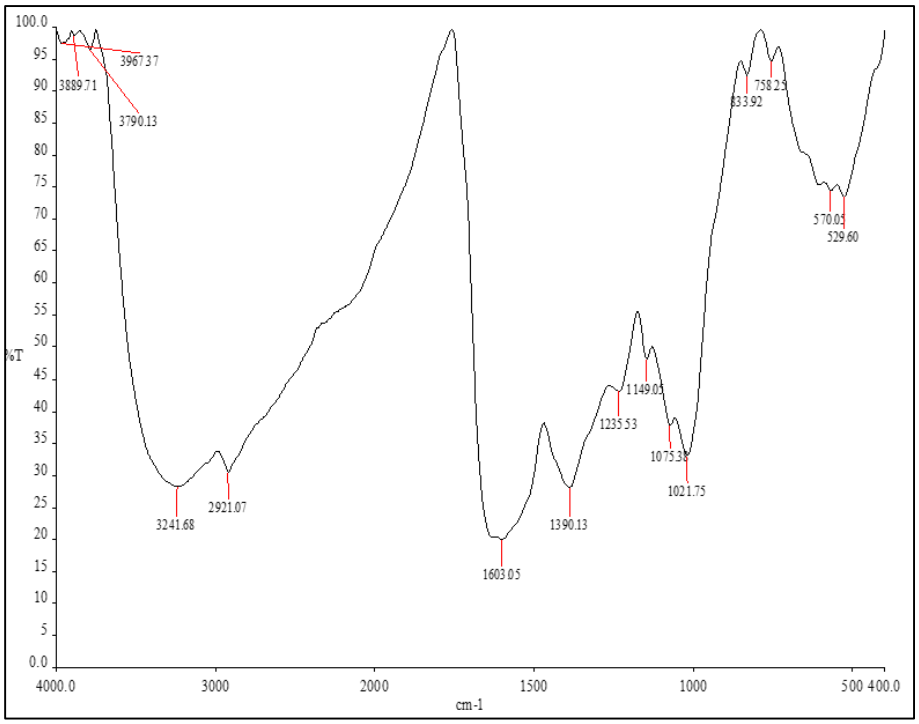

Fig 3: FTIR spectrum of silver nano-particles synthesized by *Aegle marmelos* leaf extract

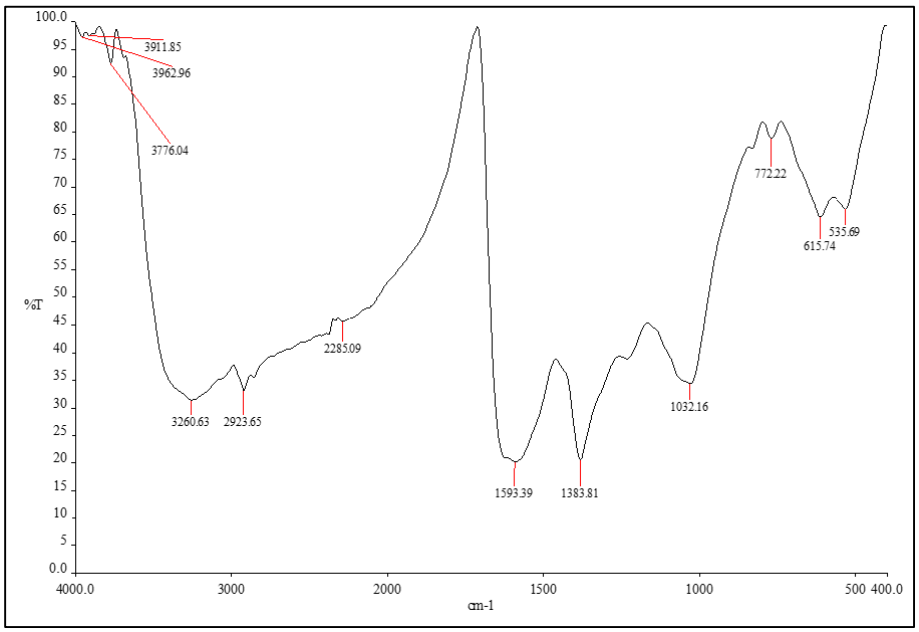

Fig 4: FTIR spectrum of silver nano-particles synthesized by *Colocasia esculenta* leaf extract

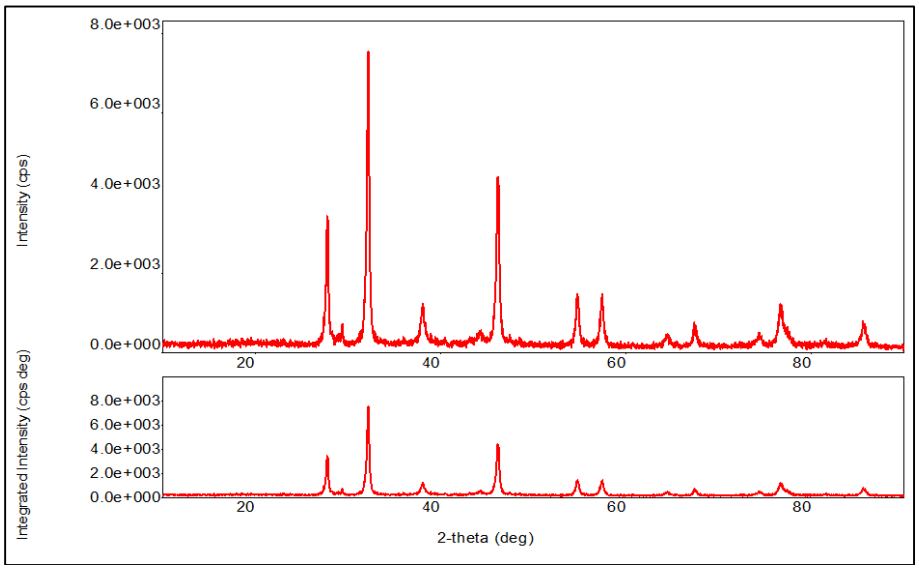

Fig 5: XRD analysis of silver nano-particles synthesized by *Aegle marmelos* leaf extract

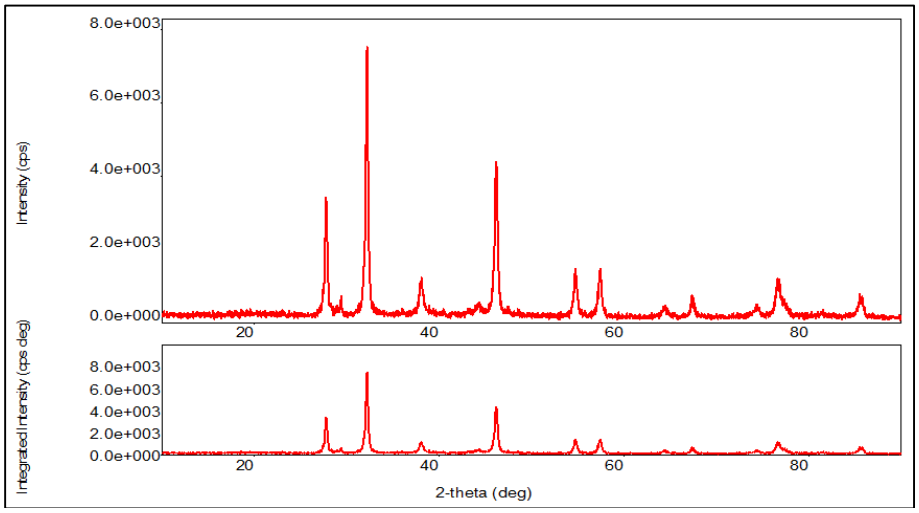

Fig 6: XRD analysis of silver nano-particles synthesized by *Colocasia esculenta* leaf extract

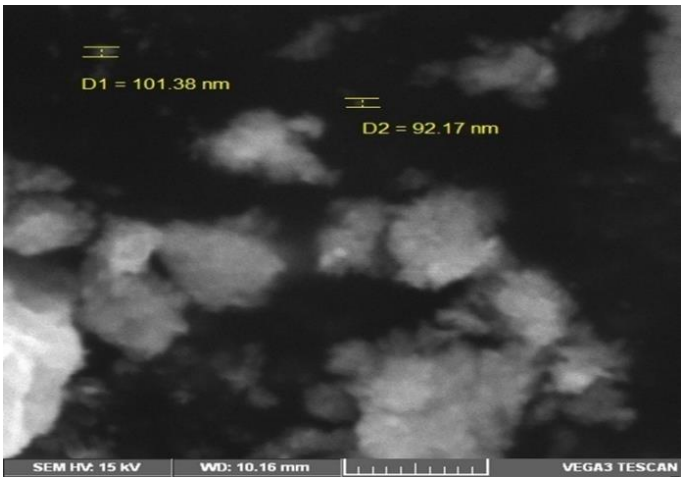

Fig 7: SEM image of silver nano-particles synthesized by *Aegle marmelos* leaf extract

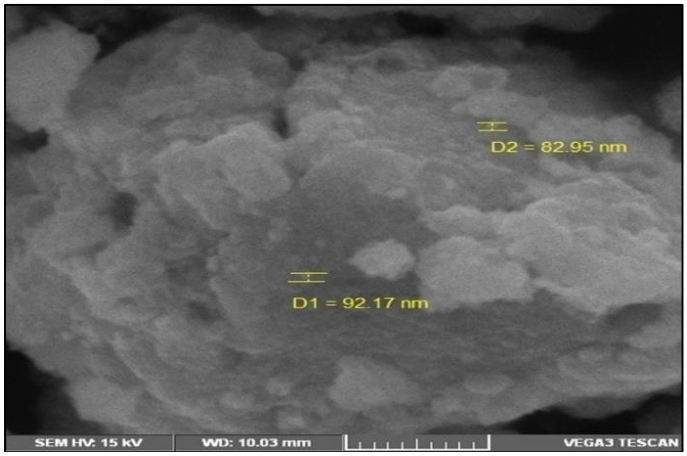

Fig 8: SEM image of silver nano-particles synthesized by *Colocasia esculenta* leaf extract

Table 1: The LC_{50} and LC_{90} values of *Aegle marmelos*, *Coleus aromaticus* synthesis of silver nanoparticles against the II, III, IV instar and pupa of *Culex quinquefasciatus* under 24 h exposure.

Plant species	Larval stages	LC_{50} (ppm) (UCL-LCL)	LC_{90} (ppm) (UCL-LCL)	$\chi 2$	Regression equation
Aegle marmelos	II instar	13.14 (17.31-11.48)	36.18 (40.35-34.52)	1.005	Y= 1.78 + 0.18 X
	III instar	16.52 (20.15-15.21)	54.54 (58.16-53.22)	.468	Y= 1.35 + 0.16 X
	IV instar	33.40 (39.36 - 31.75)	72.47 (78.44-70.82)	1.237	Y= -1.05 + 0.17 X
	Pupa	43.21 (52.83 - 41.27)	71.98 (81.60-70.04)	.520	Y= -1.26 + 0.11 X
Colocasia esculenta	II instar	9.64 (13.57-8.20)	28.89 (32.52 -27.45)	3.92	Y= 3.56 + 0.14 X
	III instar	13.88 (17.32-12.65)	48.44 (51.88-47.21)	1.383	Y= 2.19 + 0.15 X
	IV instar	32.69 (37.13-31.34)	77.22 (81.66-75.87)	.270	Y= -1.02 + 0.17 X
	Pupa	44.52 (53.29-42.96)	78.80 (87.56-77.24)	.018	Y= -1.47 + 0.13 X

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

Source of variation	SS	*df*	F	P-Value	F-crit
Green synthesized silver nanoparticles	3.836	1	3.83	1.67	0.2865
Larval stages	1397.63	3	465.87	203.04	0.0058
Error	6.88	3	2.29		
Total	1408.35	7			

Significant at $P<0.05$ level

4. Discussion

A comparative study on the larvicidal activity of *N. nucifera* leaf crude extract and silver nano-particles was performed against the malarial and filarial vectors. This study indicates that than crude extract the green synthesized of silver nanoparticle show high mortality against the malarial and filarial vectors [18]. Gnanadesigan *et al*. (2011) [20] reported that the control *Ae. aegypti*, *Cx. quinquefasciatus* larvae using *R. mucronata* nanoparticles. These green synthesized nanoparticle are highly toxic to *Cx. quinquefasciatus* than *Ae. aegypti*. Biogenic nanoparticle synthesis using fungi and bacteria such as *A. bisporus*, *Penicilium spp*, *E.coli* and *Vibrio sp* were also attempted and the results indicate that *A. bisporus* synthesized nanoparticle is promising in controlling *Cx. Quinquefasciatus* [21]. Phyto synthesized nanoparticles using for *J.gossypifolia*, *E. tirucalli*, *P.tithymaloides* and

A.macrophylla were also evaluated against IV instar of *Ae. aegypti* and *An.stephensi*. Of these *J. gossypifolia* nanoparticle is effective against *Ae. aegypti* (LC_{50} = 4.44ppm) and *An.stephensi* (LC_{50} = 4.90ppm) [22].

AgNPs synthesized nanoparticles are used for *Euphorbia hirta* leaf extract showed larvicidal activity against malarial vector, *A. stephensi* [23]. *Cocos nucifera* coir mediated AgNPs were studied against IV instar of *An. stephensi* and *Cx. quinquefasciatus* [24]. The recently reported the methanolic crude extract of *Colocasia esculenta, Eclipta prostrata* and *Wrightia tinctoria* aginst *Cx. quinquefasciatus*. *Colocasia esculenta* indicate that the LC_{50} value 165.69 ppm for IV instar [25]. The insecticidal activity of the selected plant, and a high efficiency due to the favorable surface area to volume ratio due to the small size of the particles (1–100 nm) [26, 27]. This is enhanced by the synthesis of silver nanoparticles as evident from the report.

5. Conclusion

In the present study green synthesis of silver nanoparticle using *Aegle marmelos* and *Colocasia esculenta* leaf extract were made. The green synthesized of silver nanoparticles possess larvicidal activity against *Cx. quinquefasciatus* larvae and pupa. Of the two plant extracts used *Colocasia esculenta* is effective when compared with *Aegle marmelos.* Hence it is recommended to use these green synthesis plant extract as larvicidal agent in mosquito bio control program.

5. References

1. ICMR Bulletin. Prospects of using herbal, products in the control of mosquito vectors 2003;33(1):1-10.
2. Lixin S, Huiquin D at al., Larvicidal activity of extracts of *Ginko biloba* Exocarp for three different strains of *Culex pipiens* pallens. J Med. Entomol 2006;43(2):258-261.
3. Liu HQ, Xu L *et al*., Chlorpyrifos resistance in Mosquito *Culex quinquefasciatus*. J Med. Entomol 2005;42(5):815-820.
4. Brown AWA. Insecticide resistance in mosquito's pragmatic review. J Am. Mosq. Control. Assoc 1986;2:123-140.
5. Parashar UK, Saxenaa PS *et al*., Bioinspired synthesis of silver nanoparticles. Dig. J. Nanomater. Biostruct 2009;4:159-166.
6. Subarani S, Sabhanayakam *et al*., Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stepensi* Liston and *Culex quinquefasiatus* Say (Diptera:Culicidae). Paracitol res 2013;112(2):487-499.
7. Dhankhar S, Ruhil S, Balhara M, Dhankhar S, Chillar AK. Aegle marmelos (Linn.) Correa: A potential source of phytomedicine. J Med Plant Res 2011;5:1497-1507.
8. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Corr. J Ethnopharmacol 2005;96:159-163.
9. Chatterjee A, Pakrashi SC. The Treatise of Indian Medicinal Plants. (Council of Industrial and Scientific Research, New Delhi) 2001.
10. Suganya A, Murugan K *et al*., Green synthesis of silver nanoparticles using *Murraya koenigii* leaf extract against *Anopheles stephensi* and *Aedes aegypti*. Parasitol. Res 2013;112(4):1385-1397.
11. Suganya G, Karthi S *et al*., Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae). Parasitol. Res 2011;109(3):823-831.
12. Rajkumar G, Rahuman AA. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vector. Acta. Trop 2011;188(3):196-203.
13. Kuppusamy C, Murugan K. Mosquitocidal effect of *Andrographis paniculatanees* against the malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Inter. J Integrative. Biol 2009;5:75-81.
14. Arivoli S, John Ravindran K *et al*., S. Larvicidal activity of botanicals against the filarial vector *Culex quinquefasciatus* say (Diptera: culicidae). Inter. J Res. Zoo 2012;2(1):13-17.
15. WHO. Vector resistance to pesticides, Fifteenth report of the WHO Expert Committee of Vector Biology and Control. WHO. Tech. Rep. Ser 1992;818:1-62.
16. Abbott WS. A method of computing the effectiveness of insecticides. J. Econ. Entomol 1925;(18):267-269.
17. SPSS. SPSS for windows, version 11.5.SPSS Chicago, IL 2007.
18. Finney DJ. In Probit Analysis, Cambridge University Press, London 1971,68-78.
19. Santhoskumar T, Rahuman AA *et al*., Synthesis f silver nanoparticles using *N.nucifera* leaf Extract and its larvicidal activity against Malaria and Filariasis vectors. Parasitol. Res 2011;108(3):693-702.
20. Gnanadesigan M, Ravikumar S *et al*., Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property. Asian. Pac. J Trop. Med 2011,799-803
21. Dhanasekaran D, Thangaraj R. Evaluation of larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector. Asian. Pac. J. Trop. Dis 2013;3(3):174-179.
22. Borase HP, Patil CD *et al*., Phyto-Synthesized silver nanoparticles: A Potent mosquito biolarvicidal agent nanomedicine and biotherapeutic discovery. J Nanomedine Biotherapeutic Discov 2013;3(1):1-7.
23. Priyadarshini KA, Murugan K *et al*., Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol. Res 2012;111(3):997-1006.
24. Roopan SM, Rohit G *et al*., Low coast and eco-friendly Phyto synthesis of silver nanoparticles using *Cocus nucifera* coir extract and larvicidal activity. Ind. Crop. Prod 2013;43:631-635.
25. Dass K, Mariappan P. Larvicidal activity of *Colocasia esculendum, Eclipta prostrata* and *Wrightia tinctoria* leaf extract against *Culex quinquefasciatus*. Proc.Natil. Acad Sci India Sect. B. Biol Sci 2016;86:139-143.
26. Muthukumaran U, Govindarajan M, Rajeswary M, Hoti S. Synthesis and characterization of silver nanoparticles using Gmelina asiatica leaf extract against filariasis, dengue, and malaria vector mosquitoes," Parasitology Research 2015;114(5):1817-1827.
27. Nalini M, Lena M, Sumathi P, Sundaravadivelan C. Effect of phyto-synthesized silver nanoparticles on developmental stages of malaria vector, Anopheles stephensi and dengue vector, Aedes aegypti, Egyptian Journal of Basic and Applied Sciences 2017;4(3):212-218.