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Synthesis of Silver Nanoparticles by Using Plants Extract and its Efficiency Against *Aedes aegypti* (Linn.)

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Abstract: The use of chemical pesticides to control mosquitoes generates a variety of problems, particularly developing resistance. This leads to the discovery of new techniques based on green production of silver nanoparticles. In this study we synthesized silver nanoparticles by using leaf extracts of *Aegle marmelos* and *Colocasia esculenta* and evaluated their larvicidal efficacy against *Aedes aegypti* II, III, IV instars and pupa. The green synthesized silver nanoparticles were characterized by various spectroscopy techniques, such as UV, FTIR, XRD and SEM. Thus, the data indicated that the plant extract and its nanoparticles may be a preferable alternative to chemical insecticides.

Keywords: Silver nanoparticle**,** *Aegle marmelos*, *Colocasia esculenta, Aedes aegypti,* Larvicidal activity

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Introduction

Mosquito-borne diseases are the most dangerous health concerns in the tropical and subtropical regions, and many mosquito species from various genera such as *Anopheles, Culex,* and *Aedes* transmit malaria, filariasis, Japanese encephalitis, dengue fever, hemorrhagic fever, yellow fever, chickungunya, and zika fever etc. Attempts were made to suppress mosquitoes at both the larval and adult stages (Prod and Repos, 2011; Priya and Santhi, 2014; Hajra and Mondal, 2015).

Synthetic chemical pesticides are widely used to control mosquitoes, but as these pesticides are non-biodegradable, they cause pollution and have a negative impact on non-target organisms (Karthikeyan *et al.,* 2014). Thus, plant-based bio-pesticides with promising effects are an alternative for these pesticides (Soni and Prakash, 2012). Currently, several studies used plant-based nanoparticles to control mosquito larvae and pupa. These biosynthesized nanoparticles are easily degradable, harmless to non-target species, and safe to the environment (Kalyanasundaram and Gunasekaran, 2013; Datta, 2014; Naseem *et al.,* 2016).

Aegle marmelos belongs to the family Rutaceae. Leaf, root, bark, seeds, and fruits of this plant have medicinal value and used to cure ophthalmia, deafness, inflammations, cataract, heart palpitation, asthmatic problems, diabetes, cholesterol, peptic ulcer, diarrhoea, dysentery, anticancer, cardio-protective, antibacterial, antifungal, radio-protective, antipyretic, analgesic, constipation, respiratory infection, antioxidant, hepato-protective, wound healing, and many more conditions (Uma Devi *et al.,* 2011; Kumar Sekar, 2011; Satyal *et al.,* 2012). The plant *Colocasia esculenta* is widely used in traditional medicine as antimicrobial, antihepatotoxic, anticancer, anti-lipidperoxidative, antibacterial and antifungal, antidiabetic, antitimelanogenic, and antihelmintic (Reyad-ul-Ferdous *et al.*, 2015)

In this study, *Aegle marmelos* and *Colocasia esculenta* leaf extracts were used to enhance the activity of plant-derived bioactive compounds by synthesis of nano-particles using silver nanoparticles. These were tested against *Aedes aegypti* larvae and pupa.

Materials and Methods

Collection of plant material and extract preparation:

Aegle marmelos (Linn.) (Rutaceae) and *Colocasia esculenta* (L.) Schott (Asteraceae) plant leaves were collected in Karambayam village (10.490N, 79.300E) in Thanjavur district, Tamil Nadu, India. Fresh leaves of *Aegle marmelos* and *Colocasia esculenta* were rinsed with distilled water before being pulverized with 100 ml distilled water. Whatman No. 1 filter paper (pore size 25 mm) was used to filter the extract. The filtrate was then filtered again with 0.6 mm filters (Singh *et al.,* 2013).

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 2 mM silver nitrate for reduction into Ag^+ ions and kept at room temperature for 5 h (Priya *et al.,* 2013). As a result, a brown solution forms, indicating that silver nanoparticles have been produced (Ramar *et al.,* 2014). The material was centrifuged at 5000 rpm for 20 min before being redispersed in 10 ml sterile distilled water. Centrifugation and redispersion processes were carried out three times. To obtain dry powder, the filtered suspension was freeze dried. The dried nanoparticles were used for experiment (Karthikeyan *et al.,* 2014).

Characterization of silver nanoparticles:

UV-Vis spectrum analysis:

UV-Vis spectra analysis is used to predict nanoparticle shape and stability. After 5 h of diluting a small portion of the sample into distilled water, the reduction of pure Ag^+ ions was measured by UV-Vis spectrum of the reaction medium. UV-Vis spectral analysis was performed with the UV-3024 UV-Vis spectrometer (Adesuji *et al.,* 2016).

Fourier transforms infrared spectroscopy (FTIR):

The FTIR analysis was performed at St. Joseph's College's Central Instrumentation Facility in Tiruchirappalli, Tamil Nadu. 2 mg of silver nanoparticles was added to 200 mg of spectroscopic grade KBr. A Nicolet 520P spectrometer with a detector at 4000-400 cm-1 resolution and 20 scans per sample was used to acquire FTIR spectra (Subashini *et al.,* 2016).

X-Ray Diffraction measurements:

An XPERT PRO X-ray diffract metre (Rigaku Ultima III XRD) operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in θ-2θ configuration was used to determine silver nanoparticles. Using the Debye Scherrer's formula, the crystallite domain size was determined from the width of the XRD peaks, assuming that they were free of non-uniform stains:

$$D=0.94\lambda/\beta \cos \theta$$

Where D is the average crystalline domain size perpendicular to the reflecting planes, λ denotes the X-ray wavelength, β is the line broadening at half the maximum intensity (FWHM) after subtracting the instrumental line broadening, in radians, and θ is the Bragg angle (Bhuvaneswari *et al.,* 2016).

SEM analysis of silver nanoparticles:

The Vega 3 Tescan SEM machine was used for scanning electron microscopic (SEM) analysis. A thin film of the sample was created on a carbon coated copper grid by dropping a small amount of the sample onto the grid, excess solution was removed with blotting paper, and the film formed on the SEM grid was allowed to dry for 5 min under a mercury lamp (Velayutham and Ramanibai, 2016).

Stock solution and test concentration preparation:

To begin, 50 mg of silver nanoparticles were dissolved in 10 ml distilled water (Satsangi *et al.,*2016). Test concentrations of 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were obtained from the stock solution and used in the experiment.

Maintenance of experimental animal:

The Vector Control Research Centre in Madurai, Tamil Nadu, India, provided the *Aedes aegypti* eggs. The eggs hatched into the first instar, which was kept at room temperature (27±2 C) and humidity levels of 75-85 per cent in the research lab. 3:1 mixture of dog biscuit and yeast powder was fed to the larvae (Rawani, 2017). The second, third, and fourth instars and pupa of *Aedes aegypti* were used in this experiment.

Bioassay for larvicidal and pupicidal activity:

The WHO (WHOPES, 2005) standard technique for mosquito larvicidal bioassays was followed with minor modifications. In a series of 250 ml beakers, 200 ml of tap water was collected. Silver nanoparticles from *Aegle marmelos* and *Colocasia esculenta* were tested at concentrations ranging from 5 to 50 parts per million (ppm). A separate control was kept by adding 2 ml of distilled water to 200 ml of water. 10 larvae and pupae were used in experiment for each concentration . The number of dead larvae was counted at the end of the 24 h and the mortality rate (percentage) was determined using Abbott's formula (Abbott, 1987).

Statistical analysis:

The LC_{50} and LC_{90} data for mortality were determined using the SPSS 16 (SPSS 2007) software package and the relation between concentration and mortality was subjected to regression analysis with a 95% confidence interval. The association between green produced silver nanoparticles and larval and pupa mortality was determined using Probit analysis (Finney, 1952). ANOVA was used to determine the relationship.

Results

Aqueous extracts of *Aegle marmelos* and *Colocasia esculenta* leaf extracts were filtered and incubated at room temperature for 5 h after being treated with 2 mM AgNPs solution. The solution's colour was changed to dark brown. This denotes the creation of nanoparticles. UV-Visible absorption spectroscopy was also used to check the production of silver nanoparticles. *Aegle marmelos* had a peak at 434 nm, while *Colocasia esculenta* had a peak at 456 nm (Figs. 1, 2). For *Aegle marmelos*, FTIR of manufactured Ag nanoparticles was found at 3967, 3889, 3790, 3241, 2921, 1603, 1390, 1235, 1149, 1075, 1021, 833, 758, 570, 529 cm^{-1}, and for *Colocasia esculenta*, 3911, 3962, 3776, 3260, 2923, 2285, 1593, 1383, 1032, 772, 615 cm^{-1}, respectively (Figs. 3, 4) . In X-ray diffraction the characteristic peak was exhibited between 20-80, where distinct diffraction peak was obtained at 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 nanoparticles (Figs. 5, 6). Scanning Electron Microscopic (SEM) image

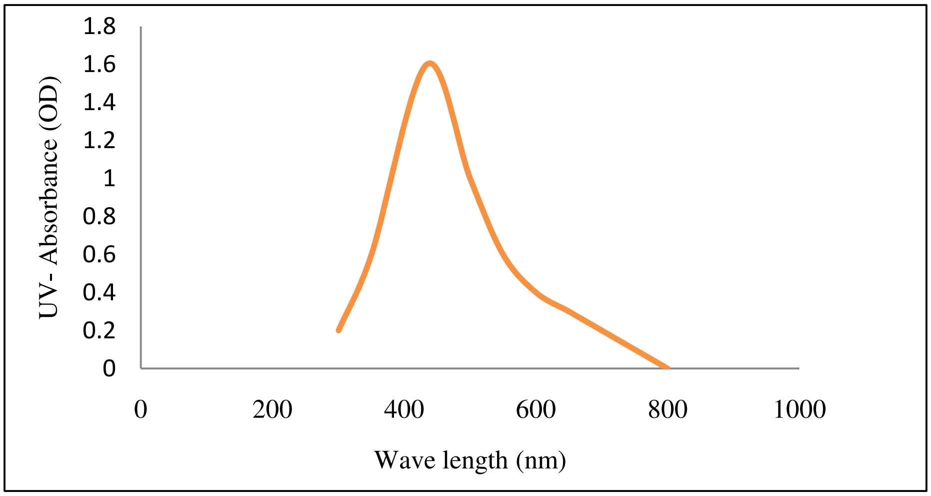

Fig. 1: UV-Vis absorption spectra of silver nanoparticles synthesized by *Aegle marmelos* leaf extract.

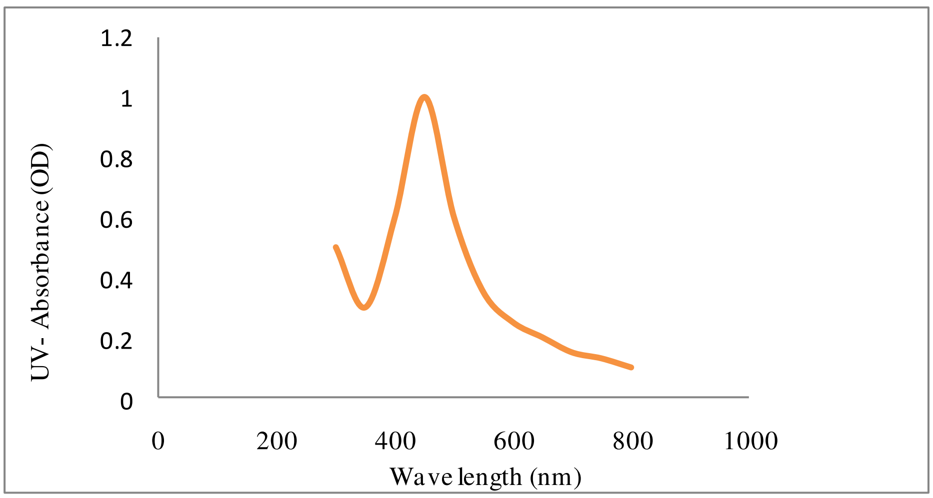

Fig. 2: UV-Vis absorption spectra of silver nanoparticles synthesized by *C. esculenta* leaf extract.

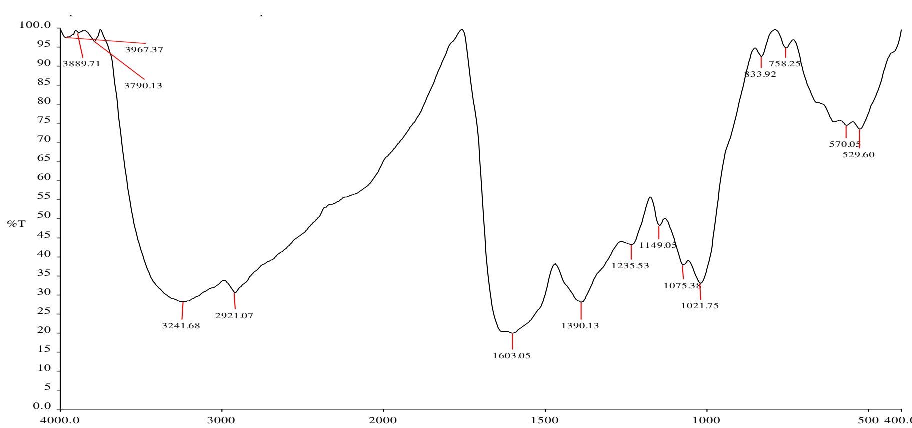

Fig. 3: FTIR spectrum of silver nanoparticles synthesized by *Aegle marmelos* leaf extract.

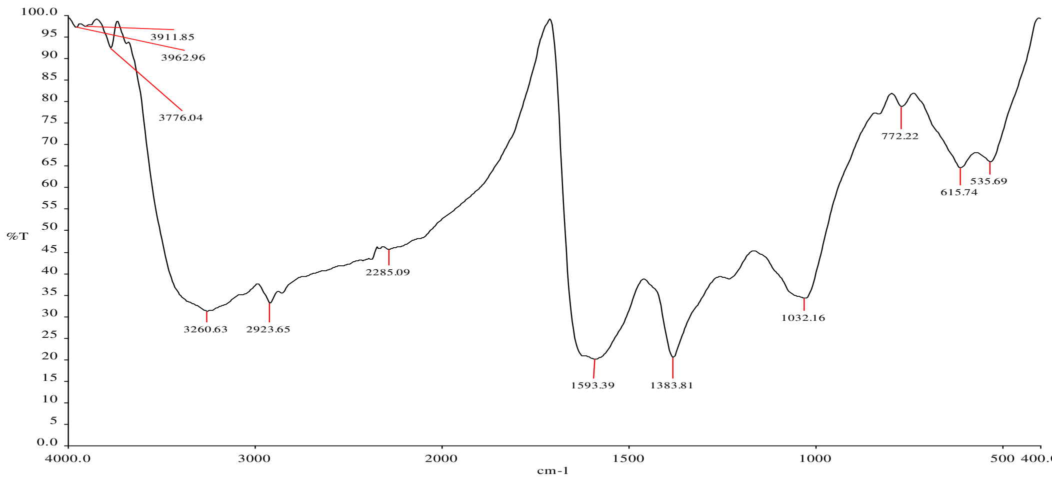

Fig. 4: FTIR spectrum of silver nanoparticles synthesized by *C. esculenta* leaf extract.

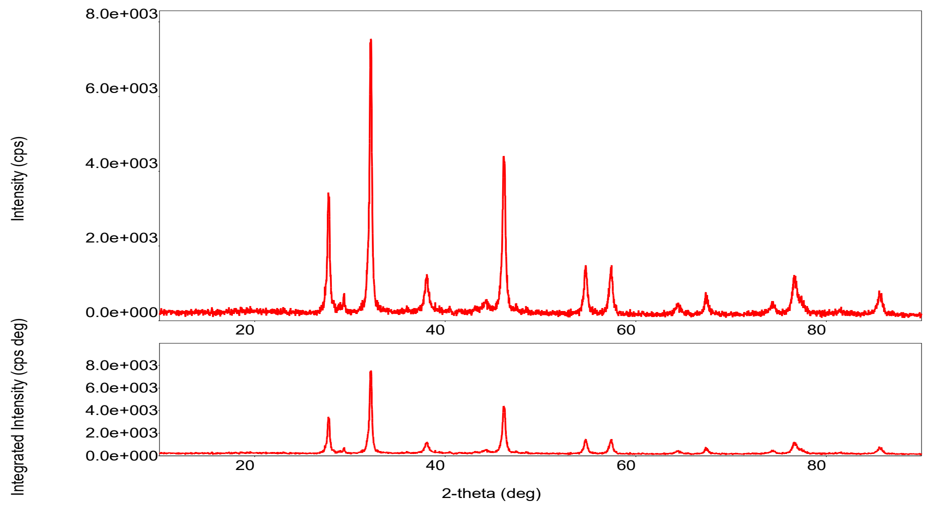

Fig. 5: XRD analysis of silver nanoparticles synthesized by *Aegle marmelos* leaf extract.

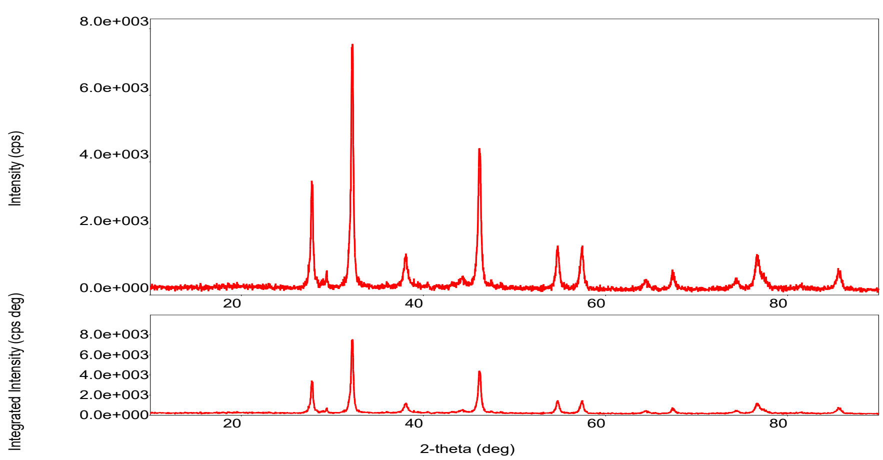

Fig. 6: XRD analysis of silver nanoparticles synthesized by *C. esculenta* leaf extract

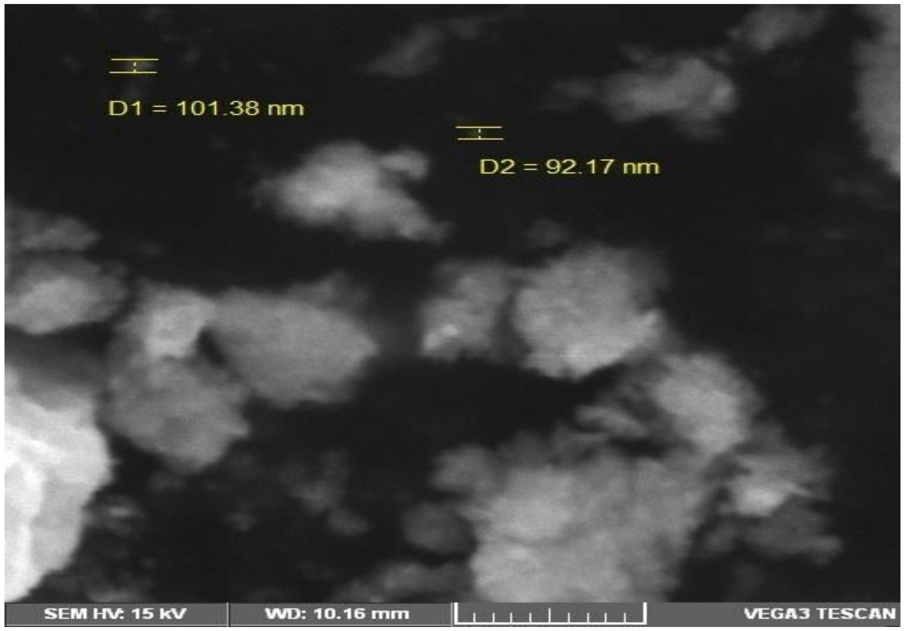

Fig. 7: SEM image of silver nanoparticles synthesized by *Aegle marmelos* leaf extract.

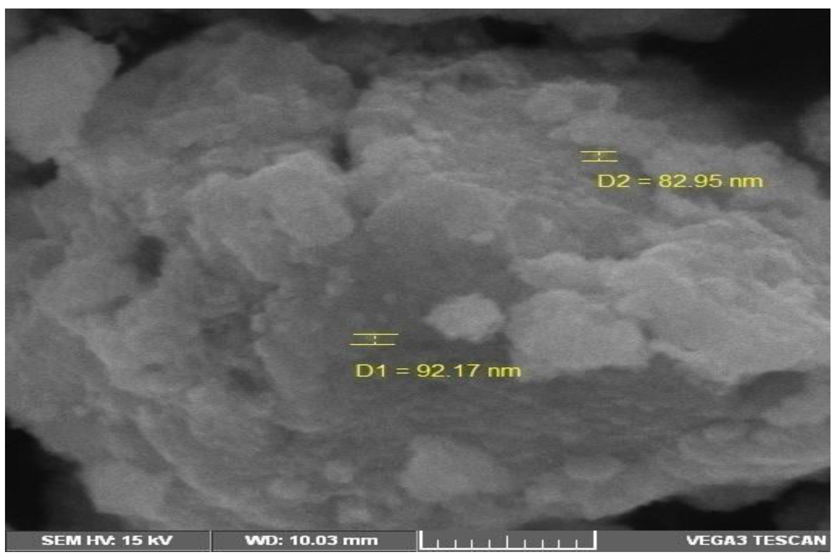

Fig. 8: SEM image of silver nanoparticles synthesized by *C. esculenta* leaf extract.

analysis showed a clear shape and size of the nanoparticles of AgNPs for *Aegle marmelos* (101.38 nm, 92.17 nm) and *Colocasia esculenta* (92.17 nm, 82.95 nm) (Figs. 7, 8).

The LC_{50} and LC_{90} values were determined by larvicidal bioassay of synthesised AgNPs against *Aedes aegypti* II, III, IV instars and pupa (Table 1). The LC_{50} values of *Aegle marmelos* green synthesis of silver nanoparticles against *Aedes aegypti* II, III, IV instars and pupa were determined as 11.99, 14.56, 28.42 and 44.31 ppm, respectively, whereas the LC_{90} values were determined as 32.77, 44.54, 57.83 and 99.63 ppm, respectively. The LC_{50} values of *Colocasia esculenta* green synthesis of silver nanoparticles against *Aedes aegypti* II, III, IV instars and pupa were determined as 10.39, 13.40, 30.03 and 55.61 ppm, respectively, whereas the LC_{90} values were determined as 29.25, 38.19, 57.23 and 108.77 ppm, respectively. There was no mortality in the control group.

According to the results of this investigation, II instar larvae were more susceptible to green

Table 1: The LC_{50} and LC_{90} values of *A. marmelos*, *C. esculenta* synthesis of silver nanoparticles against the II, III, IV instar and pupa of *Aedes aegypti under* 24 h exposure

Plant species	Larval stages	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ2	Regression equation
Aegle marmelos	II instar	11.99 (7.61-16.24)	32.77 (23.10-64.39)	0.789	Y= 2.26 + 0.17 X
	III instar	14.56 (9.20-20.19)	44.54 (30.19-100.71)	0.939	Y= 1.69 + 0.17 X
	IV instar	28.42 (21.41 - 56.68)	57.83 (43.18-123.43)	0.920	Y= -1.06 + 0.19 X
	Pupa	44.31 (34.10 - 92.57)	99.63 (61.44-189.92)	0.996	Y= -1.09 + 0.13 X
Colocasia esculenta	II instar	10.39 (6.11-14.27)	29.25 (20.76 -59.24)	0.668	Y= 3.13 + 0.15 X
	III instar	13.40 (8.43-18.393)	38.19 (26.61- 78.65)	0.754	Y= 1.88 + 0.17 X
	IV instar	30.03 (23.07-37.82)	57.23 (43.62-119.87)	0.048	Y= -1.30 + 0.20 X
	Pupa	55.61 (42.65 - 534.44)	108.77 (64.40- 244.90)	0.957	Y= -0.95 + 0.09 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	MS	F	P-value	F crit
Green synthesized silver nanoparticles	1896.534438	3	632.1781	34.98105957	0.0077	9.276
Larval stages	12.8778125	1	12.87781	0.7125832	0.4605	10.12
Error	54.2160375	3	18.07201			
Total	1963.628288	7				

Significant at P<0.05 level

produced silver nanoparticles (P<0.05). *Colocasia esculenta* outperformed the other green produced silver nanoparticles utilised in the study. The difference in mortality between plant species and larval stages was determined using a two-way analysis of variance, and the results showed that there is a significant difference (P<0.05) between larval mortality of larval stages and green produced silver nanoparticles (Table 2).

Discussion

There are few reports regarding larvicidal and pupicidal activities of various plant parts and their larvicidal potential (Korbekandi and Iravani, 1994; Rout *et al.,* 2012; Priya and Santhi, 2014; Hajra and Mondal, 2015; Naseem *et al.,* 2016; Panda, 2022). Silver nanoparticles and crude extracts were tested against malarial and filarial vectors. Larvicidal activity of *Nelumbo nucifera* silver nanoparticles had a higher mortality rate against malarial and filarial vectors (Santhoskumar *et al.,* 2011). The control of *Aedes aegypti* and *Culex quinquefasciatus* larvae by using *Rhizophora mucronata* nanoparticles has been reported by Gnanadesigan *et al.* (2011). *Culex quinquefasciatus* showed more response than *Aedes aegypti* to these green nanoparticles. Biogenic nanoparticle creation was also attempted using fungus and bacteria such as *Agaricus bisporus*, *Penicilium* spp, *Escherichia coli*, and *Vibrio* sp., with the results indicating that nanoparticles generated by *Agaricus bisporus* are promising in controlling

Culex quinquefasciatus (Dhanasekaran and Thangaraj, 2013).

Annona squamosa leaf extract with AgNPs nanoparticles showed larvicidal action against *Culex quinquefasciatus* and *Aedes aegypti* (Velayutham and Ramanibai, 2016). In the laboratory, the larvicidal activity of silver nanoparticles synthesized by *Azadirachta indica* leaf extracts against *Culex quinquefasciatus* was tested (Subashini *et al.*, 2016). According to the findings of this investigation, *Culex quinquefasciatus* larval mortality was high and the *Azadirachta indica* leaf extract was found to be safe for non-target organisms (Subashini *et al.*, 2016)

Ambrosia arborescens aqueous extracts and green-synthesised silver nanoparticles (AgNPs) were tested against *Aedes aegypti* third instar larvae. AgNPs were shown to be more effective (LC_{50} 0.28 ppm; LC_{90} 0.43 ppm) than the plant extract (LC_{50} 1844.61 ppm; LC_{90} 6043.95 ppm) (Morejon *et al.,* 2018).

In order to determine the larvicidal property of silver nanoparticles (AgNPs) against *Anopheles stephensi* and *Aedes aegypti*, an *in vitro* study was conducted using *Belosynapsis kewensis* leaf extract. These findings suggested that green produced AgNPs could be used as a contender for controlling *Anopheles stephensi* and *Aedes aegypti* in an environmentally friendly and cost-effective manner (Bhuvaneswari *et al.,* 2016).

Conclusion

The present study used *Aegle marmelos* and *Colocasia esculenta* leaf extract to create green silver nanoparticles. The green silver nanoparticles showed larvicidal activity against *Aedes aegypti* larvae and pupa. When comparing the two plant extracts used, *Colocasia esculenta* outperforms than *Aegle marmelos*. Thus, these green synthesized plant extracts are suggested for use as a larvicidal agent in mosquito biocontrol programmes.

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