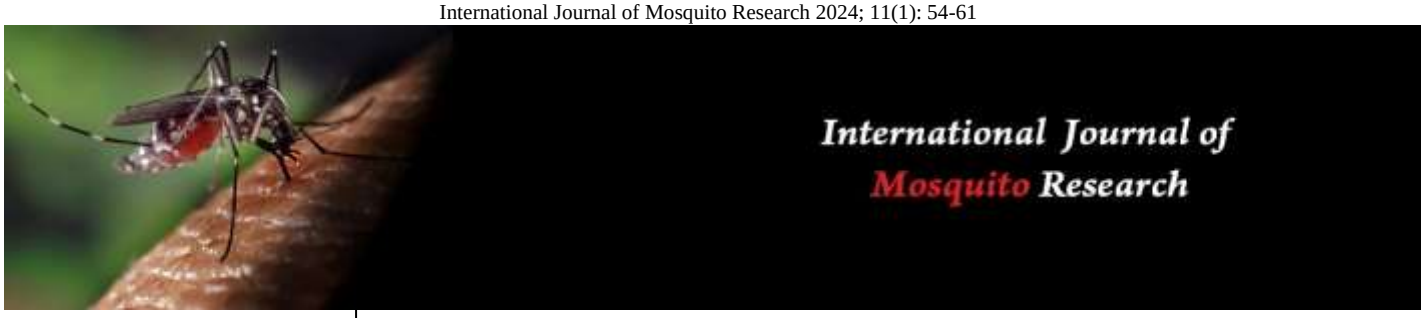

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2024; 11(1): 54-61

https://www.dipterajournal.com
Received: 26-11-2023
Accepted: 04-01-2024

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Larvicidal activity of synthesised silver nano particles from *Coccinia grandis* leaves extract

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DOI: https://doi.org/10.22271/23487941.2024.v11.i1a.752

Abstract
The goal of the current study is to synthesise silver nanoparticles (SNPs) using *Coccinia grandis* leaf extract in aqueous form and assess their effectiveness against mosquito larvicidal properties. Through the use of FTIR analysis, scanning electron microscopy, and UV visible absorption, the synthesised silver nanoparticles (SNP) were identified and verified. *Aedes aegypti* and *Culex quinquefasciatus* Ist to IVth in star larvae were assayed for 24 and 48 hours at different concentrations (50-250 ppm). Aqueous extracts from *Coccinia grandis* leaves exhibited noteworthy activity, while silver nanoparticles (SNP) demonstrated a notably greater mortality activity in *Culex quinquefasciatus* than *Aedes aegypti* larvae in the current investigation. The potential larvicidal activity of our study's *Coccinia grandis* leaf extract and synthesised SNPs suggests that this could be an excellent environmentally friendly method of control and protection. This is only an initial investigation into its larvicidal activity more research is required for deep insights of this formulation.

Keywords: *Coccinia grandis*, silver nanoparticles, *Aedes aegypti*, dengue fever

1. Introduction
More diseases can be spread by mosquitoes than by any other group of arthropods, and millions of people are impacted worldwide. Mosquito-borne diseases, such as malaria, yellow fever, dengue, chikungunya, filariasis, encephalitis, which can be fatal and increase morbidity are quite common in all most all tropical and subtropical countries [1]. According to WHO (2013), dengue fever, which is caused by the dengue virus and falls under the family Flaviviridae, is an arboviral diseases with a 390 million patients worldwide annually. India is regarded as endemic with an estimation of 34% of all cases worldwide, with all four serotypes circulating round the year in various regions of the nation [2, 3].
According to WHO (2009), in India the most common virus is *Aedes aegypti*. It also serves as a vector for yellow fever in West Africa, Central and South America, and Asia [4]. Mosquito control is crucial to reduce the frequency of diseases carried by mosquitoes and to improve public health. The World Health Organisation (WHO) stated in 1981 that the best way to reduce diseases carried by mosquitoes is to eliminate the intermediate hosts or vectors while they are still immature because of their limited habitat and low dispersal. Mosquito control assumes global significance because of its medical significance. To achieve immediate results in mosquito larvae control, the most effective method is to use synthetic insecticides. Mosquito control, the alarming emergence of physiological resistance in vectors, higher toxicities to organisms other than the target, and substantial expenditures are all issues that require attention (WHO, 1975) [5]. To manage the ever-increasing population of vectors, synthetic pesticides comprising of carbamates, pyrethrins, pyrethroids, organochlorine, and organophosphorus are commonly employed. Because of the environmental risks and development of resistance in non-target organisms, excessive use of these chemical pesticides is not safer [6]. Thus, the development of novel pesticides that are target-specific, biodegradable, and safer for the environment is desperately needed. Given this, natural products are typically favoured due to their inherent biodegradability and reduced harm to the intended organisms.

Herbal products have long been used by human communities worldwide to combat insect species and vectors. Numerous studies have discovered that phytochemicals derived from plants have deterrent properties in addition to serving as larvicides and insect growth regulators.

Noble metals like gold, silver, platinum, and lead are the primary raw materials used in the chemical production of metal nanoparticles. In the fields of biology, medicine, and living things, silver (Ag) is the most widely used of the Nobel metals. The Parashar group (2009) [7]. *Coccinia grandis* (family Cucurbitaceous) commonly known as 'little gourd 'or "scarlet gourd "is a unique tropical vine, growing abundantly everywhere in India. *Coccinia grandis* is a perennial shrub with white foliage grows quickly, spreads too many metres in length and forms a dense mat that easily covers small trees [8]. Every part of the *Coccinia grandis* plant has therapeutic value, and indigenous, universal medicinal systems have mentioned several preparations for a variety of skin conditions, including ringworm, psoriasis, ulcers, smallpox, scabies, and itchy skin eruptions [10, 9]. The herb is used as a decoction to treat gonorrhoea, diabetes, kidney disease, pyelitis, cystitis, strangulation, snakebite, and calculi in the urine [11, 12, 13].

Present study to evaluate the larvae mortality potential of aqueous leaves *Coccinia grandis* synthesised silver nanoparticles (Ag NPs) on *Aedes aegypti* and *Culex quinquefasciatus*. To the best of our statistics, this was the first attempt made on formulation and evaluation of silver nanoparticles (SNPs) using aqueous leaves extract of *Coccinia grandis* for its larvae mortality potential.

2. Materials and Methods

The extraction, silver nanoparticles synthesis and larvicidal potential against *Aedes aegypti* and *Culex quinquefasciatus* mosquitos was investigated in our college laboratory. The study was conducted in July – September 2022. Eggs, larvae and pupae of *Aedes aegypti* and *Culex quinquefasciatus* mosquitos are collected directly from ponds and surfaces where water can collect. After that, they were put on a plastic tray in the lab to be developed. Following pupal development, adult *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes are gathered and housed in an entomological cage to facilitate further reproduction. In the present study mosquito samples adult males were given sugar water to drink, whereas females were given the blood of a common poultry.

2.1 Plant Collection and authentication

In February 2022, fresh leaves of *Coccinia grandis* (Linn.) Voigt (Family: Cucurbitaceae) Syn. *Coccinea indica* (Wight & Arn) gathered from Astha Agricultural Farm in Sangli, Maharashtra, India. Dr. S.S. Kamble, a taxonomist at Shivaji University's Department of Botany in Kolhapur, Maharashtra, India, performed the taxonomic authentication. The voucher specimen was assigned a number and stored for future use in our research facility. $AgNO_3$, which was used without further purification after being purchased from Fisher Scientific Co. (Mumbai).

2.2 Plant extract preparation

The collected leaves cleaned by water and allowed to air dry in the shade, and powdered using mixer grinder. With a Soxhlet apparatus, the powdered material 10 gram used with 100 mL of distilled water was extracted and filtered. Under lowered pressure and regulated temperature, the filtrate was concentrated and dried. Standard stock solutions with a concentration of 1% were produced by dissolving the residues in the solvent, dimethyl sulphoxide (DMSO). The larvicidal activity and silver nanoparticle synthesis were conducted using different concentrations (ranging from 50-250 ppm) that were made from this stock solution using distilled water.

2.3 Synthesis of silver nanoparticles

Coccinia grandis extract was added to a 300 ml flask was used in our experiments within a week after it was boiled for five minutes, decanted, and kept at -4 ºC. After treating the filtrate in an Erlenmeyer flask with an aqueous solution of 1 mM AgNO3, it was allowed to settle at room temperature. The solution's brown-yellow colour, which suggests that plant extract reduction of silver ions to produce stable silver nanoparticles in water and indicate the formation of AgNPs. (Figure 2.1)

Fig 1: 10-3 M silver nitrate aqueous solution *Coccinea* gradins leaves extracts (a) prior and (b) four hours after addition of leaf extract

Silver Nanoparticles Characterization

Reaction mixture samples are periodically scanned by using Ultraviolet visible spectra at 200-600 nm on a (Ultraviolet 3600 Shimadzu spectrophotometer) synthesised silver nanoparticles were verified. Reaction mixture is centrifuged about 20 min. (at15,000 rpm), filtered through millipore filter (0.45 µm) and dissolved in deionized water. For SEM, EDS, and FTIR investigations, aqueous extract and aqueous silver nanoparticles were employed. Lyophilized silver particle's structure and composition are evaluated using Ultra high (10 kV) resolution scanning electron microscope (200 SEM -FEI QUANTA) sample of 25 µl was sprayed onto a copper rod and images of the nanoparticles were examined. Fourier transform infrared spectroscopy (FTIR) Stuart (2002) is used to qualitatively confirm surface groups nanoparticles. Additionally, energy dispersive spectroscopy was used to analyse the sample for the presence of metals.

2.4 Culture of test organism

In our laboratory *Culex quinquefasciatus* and *Aedes aegypti* eggs were smeared with a "O"-style brush and identified under a microscope [14]. These eggs, measuring 18 × 13 × 4 cm and housed on an enamel tray with de-chlorinated water of 500 ml for hatching cycled for 12 hours a day at 25–30 °C with relative humidity of 80–90%. Pet biscuits and a 10% sterile sucrose solution were given to the mosquito larvae. The larvae were fed continuously until they reached the pupal stage. Pupae were taken out of the culture medium, placed into 12 x 12 cm plastic containers with 500 ml of water inside. The mosquito larvae were housed in 60 x 60 x 60 cm plastic jars with a 14:10 light: dark photoperiod and constant temperature and humidity levels of 27±2 °C to 85%. For duration of three days, a 10% sterile sucrose solution is offered.

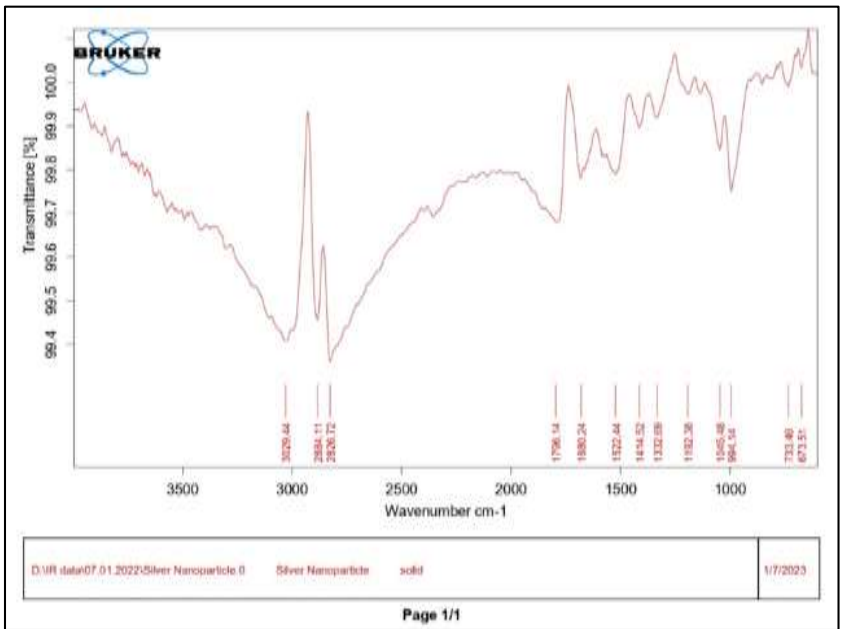


Fig 2: Synthesised silver nanoparticles leaves extract of *Coccinia grandis* by reduction silver ions using FTIR spectra

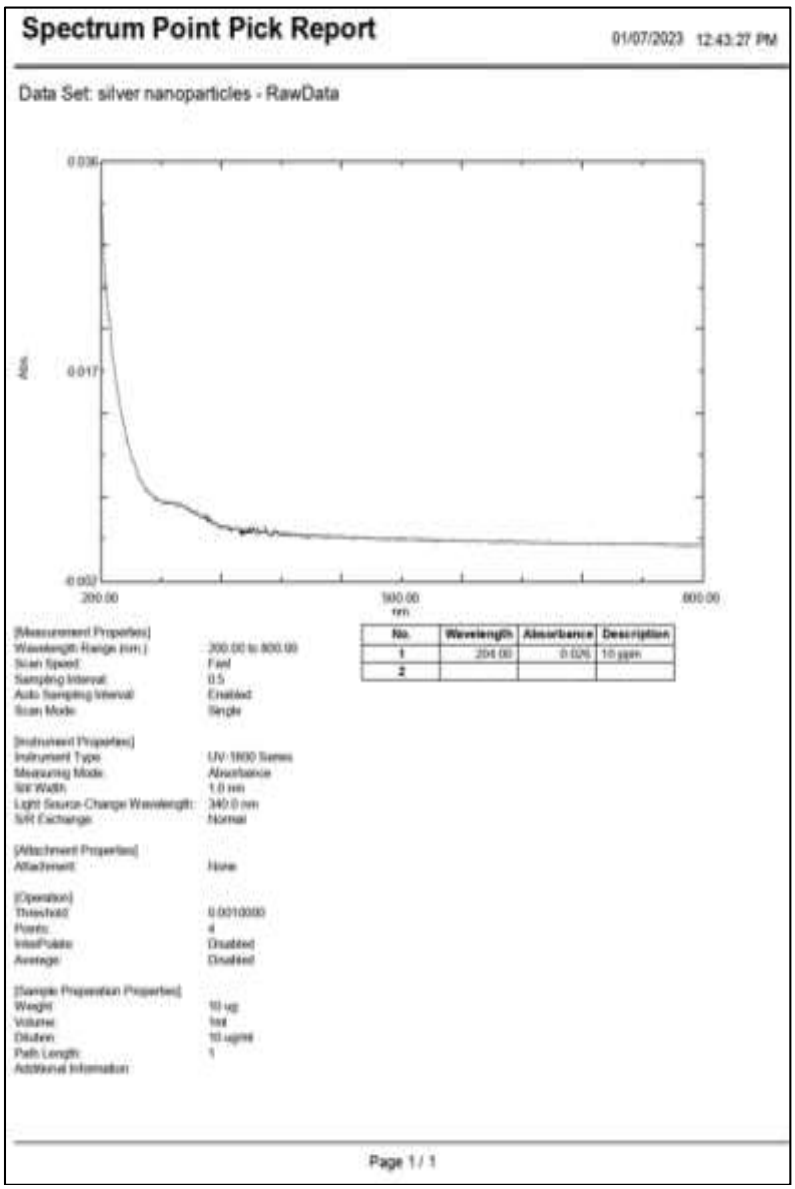


Fig 3: Synthesised silver nanoparticles leaves extract of *Coccinia grandis* by using Ultraviolet analysis

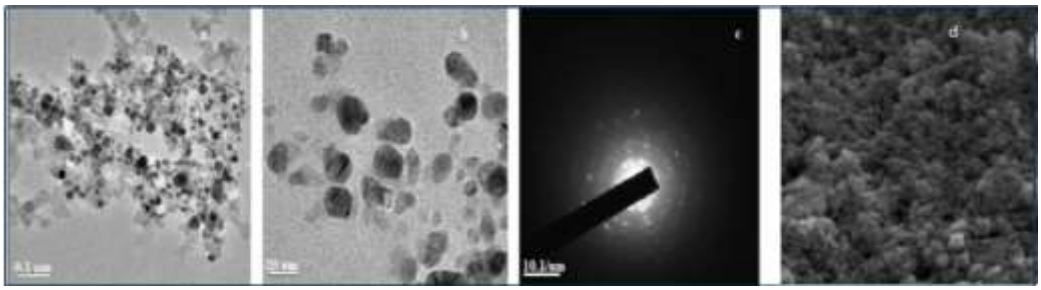


Fig 4: Synthesised silver nanoparticle microscopic image using high resolution transmission electron microscopic (a) Discrete nanoparticles. (b) Clear lattice fringes of silver nanoparticles (c) SAED pattern and (d) Spherical and agglomeration of silver nanoparticles image using SEM (Scanning Electron Microscope).

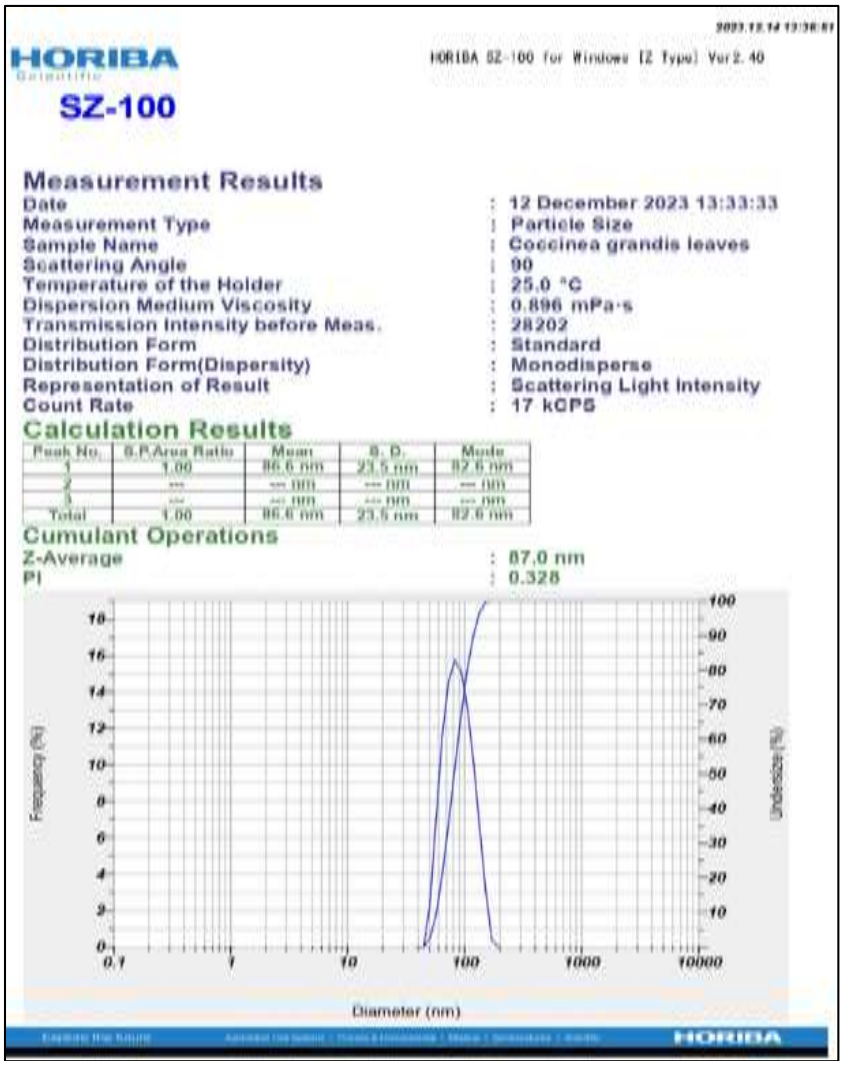
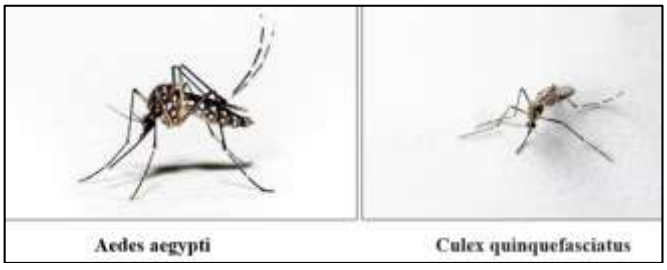


Fig 5: Synthesised silver nanoparticles leaves extract of *Coccinia grandis* nano particle analysis using zeta potential



2.5 Mosquito larvicidal assay

Both *Aedes aegypti* and *Culex quinquefasciatus* The WHO (1981) standard procedure for mosquito larvicidal activity was followed, and larvae colonies used. Glass beaker filled with 500 ml de-chlorinated water is filled with 25 larvae in their Ist to IVth in stars. The desired plant extract concentrations and synthesised silver nanoparticles of *Coccinia grandis* leaf extract were added, totalling 1 millilitre. Five replicates of each tested concentration were used, and each replicate was also run in parallel with the control experiments. After the exposure period of 24 and 48 hours, the larval mortality was determined. The larvae were not fed any food at all during the experiment. The Abbott formula was used to adjust for mortality in the control group (Abbott, 1925) [15]. Probit analysis was used to determine LC50 and LC90 from toxicity data (Finney, 1971) [16].

2.6 Ovicidal Activity

The ovicidal activity is performed as per Su & Mulla (1998) method [17] and appropriate solvent is used to dilute the leaf extract to various concentrations. Using a hand lens, the newly laid egg raft of *Aedes aegypti* and *Culex quinquefasciatus* were counted to 100 eggs in a container. One hundred freshly hatched eggs were subjected to six replications of leaves extract of each concentration and silver nanoparticles till hatched or perished. Water served as control solvent in the study. Forty-eight hours after treatment, the growth rate was determined using formula as given below:

$$\text{Egg mortality \%} = \frac{\text{No. of hatched larvae} * 100}{\text{Total number of eggs in egg raft}}$$

2.7 Adulticidal activity

Adult female mosquitoes of six days old are used and fed with10% sterile sucrose solution. From the stock solution of aqueous leaves extract, synthesised silver nanoparticles of *Coccinia grandis* (1% DMSO), different concentrations (50-250 ppm) were prepared and used for studies. Filter papers measuring 140 by 120 mm were covered with the diluted plant extracts. As a control, blank paper composed only of distilled water was employed. Papers are dried at the room temperature for entire night. Only brand-new exam papers are used for all tests. Using the WHO method, experiment is carried out using a kit made up of two 125*44 mm plastic tubes having cylindrical assembly (WHO, 1981). The mosquitoes were held in one tube prior to and following the exposure periods, while another tube was utilised to expose them to the test sample under investigation. After being rolled, the impregnated papers were put inside the exposure tube. Each tube had a wire screen with a mesh size of sixteen to close off one end. 25 mosquitoes that had been fed a sterile sucrose solution (10%) and starved of blood were released into the tube. During a three-hour exposure period, the extracts' and the synthesised silver nanoparticles' mortality effects were noted every ten minutes. After being exposed for one, two, and three hours, mosquitoes were put inside the holding tube. In 24-hour study period, immersed cotton swabs in a 10% sucrose solution containing multi-vitamins in the tube. For every concentration, afore mentioned process was done in triplicate while the death rate was noted. To determine adulticidal activity, the number of dead mosquitoes from introduced mosquitoes was taken into consideration. If a mosquito remained motionless after being repeatedly pushed with a soft brush, it was deemed dead.

2.9 Statistical analysis

Adulticidal rate was recorded after, 12, 24 and 48 h of exposure time and average adult mortality rate is calculated using Probit statistical software Stats Direct 2.8.0 was used to compute LC_{50}, LC_{90}, and 95% confidence intervals for the upper and lower confidence limits using chi-square values. $P \leq 0.05$ results are considered as statistically significant.

3. Results

Aqueous extract of *Coccinia grandis* leaves exhibits larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* larvae indicating that the extract has higher mortality rates than the control. Below Table 3.1indicates the larval mortality of Ist to IVth instars. After 24 hours, the larvicidal potency with LC50 and LC90 was at its highest as 135.35, 117.62, 100.27, 108.64 ppm of AQCG (IC50 =115.47±15.08 PPM on Ae. aegypti) &93.73, 108.26, 131.89, 189.74ppm AQCG IC 50 119.93 PPM on Cx. quinquefasciatus larvae. The aqueous extract of *Coccinia grandis* (AGCG) contains silver nanoparticles with a significant larvicidal activity (IC50 Value 156.3 from AQCG IC 50 = 115.33 PPM) against *Aedes aegypti* larvae (Table 3.2). The larvicidal activity of silver nanoparticle of aqueous extract of *Coccinia grandis* is significantly increased IC50 Value = 181 from IC50 = 119.93 PPM on *Culex quinquefasciatus* as compare to the aqueous extraction mortality in *Aedes aegypti* (% mortality). Here the larvicidal effect of silver nano particle of *Coccinia grandis* is more significant than aqueous extract *Coccinia grandis* on the both strain of larva *Aedes aegypti* & *Culex quinquefasciatus*. Table3.2 signifies larvicidal effect on Ist to IVth instars. Maximum larvaemortality LC_{90} was depicted after 24 hours was 127.8, 137.7, 165.75, 193.8 ppm and 147.9, 177.1, 189.79, 2s09.48 ppm. Whereas, after 48 hours the LC_{50} and LC_{90} values indicated 64.43, 72.64, 91.73, 108.93, 114.62 ppm and 76.95, 92.72, 123.20, 122.65, 125.16 ppm. The LC50 and LC_{90} (LFL – UFL) 95% Confidence intervals were computed. Larvae mortality results signifies that the % mortality of the larvae of *Aedes aegypti* and *Culex quinquefasciatus* have direct associated with the concentration of the silver nanoparticle of *Coccinia grandis* aqueous extract significant larvicidal activity is exhibited by the silver nanoparticle of the aqueous extract of *Coccinia grandis* (AGCG). IC_{50} Value 156.3 from AQCG IC_{50} =115.33 PPM against Ae. Aegypti larvae (Table 3.2)

Table 1: Aq Extract of CG on Mortality in *Aedes aegypti* larvae & *Culex quinquefasciatus* larvae (% mortality)

AqExtact CG PPM	Aqextract of CG on mortality in *A. aegypti* Larvae (% mortality)				Mean +SD	LC_{50} Value (PPM)	LC_{90} Value (PPM)	DF (DFn, DFd) & P value
	LS-1	LS-2	LS- 3	LS-4				
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Raw factor RF (1.684, 10.10) = 241.3 p<0.0001
50	12.97	27.06	24.06	20.60	21.17±3.03	11.51	30.834	
100	36.96	42.99	51.05	46.20	44.3±2.95	34.89	53.70	
150	54.20	56.34	70.77	70.77	63.02±4.49	48.71	77.32	
200	75.36	81.87	84.50	72.80	78.63±2.73	69.93	87.33	
250	86.81	91.37	94.73	84.20	89.27±2.34	81.83	96.73	
AQ LS IC_{50}	135.35	117.62	100.27	108.64	115.47±15.08	AQCGIC 50 =115.33 PPM on *A. aegypti*		Column Factor CF (1, 6) = 0.06101 P=0.8131
AqExtact CG PPM	Aq extract of CGon mortality in Cx. Quinquefasciatus larvae (% mortality)				Mean +SD	LC50 Value (PPM)	LC90 Value (PPM)	
	LS-1	LS-2	LS- 3	LS-4				
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

50	34.80	25.20	22.20	18.20	25.1±3.53	13.84	36.35	95% CI of difference is -12.70 to 15.55 PPM
100	54.20	42.80	32.20	26.40	38.9±3.12	19.40	58.39	
150	82.20	71.20	58.80	34.20	61.6±2.85	28.79	94.40	
200	91.60	82.20	72.80	58.80	76.35±3.99	54.08	98.61	
250	96.60	94.20	82.40	70.40	85.9±2.03	66.71	105.08	
AQ LS IC 50	93.73	108.26	131.89	189.74	119.40±45.37	AQCG IC 50 =119.93 PPM on Cx. Quin		

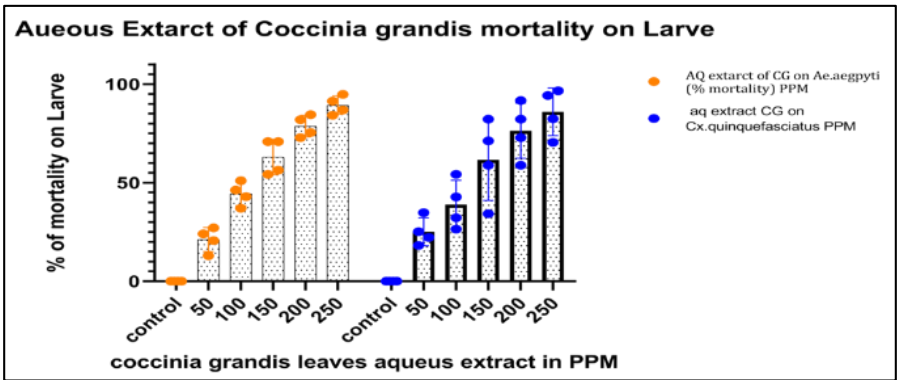


Fig 6: Aqueous extracts of CG on % Mortality on *Aedes aegypti* larvae & *Culex quinquefasciatus* larvae (% mortality)

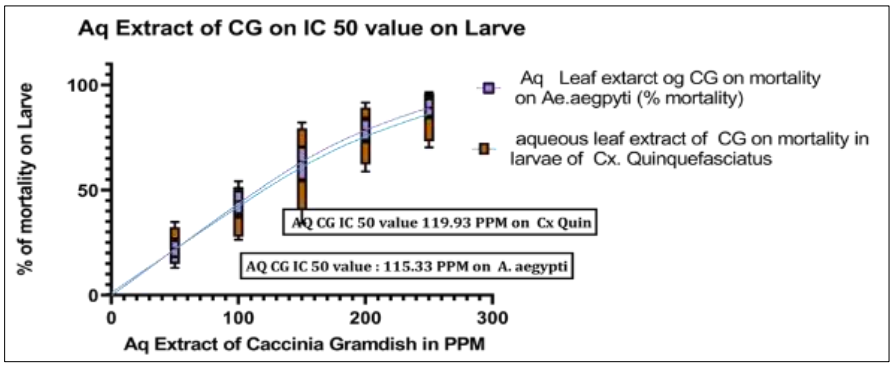


Fig 7: Aq extract of CG on IC50 value in PPM on *Aedes aegypti* larvae & *Culex quinquefasciatus* larvae (% mortality)

Table 2: % Mortality on *Aedes aegypti* & *Culex quinquefasciatus* larvae (% mortality) of *Coccinia grandis* silver nanoparticles

SNPs CG Con PPM	**Silver nanoparticles of C.G on mortality in Ae. aegypti Larve (% mortality)**				**Mean +SEM**	**LC50 Value (PPM)**	**LC90 Value (PPM)**	**DF (DFn, DFd) & P value**
	LS-1	**LS-2**	**LS- 3**	**LS-4**				
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Raw Factor (1.530, 9.180) = 241.1 p<0.0001 Column Factor F (1, 6) = 0.1228 P=0.7380 95% CI of difference -15.70 to 11.77 PPM
50	59.96	62.06	56.06	48.60	56.54±3.08	45.90	64.43	
100	85.60	82.40	74.40	59.60	75.5±5.8	61.35	72.64	
150	92.20	90.40	76.70	72.00	82.83±5	71.95	91.73	
200	110.6	103.0	88.40	85.20	96.8±6.01	82.66	108.93	
250	116.8	113.2	100.20	99.20	107.3±4.48	98.08	114.62	
IC 50	127.8	137.7	165.75	193.80	156.3±14.87	IC50 Value 156.41 PPM		
SNPs CG Con PPM	Silver nanoparticles of C. G on mortality in *Cx. Quinquefasciatus* larve (% mortality)				Mean +SEM	LIC_{50} Value (PPM)	LC_{90} Value (PPM)	
	LS-1	LS-2	LS- 3	LS-4				
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
50	74.80	70.20	64.06	62.40	67.86±2.85	58.77	76.95	
100	84.20	88.80	78.20	71.20	80.6±3.81	68.47	92.72	
150	116.6	104.2	92.40	80.20	98.35±7.81	73.49	123.20	
200	123.6	112.0	108.40	88.40	108.1±7.32	84.64	122.65	
250	124.0	107.0	113.00	112.00	116.3±3.86	107.83	125.16	
IC 50 PPM	147.9	177.1	189.79	209.48	181±12.91	IC50 Value 181.91PPM		

The larvicidal activity of silver nanoparticle of aqueous extract of *Coccinia grandis* is significantly increased IC50 Value = 181 from IC50 = 119.93 PPM on *Culex quinquefasciatus* as compare to the aqueous extract on mortality in *Culex quinquefasciatus* larvae (% mortality). Here the larvicidal effect of silver nano particle of *Coccinia*

grandis is more significant than aqueous extract *Coccinia grandis* on the both strain of larva *Aedes aegypti* & *Culex quinquefasciatus*. Table 3.2 signifies larvicidal effect on instar Ist to IVth, after a 24-hour period, the maximum larvicidal potency LC90 was shown to be 127.8, 137.7, 165.75, 193.8 ppm, and 147.9, 177.1, 189.79, 209.48 ppm. In contrast, the LC50 and LC90 values after 48 hours were 64.43, 72.64, 91.73, 108.93, 114.62 ppm and 76.95, 92.72, 123.20, 122.65, 125.16 ppm, respectively.

Percentage mortality or mortality of larvae results of *Aedes aegypti* & *Culex quinquefasciatus* indicates it has a direct correlation to the concentration of the silver nanoparticle of aqueous extract of *Coccinia grandis*. The 95% confidence limits, LC_{50} and LC_{90} (LFL – UFL) were calculated. This demonstrates how concentration affects larvicidal activity. At the $p \leq 0.05$ level, chi-square values were significant. A control group was included in each test for each concentration. The result values are slightly higher in fourth instar larvae of *Culex quinquefasciatus* than *Aedes aegypti* under similar conditions (Table 3.2).

4. Discussions

AgNPs, or green synthesised silver nanoparticles, are a new method of controlling vector mosquito larvae. [18] In order to control *Aedes aegypti* and *Culex quinquefasciatus*, current study achieved synthesis of silver nanoparticles using leaves extract of *Coccinia grandis*. Current studies on silver nanoparticles made from plants that contain significant chemicals like tannins, alkaloids, steroids, saponins, and iso flavonoids Seeds, leaves, fruits, bark, and twig.

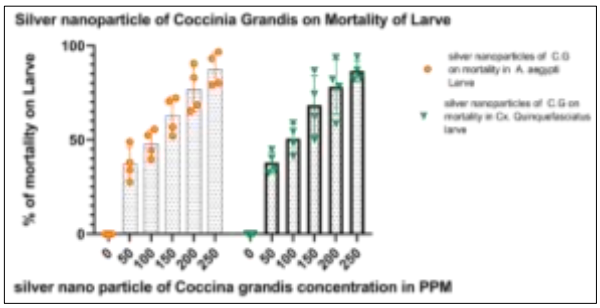


Fig 8: Silver nanoparticle of CG on % Mortality on *Aedes aegypti* larvae & *Culex quinquefasciatus* larvae (% mortality)

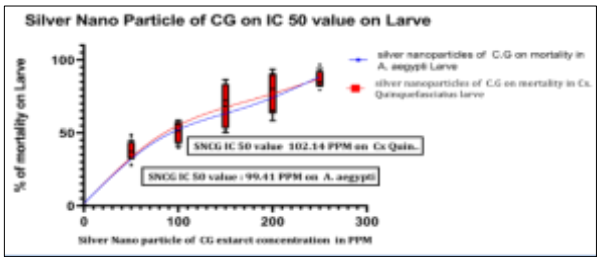


Fig 9: Silver nanoparticle of CG on IC_{50} value in PPM on *Aedes aegypti* larvae & *Culex quinquefasciatus* (% mortality)

extracts have the potential to function as insecticides, larvicides, repellents, antifeedants, moulting hormones, and antimoulting hormones [19].

SEM analysis of the *Coccinia grandis* synthesised stable silver nanoparticles (SNPs) revealed the formation of nanoparticles, the majority of which have smooth edges and a spherical shape. According to studies on the distribution of particle sizes, a mixture of nanoparticle sizes ranging from 100 to 150 nm was formed. As per definitions nanoparticles are those having size range 10 to 100nm solid particles or particulate dispersions [20]. Agglomeration in our studies explains nanoparticle size decreased as the concentration of plant material increased.

The current study demonstrated the larvae mortality effect of silver nanoparticles *Coccinia grandis* aqueous extract against *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae, as evidenced by a high percentage of mortality relative to those in the control treatment. This indicates that the extract's chemical composition and the solution's silver nanoparticle content are to blame for the death of mosquito larvae. According to the current study *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae are toxic to *Coccinia grandis* silver nanoparticles (LC_{50} = 156.41 PPM and LC_{50} = 181.91 PPM after 24 h exposure) greater than aqueous plant extract: (LC_{50} = 115.33PPM; 119.93 PPM respectively). Larval mortality is increased with the exposure time [21].

The capacity of the nanoparticles to pierce the exoskeleton may be connected to their toxicity against mosquito larvae in their early stages of development [22]. Additionally, nanoparticles have the ability to bind intracellularly to phosphorus from DNA or sulphur from proteins. This causes organelles and enzymes to quickly denaturate, which kills mosquito larvae and pupae. Moreover, cellular death and loss of function may result from disturbed proton motive force and decreased membrane permeability [23]. The extract from *Coccinia grandis* leaves may target a variety of proteins, including structural proteins, ion channels, signalling molecules, enzymes, receptors, and bio membranes, among other cellular constituents [24].

5. Conclusion

This research represents the first attempt at synthesising silver nanoparticles (AgNPs) with aqueous plant extract from *Coccinia grandis*. *Aedes aegypti* and *Culex quinquefasciatus* mosquitos are the primary vectors of dengue, and silver nanoparticles (AgNPs) have a potent larvae mortality effect. Our findings imply that synthetic SNPs may be a perfect, environmentally friendly method of protecting against and controlling *Aedes aegypti* and *Culex quinquefasciatus* mosquitos. For its larvicidal activity, more research is required as this is only a preliminary investigation.

6. Reference

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