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Larvicidal and adulticidal activities of *Cymbopogon citratus* synthesized silver nanoparticles in different mosquito vectors

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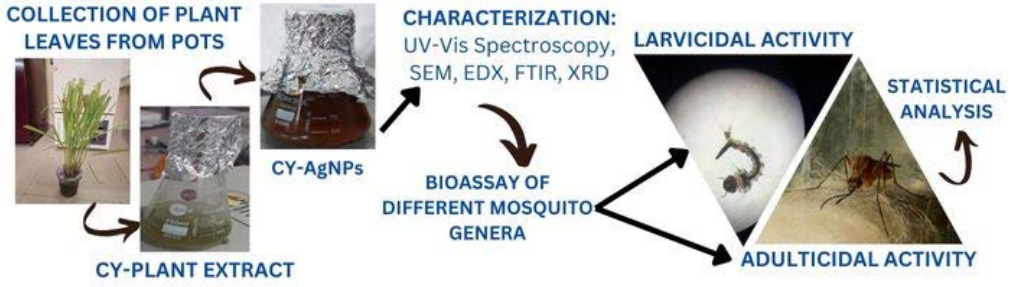
Article

ABSTRACT



Mosquito-borne diseases are checked by controlling the mosquito population with innovative scientific interventions. The present study aims to synthesize silver nanoparticles using aqueous leaf extract of *Cymbopogon citratus* (CY-AgNPs) and testing their larvicidal and adulticidal potential against medically important genera of mosquitoes- *Anopheles spp., Aedes spp.,* and *Culex spp*. The synthesized nanoparticles were characterized by UV/Visible spectroscopy, FESEM, EDX, XRD, and FTIR. Larvicidal and adulticidal assays were performed against different mosquito genera as per WHO (2005 and 2022) standard protocols with slight modifications. The mortality data was subjected to One-way ANOVA and probit plane regression analysis to calculate LC_{50}, LC_{90}, and LC_{95} values. The results with $p<0.05$ were considered statistically significant. The green synthesized CY-AgNPs demonstrated substantial larvicidal activity against *Anopheles stephensi* (LC_{50} = 46.55 ppm), *Aedes aegypti* (LC_{50} = 40.94 ppm), and *Culex quinquefasciatus* (LC_{50} = 41.45 ppm). Similarly, in the adulticidal assay, the silver nanoparticles showed potency against all genera- *An. stephensi* (LC_{50} = 22.48 ppm), *Ae. aegypti* (LC_{50} = 20.94 ppm), *Cx. quinquefasciatus* (LC_{50} = 25.34 ppm). As per the results, *C. citratus*-mediated silver nanoparticles showed the highest mosquitocidal activity against *Aedes* mosquitoes.

Keywords: Silver Nanoparticles, Cymbopogon, Mosquito vector, larvicidal, adulticidal

INTRODUCTION

Mosquitoes act as vectors for various deadly diseases which contribute to social and economic burdens and even pose life-threatening situations in human societies. Deadly pathogens like viruses, protozoans, and helminths are transmitted by mosquitoes to humans causing various diseases in humans and livestock[1]. Mosquito species from the genus, *Anopheles* are known to cause malaria. Year 2022 witnessed 249 million malaria cases in 85 countries with an increase of 5 million cases compared to the cases reported in 2021. World malaria reported 2.1 billion malaria cases between the years 2000-2022 with 11.7 million deaths[2]. *Aedes* mosquitoes are the culprit behind the spread of various life-threatening diseases like dengue fever, yellow fever, Chikungunya, Zika virus, etc[3]. Dengue fever is prevalent in over 100 countries, affecting 2.5 billion people worldwide. Annually, 80 million cases of dengue fever are recorded worldwide[4]. Dengue fever decreases in platelet ratio and leads to dengue hemorrhagic fever in severe conditions leading ultimately to death. Seventy percent of the dengue infections are recorded in Asia, out of them 34% are estimated in India alone[5]. Yellow fever spread through the *Aedes* mosquito, is associated with jaundice, hemorrhage, and death in severe conditions endemic in Central America, South America, and Africa, especially in tropical regions of the continent[6]. *Ae. aegypti* and *Ae. albopictus* are responsible for spreading chikungunya characterized by headache, arthlagia, nausea, high fever, and severe cases that may cause neurological complexities[7]. Zika virus disease mainly spreads through *Aedes* mosquitoes and is predominantly reported in South Asia, America, and the Pacific islands. WHO declared a Global health emergency of international concern in 2016 relating to the spread of Zika virus disease[9].

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Genus *Culex* is associated with some notorious diseases like Filariasis, West Nile fever, and Japanese Encephalitis. It is estimated that around 31 million people in India are harboring the filarial worm infection transmitted by mosquitoes and 23 million are suffering from its clinical manifestations[10]. About 450 million people in India are at direct risk of filarial infection[10-11]. *Culex tarsalis* has been reported as a vector of West Nile Virus transmission in the western United States[12]. *Culex quinquefasciatus, Culex thriambus, Culex nigripalpus, and Culex pipiens have also been reported to spread West Nile Virus infection as primary vectors[13]. Though Aedes mosquitoes have also been reported as the vector for West Nile Virus however, Aedes is not considered the primary vector of the disease[14-15]. Culex vishnui, Culex gelidus, Culex sitiens are known as the main vectors of Japanese encephalitis along with some species of Genus Anopheles[16].* It is also reported that newborn babies are the more vulnerable group to Japanese encephalitis in facing neurological complications when compared to adults[17]. As per the reports around 1,00,308 cases were recorded in 2015, which were accompanied by nearly 30,000 deaths worldwide due to Japanese encephalitis transmission[18].

Such menaces that bring havoc to mankind should be checked and controlled scientifically to save the lives of the millions worldwide who are lost due to a lack of appropriate research and knowledge about these death-carrying mosquitoes. Traditional methods involve using chemical-based insecticides to control mosquitoes but have many shortcomings making them inefficient in curbing the outbreaks of these diseases. Outdoor spraying and indoor residual spraying programs generally rely on synthetic pyrethroids. However, resistance has been developed in most mosquitoes against them failing various chemical insecticide-based vector control programs. Molecular, metabolic, and physiological resistances have been reported in mosquitoes against various chemical insecticides[19]. Elevated P450 monooxygenase enzyme activity has been seen as one of the causes of resistance in insects. Pyrethroid resistance has been associated with the *Cyp* gene in many insects[20].

However, insect resistance is not the only challenge to chemical-based mosquito control methods. Chemical insecticides have an adverse impact on the health of terrestrial and aquatic life leading to the overall deterioration of the environment and ecosystem. Environmental problems such as bioaccumulation and biomagnification are also associated with the use of chemical insecticides in vector control programs. Various chemicals like malathion, parathion, and dimethoate are associated with endocrine disruptions leading to hormonal imbalances, neurological complications, oxidative stress, and enzymatic malfunctions in humans[21]. Genetic abnormalities have been reported leading to tumors, hormonal imbalances, and even cancers[22]. Soil and water pollution caused by chemical insecticides further add to agriculture-related problems and food security burdens[23]. Leaching, surface runoff, and adsorption of these chemical insecticides lead deterioration of fertile soil as well as nearby water bodies affecting floral and faunal biodiversity[24]. Biological control methods involve the use of natural enemies of mosquitoes like *Gambusia* fish and Copepods to control the mosquito population but this method is also not very helpful due to geographical constraints related to the natural enemies as breeding habitats of mosquitoes in human habitations are very much diverse and unsuitable to their natural enemies[25].

Novel approaches need to be incorporated into our mosquito vector control programs to deal with the mosquito populations to better curb outbreaks of deadly diseases in human habitations. Nanotechnology is one such new and revolutionary approach that utilizes nanoscale products in the fields of bioengineering, biophysics, and biochemistry[26-27] and provides a wide range of scientific applications in drug discoveries, cancer treatments, and disease diagnosis[28]. Many researches are being carried out exploring other potential applications of nanotechnology that can benefit the environment and mankind at large.

Nanoparticle is a general term that refers to any object which has its size ranging in nanometre (10^{-9} meter) dimensions. Their distinct size, shape, and high surface-to-volume ratio provide them with unique chemical and physical characteristics that are utilized by modern researchers to bring about interdisciplinary and multidimensional applications[29]. Metal nanoparticles of silver, zinc, silicon, tin, iron, gold, platinum, etc. have been successfully synthesized in laboratories, and their industrial and medical applications are also reported[30]. Chemical, physical, and mechanical methods are generally used in the synthesis of nanoparticles[31]. Recent researches focus on green nanosynthesis methods that utilize biological products such as microorganisms[32], plant extract from leaves[33], flowers and seeds derivatives[34] as reducing and stabilizing agents in the process. As biological products are used in the synthesis, the nanoparticles thus formed are environment friendly and are equipped with the benefits of such products which increases their potency in various applications[35]. The synthesis and characterization of silver nanoparticles have been reported by using leaf extract of the plant *Moringa oleifera*[36], *Euginea jambolana*[37], and *Eugenia roxburghii*[38].

Cymbopogon citratus, commonly known as lemongrass is a perennial herb of Poaceae family[39] and has been reported to possess a cyclic monoterpene called citral which gives it a characteristic lemon-like smell[40]. The leaf contains essential oils like myrcene, geraniol, α-oxo bisabolene, citronella, methyheptenone, and elemol[41-42]. The presence of various secondary metabolites and other phytochemicals like anthocyanins, furfurol, coumaric acid, fumesol, and isopulegol have also been reported. *Cymbopogon citratus* is rich in bioactive components such as alkaloids, ketones, tannins, flavonoids, terpenoids, phenol, alcohol, and ketones[43]. These biological agents can reduce inorganic metal ions to nanoparticles. Further, extracts of *Cymbopogon citratus* have been shown to possess insecticidal activity against *Musca domestica*[44], *Phemacoccus solenopsis*[45], and *Ae. aegypti*[46].

The objective of the present study is to synthesize, and characterize the silver nanoparticles using aqueous leaf extract of the *Cymbopogon citratus* and to test their larvicidal and adulticidal potential against mosquito vectors of deadly diseases like Malaria, dengue, and filariasis. The findings of the study provide an alternative to currently used pollution-causing toxic chemical insecticides in mosquito vector control programs.

MATERIAL AND METHODS

Collection of plant Material

Cymbopogon citratus (CY) was purchased from the local nursery and was subjected to taxonomic identification in the Department of Botany, University of Rajasthan, Jaipur. The specimen was given voucher number RUBL21686 by the Herbarium when deposited in the Department of Botany, University of Rajasthan, Jaipur. Leaves were washed properly with fast-flowing tap water followed by distilled water to avoid contamination. Then leaves were kept for air drying at room temperature. Dried leaves were then meshed to form leaf powder which was further used to prepare leaf extract.

Preparation of leaf extract

Fifty grams of leaf powder was poured into 200 ml of distilled water. The above mixture was kept in a beaker covered with aluminum foil with a magnetic stirrer at 500 rpm and 40ºC temperature for three hours. The obtained mixture was then allowed to cool at room temperature. The concentration of the stock solution was noted to be 250mg/ml. The mixture was filtered through Whatman filter paper no.1 and was stored at 4ºC temperature for further use[47].

Synthesis of Silver Nanoparticles

The analytical-grade silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich. By blending 17 mg of silver nitrate powder in 100 ml of distilled water and stirring with a magnetic stirrer, an aqueous 1 mM silver nitrate solution was obtained. Ninety milliliters of silver nitrate solution were taken in a beaker kept on a magnetic stirrer set at 500 rpm and 25ºC temperature covered with aluminum foil. Now, ten milliliters from the stocked leaf extract of CY were added to the ninety milliliters of silver nitrate solution dropwise under continuous stirring to procure CY-based silver nanoparticles (AgNPs). The above mixture was kept at a magnetic stirrer at 500 rpm and 25ºC temperature for three hours. Dark conditions were maintained during the complete procedure[48].

Characterization of CY-AgNPs

An aliquot of the prepared solution was taken and subjected to the UV/Vis Spectrophotometer (Thermo Scientific Multiskan Go) under the 300 nm - 600 nm range. For further characterization, the reaction mixture was centrifuged at 5000 rpm for 15 minutes. The pellets were collected and dissolved in deionized water and ethanol. The obtained pellets were subjected to Field Emission Scanning Electron Microscopy (FESEM) using Thermofisher Scientific Model Apreo 25 High Vac and images were obtained to determine the size and shape of nanoparticles. The nanoparticle sample was also examined by Energy Dispersive X-ray Spectroscopy (EDX) using OXFORD EDX to determine their elemental composition. The atomic arrangement and crystal structure of the synthesized nanoparticles were determined by X-ray diffraction (XRD-Panalytical Xpert Pro). Fourier Transform Infrared Spectroscopy (FTIR) using the Perkin Elmer 95163 model also examined the functional groups in the prepared CY-AgNPs.

Rearing of mosquito stages

Mosquito larvae of three medically important species *An.stephensi*, *Ae.aegypti*, and *Cx. quinquefasciatus* were reared in enamel trays (40 x 30 x 8 cm) in the laboratory at a set temperature of 28ºC. They were fed with dog biscuits and yeast extract in a 3:1 ratio. Adult mosquitoes from the same genera and species were reared in the laboratory's rearing cages ($1x1x1m^3$). Temperature was maintained in the insectary between 25-28ºC with 75-80% relative humidity. Cotton plugs soaked with 10% aqueous glucose solution were provided to feed upon. Blood feeding was performed per day by inserting hands in the rearing cage. The alternate period of light and dark was maintained for 14:10 hours[49].

Larvicidal Assay

WHO guidelines (2005) for the laboratory testing of CY-AgNPs against mosquito larvae followed for the larvicidal assay with a slight modification[50]. Mosquito larvae (III^{rd} and IV^{th} instars) were exposed to 0.5ppm, 5ppm, 15ppm, 25ppm, 35ppm, and 45ppm concentrations of leaf extract (CY-Group I) and green synthesized silver nanoparticles (CY-AgNPs-Group II). Distilled water was used for the placebo group (UN-Group III). Equal dispersion of nanoparticles was ensured by using Ultrasonication of nanoparticles before the preparation of doses. The doses of Group I and Group II concentrations were prepared in 250 ml of distilled water. Twenty-five mosquito larvae (third and fourth instars) from each species- *An.stephensi*, *Ae.aegypti*, and *Cx. quinquefasciatus* were exposed to different doses in Groups I, II, and III in plastic cups. Each genera of mosquito larvae were exposed to the test concentration in triplicates for 24 hours. Mortality data of treated groups were recorded by observing the motionless larvae after the culmination of the exposure period. The mortality percentage higher than 5% in the control group was corrected by the Abbott formula[51];

$$\text{Corrected mortality} = \frac{\%\text{exposed mortality} - \%\text{control mortality}}{(100 - \%\text{control mortality})} \times 100$$

Adulticidal assay

Freshly emerged adult stages of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* collected (F1 generation from rearing stock) were exposed to 5 ppm, 20 ppm, 35ppm, 75 ppm, and 95 ppm concentrations of *Cymbopogon citratus* leaf extract (CY-Group I) and green synthesized silver nanoparticles (CY-AgNPs-Group II). Mosquitoes were exposed to distilled water in the control group (UN-Group III). Mosquitoes were kept in the holding tubes for 1 hour to acclimatize them in tubes. For adulticidal experiments, Whatman filter papers (Analytical grade A) were treated with varying test concentrations of leaf extract and silver nanoparticles, which were tested using WHO tubes assay as per WHO guidelines (2022)[51]. In control groups, filter papers were with distilled water only. Twenty-five adult mosquitoes were inserted in test tubes containing treated filter papers. Mosquitoes were kept in the exposure tubes containing treated filter paper for an exposure period of 1 hour. After the exposure period, mosquitoes were kept in post-exposure holding tubes for the post-exposure holding period of 24 hours. Mortality data was recorded by observing the knockdown adult mosquitoes lying on the floor of the test tube after the completion of the exposure period[52].

Statistical analysis

The average mean of larval and adult mortality data were subjected to probit analysis to calculate LC_{50}, LC_{90}, and LC_{95} values with 95% confidence limits, and the mean difference among the experimental groups was analyzed by One-way Analysis of

Variance (ANOVA) using MS Excel. Results obtained with $p<0.05$ and $p<0.01$ were considered significant and highly significant.

RESULTS

Characterization analysis

Characterization studies using different analytical instruments revealed the following details about the C/Y-AgNPs;

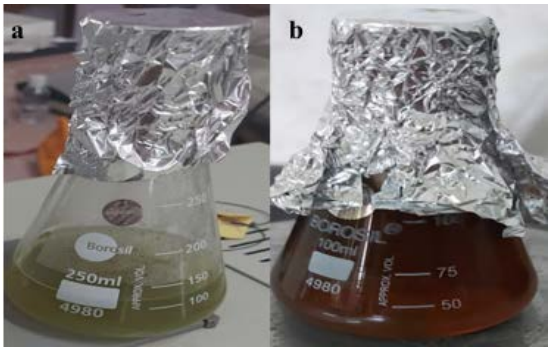


Figure 1: a) CY-leaf extract and b) Green synthesized CY-AgNPs show a change in coloration.

UV/Vis Spectrophotometer

The milky white silver nitrate solution turned greenish yellow (Figure 1a) upon the addition of CY leaf extract and colloidal brown (Figure 1b) after the completion of the reaction. The color change was considered as the primary sign of nanoparticle synthesis. For the preliminary analysis, the nanoparticle sample was subjected to UV/Visible spectrophotometry. The absorbance peak was recorded at 435 nm (Figure 2), which confirmed the synthesized silver nanoparticles.

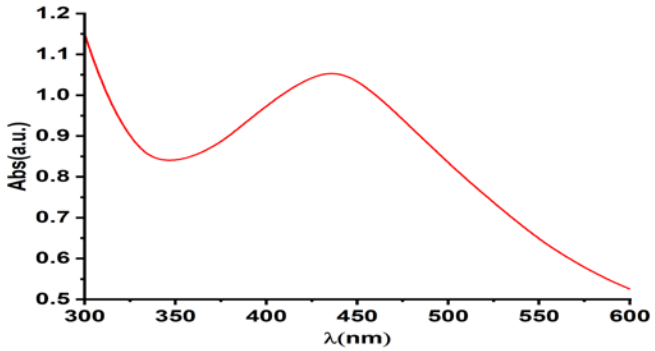


Figure 2: UV/Visible Spectroscopy of CY-AgNPs.

Scanning electron microscopy

Analysis of SEM images (Figure 3a) revealed the shape and size of the synthesized CY-AgNPs. The data showed that nanoparticles were spherical shaped with sizes ranging between 27 nm to 54 nm, with a mean average size of 39.85 nm (Figure 3b).

EDX analysis

The strong peak of silver at 3KeV was recorded in the present sample of the EDX spectrum (Figure 4), which showed the presence of silver in our sample as recorded in another study[52]. Along with the silver (Ag), the presence of Nitrogen (N) and Oxygen (O) were also recorded through weak peaks in the EDX spectrum. The weight % and atomic % and of Silver, Oxygen, and Nitrogen were determined as 88.31, 10.12, 1.57, and 52.36, 40.18, 7.16 respectively.

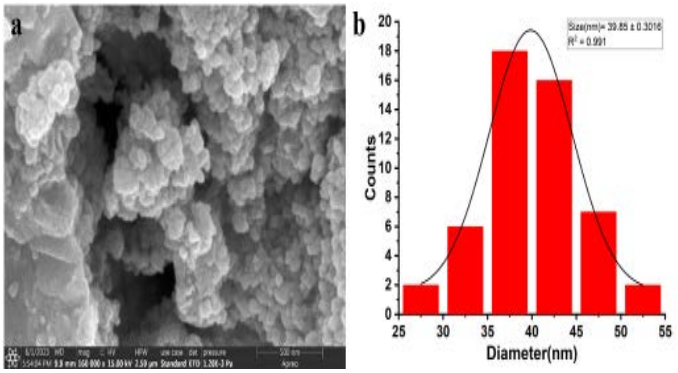
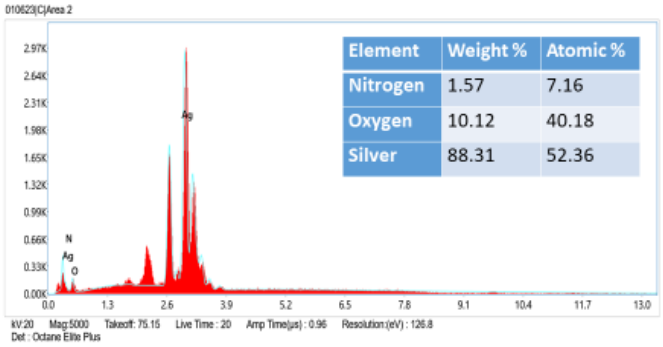


Figure 3: a) Scanning Electron Microscopy Image of CY-AgNPs and b) Size distribution histograms for CY-AgNPs.



Element	Weight %	Atomic %
Nitrogen	1.57	7.16
Oxygen	10.12	40.18
Silver	88.31	52.36

Figure 4: EDX characterization results of CY-AgNPs.

X-ray diffraction pattern

The XRD data demonstrated different diffraction peaks corresponding to the 2θ values of 38.2°, 44.3°, 64.5°, 76.8° which can be assigned to planes of (1 1 1), (2 0 0), (2 2 0), and (3 1 1) respectively (Figure 5). The data shows that the synthesized CY-AgNPs are fcc in nature.

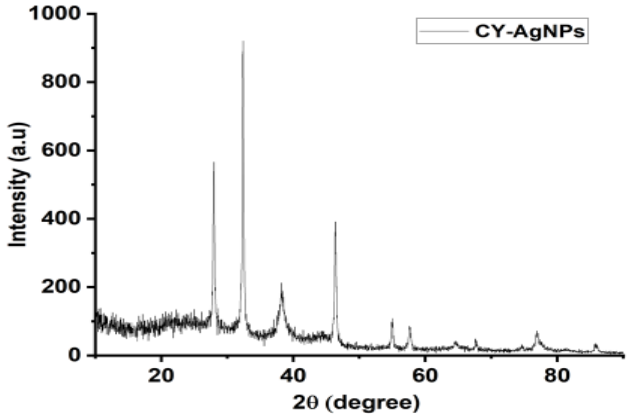


Figure 5: XRD pattern of silver nanoparticles of CY-AgNPs.

Table 1: Effect of plant extract and CY-AgNPs on larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

G	C	*An. stephensi*		*Ae. aegypti*		*Cx. quinquefasciatus*	
		MA	% Mortality	MA	% Mortality	MA	% Mortality
G-I	0.5	0.67±0.33	2.67	0.33±0.33	1.33	0.33±0.33	1.33
	5	1.33±0.33	5.33	1.00±0.00	4.00	1.00±0.00	4.00
	15	1.67±0.33	6.67	2.67±0.33	10.67	2.67±0.33	10.67
	25	3.33±0.33	13.33	4.67±0.33	18.67	4.67±0.33	18.67
	35	5.33±0.33	21.33	10.67±0.33	42.67	10.33±0.33	41.33
	45	7.00±0.57	28.00	13.00±1.00	52.00	12.67±0.33	50.67
G-II	0.5	4.33±0.33	17.33	3.33±0.33	13.33	3.33±0.33	13.33
	5	7.33±0.33	29.33	6.67±0.33	26.67	6.33±0.33	25.33
	15	10.67±0.33	42.67	10.67±0.33	42.67	10.33±0.33	41.33
	25	14.67±0.33	58.67	13.00±1.00	52.00	13.33±0.33	53.33
	35	19.33±0.33	77.33	18.67±0.33	74.67	18.33±0.33	73.33
	45	22.67±0.33	90.67	22.33±0.33	89.33	21.67±0.33	86.67
G-III	Control	0.00	0.00	0.00	0.00	0.00	0.00

G-I: CY-Plant extract group, G-II: CY-AgNPs group, G-III: Placebo group, C- Concentration (in ppm), MA-Mean Average

Note- Three replicates with 25 larvae taken for each species each replicate

Table 2: Effect of plant extract and CY-AgNPs on adults of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

G	C	*An. stephensi*		*Ae. aegypti*		*Cx. quinquefasciatus*	
		MA	% Mortality	MA	% Mortality	MA	% Mortality
G-I	5	0.33±0.33	1.33	0.33±0.33	1.33	0.33±0.33	1.33
	20	1.33±0.33	5.33	1.33±0.33	5.33	1.00±0.00	4.00
	35	3.67±0.33	14.67	4.33±0.33	17.33	4.00±0.00	16.00
	55	6.00±1.00	24	7.67±0.33	30.67	7.33±0.33	29.33
	75	14.67±0.33	58.67	11.67±0.33	46.67	11.33±0.33	45.33
	95	17.33±0.33	69.33	13.67±0.33	54.67	12.67±0.33	50.67
G-II	5	3.33±0.33	13.33	3.33±0.33	13.33	3.33±0.33	13.33
	20	6.33±0.33	25.33	6.33±0.33	25.33	5.67±0.33	22.67
	35	10.33±0.33	41.33	10.33±0.33	41.33	10.00±1.00	40.00
	55	13.33±0.33	53.33	13.33±0.33	53.33	12.66±0.33	50.67
	75	18.33±0.33	73.33	17.67±0.33	70.67	17.66±0.33	70.67
	95	24.67±0.33	98.67	23.33±0.33	93.33	22.33±0.33	89.33
G-III	Control	0.00	0.00	0.00	0.00	0.00	0.00

G-I: CY-Plant extract group, G-II: CY-AgNPs group, G-III: Placebo group, C- Concentration (in ppm), R- Replicate, MA-Mean Average, Note- Three replicates with 25 larvae taken for each species, each replicate

Fourier Transformation Infrared Spectroscopy

The FTIR band at 3427.44 cm^{-1} corresponds to O-H stretching in the sample, reflecting the presence of the alcoholic group. The band at 2925.76 cm^{-1} corresponds to C-H stretching signifies the aromatic compounds in the sample. Proteins were indicated by the band at 1648 cm^{-1} which corresponds to C-N (amines) stretching. The band at 1075 cm^{-1} signifies C-O stretch vibrations of phenolic compounds. The presence of alkyl halides is indicated by the band at 529 cm^{-1} which is shown by C-Br stretching (Figure 6).

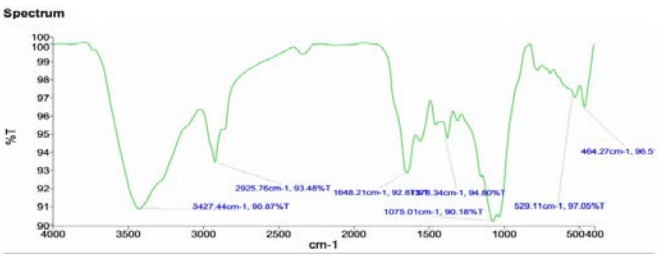


Figure 6: FTIR spectrum of silver nanoparticles of CY-AgNPs.

Larvicidal activity

Table 1 shows the larvicidal activity in all three test groups against the larval stages of *An.stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* mosquitoes respectively. Mortality percentages at 0.5 ppm concentration against *An.stephensi* in Group I and Group II were found to be 2.67% & 17.33% while *Ae. aegypti*, and *Cx. quinquefasciatus* were 1.33% & 13.33 % respectively. At the highest dosage of 45 ppm concentration treated against *An.stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* in both treated groups recorded mortality percentages of 28%, 52%, 50.67 % (G-I) and 90.67%, 89.33%, 86.67% (G-II) respectively. Zero mortality was recorded in the control group therefore; Abbott's corrected mortality wasn't obtained. The highest mortality data was recorded in group II for all three Genera which signifies the high efficacy of CY-AgNPs as compared to CY leaf extract against mosquito larvae.

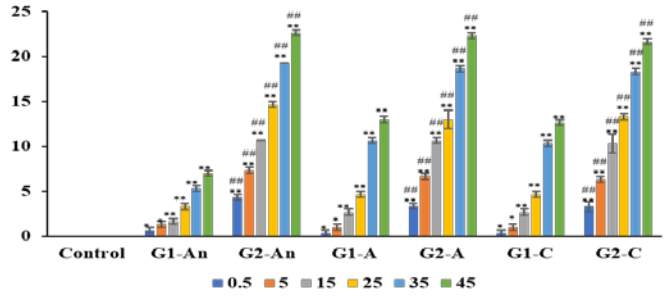


Figure 7: Bioassay of larvicidal efficacy of CY-AgNPs. *Where An-Anophels, A-Aedes, C-Culex, G-Group, *As compared with control; # As compared with G1, *P< 0.05; **P <0.01, #P< 0.05; ##P <0.01*

Adulticidal activity

Table 2 shows the adulticidal activity in both the test groups against all genera- *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* mosquitoes respectively. Adult mortality percentages at 5 ppm concentration against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* in Group I and Group II were found to be exactly the same 1.33 %, and 13.33 % respectively. However, the mortality percentages at the highest dose of 95 ppm concentration were distinct for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* and recorded as 69.33%, 54.67%, 50.67 % (Group I), and 98.66%, 93.33%, 89.33% (Group II) respectively. Zero mortality was recorded in the control group therefore; Abbott's corrected mortality was not recorded. Green synthesized silver nanoparticles were observed to be highly potent against adult stages of all three mosquito genera compared to leaf extract.

Statistical analysis

In the larvicidal assay, LC_{50}, LC_{90} and LC_{95} values in Group I for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were 77.75ppm, 144.11 ppm, and 152.40ppm; 44.12ppm, 82.43ppm, and 87.22ppm; 45.26ppm, 84.55ppm and 89.46ppm respectively whereas LC_{50}, LC_{90} and LC_{95} in Group II noted were 46.55ppm, 87.10 ppm, and 92.16ppm for *An. stephensi*; 40.94ppm, 75.94 ppm, and 79.94ppm for *Ae. aegypti*; 41.45ppm, 76.55 ppm, and 80.93ppm for *Cx. quinquefasciatus* respectively. The lower LC_{50} and LC_{90} values in Group II as compared to Group I validate that CY-AgNPs are more potent than CY-extract in their larvicidal activity. Among all the genera lowest lethal concentrations (G-II) were recorded in *Aedes spp.* followed by *Culex spp.* and *Anopheles spp.*

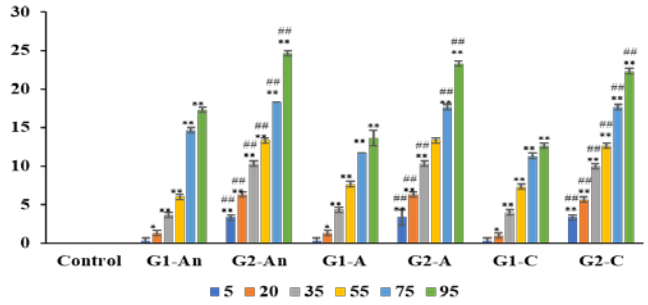


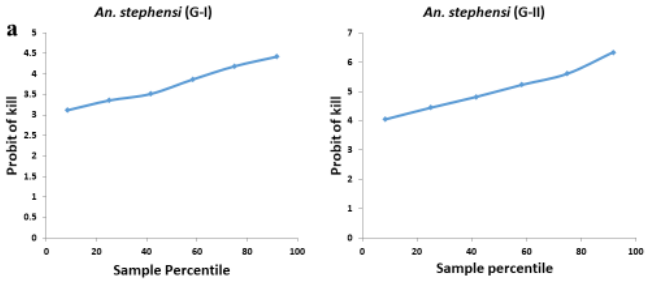
Figure 8: Bioassay of adulticidal efficacy of CY-AgNPs. *Where An-Anophels, A-Aedes, C-Culex, G-Group, *As compared with control; # As compared with G1, *P< 0.05; **P <0.01, #P< 0.05; ##P <0.01*

Similar trends were also observed in the adulticidal assay and estimated values of LC_{50}, LC_{90}, and LC_{95} for CY-AgNPs. Adulticidal assay of *Ae. aegypti* calculated to be 24.76, 45.01, 47.54 ppm, and 20.94, 38.17, 40.32 ppm in Group I and Group II respectively which were recorded lower than *Anopheles* (LC_{50}=24.61, LC_{90}=47.01, LC_{95}=51.31 ppm for Group I, and LC_{50}=22.48, LC_{90}=41.19, LC_{95}=43.52 ppm for Group II), and *Cx. quinquefasciatus* (LC_{50}=28.48, LC_{90}=52.42, LC_{95}=55.41 ppm for Group I assay, and LC_{50}= 25.34, LC_{90}=46.07, LC_{95}= 48.66 ppm for Group II assay) suggesting that CY-AgNPs are more potent against *Ae. aegypti* mosquitoes as compared to *An. stephensi* and *Cx. quinquefasciatus* mosquitoes. The histograms comparing the larval and adult mortalities between both the treated groups with placebo and their level of significance are presented in Figures 7-8.

Table: 3 Probit regression equation of both the treated groups (G-I, G-II) for larvae and adults against various genera

G	*An. stephensi*	*Ae. aegypti*	*Cx. quinquefasciatus*
G-I (L)	Y=0.602+3.126	Y=1.044x+3.931	Y=1.018x+3.918
G-II (L)	Y=0.9862x+4.068	Y=1.153x+2.760	Y=1.139x+2.763
G-I (A)	Y=2.173x+.844	Y=1.974x+1.097	Y=1.929x+1.108
G-II (A)	Y=2.138x+1.90	Y=2.322x+1.356	Y=1.671x+2.403

Where G-Group, L-Larva, A-adult



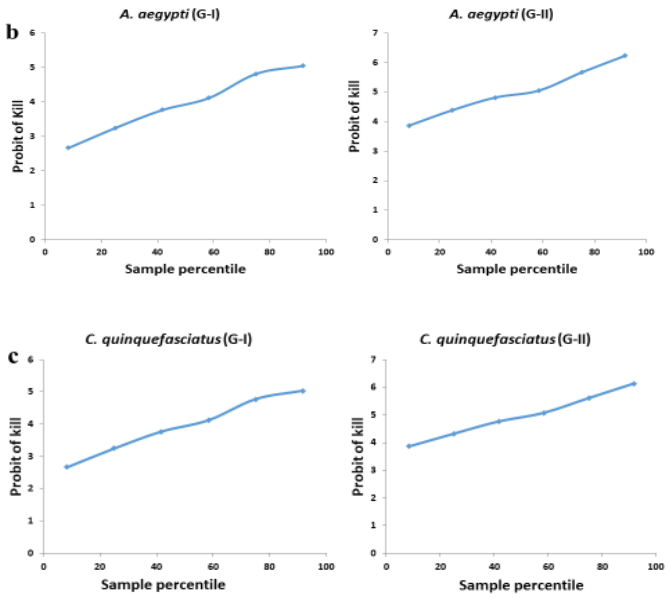
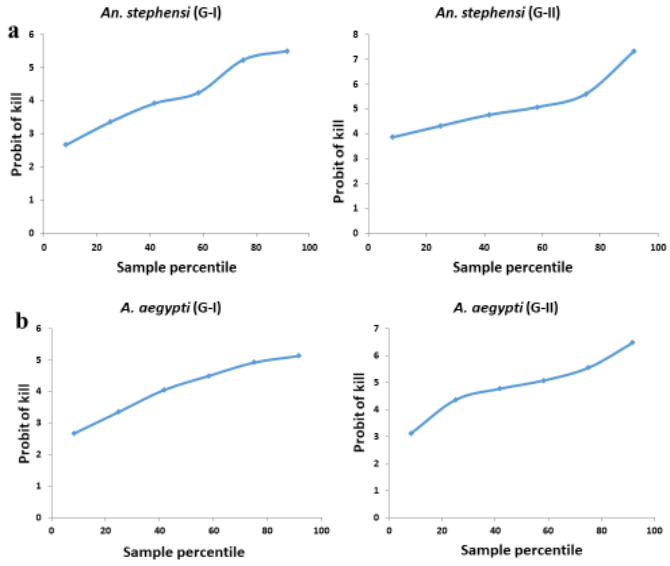


Figure 9: Probit graphs plotted for larvae of different genera for both the groups- Group I (CY-leaf extract) and Group II (CY-AgNPs) a) *An. stephensi*, b) *Ae. Aegypti*, and c) *Cx. Quinquefasciatus*.

The linear regression equation of both the groups (G-I, G-II) for their larval and adult stages are presented in Table 3. Probability output in the form of graphs with probit analysis and sample percentile are depicted in Figures 9-10 where each of the groups is compared with the control for their larvicidal and adulticidal efficiencies.



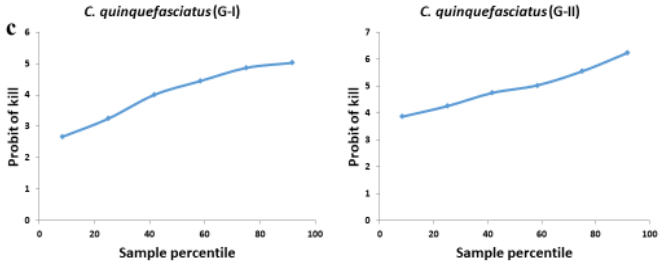


Figure 10: Probit graphs plotted for adults of different genera for both the groups- Group I (CY-leaf extract) and Group II (CY-AgNPs) a) *An. stephensi*, b) *Ae. Aegypti*, and c) *Cx. Quinquefasciatus*.

DISCUSSION

Synthesis of nanoparticles using plant extracts is a cost-effective and environment-friendly approach in which extracts from leaves[53-55], seeds[56], flowers[57], fruits[58], etc. are mixed with metal salts and oxides to produce metal nanoparticles in the laboratory. An intense brown color was observed in the reaction mixture of silver nanoparticles synthesized using leaf extract of *Ocimum sanctum*[59]. Silver nanoparticles were synthesized using *Tagetes erecta* flower extract and characterized using UV/Visible spectroscopy, XRD, and FTIR[57]. Silver nanoparticles synthesized using the leaf extract of *Azadirachta indica* showed colloidal brown color in the reaction mixture[60]. An analogous color change was observed in the present study with an absorption peak found at 435 nm in UV/Vis spectroscopy analysis. Similarly, the UV/Visible spectrum of silver nanoparticles using *Plantago lanceo*lata extract showed an absorption peak at 432 nm[61]. A similar result was shown by silver nanoparticles synthesized using *Polyalthia longifolia* showing an absorbance peak at 435 nm[62]. Leaf extract of *Catharanthus roseus* was used to synthesize green silver nanoparticles of particle size 33-55 nm in diameter as determined by the SEM images[63]. SEM analysis of silver nanoparticles synthesized in the present investigation revealed the particle size in the 27– 54 nm range. The presence of carbon (C) and oxygen (O) was detected in the EDX spectrum of silver nanoparticles synthesized using the leaf extract of *Cucumis prophetarum* which were related to the organic constituents of the leaf extract[64]. Beside strong signals of silver (Ag), weak signals of Nitrogen (N), Oxygen (O), and Chloride (Cl) were detected in the EDX spectrum of silver nanoparticles synthesized using *Moringa lucida* leaf extract. EDX analysis of the synthesized nanoparticles in the present study indicated a strong peak of Silver (Ag) along with weak peaks of Oxygen (O) and Nitrogen (N), which must be the constituents of the organic compounds attributed to the silver nanoparticles by the *Cymbopogon citratus* leaf extract. In our study, the X-ray diffraction pattern showed strong peaks at 2θ angle at 27.96, 32.36, 38.25, and 46.34 with intense counts 474.99, 821.22, 120.85, and 313.86 respectively which is in concurrence with strong peaks at 27.41, 31.82, and 45.83 with intense counts 154, 391, and 216 respectively in the XRD pattern of silver nanoparticles synthesized using Pseudomonas fluorescens[65]. FTIR spectrum of the silver nanoparticles synthesized using leaf extract of *Holoptelea integrifolia* showed bands at 3420 cm^{-1}, 2915 cm^{-1}, and 1654 cm^{-1},

corresponding to O-H, C-H, and N-H stretching respectively[66]. The intense peak at 1019 cm-1 represents the C-O stretching of phenols[67]. The band at 596 cm^{-1}in the FTIR spectrum of the silver nanoparticles synthesized for using *Urtica dioica* leaf extract corresponds to C-Br stretching signifying the presence of alkyl halides[53]. FTIR analysis of the synthesized nanoparticles in the current characterization results demonstrated bands at 3427 cm^{-1}, 2925 cm^{-1}, 1075 cm^{-1}, and 529 cm^{-1}which signifies functional groups related to amines, halides, alcoholic, and phenolic groups on the surface of the synthesized nanoparticles.

Aqueous leaf extract of *Cymbopogon citratus* showed considerable larvicidal activity with LC_{50}, $LC_{90,}$ and LC_{95} values as 77.75 ppm, 144.11 ppm, and 152.40 ppm for *An. stephensi*; 44.12 ppm, 82.43 ppm, and 87.22 ppm for *Ae. aegypti*; and 45.26 ppm, 84.55 ppm, and 89.46 ppm for *Cx. quinquefasciatus* mosquitoes. Similar results were obtained from larvicidal experiments of aqueous leaf extract *of Feronia elephantum* which showed LC_{50} and LC_{90} values of 54.88ppm and 97.38 ppm for *Anopheles*; 62.02 ppm and 110.71 ppm for *Aedes*; and 62.02 ppm and 110.71 ppm against *Culex* mosquito[87]. Aqueous extract of *Eclipta prostata* demonstrated larvicidal activity with LC_{50} and LC_{90} values of 27.85 and 71. 45 ppm for *Anopheles*; 27.49ppm and 70.38ppm for *Culex* mosquitoes[69]. The CY-AgNPs showed significant larvicidal activity against *An. stephensi* with LC_{50}, LC_{90}, and LC_{95} values of 46.55 ppm, 87.10 ppm, and 92.16ppm, *Ae. aegypti* with values of 46.94 ppm, 75.60 ppm, and 79.94 ppm, and *Cx. quinquefasciatus* with values of 41.46 ppm, 76.55 ppm, and 80.93 ppm respectively. However, when silver nanoparticles synthesized by *Bacillus marisflavi* were tested against larvae of *Anopheles* (LC_{50}= 52.54 ppm and LC_{90}= 95.24 ppm), *Aedes* (LC_{50}= 28.47 ppm and LC_{90}= 73.79 ppm), and *Culex* (LC_{50}= 52.54 ppm and LC_{90}= 95.24 ppm) showed efficient results[70]. Silver nanoparticles synthesized by *Plumbago auriculata* were also reported to have larvicidal activity against larval stages of *Aedes* and *Culex* mosquitoes with LC_{50} values of 45.1 ppm and 41.1 ppm respectively[71]. These results are in concurrence with the adulticidal activity shown by silver nanoparticles synthesized using *Ipomoea batatas* with LC_{50} and LC_{90} values of 12.56 and 19.51 ppm for *Anopheles*, 17.578 and 26.04 ppm for *Aedes*, and 10.06 and 15.65 ppm for *Culex* mosquitoes[72]. Silver nanoparticles synthesized using *Phyllanthus niruri* showed LC_{50} and LC_{90} values of 6.68 and 23.58 ppm respectively against *Aedes* mosquitoes[73]. In adulticidal assay, CY-AgNPs demonstrated LC_{50}, $LC_{90,}$ and LC_{95} values as 22.48, 52.42, and 43.52 ppm for *An. stephensi*, 20.94, 38,17, and 40.32 ppm for *Ae. aegypti*, and 25.34, 46.07, and 48.66 ppm for *Cx. quinquefasciatus* mosquitoes. Although, the literature cited supported the present investigation in terms of the efficacy of green synthesized nanoparticles against larvae and adult stages of different mosquito vectors, still much research is needed to explore the factual status of the eco-friendly claims.

CONCLUSION

The study demonstrated the successful synthesis of silver nanoparticles using an aqueous leaf extract of *Cympopogon citratus* in the laboratory. The CY-AgNPs showed significant larvicidal and adulticidal activity against malaria-causing *Anopheles*, dengue-causing *Aedes*, and elephantiasis-causing *Culex* mosquitoes. *Ae. aegypti* mosquitoes were found more susceptible to the CY-AgNPs compared to *An. stephensi* and *Cx. quinquefasciatus* mosquitoes. Results of the study revealed that using silver nanoparticles synthesized using plant leaf extract of CY could be viewed as an eco-friendly vector control approach that could be incorporated into the vector control programs aimed at reducing the mosquito vector population in human habitations.

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CONFLICT OF INTEREST STATEMENT

It is declared that the authors do not have any conflict of interest regarding this research article.

REFERENCES AND NOTES

1. B.R. Naik, B.K. Tyagi, R. De Xue. Mosquito-Borne Diseases in India Over the Past 50 Years and Their Global Public Health Implications: a Systematic Review. *J. Am. Mosq. Control Assoc.* **2023**, 39 (4), 258–277.
2. P. Venkatesan. The 2023 WHO World malaria report. *The Lancet Microbe* **2024**, 5 (3), e214.
3. S. Nasir, I. Ahmed, B. Hussain, et al. A study on the role of aedes mosquitoes in arboviruses and SARS-CoV-2 infection: A new challenge. *J. King Saud Univ. - Sci.* **2022**, 34 (6), 102179.
4. C. Pancharoen, T. Tantawichien, C. Thisyakorn, W. Kulwichit, U. Thisyakorn. Dengue infection: A global concern. *J. Med. Assoc. Thail.* **2002**, 85 (SUPPL. 1), 85.
5. S. Bhatt, P.W. Gething, O.J. Brady, et al. The global distribution and burden of dengue. *Nature* **2013**, 496 (7446), 504–507.
6. T. Garske, M.D. Van Kerkhove, S. Yactayo, et al. Yellow Fever in Africa: Estimating the Burden of Disease and Impact of Mass Vaccination from Outbreak and Serological Data. *PLoS Med.* **2014**, 11 (5), 1001638.
7. H. Kang, M. Auzenbergs, H. Clapham, et al. Chikungunya seroprevalence, force of infection, and prevalence of chronic disability after infection in endemic and epidemic settings: a systematic review, meta-analysis, and modelling study. *Lancet Infect. Dis.* **2024**, 24 (5), 488–503.
8. Z. Guo, W. Jing, J. Liu, M. Liu. The global trends and regional differences in incidence of Zika virus infection and implications for Zika virus infection prevention. *PLoS Negl. Trop. Dis.* **2022**, 16 (10), 10812.
9. J. Ikejezie, C.N. Shapiro, J. Kim, et al. Zika Virus Transmission — Region of the Americas, May 15, 2015–December 15, 2016. *MMWR. Morb. Mortal. Wkly. Rep.* **2017**, 66 (12), 329–334.
10. V.K. Agrawal, V.K. Sashindran. Lymphatic filariasis in India: Problems, challenges and new initiatives. *Med. J. Armed Forces India* **2006**, 62 (4), 359–362.
11. P.K. Das, K.D. Ramaiah, D.J. Augustin, A. Kumar. Towards elimination of lymphatic filariasis in India. *Trends Parasitol.* **2001**, 17 (10), 457–460.
12. A.M. Kilpatrick, L.D. Kramer, S.R. Campbell, et al. West Nile virus risk assessment and the bridge vector paradigm. *Emerg. Infect. Dis.* **2005**, 11 (3), 425–429.
13. G.L. Hamer, U.D. Kitron, J.D. Brawn, et al. Culex pipiens (Diptera: Culicidae): A Bridge Vector of West Nile Virus to Humans . *J. Med. Entomol.* **2008**, 45 (1), 125–128.
14. E.W. Cupp, H.K. Hassan, Y. Xin, et al. West Nile virus infection in mosquitoes in the Mid-South USA, 2002-2005. *J. Med. Entomol.* **2007**, 44 (1), 117–125.

15. A. Farajollahi, M.P. Nelder. Changes in aedes albopictus (Diptera: Culicidae) populations in New Jersey and implications for arbovirus transmission. *J. Med. Entomol.* **2009**, 46 (5), 1220–1224.
16. K.S. Srivastava, V. Jeswani, N. Pal, et al. Japanese Encephalitis Virus: An Update on the Potential Antivirals and Vaccines. *Vaccines* **2023**, 11 (4), 742.
17. J.C. Pearce, T.P. Learoyd, B.J. Langendorf, J.G. Logan. Japanese encephalitis: The vectors, ecology and potential for expansion. *J. Travel Med.* **2018**, 25 (Suppl_1), S16–S26.
18. T.M. Quan, T.T.N. Thao, N.M. Duy, T.M. Nhat, H.E. Clapham. Estimates of the global burden of japanese encephalitis and the impact of vaccination from 2000-2015. *Elife* **2020**, 9, 1–187.
19. J. Hemingway, H. Ranson. Insecticide resistance in insect vectors of human disease. *Annu. Rev. Entomol.* **2000**, 45 (1), 371–391.
20. J. Hemingway, N.J. Hawkes, L. McCarroll, H. Ranson. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem. Mol. Biol.* **2004**, 34 (7), 653–665.
21. R. Kaur, D. Choudhary, S. Bali, et al. Pesticides: An alarming detrimental to health and environment. *Sci. Total Environ.* **2024**, 915, 170113.
22. N. Calista, M.D. Haikael, M.O. Athanasia, K. Neema, K. Judith. Does Pesticide exposure contribute to the growing burden of non - communicable diseases in Tanzania. *Sci. African* **2022**, 17, 1276.
23. B. Pokhrel, P. Gong, X. Wang, et al. Distribution, sources, and air–soil exchange of OCPs, PCBs and PAHs in urban soils of Nepal. *Chemosphere* **2018**, 200, 532–541.
24. M. Tudi, H.D. Ruan, L. Wang, et al. Agriculture development, pesticide application and its impact on the environment. *Int. J. Environ. Res. Public Health* **2021**, 18 (3), 1–24.
25. S. Hales, W. van Panhuis. A new strategy for dengue control. *Lancet* **2005**, 365 (9459), 551–552.
26. P.K.D. Pramanik, A. Solanki, A. Debnath, et al. Advancing Modern Healthcare with Nanotechnology, Nanobiosensors, and Internet of Nano Things: Taxonomies, Applications, Architecture, and Challenges. *IEEE Access* **2020**, 8, 65230–65266.
27. B. Bhushan. Introduction to nanotechnology; Springer Handbooks, **2017**.
28. V. Vaishampayan, A. Kapoor, S.P. Gumfekar. Enhancement in the limit of detection of lab-on-chip microfluidic devices using functional nanomaterials. *Can. J. Chem. Eng.* **2023**, 101 (9), 5208–5221.
29. S.H. Lee, B.H. Jun. Silver nanoparticles: Synthesis and application for nanomedicine. *Int. J. Mol. Sci.* **2019**, 20 (4), 865.
30. E.C. Dreaden, A.M. Alkilany, X. Huang, C.J. Murphy, M.A. El-Sayed. The golden age: Gold nanoparticles for biomedicine. *Chem. Soc. Rev.* **2012**, 41 (7), 2740–2779.
31. V. Harish, M.M. Ansari, D. Tewari, et al. Nanoparticle and Nanostructure Synthesis and Controlled Growth Methods. *Nanomaterials* **2022**, 12 (18), 3226.
32. F. Arsiya, M.H. Sayadi, S. Sobhani. Green synthesis of palladium nanoparticles using Chlorella vulgaris. *Mater. Lett.* **2017**, 186, 113–115.
33. A. Chahardoli, N. Karimi, A. Fattahi. Nigella arvensis leaf extract mediated green synthesis of silver nanoparticles: Their characteristic properties and biological efficacy. *Adv. Powder Technol.* **2018**, 29 (1), 202–210.
34. J.F. Gao, H.Y. Li, K.L. Pan, C.Y. Si. Green synthesis of nanoscale zero-valent iron using a grape seed extract as a stabilizing agent and the application for quick decolorization of azo and anthraquinone dyes. *RSC Adv.* **2016**, 6 (27), 22526–22537.
35. S. Ying, Z. Guan, P.C. Ofoegbu, et al. Green synthesis of nanoparticles: Current developments and limitations. *Environ. Technol. Innov.* **2022**, 26, 102336.
36. A. Srinivasan, K. Manickavasagam, K. Malaichamy, et al. Impact of silver nanoparticles against stored product pest Sitophilus oryzae (L.) and effect on agromorphological characteristics of Zea mays (maize seeds). *Process Saf. Environ. Prot.* **2023**, 177, 1336–1350.
37. S. Gomathi, J. Firdous, V. Bharathi, et al. Phytochemical screening of silver nanoparticles extract of eugenia jambolana using fourier infrared spectroscopy. *Int. J. Res. Pharm. Sci.* **2017**, 8 (3), 383–387.
38. A.K. Giri, B. Jena, B. Biswal, et al. Green synthesis and characterization of silver nanoparticles using Eugenia roxburghii DC. extract and activity against biofilm-producing bacteria. *Sci. Rep.* **2022**, 12 (1), 8383.
39. V. Francisco, A. Figueirinha, B.M. Neves, et al. Cymbopogon citratus as source of new and safe anti-inflammatory drugs: Bio-guided assay using lipopolysaccharide-stimulated macrophages. *J. Ethnopharmacol.* **2011**, 133 (2), 818–827.
40. Tajidin, N. E. Chemical composition and citral content in lemongrass (Cymbopogon citratus) essential oil at three maturity stages. *African J. Biotechnol.* **2012**, 11 (11), 2685–2693.
41. A.V. Costa, P.F. Pinheiro, V.M. Rondelli, et al. Cymbopogon citratus (Poaceae) essential oil on Frankliniella schultzei (Thysanoptera: Thripidae) and Myzus persicae (Hemiptera: Aphididae). *Biosci. J.* **2013**, 29 (6), 184–187.
42. R. M., A.H. Nour, Z.A. Sulaiman, A.H. Nour, T.R. S. A Comparative Study of Lemongrass (Cymbopogon Citratus) Essential Oil Extracted by Microwave-Assisted Hydrodistillation (MAHD) and Conventional Hydrodistillation (HD) Method. *Int. J. Chem. Eng. Appl.* **2014**, 5 (2), 104–108.
43. M. Asif, E. Khodadadi. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. *J. Paramed. Sci.* **2013**, 4 (3), 119–138.
44. Z.T. Pinto, F.F. Sánchez, A.R. dos Santos, et al. Composição química e atividade inseticida do óleo essencial de Cymbopogon citratus de Brasil e Cuba contra mosca doméstica. *Rev. Bras. Parasitol. Vet.* **2015**, 24 (1), 36–44.
45. A. Banu K, A. Devaraj. a Novel Herbal Pesticide To Control Adult Scale Insects. *J. Pharm. Sci. Innov.* **2013**, 4 (4), 22–25.
46. S.S. Vera, D.F. Zambrano, S.C. Méndez-Sanchez, et al. Essential oils with insecticidal activity against larvae of Aedes aegypti (Diptera: Culicidae). *Parasitol. Res.* **2014**, 113 (7), 2647–2654.
47. S.A. Masurkar, P.R. Chaudhari, V.B. Shidore, S.P. Kamble. Rapid Biosynthesis of silver nanoparticles using Cymbopogan citratus (Lemongrass) and its Antimicrobial Activity. *Nano-Micro Lett.* **2011**, 3 (3), 189–194.
48. Z. Haris, I. Ahmad. Green synthesis of silver nanoparticles using Moringa oleifera and its efficacy against gram-negative bacteria targeting quorum sensing and biofilms. *J. Umm Al-Qura Univ. Appl. Sci.* **2024**, 10 (1), 156–167.
49. H. Imam, G. Sofi, Zarnigar, S. Aziz. The basic rules and methods of mosquito rearing (Aedes aegypti). *Trop. Parasitol.* **2014**, 4 (1), 53.
50. M. Larvicides. Guidelines for laboratory and field testing of mosquito larvicides. In *World Health Organization*; **2005**; pp 1–41.
51. WHO. Standard operating procedure for determining the ability of PBO to restore susceptibility of adult mosquitoes to pyrethroid insecticides in WHO tube tests. *World Heal. Organ.* **2022**, 29 (7), 283–286.
52. K. Veerakumar, M. Govindarajan, S.L. Hoti. Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes. *Parasitol. Res.* **2014**, 113 (12), 4567–4577.
53. K. Jyoti, M. Baunthiyal, A. Singh. Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. *J. Radiat. Res. Appl. Sci.* **2016**, 9 (3), 217–227.
54. N. Karim, S. Liu, A.K. Rashwan, et al. Green synthesis of nanolipo-fibersomes using Nutriose® FB 06 for delphinidin-3-O-sambubioside delivery: Characterization, physicochemical properties, and application. *Int. J. Biol. Macromol.* **2023**, 247, 125839.
55. A.A. Zahir, A.A. Rahuman. Evaluation of different extracts and synthesised silver nanoparticles from leaves of Euphorbia prostrata against Haemaphysalis bispinosa and Hippobosca maculata. *Vet. Parasitol.* **2012**, 187 (3–4), 511–520.
56. A. Rautela, J. Rani, M. Debnath (Das). Green synthesis of silver nanoparticles from Tectona grandis seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. *J. Anal. Sci. Technol.* **2019**, 10 (1), 5.
57. H. Padalia, P. Moteriya, S. Chanda. Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. *Arab. J. Chem.* **2015**, 8 (5), 732–741.

58. B. Ankamwar, C. Damle, A. Ahmad, M. Sastry. Biosynthesis of gold and silver nanoparticles using Emblica Officinalis fruit extract, their phase transfer and transmetallation in an organic solution. *J. Nanosci. Nanotechnol.* **2005**, 5 (10), 1665–1671.
59. D. Philip, C. Unni. Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (Ocimum sanctum) leaf. *Phys. E Low-Dimensional Syst. Nanostructures* **2011**, 43 (7), 1318–1322.
60. P. Banerjee, M. Satapathy, A. Mukhopahayay, P. Das. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour. Bioprocess.* **2014**, 1 (1), 3.
61. M.Z. Shah, Z.H. Guan, A.U. Din, et al. Synthesis of silver nanoparticles using Plantago lanceolata extract and assessing their antibacterial and antioxidant activities. *Sci. Rep.* **2021**, 11 (1), 20754.
62. A. Dashora, K. Rathore, S. Raj, K. Sharma. Synthesis of silver nanoparticles employing Polyalthia longifolia leaf extract and their in vitro antifungal activity against phytopathogen. *Biochem. Biophys. Reports* **2022**, 31, 101320.
63. S. Ponarulselvam, C. Panneerselvam, K. Murugan, et al. Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. *Asian Pac. J. Trop. Biomed.* **2012**, 2 (7), 574–580.
64. Hemlata, P.R. Meena, A.P. Singh, K.K. Tejavath. Biosynthesis of Silver Nanoparticles Using Cucumis prophetarum Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity against Cancer Cell Lines. *ACS Omega* **2020**, 5 (10), 5520–5528.
65. D. Kalaimurugan, P. Vivekanandhan, P. Sivasankar, et al. Larvicidal Activity of Silver Nanoparticles Synthesized by Pseudomonas fluorescens YPS3 Isolated from the Eastern Ghats of India. *J. Clust. Sci.* **2019**, 30 (1), 225–233.
66. V. Kumar, S. Singh, B. Srivastava, R. Bhadouria, R. Singh. Green synthesis of silver nanoparticles using leaf extract of Holoptelea integrifolia and preliminary investigation of its antioxidant, anti-inflammatory, antidiabetic and antibacterial activities. *J. Environ. Chem. Eng.* **2019**, 7 (3), 103094.
67. U.P. Manik, A. Nande, S. Raut, S.J. Dhoble. Green synthesis of silver nanoparticles using plant leaf extraction of Artocarpus heterophylus and Azadirachta indica. *Results Mater.* **2020**, 6, 100086.
68. K. Veerakumar, M. Govindarajan, M. Rajeswary, U. Muthukumaran. Low-cost and eco-friendly green synthesis of silver nanoparticles using Feronia elephantum (Rutaceae) against Culex quinquefasciatus, Anopheles stephensi, and Aedes aegypti (Diptera: Culicidae). *Parasitol. Res.* **2014**, 113 (5), 1775–1785.
69. G. Rajakumar, A. Abdul Rahuman. Larvicidal activity of synthesized silver nanoparticles using Eclipta prostrata leaf extract against filariasis and malaria vectors. *Acta Trop.* **2011**, 118 (3), 196–203.
70. J. Thelma, C. Balasubramanian. Ovicidal, larvicidal and pupicidal efficacy of silver nanoparticles synthesized by Bacillus marisflavi against the chosen mosquito species. *PLoS One* **2021**, 16 (12 December), 260253.
71. L. Govindan, S. Anbazhagan, A.B. Altemimi, et al. Efficacy of antimicrobial and larvicidal activities of green synthesized silver nanoparticles using leaf extract of plumbago auriculata lam. *Plants* **2020**, 9 (11), 1–13.
72. V. Pavithra Bharathi, C. Ragavendran, N. Murugan, D. Natarajan. Ipomoea batatas (Convolvulaceae)-mediated synthesis of silver nanoparticles for controlling mosquito vectors of Aedes albopictus, Anopheles stephensi, and Culex quinquefasciatus (Diptera:Culicidae). *Artif. Cells, Nanomedicine Biotechnol.* **2017**, 45 (8), 1568–1580.
73. U. Suresh, K. Murugan, G. Benelli, et al. Tackling the growing threat of dengue: Phyllanthus niruri-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector Aedes aegypti (Diptera: Culicidae). *Parasitol. Res.* **2015**, 114 (4), 1551–1562.