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Ananas Comosus fruit peel extract-Mediated green synthesis of silver nanoparticles and their larvicidal potential

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Abstract
Silver nanoparticles are useful in managing the prevalence of mosquitoes and multidrug-resistant infections without posing a significant risk to human health. The current work concentrated on the environmentally friendly manufacturing of silver nanoparticles to combat mosquito vectors. The current work uses fruit peels from *Ananas comosus* to demonstrate the mosquito-larvicidal potential of green produced silver nanoparticles. AgNPs that were synthesized were examined using UV-Vis, FTIR, XRD, SEM, and HRTEM. The start synthesis of AgNPs was revealed by the color shift of the AgNO3 solution and the aqueous extract of *A. comosus* fruit peel from pale yellow to dark red. The biosynthesis of AgNPs has been confirmed by the UV-Vis Surface Plasmon Resonance (SPR) band at 460 nm. The produced AgNPs were spherical and rectangular in form, according to SEM and HRTEM pictures. Larvicidal bioassays were performed on fourth-instar larvae of the vectors of malaria, dengue, and filariasis, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*, using varying doses of AgNPs and crude aqueous peel extract (5 ppm to 25 ppm). After being exposed for 24 hours, synthesized AgNPs showed extremely significant larvicidal efficacy against every mosquito vector tested. Larvicidal activity of AgNPs synthesized from *A. comosus* peel aqueous extract is superior to the crude aqueous peel extract of *A. comosus*. Based on these findings, we strongly suggested that face centered cubic structured *A. comosus* AgNPs is an eco-friendly and potent bio-medical agent and can be apply in wide range of application an alternative chemically synthesized metal nanoparticles to control vector mosquitoes.

Keywords: *Ananas comosus*, green synthesis of silver nanoparticles, larvicidal activity, *A. aegypti*

Introduction

Malaria, Filaria, Yellow Fever, Dengue, Chikunguniya, Japanese encephalitis, and other diseases transmitted by insects are major causes of morbidity and mortality worldwide (Doloi, 2021) [1]. An estimated 1.5 million cases of malaria and 29 million cases of filariasis are reported each year in India (Mbacham *et al.*, 2019; Wilairatana *et al.*, 2022) [2, 3]. Mosquitoes belong to the order Diptera and family Culicidae (Muñoz-Gamba *et al.*, 2021) [4]. Currently, 44 genera contain about 3,490 officially recognized species. Less than 150 species of female mosquitoes, mostly found in the genera Anopheles, Aedes, Culex, Culiseta, Haemagogus, and Mansonia, are carriers of the pathogens that cause these illnesses (Dar, 2018) [5].

The elimination of the infectious organism or controlling the mosquito vectors can help prevent diseases spread by mosquitoes (Niang *et al.*, 2018; Dahmana and Mediannikov, 2020) [6, 7]. One strategy to prevent diseases spread by mosquitoes is to stop the aquatic stages or use chemical insecticides to kill the adult mosquitoes (Engdahl *et al.*, 2022) [8]. Continued use of insect growth regulators (diflubenzuron and methoprene) and organophosphates (chloropyrifos, temephos, and fenthion) is often necessary to control mosquito larvae (Abbas *et al.*, 2014; Choi and Wilson, 2017; Bhuyan *et al.*, 2020) [9, 10, 11]. Insecticide resistance, the recovery of pest species, and environmental contamination are the results of the efficient and frequent application of controlling agents, which have upset the natural, biological control systems (Iftikhar *et al.*, 2023) [12]. However, there are significant disadvantages to the widespread and careless use of pesticides, including toxicity risks to people, animals, and wildlife (Mahmood *et al.*, 2016) [13].

To address these issues with traditional mosquito control, significant work is needed to create new or complementary methods of controlling mosquito species. This has led to the hunt for environmentally friendly, economically viable, biodegradable, and species-specific insecticides (Oliva *et al.*, 2021) [14]. Plant-mediated synthesis of metal nanoparticles is gaining popularity because it is easy to use, produces nanoparticles quickly, has a variety of morphologies, eliminates the need for meticulous cell culture maintenance, and is environmentally friendly (Johnson and Uwa, 2019; Alprol *et al.*, 2023) [15, 16]. Extracts of neem (Azadirachta indica) (Asimuddin *et al.*, 2020) [17], citrus lemon (Mohapatra *et al.*, 2015) [18], pineapple leaf (Odeja *et al.*, 2014) [19], Thevetia peruviana (Oluwaniyi *et al.*, 2015) [20], Piper nigrum (Mohapatra *et al.*, 2015) [18], and Ocimum santum (Singhal *et al.*, 2011) [21] have all been used in the biosynthesis of AgNps.

Ananas cosmosus

According to De Corato *et al.*, (2018) [22], biowaste products from a variety of sources, including fruits, have so far been extensively recycled into useful goods that include agricultural compost, citric acid synthesis, biofuel, pigment, and bioactive compounds. Additionally, this recycling technique can successfully correct an imbalance in the ecosystem (Ghisellini *et al.*, 2016) [23]. A few research have demonstrated that pineapple peel extracts can scavenge oxidants (Emmanuel *et al.*, 2016) [24].

Therefore, the current study demonstrates the larvicidal activity of produced silver nanoparticles (AgNPs) utilizing peel extracts from *A. comosus* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* larvae in their fourth instar.

Materials and Methods

Selection and procurement of fruit peels

Fruits and agricultural waste from the Bromeliaceae family that are healthy and free of illness were purchased from Koyambedu market in Chennai, Tamil Nadu, India. *Ananas comosus* (L.) Hepper fruit peels and agricultural waste were used for the investigation. The Plant Anatomy Research Centre (PARC) in Chennai, Tamil Nadu, recognized and verified the fruit waste.

Fig 1: *A. comosus* fruit and its peel

Selection of mosquito species

The fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* were the mosquito species chosen for this investigation (Fig). *Aedes aegypti* is widely distributed, extremely domesticated, and anthropophilic. The species Aedes is in charge of spreading the arbovirus that causes dengue and dengue hemorrhagic fever (Zannoli *et al.*, 2017). Plasmodium falciparum, the causative agent of the most severe type of malaria, is one of the four parasites that can be spread by Anopheles species (Saif, 2017). Widely found in trophical locations, *C. quinquefasciatus* is a vector of Wuchereria species that cause lymphatic filariasis (Babu *et al.*, 2018).

AgNPs synthesis from *A. comosus* aqueous peel extract

After properly washing the *A. comosus* peels in tap water for ten minutes to get rid of any dust, they were briefly rinsed in deionized water. In a 250 ml Erlenmeyer flask, 10 g of cleaned and finely ground peel powder and 100 ml of deionized water were combined to create the aqueous solution, which was then heated for 15 minutes at 60°C. This extract was used for additional tests after being filtered through a millipore hydrophilic filter (0.22m) and nylon mesh (spectrum) (Parashar *et al.*, 2009) [29].

UV- visible spectral analysis

A UV-vis spectrophotometer (Shimadzu 1601 model, Japan) was used to characterize the surface plasmon resonance of silver nanoparticles at intervals of one milliliter, with a resolution of one nanometer between 200 and 800 nm.

Fourier transform infrared (FTIR) spectroscopy

The functional group of the *A. comosus* peel aqueous extract involved in stabilizing the silver as a silver nanoparticle was identified using Fourier transform infrared spectroscopy, a quick and accurate analytical method. Shimadzu's IR Infinity 1 Fourier Transformation Infrared spectrophotometer was used to acquire the silver nanoparticles' FT-IR spectra.

X-ray diffraction studies

The diffractometer instrument from X'Pert Pro Materials Research was used to examine the compounds' production and quality. CuKα radiation, amplitude wave k = 1.5418 Å, a 40-kV voltage, and a 30-mA current were used to measure the X-ray diffraction (XRD) pattern using drop-coated films of AgNO3 on a glass plate. The characteristic radiation was in the range of 20-90˚, and the scan rate was 0.05° /min with a time constant of 2 s. The average crystallite size of the nanoparticles was calculated using Scherrer's equation and the full-width at half-maximum (FWHM) from three distinct peaks.

Scanning electron microscopy

A Hitachi SU6600 JEOL JSM 7500F was used to create a scanning electron microscopy (SEM) image. Using a backscattered electron (BSE) detector (designated as COMPO) for higher resonance emission scanning. The portion of the sample was coated with chromium using a K575X Turbo Sputter Coater (deposited film thickness: 20 nm).

High Resonance Transmission electron microscopy (TEM) of silver nanoparticles

The JEOL model 3010 apparatus, which was operated at 200 kV and a beam current of 104.1µA, was used to obtain the transmission electron microscopy (TEM) images. Aqueous AgNPs were deposited on carbon-coated copper grids (300 mesh size) using slow evaporation to create the sample for this study. The grids were then left to dry in a vacuum at 25°C for the entire night.

Mosquito larvicidal activity of synthesized nanoparticles

First, 100 milliliters of distilled water (stock solution) were used to dissolve one gram of aqueous peel extract. For the bioassay test, 100 mg/L of the stock solution was made using dechlorinated tap water. With minor adjustments, the WHO's (2005) approach was used to evaluate the larvicidal activity. Larvae were collected in five batches of 20 in 199 milliliters of water for the bioassay test, and 1.0 milliliter of aqueous peel extract was added. Following a 24-hour exposure period, the number of dead larvae was tallied, and the average of five replicates was used to calculate the % mortality.

Dose response bio assay

For the dose-response bioassay, the experimental media where 100% larval mortality occurs were chosen. Twenty mosquito larvae were placed in 200 milliliters of sterile double-distilled water containing nanoparticles to investigate the toxicity of synthesized AgNPs. Double-distilled water was used as a solvent to dilute the nanoparticle solutions to the appropriate concentrations (15, 10, 5, 2.25, and 1.25 mg/L). Five replicates of each concentration were included in each test, along with a set of control groups (distilled water and silver nitrate). To ascertain the acute toxicities against *Aedes aegypti*, *Anopheles stephensi*, and *C. quinquefasciatus* larvae in their fourth instar, mortality was evaluated after 24 hours. Before adding mosquito larvae, all treatments were sonicated for an extra five minutes to prevent particle settling, particularly at higher doses. The settling of particles seems to be much reduced by this extra sonication.

Statistical analysis

The software created by Reddy *et al.*, (1992) [30] was used to calculate chi-square values and perform probit analysis on the average larval mortality data in order to determine LC50, LC90 (Finney, 1971) [27], and other statistics at 95% confidence intervals of the upper and lower confidence limits (Busvine, 1971) [28]. Statistical significance was defined as results with $p < 0.05$ (SPSS, 11.5).

Results

Synthesis and characterization of silver nanoparticles

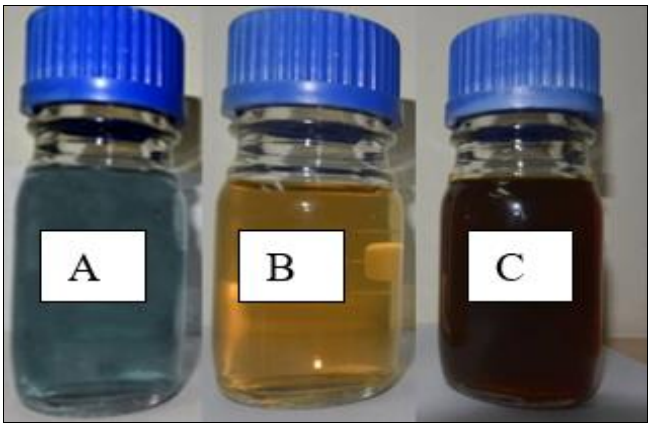

Fig 2: (A) $AgNO_3$ solution (B) *Ananas comosus* peels aqueous extract (C) (AgNPs) Discolouration of *Ananas comosus* peels aqueous extract by the reduction of $AgNO_3$ to AgO

UV–vis analysis of phyto-synthesised solutions

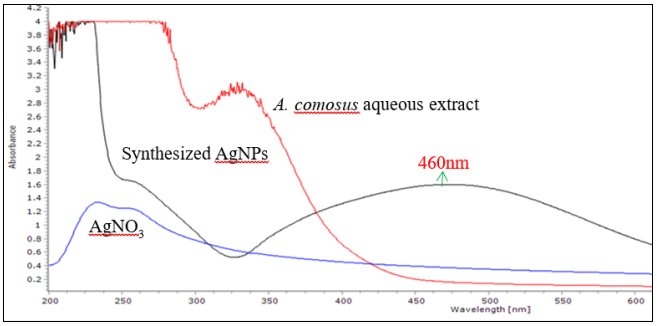

Fig 3: UV-vis spectra of synthesized AgNPs using *A. comosus* peels aqueous extract

Although the extract treated with silver nitrate turned brown following the creation of AgNPs, the aqueous peel extract of *A. comosus* maintained its natural color, which was yellow (Figure). Within 45 minutes of exposing the peel extract to AgNO3, a color shift was noticed and the biosynthetic reaction began. The creation of matching nanoparticles was indicated by the clear AgNO3 solution turning brown. The creation of the silver nanoparticles is confirmed by their brown appearance. The primary cause of this color is the silver nanoparticles' Surface Plasmon Resonance.

The silver nanoparticles were examined using a UV-visible double beam spectrophotometer that scanned wavelengths from 200 nm to 800 nm. The silver nanoparticle's surface Plasmon resonance is shown by the UV maxima in the spectrum at nm (Figure 3).

FT-IR analysis of synthesized silver nanoparticles

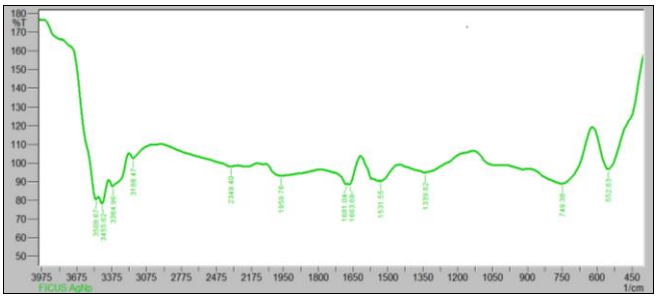

Fig 4: FT-IR spectrum of synthesized AgNPs using *A. comosus* peels aqueous extract

The existence of several functional groups in biomolecules that are in charge of the bioreduction of Ag+ and the capping/stabilization of silver nanoparticles was determined by FT-IR studies. The functional groups were identified by comparing the observed intensity bands with standard values. The presence of a capping agent with the nanoparticles is shown by the FTIR spectrum's absorption bands at 3455, 3364, 3188, 2344, 1959, 1681, 1663, 1531, and 1339 cm−1, respectively. The FTIR analysis of this study reveals various bond stretches at various peaks: the presence of a hydroxy group is represented by 3364, 3455, 3508, and 3188 cm−1; the presence of an H-bonded strong OH stretching of alcohols intermolecularly bonded is represented by 2349 cm−1; the presence of a strong O=C=O stretching of carbon dioxide is represented by 2349 cm−1; the presence of a weak C-H bending aromatic compound is represented by 1959, 1681 & 1663 cm−1; the presence of a strong N-O stretching nitro compound is represented by 1531 cm−1; and the presence of a medium C-H bending alkane methylene group is represented by 1339 cm−1 (Figure 4).

X-ray diffraction (XRD) of synthesized silver nanoparticles

According to the (fcc) structure of AgNPs, the nanoparticles' XRD investigation revealed a strong peak that corresponded to Bragg's reflections (110), (111), (121) and (200). The development of nanoparticles is shown by the broadening of the Braggs peak. Strong X-ray scattering centers in the crystalline space are suggested by intense Bragg's reflection, which may be caused by a capping agent. Thus, XRD data indicate that the bioorganic phase crystallizes on the silver nanoparticle surface.

Scanning Electron Microscopy analysis (SEM)

The nanoparticles' structure was examined using a scanning electron microscope. High resonance displays the produced silver nanoparticles' SEM picture. This instrument's analytics are quick, simple, and have a remarkably high throughput. The polydisperse, spherical, oval-shaped silver nanoparticles (AgNPs) with an average size of 120 nm and a range of 100 to 150 nm were measured after being magnified to 5000X.

HR-TEM analysis of synthesized silver nanoparticles

Transmission electron microscopy (TEM) images were used to assess the size and shape of AgNPs. The size and morphology of the biogenically stable Ag nanoclusters were examined using TEM examination. The majority of the

nano-crystals made from *A. comosus* peel extract are spherical in shape, rather homogeneous, and exhibit a modest degree of particle size variation, as illustrated in Fig. The majority of nanoparticles fell between 20 and 50 nm in size distribution (Figure 5).

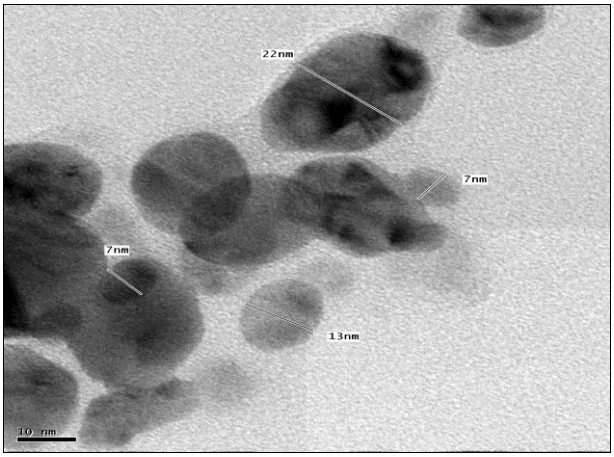

Fig 8: HRTEM and SAED image of synthesized *AgNPs* using *A. comosus* peel aqueous

Mosquito larvicidal activity of aqueous peel extract of *A. comosus* and their synthesized silver nanoparticles

The aqueous peel extract of synthetic silver nanoparticles of *A. comosus*'s larvicidal activity against the three major mosquito vectors—*A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*—is shown in the table. Synthesized silver nanoparticles of *A. comosus* shown strong larvicidal action against *Aedes aegypti*, *Anopheles stephensi*, and *C. quinquefasciatus* larvae.

Table 1: Larvicidal activity of Synthesized AgNPs from *A. comosus* against the fourth instar larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
A. aegypti	5 10 15 20 25	23 48 75 100 100	11.050 10.119±11.984	24.911 22.103±29.069	28.455
A. stephensi	5 10 15 20 25	18 35 56 83 100	8.747 7.995±9.476	17.727 16.072±20.009	26.049
C. quinquefasciatus	5 10 15 20 25	28 59 88 100 100	7.563 6.863±8.230	15.044 13.660±16.939	14.216

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50} - Lethal concentration that kills 50% of the exposed larvae LC_{90} – Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

Table 2: Larvicidal activity of *A. comosus* peel aqueous extract against the fourth instar larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
A. aegypti	25 50 75 100 150	17 35 59 81 100	63.327 59.515±78.216	128.635 113.702±150.796	19.185
A. stephensi	25 50 75 100 150	19 37 64 87 100	52.537 47.990±57.091	117.639 104.601±136.661	18.140
C. quinquefasciatus	25 50 75 100 150	16 33 61 84 100	56.163 51.548±60.846	122.667 109.245±142.200	18.520

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50} - Lethal concentration that kills 50% of the exposed larvae LC_{90} – Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

After tested against the indicated mosquito species, the green produced silver nanoparticles were more poisonous than the aqueous extracts of *A. comosus*. All three of the examined mosquito species' fourth-instar larvae were extremely vulnerable to *A. comosus* AgNPs. After 24 hours following treatment, 100% mortality was noted at a 20-ppm concentration. After 24 hours after treatment, the LC50 and LC90 values of synthesized AgNPs treated against fourth instar larvae of *C. quinquefasciatus* were 7.563 ppm and 15.044 ppm, *A. stephensi* was 8.747 ppm and 17.727 ppm, and *A. aegypti* was 11.050 ppm and 24.911 ppm. In contrast, the LC50 and LC90 values of aqueous peel extract treated against fourth instar larvae of *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti* were 56.163 ppm, 122.667 ppm, 52.537 ppm, 117.639 ppm, and 63.327 ppm, respectively. The green synthesized AgNPs of *A. comosus* showed high toxicity against the treated larvae at very low concentrations.

Discussion

One straightforward, affordable, and environmentally benign synthesis approach for NPs is green synthesis. In the green production of NPs, several biomolecules serve as capping and reducing agents (Patel, 2021) [31]. These natural materials have low cost, low toxicity, and low energy, pressure, and temperature needs. Consequently, in contrast to chemically produced silver nanoparticles, these synthesized nanoparticles could find application in biological systems (Garg *et al.*, 2020) [32].

According to earlier research, the primary groups that reduce Ag+ to Ag0 and cap the AgNPs are the carbonyl and hydroxyl groups found in tannins, flavanoids, alkaloids, steroids, and glycosides. The reduction process and AgNP stability may be attributed to the phenolic group of compounds (Wang *et al.*, 2007). The existence of silver nanoparticles was detected by UV-vis spectroscopy, and the metal's UV spectra showed that the particles had free electrons, which caused the combined wave to produce an SPR absorption band. The SPR of AgNPs, as determined by Ramanibai and Velayutham (2015) [34] at 460 nm, is caused by collective electron oscillation around the wavelength of the UV-Vis spectra in the current work.

The FTIR result is consistent with the Suresh *et al.*, (2014) [35] findings. A large peak at about 1645 cm-1 is caused by polyols and phenols reducing silver ions and oxidizing unsaturated carbonyl groups. This suggests that phenolic compounds (tannins, terpenoids, and flavanoids) may supply functional groups that are involved in the biosynthesis and capping of AgNPs. Furthermore, according to Huang *et al.*, (2012) [36], the broad bands at 3200–3400 cm-1 correspond to the OH functional groups of tannin, flavanoids, and other phenolic chemicals.

HRTEM analysis was used to look at the size and form of the produced silver nanoparticles. The nanoparticles had an average size of 14 nm and were nearly spherical in shape, which is comparable to the previous findings of Ramanibai and Velayutham *et al.*, (2016) [37].

Strong larvicidal action was demonstrated by synthesized AgNPs made from *A. comosus* fruit peels against *A. aegypti*, A. stephenesi, and *C. quinquefasciatus* larvae in their fourth instar. When compared to aqueous extract, synthetic AgNPs showed the highest mortality rate against all three tested mosquito species. The larvicidal activity of synthetic silver nanoparticles utilizing 3,5-di-t-butyl-4-hydroxyanisole from Cynodon dactylon against *A. aegypti* and *C. quinquefasciatus* was reported by Ramanibai and Velayutham (2016) [37], and these results are similar to their prior findings. Velayutham and Ramanibai (2015) [34] noted similar outcomes.

According to Soni and Prakash (2012) [38], silver nanoparticles synthesized by Chrysosporium tropicum were efficient against *Aedes aegypti* larvae in their third instar. Likewise, silver nanoparticles produced with the filamentous fungus Cochliobolus lunatus were extremely poisonous to *A. aegypti* larvae in their second, third, and fourth instars. Silver nanoparticles may be hazardous to mosquito larvae because of their small size, which allows them to enter cells and disrupt physiological functions including moulting (Pathipati and Kanuparthi, 2021) [39].

Conclusion

According to the current study, *A. comosus* fruit peel extract is a renewable and environmentally safe source that may be utilized as an efficient capping and reducing agent for AgNP production. Silver nanoparticles can be easily and effectively synthesized at ambient temperature without the use of hazardous reducing chemicals by employing a green chemistry methodology. The creation of a clean, non-toxic, environmentally acceptable "green approach" to producing metal nanoparticles using peel extracts would benefit greatly from the biological reduction of metal. The resulting silver nanoparticles are extremely stable and shown notable larvicidal efficacy against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* mosquito larvae. The findings demonstrate AgNPs' excellent effectiveness as a potent larvicidal agent. These functionalized nanoparticles are attractive options for vector control because of the surface reactivity made possible by capping.

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