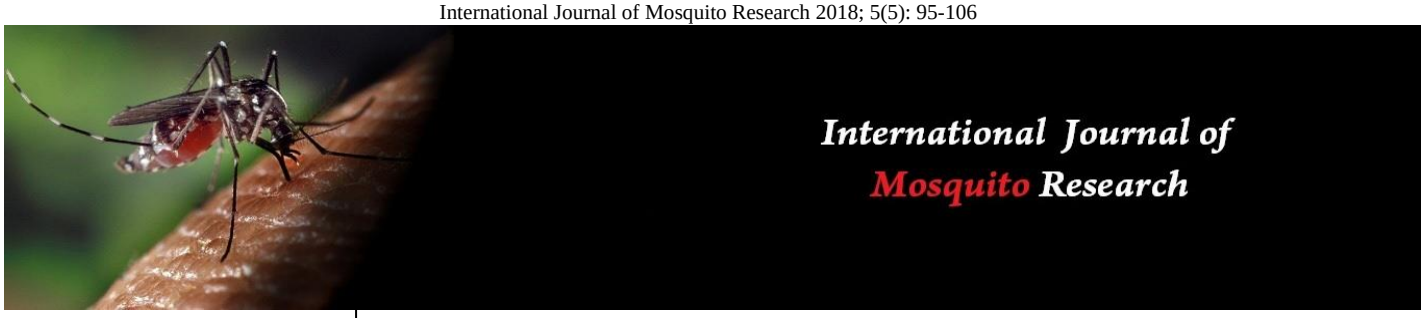

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Novel insecticides of *Syzygium cumini* fabricated silver nanoparticles against filariasis, malaria, and dengue vector mosquitoes

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Abstract
In the present study, the mosquito ovicidal, larvicidal, adulticidal activity of silver nanoparticles (AgNPs) synthesized using *Syzygium cumini* seed extract against three important adult female mosquitoes of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* was determined. The results were recorded from UV–visible spectroscopy; Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), energy-dispersive X-ray Vector control methods involving use of chemical insecticides are becoming less effective due to the development of insecticides resistance, biological magnification of toxic substances through the food chain, and adverse effects on environmental quality including human health. Today, nanotechnology is a promising research domain which has a wide ranging application in vector control programs. Energy-dispersive X-ray spectroscopy (EDX) and X-ray diffraction and dynamic light scattering analysis were carried out. AgNPs were highly effective against the larvae of *An. stephensi* (LC_{50} = 14.58 µg/ml), *Ae. aegypti* (LC_{50} = 16.45 µg/ml) and *Cx. quinquefasciatus* (LC_{50} = 18.83 µg/ml), respectively. Combined treatments testing *S. cumini* with AgNPs were also effective against *An. stephensi* (LC_{50} = 35.51 µg/ml), *Ae. aegypti* (LC_{50} = 47.94 gµ/ml) and C*x. quinquefasciatus* (LC_{50} = 61.79 µg/ml). Overall, this study suggests that the synthesized AgNPs can be a rapid, environmentally safer bio-pesticide to be used in synergy with *S. cumini* to control mosquito vectors.

Keywords: *Syzygium cumini*, Mosquitocidal activity, AgNPs, TEM, XRD, FTIR, SEM

1. Introduction

Mosquito-borne diseases, such as malaria, yellow fever, dengue, West Nile and Zika virus are of huge medical and veterinary importance [1-4]. Despite the recent positive results in limiting malaria diffusion (reduction in malaria mortality rates by more than 25% globally since 2000 and by 33% in the WHO African Region), this plague still has predominant importance in number of infections (2013: about 198 million cases of malaria), deaths (an estimated 584,000 deaths) and public concern [5, 6]. *Aedes aegypti* is the primary carrier for viruses that cause dengue fever, dengue haemorrhagic fever, chikungunya fever, and yellow fever, and is widespread over large areas of the tropics and subtropics [7]. *Culex quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestation [8]. In India alone, 25 million people harbour microfilaria and 19 million people suffer from filarial disease manifestations [9]. More than 1.3 billion people in 81 countries worldwide are threatened by lymphatic filariasis (WHO, 2010) [10].

Nanobiotechnology has the potential to revolutionize a wide array of applications, including drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, tissue engineering, pest management, and parasitology [11]. The plant-mediated biosynthesis (i.e., green synthesis) of metal nanoparticles is advantageous over chemical and physical methods, since it is cheap, single-step, and does not require high pressure, energy, temperature, the use of highly toxic chemicals. In latest years, biological routes for fabrication of nanoparticles have been suggested as possible eco-friendly alternatives to classic chemical and physical methods (Mohanpuria *et al*., 2005; Rajan *et al*., 2015) [12, 13].

Syzygium cumini belongs to the family Myrtaceae and commonly called as King of medicine and the active ingredient have been listed with hypoglycaemic properties throughout the world. The seed extract of the reported pharmacological activities of the plant are antibacterial, antifungal, antiviral, antidiabetic, antioxidant [14]. In the same way, [15] reported the antidiabetic potential of jamun seeds botanically named as *S. cumini*, acknowledged to be very high quality for its curative function chiefly against diabetes because of its effect on pancreas [16]. Jamun holds antihypercholesterolemic properties and helps in regulating the blood lipid profile due to presence of bioactive components [17]. Studies accomplished in last twenty years have explored that seed have got good complex of naturally present antioxidant compounds [18]. These bioactive compounds are helpful in preventing different metabolic syndromes. In this study, we biofabricated silver nanoparticles (AgNPs) using the seed extract of *S. cumini* as reducing agent. We tested the nanoparticles against the eggs, larvae and adults of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The green synthesis of AgNPs was confirmed analyzing the excitation of surface Plasmon resonance using ultraviolet–visible (UV–Vis) spectrophotometry. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed the irregular shapes of AgNPs. The presence of silver was determined by energy dispersive X-ray (EDX) spectroscopy. Fourier transforms infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and dynamic light scattering (DLS) analyses were also carried out.

2. Materials and methods

2.1 Collection of plant material

The fully developed fresh fruit seeds of *S. cumini* were collected from in and around Chidambaram, Annamalai Nagar and Annamalai University Campus, Cuddalore, Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany and Voucher specimens were deposited in Vector Biology and Entomology laboratory and are available upon request.

2.2 Mosquitoes

Experiments were conducted using laboratory-reared strains of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Colonies were originally established as described by Dinesh *et al.* [19] respectively. Mosquito larvae were maintained at 27 + 2 ˚C, 75–85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% (w:v) sugar solution was provided for a period of three days before blood feeding Suresh *et al.* [20].

2.3 Preparation of plant extracts

The seed *S. cumini* were dried in shade and ground to fine powder in an electric grinder. Aqueous seed extract was prepared by mixing 50 g of dried seed powder with 500 ml of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer. The suspension of dried seed powder in water was left for 3 h, filtered through Whatman no.1 filter paper, and the filtrate was stored in amber-colored air-tight bottle at 10 °C temperature until for use.

2.4 Synthesis of Silver nanoparticles

The broth solution of fresh *S. cumini* seed was prepared by taking 10 g of thoroughly washed and finely cut seed in a 300-ml Erlenmeyer flask along with 100 ml of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The seed extracts was filter with Whatman no.1 filtered paper and stored at -15°C; it could be used within 1 week. The filtrate was treated with aqueous 1mM $AgNO_3$ (21.2 mg of $AgNO_3$ powder in 125 ml water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight milliliters of an aqueous solution of 1 mM silver nitrate was reduced using 12 ml of seed extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of AgNPs.

Synthesis of silver nanoparticles solution with *S. cumini* seed extract may be easily observed by UV–Vis spectroscopy. The bio-reduction of the Ag+ ions in solutions was monitored by periodic sample of aliquots (1ml) of the aqueous component after 20 times dilution and measuring the UV–Vis spectra of the solution. UV–Vis spectra of these aliquots were monitored as a function of time the reaction on a Shimadzu 1601 spectrophotometer in the 300–900-nm wavelength range operated at a resolution of 1nm. The reaction mixture was subjected to centrifugation at 60,000 g for 30 min; the resulting pellet was dissolve in deionized water and filtered through Millipore filtrate (0.45 µm). After freezedrying of the purified AgNPs, the structure, size and composition were analyzed by 30-kV ultra-high resolution SEM (FEI QUANTA-200 SEM), An aliquot of this filter containing silver nanoparticles was used for Fourier transform infrared (FTIR) and transmission electron microscopy. The AgNPs was analyzed by using transmission electron microscope (JEOL, model 100CX II with an accelerating voltage of 100 kV).Silver nanoparticles for TEM analysis was prepared by coating of aqueous solution of AgNPs drops on carbon-coated copper grids and left to dry for 5 min; the extra solution was removed using blotting paper at room temperature. FTIR spectra were recorded in the 4000-400 cm^{-1} with a Shimadzu IR-470 Spectrometer, equipped with data station. Dried samples of about 100 mg were mixed with 100 mg of spectral grade KBr and pressed into discs under hydraulic pressure. X-ray diffraction analysis was detected examine the crystallographic structure of the purified AgNPs. The XRD grids were coated with dried biosynthesized nanoparticles and the synthesized nanoparticles diffraction pattern was measured by X-ray diffract meter. (Shimadzu XD-3A). Energy dispersive X–ray spectroscopy (EDX) analysis for the confirmation of elemental silver was carried out for the detection of elemental silver. Particle size and zeta potential of AgNPs was analyzed on particle size analyzer system (Zeta sizer, Malvern Instruments Ltd.). The average distribution of nanoparticles on the basis of intensity, volume and number weighting was studied comparatively.

2.5 Larvicidal bioassay

The larvicidal activity of the *S. cumini* seed extracts was evaluated as per the method recommended by WHO [21]. Batches of 25 third instars larvae were transferred to small disposable paper cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration of the seed extract (50, 100, 150, 200,250 µg/ml) or (10, 15, 20, 25 and 30) green synthesized

(AgNPs). Five replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of ethanol was added. The LC_{50} (lethal concentration that kills 50% of the exposed larvae) and LC_{90} (lethal concentration that kills 90% of the exposed larvae) values were calculated after 24 h by probit analysis [22].

Percentage mortality = (number of dead individuals) x 100

2.6 Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla [23] was performed. *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* eggs were collected from vector control laboratory, Department of Zoology, Annamalai University. The seed extracts were diluted in the ethanol to achieve various concentrations ranging from 50 to 300 µg/ml. Eggs of these mosquito species (100) were exposed to each concentration of seed extracts. After 24 h treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following the formula:

Egg hatchabilty (%) = (Number of hatched larvae = Number of total eggs), x 100

2.7 Adulticidal activity

Adulticidal bioassay was performed by WHO method [24]. Based on the wide range and narrow range tests, aqueous seed extract was tested at 80, 160, 240, 320 and 400 µg/ml concentrations, and AgNPs were tested at 12, 24, 36, 48 and 60 µg/ml concentrations. Aqueous seed extract and silver nanoparticles were applied on Whatman no.1 filter papers (size 12×15 cm). Control papers were treated with silver nitrate and distilled water. Twenty-five female mosquitoes were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1h and then exposed to test paper for 1h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept for 24-h recovery period. A pad of cotton soaked with 10% glucose solution was placed on the mesh screen. Each test included a set control groups ($AgNO_3$ and distilled water) with five replicates for each individual concentration.

2.8 Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the Statistical Package of Social Sciences 12.0 software. Results with $p<0.05$ were considered to be statistically significant.

3. Results

3.1 Larvicidal, Ovicidal and Adulticidal Potential

S. cumini seed extract was highly effective against third instars of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* (Table 1). The *S. cumini* achieved good LC_{50} and LC_{90} values on *An. stephensi* (LC_{50} = 121.80 and LC_{90} = 283.47 µg/ml) followed by *Ae. aegypti* (LC_{50} = 139.02 and LC_{90} = 313.13 µg/ml) and *Cx. quinquefasciatus* (LC_{50} = 152.22 and LC_{90} = 324.03 µg/ml). In our work, synthesized AgNPs were highly effective against mosquitoes; larvicidal activity was highlighted by LC_{50} and LC_{90} values on *An. stephensi* (LC_{50} = 21.86 and LC_{90} = 41.72 µg/ml) followed by *Ae. aegypti* (LC_{50} = 19.86 and LC_{90} = 35.56 µg/ml) and *Cx. quinquefasciatus* (LC_{50} = 20.93 and LC_{90} = 36.43 µg/ml) (Table 2). *S. cumini* seed extract and synthesized AgNPs was tested against eggs of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. The *S. cumini* seed extract treatment achieved 100% mortality after exposure to 350 µg/ml (Table 3). Synthesized AgNPs had 100% mortality post-treatment at 125, 150 and 175 µg/ml respectively, against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The synthesized AgNPs were highly effective when compared with *S. cumini* seed extract against eggs of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Control eggs showed 100% hatchability (Table 4). The synthesized AgNPs were highly effective when compared with *S. cumini* seed extract against eggs of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Control eggs showed 100% hatchability (Table 5 and 6). *S. cumini* against tested against mosquito adults showed good efficacy on *An. stephensi* (LC_{50} 162.77 µg/ml and LC_{90} 464.21 µg/ml) followed by *Ae. aegypti* (LC_{50} 203.25 µg/ml and LC_{90} 519.49 µg/ml) and *Cx. quinquefasciatus* (LC_{50} 250.29 and LC_{90} 584.46 µg/ml), respectively (Table 7). *An. stephensi* had LC_{50} and LC_{90} values of 19.86 and 50.51µg/ml; *Ae. aegypti* had LC_{50} and LC_{90} values of 24.28 and 63.56 µg/ml; and *Cx. quinquefasciatus* had LC_{50} and LC_{90} values of 29.08 and 74.75 µg/ml, respectively (Table 8). Moreover, combined treatments of *S. cumini*-synthesized AgNPs adulticidal showed higher mortality if when compared to the individual experiment with *S. cumini* alone, indeed highest toxicity was found for *An. stephensi* (LC_{50} = 67.33 and LC_{90} = 196.40 µg/ml) followed by *Ae. aegypti* (LC_{50} = 66.82 and LC_{90} = 195.94 µg/ml) and *Cx. quinquefasciatus* (LC_{50} = 79.62 and LC_{90} = 236.06 µg/ml), respectively (Table 9).

3.2 Characterization of AgNPs

The colour change was noted by visual observation of the *S. cumini* seed extracts which were incubated with which no significant change occurred Fig. 1A. The absorption spectrum of *S. cumini* seed extract at different wavelengths ranging from 300 to 800 nm revealed a peak at 420 nm Fig. 1B. TEM observations showed different shapes of *S. cumini* seed biosynthesized AgNPs, including spherical, round, triangular, and hexagonal under the magnification of average size of 50 nm Fig. 2. The shape of metal nanoparticles considerably changes their optical and electronic properties. EDX spectrum recorded from *S. cumini* synthesised AgNPs showed a strong Ag signal, confirming the formation of AgNPs Fig. 3. The EDX spectrum recorded from *S. cumini*-synthesized AgNPs revealed a distinct signal and high atomic percent values for silver, thus confirming formation of AgNPs. Our samples showed an optical absorption peak at 3 keV due to the surface plasmon resonance, which is typical of silver nanostructures. The Cu, C signal was probably due to the X-ray emission from different minerals present in the *S. cumini* seed extract. XRD patterns indicated that the AgNPs formed by the reduction of $AgNO_3$ using *S. cumini* seed extract were crystalline in nature they showed intense peaks at 2h = 28.60°, 40.80°, 50.30°, 58.40°, and 66.50°, corresponding to the (111), (200), (220), (311) and (222) sets of lattice planes Fig. 4. FT-IR analysis of synthesized AgNPs was carried out to identify the potential functional groups, responsible for its

reduction, capping and stabilization Fig. 5. The FT-IR spectrum of the silver nanoparticles ($AgNP_3$) showed broad and strong absorbance peaks at 3451.76, 2074.53, 1637.64 and 417.61cm-^{1}, The observed peaks denote C-N stretch (aromatic amines), C-C stretch (aromatics), N-H bend (1°amines), C-O stretch (carboxylic group), and O-H stretch, H- bonded (alcohols, phenol). Zeta potential is the electric potential in the interfacial double layer or the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A value of 17.9 mV (positive) was obtained as the arbitrary value that separates low-charged surfaces from highly-charged surfaces Fig. 6. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions.

4. Discussion

Our results showed that synthesized AgNPs of *S. cumini* have significant larvicidal activity against *An.stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. This result is also comparable to earlier reports of Veerakumar *et al*. [25] reported that synthesized AgNPs from *Feronia elephantum* against the larvae of *An. stephensi* (LC_{50}=26.712 µg/ml; LC_{90}= 49.061 µg/ml), *Ae. aegypti* (LC_{50}=29.626 mg/l; LC_{90}= 54.269 mg/l), and *Cx. quinquefasciatus* (LC_{50}=32.077 mg/l ; LC_{90}=58.426 mg/l), respectively. The ethanol extract of flower of *Rhodomyrtus tomentosa* have significant larvicidal as well as ovicidal properties against two important vector mosquitoes *Ae. Aegypti* (LC_{50}=37.93 mg/l; LC_{90}= 80.83 mg/l), and *An. Stephensi* (LC_{50}=34.42 mg/l; LC_{90}= 64.69 mg/l), (Kanthammal *et al.*, 2018).The crude benzene, hexane, ethanol and methanol extracts of seed of *Syzygium cumini* have significant larvicidal as well as ovicidal properties against two important vector mosquitoes *An. Stephensi* than *Ae. Aegypti*. The LC_{50} values were 113.86, 125.25, 93.74, 76.89 ppm and 129.42, 124.84, 100.79, 83.02 ppm, respectively, [26]. Velayutham *et al.* [27] studied the larvicidal activity of AgNPs fabricated with the aqueous bark extract of *Ficus racemosa*, testing them against fourth instar of the filariasis vector, *Cx. quinquefasciatus* and the Japanese encephalitis vector, *Cx. gelidus*. (Rawani *et al*., 2013) [28] also reported the larvicidal activities of AgNPs synthesized using extracts of fresh leaves, dry leaves and green berries of *Solanum nigrum*, against larvae of *Cx. quinquefasciatus* and *An. stephensi*. . Haldar *et al*. [29] also showed the mosquito larvicidal potential of AgNPs synthesized by dried green fruits of *Drypetes roxburghii* against two mosquitoes, namely *Cx. quinquefasciatus* and *An. stephensi*. Ovicidal activity of AgNPs fabricated using the *Carissa carandas* leaf extract against the malaria vector, *An. stephensi*, the dengue vector, *Ae. aegypti* and the filariasis vector, *Cx. quinquefasciatus* [30]. Recently, Suresh *et al*. [31] have reported that egg hatchability of *Ae. aegypti* was reduced by 100% after treatment with 25 and 30 ppm of AgNPs; The *Lysinibacillus sphaericus* extract exerted 100% mortality post-treatment with 250 ppm, while control eggs showed the 100% hatchability. Further, Munusamy *et al*. [32] showed that the methanol extract of *Rubia cordifolia* root had good ovicidal activity (82.40 and 70.40%) against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, at 500 mg/l, when compared to other plants, such as *Gymnema sylvestre*, *Scilla peruviana*, *S. cordifolia* and *Elytraria acaulis* [33, 34]. The adult mortality exerted by the ethanol extract of *Citrus sinensis* showed LC_{50} and LC_{90} on *An. stephensi* of 289.62 and 494.88 ppm, and on *Ae. aegypti* of 320.38 and 524.57 ppm, respectively [35]. The adult mortality of methanol extract of *Andrographis paniculata* on the adults of *Cx. quinquefasciatus* and *Ae. aegypti* led to LC_{50} and LC_{90} of 149.81, 172.37 ppm and 288.12, 321.01 ppm, respectively [36]. The LC_{50} and LC_{90} values of *Amelanchier alnifolia* leaf extracts (hexane, benzene, ethyl acetate, acetone and methanol) on *Cx. quinquefasciatus* adults were rather high, 383.59, 354.13, 327.74, 314.33 and 291.71 ppm, respectively [37]. Similarly, the LC_{50} and LC_{90} values of *Cassia tora* leaf extracts (hexane, chloroform benzene, acetone, and methanol) against *Cx. quinquefasciatus* adults led to LC_{50} values of 338.81, 315.73, 296.13, 279.23, and 261.03 ppm and LC_{90} values were 575.77, 539.31, 513.99, 497.06, and 476.03 ppm, respectively [38].

4.1 Green Synthesized and Characterization of Silver Nanoparticles

The absorption spectra of AgNPs at different time intervals showed highly symmetric absorption bands. A maximum absorption peak was observed at 340 nm [39]. The UV–Vis spectrum showed maximum absorbance at 450 nm, in visible light regions which increased with time of incubation of silver nitrate with the plants extract [40]. The synthesis of AgNPs was confirmed through visual assessment. Similarly, the *S. muticum* aqueous extract (5% w/v) changed from yellowish light brown to dark brown after the addition of 1 mM $AgNO_3$. [41], recently reported that lemongrass and *Aristolochia indica* leaf extracts can be used for effective synthesis of Au and AgNPs with size lower than 30 nm. Also, AgNPs produced using *Emblica officinalis* were predominantly spherical with an average size of 16.8 9nm, ranging from 7.5 to 25 nm [42]. This is important for biological applications. Indeed, it is known that the size and shape of metal nanoparticles considerably change their optical and electronic properties [43]. The EDX spectrum recorded from *Z. gracilis* synthesized AgNPs revealed a distinct signal and high atomic percent values for Ag. EDX spectroscopy confirmed the presence of Ag in the analyzed samples, showing a sharp optical absorption band peak at 5 keV, typical for the absorption of metallic silver nanocrystallites [44, 45]. For example, a recent TEM study shows fine configuration of crystalline, spherical AgNPs, with size slightly higher of that recorded in SEM assays, since it ranged between 27 and 49 nm [46]. Also, AgNPs bio-fabricated using *Annona squamosa* leaf extract were spherical in shape with an average size ranging from 20 to 100 nm [47] while Thirunavokkarasu *et al*. [48] reported the production of spherical metal nanoparticles with size ranging from 8 to 90 nm in *Desmodium gangeticum*-based synthetic routes. XRD analysis was helpful to shed light on the crystalline nature of the AgNPs. The sharp Bragg's peaks may be due to the presence of capping agent stabilizing the AgNPs [49]. The diffraction peaks were detected in the 2q angles in a range of 35˚C 80˚C which can be indexed to the (111), (200), (220), and (311). Noghabi *et al*., 2017; Dubey *et al*. [50, 51] showed that the size of Ag nanocrystals, as estimated from the full width at half maximum of (111) peak of silver using the Scherrer's formula, was 20–60 nm. FTIR spectrum of aqueous AgNPs prepared from the *Z. gracilis* leaf extract have major transmittance peaks at 3351.87, 2111.40, 1638.77, 1240.98, 658.15, 597.15 and 555.99 cm-^{1}. They indicate that these different functional groups from biomolecules have probably capped AgNPs to prevent agglomeration, thus stabilizing the medium [52, 53]. In detail, the peaks at 1620–

1636 cm-1 may represent carbonyl groups from polyphenols such as catechin gallate, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin gallate and theaflavin; the results suggest that molecules attached with AgNPs have free and bound amide groups. These amide groups may also be in the aromatic rings. This concludes that the compounds attached with the AgNPs could be polyphenols with an aromatic ring and bound amide region [54]. Mahitha *et al.* [55] studied the peak at 1381 cm^{-1} that is probably due to the C–N stretching of the aromatic amine group. The peaks at 1027–1092 cm^{-1} could correspond to the C–N stretching vibration of aliphatic amines or to alcohols/phenols representing the presence of polyphenols [56]. In particular, the broad intense band close to 3400 cm^{-1} may be assigned to the N–H stretching arising from peptide linkages present in the plant extract proteins [57], while the peak located close to 1640 cm^{-1} could be assigned to the C=O stretching in carboxyl groups or C=N bending in amide groups [58].

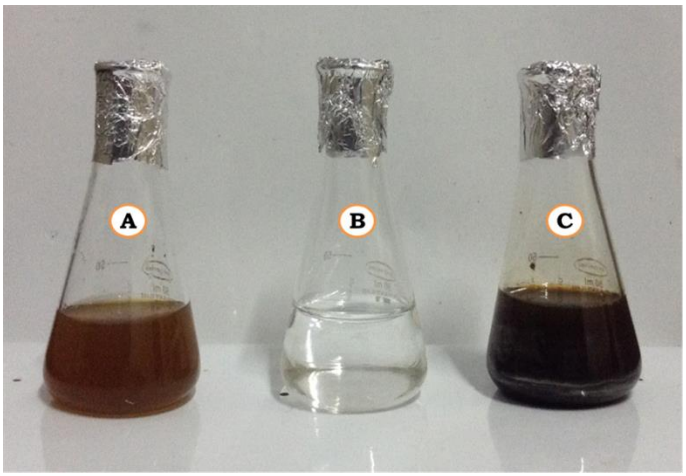


Fig 1A

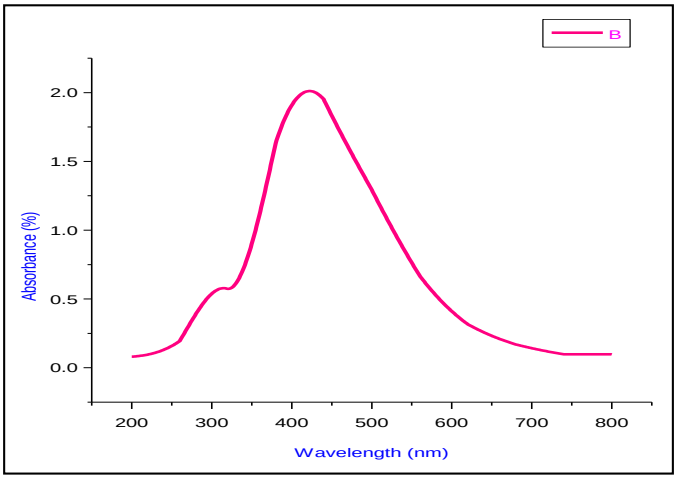


Fig 1B

Fig 1 a, b, c: Chromatic variations of the seed extract of *Syzygium cumini* (*Eugenia jambolana*) before and after the process of reduction of Ag+ to Ag nanoparticles (1**B**) UV-visualization of the absorption spectra of silver nanoparticles synthesized using *S cumini* seeds

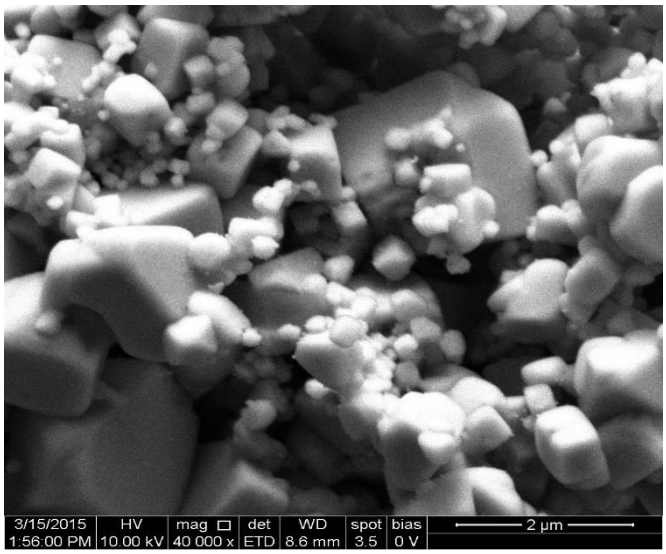


Fig 2: Scanning electron microscopy (SEM) of green-synthesized silver nanoparticles obtained by reduction of $AgNO_3$ with the seed extract of *Syzygium cumini*

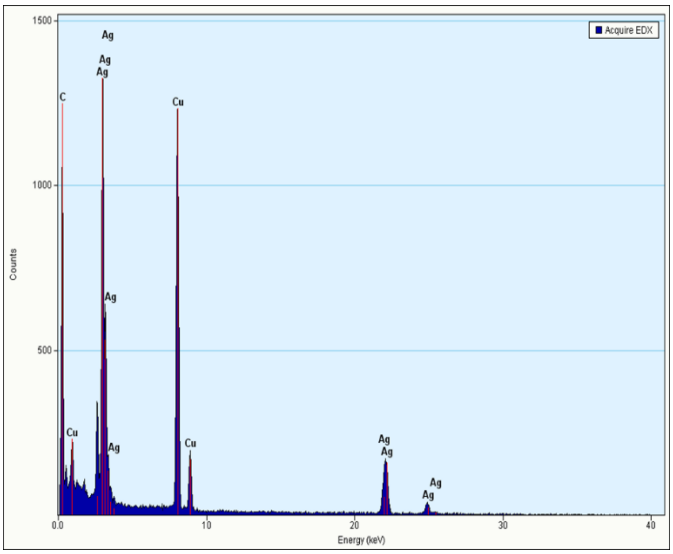


Fig 3: Energy dispersive X-ray (EDX) profile of silver nanoparticles synthesized using the seed extract of *Syzygium cumini*

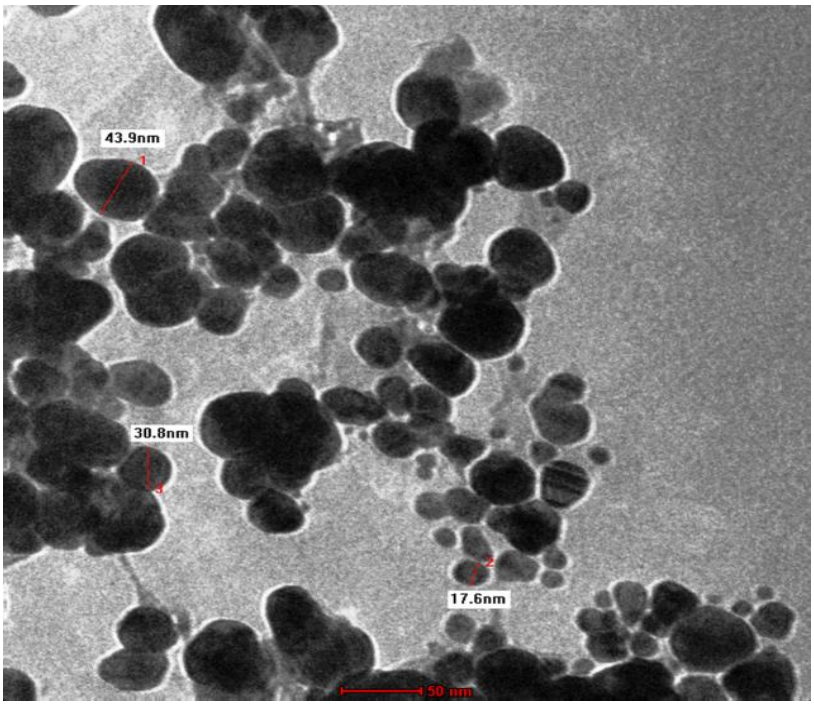


Fig 4: Transmission electron micrograph (TEM) of green-synthesized silver nanoparticles obtained by reduction of $AgNO_3$ with the seed extract of *Syzygium cumi*

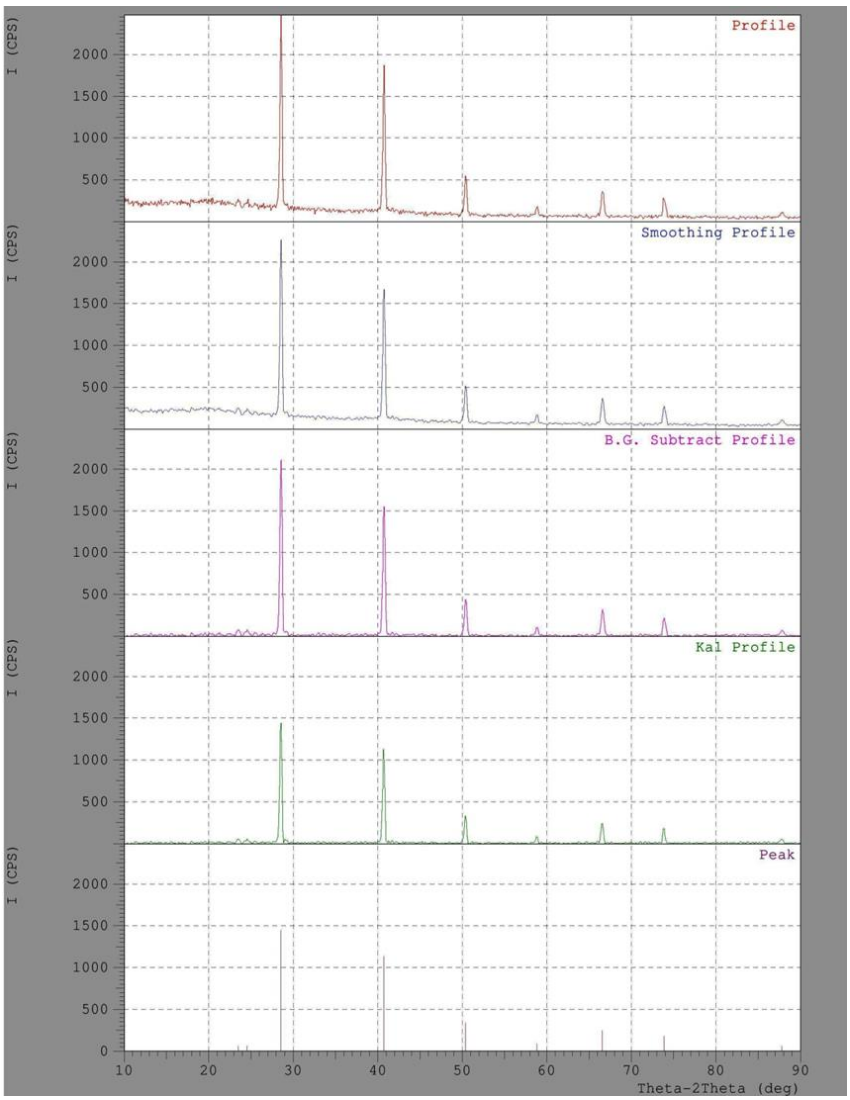
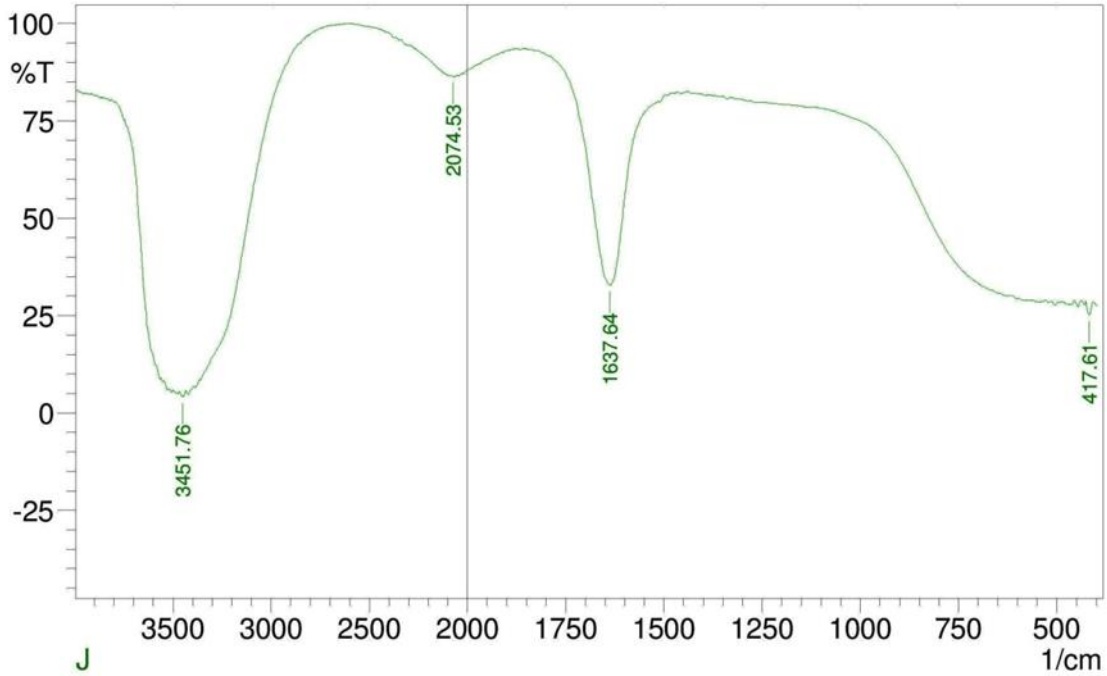


Fig 5: X-ray diffraction (XRD) pattern of silver nanoparticles biosynthesized using the seed extract of *Syzygium cumini*



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	417.61	25.2	0.36	426.29	416.64	5.57	0.07
2	1637.64	32.85	49.24	1821.84	1550.83	52.1	32.41
3	3451.76	86.44	0.04	2082.25	2072.6	0.61	0
4	3451.76	4.16	0.4	3468.16	3446.94	28.15	0.28

Fig 6: Fourier transform infrared (FTIR) spectra of vacuum-dried powder of silver nanoparticles synthesized using the seed extract of *Syzygium cumini*

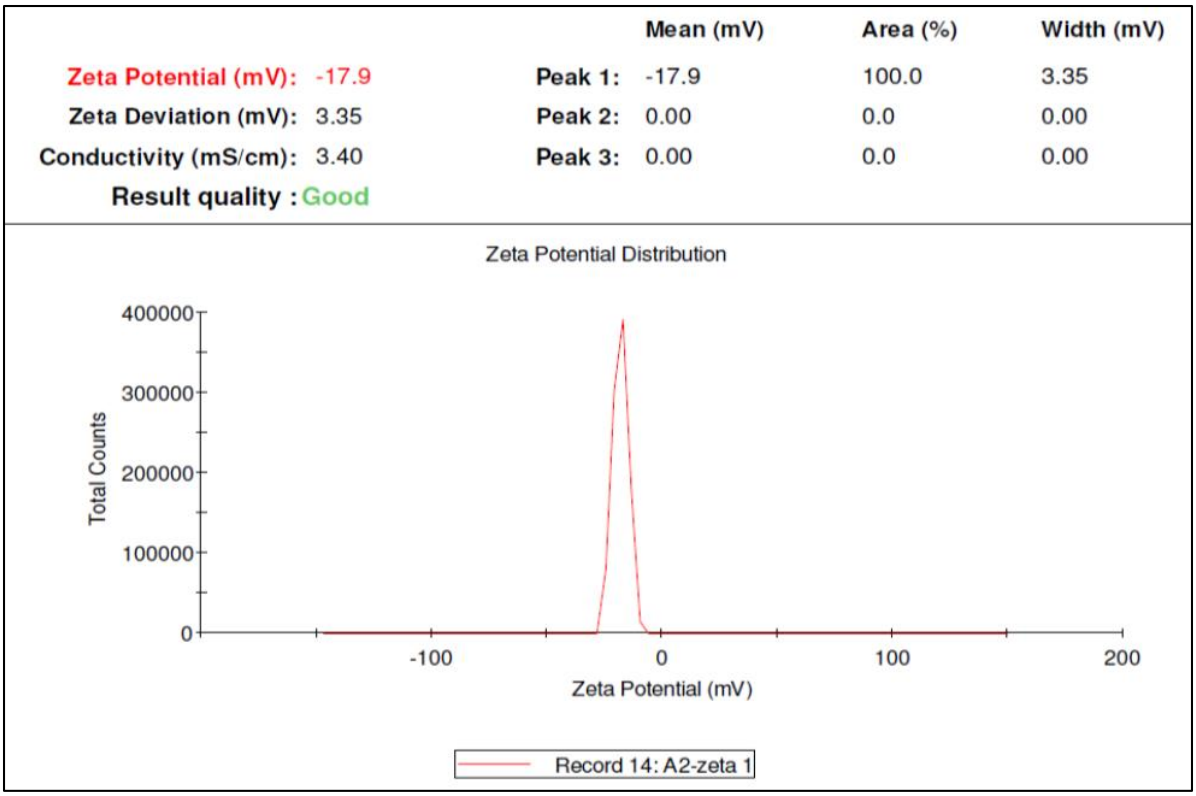


Fig 7: Zeta potential and particle **s**ize analysis of silver nanoparticles synthesized using the seed extract of *Syzygium cumini*

Table 1: Larvicidal activity of *S. cumini* aqueous seed extract against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquitoes	Concentration (µg/ml)	24 h mortality (%)	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
An. stephensi	Control	0.0±0.0	127.73(109.50-143.83)	306.33(272.45-359-06)	2.58
	50	30.61			
	100	41.39			
	150	55.72			
	200	71.36			
	250	81.48			
Ae. aegypti	Control	0.0±0.0	141.84(124.40-158.47)	325.58(288.19-384.67)	1.38
	50	27.15			
	100	38.62			
	150	51.58			
	200	66.35			
	250	78.14			
Cx. quinquefasciatus	Control	0.0±0.0	171.85(152.16-194.87)	394.17(337.93-492.96)	6.34
	50	23.64			
	100	34.71			
	150	46.53			
	200	59.16			
	250	65.87			

LCL lower confidence limits, UCL upper confidence limits, χ^2 chi-square test
*P<0.05, level of significance
Values are mean of five replicates

Table 2: Larvicidal activity synthesized silver nanoparticles of *S. cumini* against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquitoes	Concentration (µg/ml)	24 h mortality (%)	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
An. stephensi	Control	0.0±0.0	14.58(12.57-16.65)	30.39(27.88-34.10)	1.41
	10	39.14			
	15	48.32			
	20	65.72			
	25	80.53			
	30	91.42			
Ae. aegypti	Control	0.0±0.0	16.45(14.42-18.12)	34.62(31.15-39.80)	5.89
	10	32.71			
	15	45.27			
	20	63.51			
	25	71.18			
	30	83.62			
Cx. quinquefasciatus	Control	0.0±0.0	18.83(16.81-20.70)	39.70(35.34-41.02)	0.61
	10	30.26			
	15	39.43			
	20	55.91			
	25	63.92			
	30	76.18			

LCL lower confidence limits, UCL upper confidence limits, χ^2 chi-square test
*P<0.05, level of significance
Values are mean of five replicates

Table 3: Combined treatment of *S. cumini* green-synthesized silver nanoparticles against larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*

Mosquitoes	Seed Extract and AgNPs Concentration (µg/ml)	24 h mortality (%)	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
An. stephensi	Control	0.0±0.0	35.51(25.14-43.31)	94.70(86.63-105-58)	2..54
	25 + 5	48.72			
	50 + 7.5	66.61			
	75 + 10	85.12			
	100 +10.5	100.00			
	125+15	100.00			
Ae. aegypti	Control	0.0±0.0	47.94(37.50-56.11)	122.42(111.77-137.29)	3.34
	25+5	43.67			
	50+7.5	50.41			
	75+10	73.92			
	100+10.5	89.65			
	125+15	95.48			

Cx. quinquefasciatus	Control	0.0±0.0	61.79(48.82-71.90)	170.20(150.11-202.79)	5.38
	25+5	37.47			
	50+7.5	47.32			
	75+10	60.18			
	100+10.5	72.72			
	125+15	84.53			

LCL lower confidence limits, UCL upper confidence limits, χ^2 chi-square test
*P<0.05, level of significance
Values are mean of five replicates

Table 4: Ovicidal activity of *S. cumini* aqueous seed extract against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Treatment	Mosquito species	Egg hatchability (%)						
		Control	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	300 µg/ml	350 µg/ml
S. cumini seed extract	*An. stephensi*	100	54.6	49.7	39.5	33.4	NH	NH
	Ae. aegypti	100	61.5	57.5	43.2	36.5	23.0	NH
	Cx. quinquefasiatus	100	72.8	65.3	50.1	40.8	32.4	NH

NH no hatchability (100% mortality)

Table 5: Ovicidal activity synthesized silver nanoparticles of *S. cumini* against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Treatment	Mosquito species	Egg hatchability (%)						
		Control	50 µg/ml	75 µg/ml	100 µg/ml	125 µg/ml	150 µg/ml	175 µg/ml
Silver nanoparticles	*An. stephensi*	100	65.8	52.7	41.5	NH	NH	NH
	Ae. aegypti	100	72.5	61.1	49.3	33.2	NH	NH
	Cx. quinquefasiatus	100	81.5	73.6	55.4	42.7	35.8	NH

NH no hatchability (100% mortality)

Table 6: Combined treatment of *S. cumini* green-synthesized silver nanoparticles against ovicidal of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*

Treatment	Mosquito species	Egg hatchability (%)						
		Control	50+25 µg/ml	75+37.5µg/ml	100 +50µg/ml	125+67.5µg /ml	150+75µg/ ml	175+87.5µg/ ml
S. cumini	*An. stephensi*	100	78.6	69.7	NH	NH	NH	NH
seed extract	*Ae. aegypti*	100	85.5	77.5	63.9	NH	NH	NH
and AgNPs	*Cx. quinquefasiatus*	100	97.8	89.4	75.2	NH	NH	NH

NH no hatchability (100% mortality)

Table 7: Adulticidal activity of *S. cumini* aqueous seed extract against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquitoes	Concentration (µg/ml)	24 h mortality (%)	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
An. stephensi	Control	0.0±0.0	162.77 (125.20-191.64)	464.21 (410.95-548.97)	1.38
	80	35.71			
	160	48.36			
	240	67.51			
	320	76.25			
	400	82.68			
Ae. aegypti	Control	0.0±0.0	203.25(170.47-231.51)	519.49 (456.13-622.97)	1.12
	80	28.30			
	160	45.13			
	240	59.85			
	320	68.04			
	400	77.61			
Cx. quinquefasciatus	Control	0.0±0.0	250.29 (220.08-281.80)	584.46 (507.24-715.02)	9.09
	80	23.42			
	160	39.35			
	240	50.64			
	320	66.10			
	400	70.52			

LCL lower confidence limits, UCL upper confidence limits, χ^2 chi-square test
*P<0.05, level of significance
Values are mean of five replicates

Table 8: Adulticidal activity of synthesized silver nanoparticles of *S. cumini* against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Mosquitoes	Concentration (µg/ml)	24 h mortality (%)	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
An. stephensi	Control	0.0±0.0	19.86 (15.59-23.24)	50.51 (46.16-56.52)	1.09
	12	40.23			
	24	54.87			
	36	73.15			
	48	89.72			
	60	96.3			
Ae. aegypti	Control	0.0±0.0	24.28 (19.41.-28.08)	63.56 (57.13-73.20)	1.35
	12	34.25			
	24	50.43			
	36	66.12			
	48	77.81			
	60	88.72			
Cx. quinquefasciatus	Control	0.0±0.0	29.08 (24.86-33.14)	74.75 (65.98-88.82)	3.32
	12	30.52			
	24	46.43			
	36	59.28			
	48	70.32			
	60	80.47			

LCL lower confidence limits, UCL upper confidence limits, χ^2 chi-square test
*P<0.05, level of significance
Values are mean of five replicates

Table 9: Combined treatment of *S. cumini*-synthesized silver nanoparticles against adults of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*

Mosquitoes	Seed Extract and AgNPs Concentration (µg/ml)	24 h mortality (%)	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2
An. stephensi	Control	0.0±0.0	67.33 (45.57-93.72)	196.40 (178.15-227.35)	3.48
	40+6	47.52			
	80+12	65.43			
	120+18	80.12			
	160+24	94.84			
	200+30	100.0			
Ae. aegypti	Control	0.0±0.0	66.82 (46.87-81.77)	195.94 (177.79-221.71)	6.54
	40+ 6	43.16			
	80+12	60.73			
	120+18	74.06			
	160+24	87.42			
	200+30	96.37			
Cx. quinquefasciatus	Control	0.0±0.0	79.62 (57.92-95.91)	236.06 (211.28-273.79)	2.48
	40+6	39.18			
	80+12	55.72			
	120+18	68.12			
	160+24	79.86			
	200+30	90.52			

LCL lower confidence limits, UCL upper confidence limits, χ^2 chi-square test
*P<0.05, level of significance
Values are mean of five replicates

5. Conclusions

The synthesized AgNPs fabricated in this research are hydrophilic in nature, disperse uniformly in water, and highly stable. They also exhibit significant toxic activity against young instars of three important mosquito vectors. The effectiveness of green-fabricated AgNPs against the mosquito vectors was confirmed in ovicidal, larvicidal and adulticidal tests. Overall, this study suggests that the synthesized AgNPs can be a rapid. These seed extracts of *S. cumini* have the potential to be used as an ideal eco-friendly approach for the vector control programs.

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