Research Article

Abdullah F. Shater, Fayez M. Saleh, Zuhair M. Mohammedsaleh, Hattan Gattan, Bassam M. Al-Ahmadi, Nizar H. Saeedi, Mohammed M. Jalal, and Chellasamy Panneerselvam*

Green nanoarchitectonics of the silver nanocrystal potential for treating malaria and their cytotoxic effects on the kidney Vero cell line

https://doi.org/10.1515/gps-2022-8111 received September 30, 2022; accepted March 07, 2023

Abstract: This study focused on testing manufactured silver nanoparticles (AgNPs) against the malaria pathogen Plasmodium falciparum and the malaria vector Anopheles stephensi using the plant filtrate from Madhuca longifolia. The M. longifolia leaf extracts were used to synthesize the AgNPs, which were then subjected to several physicochemical methods to determine their characteristics. To evaluate the effectiveness of the green produced AgNP therapy, the mosquitocidal activity of A. stephensi, cytotoxicity assay in Vero cells, and antiplasmodial activity assay were performed. The larval and pupal toxicity of biosynthesized AgNPs against the malarial vector A. stephensi is 90% promising in laboratory settings at low dosages (10 ppm). When tested on African green monkey kidney cells, the cytotoxic effect of biosynthesized materials was found to be inappropriately damaging up to 100 g⋅mL⁻¹. The antimalarial efficacy of AgNPs was evaluated against P. falciparum strains. The parasites that were restrained by AgNPs at 100 ppm had the highest parasitemia restraint rate (80.4%). AgNPs then showed significant in vitro antimalarial activity against P. falciparum. Our findings suggested that the biosynthesized AgNPs might function as a novel antimalarial agent that is both safer for the environment and a barrier to infections spread by mosquitoes.

Keywords: *Anopheles stephensi*, antimalarial activity, IC₅₀, *Madhuca longifolia*, silver nanoparticles

1 Introduction

Mosquitoes (Diptera: Culicidae) are the fundamental and first parasitic vectors and they can transmit parasites, pathogens, and a larger number of infections than a few other species of arthropods, which have a devastating effect on many individuals throughout the world. Every year, about 1 billion people are affected, and nearly 1 million people die due to vector-borne insects. Lymphatic filariasis has an impact of over 120 million individuals in 73 nations, including southeast Asia, India, Pacific Islands, and Africa. These diseases demonstrate not only to have an impact over mortality and horribleness, but also have unlimited monetary misfortunes and social obstruction in many nations, including countries in middle east, India, and so forth. India has around 40% of worldwide filariasis presence and is evaluated to cause a yearly annual income loss of 720 crore [1]. The mosquito, Anopheles stephensi (A. stephensi), is the key vector that transmits intestinal sickness among various fields and urban zones of India and other West Asian nations [2,3]. Jungle fever is a mosquito-borne ailment caused by Plasmodium pathogens; besides, it affects directly or indirectly the newborns, youngsters, and causes death in adults. Around 3,000,000 individuals are in danger of intestinal sickness worldwide and 1,000 deaths are caused yearly [4]. Even though World Health Organization has assessed 15 million cases and 20,000 mortalities by the Southeast Asia Regional Office, of all jungle fever cases in Southeast Asia [5], over

Abdullah F. Shater, Zuhair M. Mohammedsaleh, Nizar H. Saeedi, Mohammed M. Jalal: Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk 71491, Saudi Arabia

Fayez M. Saleh: Department of Medical Microbiology, Faculty of Medicine, University of Tabuk, Tabuk 71491, Saudi Arabia Hattan Gattan: Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; Special Infectious Agents Unit, King Fahad Medical Research Center, Jeddah, Saudi Arabia

Bassam M. Al-Ahmadi: Department of Biology, Faculty of Science, Taibah University, Taibah, Saudi Arabia

^{*} Corresponding author: Chellasamy Panneerselvam, Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia, e-mail: cpselva@ymail.com

about 77% of cases were contributed by India. Human intestinal sickness cases are typically because of five Plasmodium sp., specifically P. falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi [6]. Their control is a significant objective for the improved overall well-being of humans. A few environmental issues, for example, ecological maintainability, unsafe impact on the well being of humans, and other non-target populaces, their non-biodegradable nature, higher pace of organic amplification through biological system, and advancement of obstruction in targeting vectors because of their tenacious gathering on the earth [7,8]. Under these circumstances, eco-accommodating approaches have recently emerged to build control management methods in contrast to mosquito vectors, with unique reference to organic mosquitocidals [9,10]. Therapeutic plants might be an excellent operator for regulation of mosquito menace, since they are rich in optional bioactive compounds, which are dynamic against the predetermined quantity of animal groups and found to be bio-degradable in nature; they are possibly appropriate for use in various control management methods of animals and insects [11]. Recently, scientists have been focusing on plant-related compounds, including their bioactive extracts, which play a pivotal role in targeting mosquitoes. They have mosquitocidal and ovicidal effects and are used as oviposition obstacles, in the development or potentially multiplication inhibitors, and as anti-agents [12-16].

The majority of researchers work to prevent and kill adult female mosquitoes while they are still larval. Paris green and petroleum oil are two substances that successfully kill adult female mosquitoes. While effective, synthetic pyrethroid and many organophosphates are dangerous to unintended aquatic organisms, primarily fish. Using insecticides for malaria sickness is seriously hampered by their harmful and long-lasting effects. The increase in mosquitoes that are resistant to pesticides is a significant obstacle.

In recent years, nanoscience technology research has been extensively found in various field areas including electronics, antibiotics, biomedicine, sensors, catalysts, optical fibers, etc. Nanoparticles have been utilized in many applications, for example, electrochemistry, photochemical, and biomedical. Plants, microorganisms, and organisms have been utilized for the development of nanoparticles. Plant-based green blends of nanoparticles are fast, require minimal effort, are eco-accommodating, and a solitary advance strategy for biosynthesis process [17]; they offer various advantages of financially amiable, ecologically harmonious, and are used for human applications [18]. Until now, metal nanoparticles are generally organized of noble metals like gold (Au), platinum (Pt), lead (Pb), and silver (Ag); among these metals silver (Ag) is one of the best metals for assessing the

organic framework [19]. AgNPs are more averse to cause environmental harm and have been distinguished as an expected substitution for manufacturing synthetic bug sprays. Besides, developing numerous plants and shrubs have been planned for productive and fast extracellular production of silver nanoparticles (AgNPs) [20], which show phenomenal mosquitocidal activities in a field environment [21,22].

Madhuca longifolia (normally known as Mahua) belongs to the family Sapotaceae, and is a medium estimated deciduous tree found in many countries, including Nepal, India, and Sri Lanka [23]. Blossoms are light vellow in color and beefy. Further, they are a good source of sugars, nutrients, thiamine, ascorbic acid, calcium, potassium, iron, magnesium, copper, riboflavin, betaines, anthocyanins, and salts of succinic and malic acids [24]. The bark of this plant comprises 17% tannins and utilized for the treatment of stiffness, ulcer, tingles, draining, and light gums [25]. This species is known for their cell reinforcement, antibacterial, antimicrobial, mitigating, anticancer, and antifungal properties [26,27]. The use of large-sized materials in the therapeutic process by herbal medicines has drawbacks, including in vivo instability, poor solubility, low bioavailability, poor absorption in the body, problems with target-specific delivery, and tonic efficacy. Hence, utilizing novel disease-treating systems based on nanotechnology and nanomedicine can be a successful solution to these problems. Effective agents are used in nanotechnology for tissue engineering, drug delivery, biosensors, disease prevention, and remediation. Particles with a nanoscale size exhibit special biological, chemical, mechanical, magnetic, and structural features. Additionally, it clarifies how to use nanomaterials, such as biomimetic materials in living cells and nanosensors for diagnosis and medicine delivery.

Thus, this study mainly focused on the following: (i) characterization of AgNPs biosynthesized from the leaf extract of *Madhuca longifolia* by utilizing various biophysical approaches including FESEM, UV-vis spectroscopy, EDAX, FTIR, and XRD; (ii) efficacy of the *Madhuca longifolia* leaf extract and biosynthesized AgNPs against larval and pupal population activity of jungle fever vector *A. stephensi*. (iii) Furthermore, the counter plasmodial movement of *M. longifolia* and AgNPs was assessed toward the CQ-safe (CQ-r) and CQ delicate (CQ-s) strains of the jungle fever parasite *P. falciparum*.

2 Materials and methods

2.1 Madhuca longifolia plant extract

The leaves of *M. longifolia* were collected from around the Bharathiar University campus and the materials were

identified by the taxonomist of the Botany Department, Bharathiar University, and Coimbatore. M. longifolia leaves were desiccated at room temperature and pulverized utilizing an electronic processor. About 500 g of the plant materials was extracted using 1.5 L of methanol (72 h), and the crude plant extract was concentrated using a rotary evaporator and stored at 4°C in the refrigerator. About 1 g of the plant residue was dissolved in 100 mL of acetone, as 1% (w/v) stock solution.

DE GRUYTER

2.2 Synthesis of AgNPs from M. longifolia

About 10 g of the washed leaves was minced into slight pieces in a 300 mL 4088 Erlenmeyer flask containing 100 mL of disinfected two-fold refined water. It was mixed with silver nitrate and boiled for 5 min. Then, it was filtered using Whatman filter paper and stored at -4°C. An earthy-colored yellow formation demonstrated the development of AgNPs. The watery AgNPs were condensed by M. longifolia extract forming stable AgNPs in water [28].

2.3 Characterization of AgNPs

The morphological and physicochemical properties of the synthesized nanomaterials were characterized by different methods. The development of M. longifolia-synthesized AgNPs was observed in the UV-Vis band range of 200-800 nm followed by centrifugation at 15,000 rpm for about 20 min. The obtained pellets were mixed with deionized water. The morphological nature of Ag nanoparticles was confirmed under a scanning electron microscope. Furthermore, the size and functional groups of the nanoparticles were observed using FTIR spectroscopy and, finally, the phase purity of nanoparticles was determined by XRD and EDAX [28].

2.4 Larval morphological changes

A. stephensi eggs were collected from the neighborhood water body (Tabuk region, Saudi Arabia). Eggs were moved to research facility conditions (28 \pm 3°C, 76–88% RH, 13:11 photoperiod) and set in $20 \text{ cm} \times 12 \text{ cm} \times 5 \text{ cm}$ plastic compartments containing 500 mL of distilled water. Hatchlings were taken care with a blend of canine scones and hydrolyzed yeast. Hatchlings were gathered, moved to a glass

dish loaded with 500 mL of DD water, and subjected to ensuing tests. The transferred hatchlings were treated with different convergences of the biosynthesized material for 24 h incubation, after brooding over the rewarded and untreated hatchlings were broken down under minute conditions.

2.5 Larval/pupal toxicity assessment

Following the methods reported by Kovendan et al. [29] A. stephensi larvae (I-IV instar) or pupae (25 nos) were taken from the conical flask containing 250 mL of distilled water, 20, 40, 60, 80, and 100 ppm (individually) concentrations of the M. longifolia leaf extract, and 2, 4, 6, 8, and 10 ppm of synthesized AgNPs (individually). The control was separately maintained in this experiment against A. stephensi [29]. All treatments were repeated five times against larva and pupa. Mortality rate (%) was calculated as follows:

Death rate =
$$\frac{\text{No. of larvae dead}}{\text{No. of larvae treated}} \times 100$$
 (1)

2.6 Cytotoxic effect of AgNPs on Vero cells

Vero cells were obtained from NCCS (National Centre for Cell Sciences) located in Pune, India. Cells were maintained in a Dulbecco's modified Eagle's medium (DMEM) supplemented with balanced salt solution and 2 mM L-glutamine, which contained 1.5 g·L⁻¹ Na₂CO₃, 1.5 g·L⁻¹ glucose, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES), 1 mM sodium pyruvate, and 10% fetal bovine serum. Also, 100 IU·of 100 µg⁻¹ streptomycin and 1 mL·L⁻¹ penicillin were used. Further, the cells were retained at 5% CO₂ in a humidified CO₂ atmosphere at 37°C. The cells were treated with varied concentrations of AgNPs for about 24 h.

2.7 Assessment of cytotoxicity

Cell cytotoxicity assay (MTT, Hi-Media) was done to measure the inhibitory concentration (IC_{50}) values. Vero cells were developed in 96-well plate $(1 \times 10^4 \text{ cells-well}^{-1})$ to obtain 80% confluency for 48 h. The medium was then substituted with AgNPs comprising fresh medium at different concentrations (10, 20, or 50 µM); similarly, they were treated with *M. longifolia* extract followed by incubation for 48 h. Consequently, the culture medium was observed, and $100\,\mu\text{L}$ of MTT was then added to each of the well. Then, the temperature was increased to 37°C for 4 h, accompanied by the supernatant elimination. Further, $50\,\mu\text{L}$ of DMSO was added to each well and then reared approximately for $10\,\text{min}$ to formazan crystal solubilization. The optical density (OD) of the cells was read using an Elisa plate reader (Thermo Multiskan EX, USA) fixed at 490 nm. Further, the cytotoxicity of the AgNPs was calculated using the following formula:

Percentage cell viability
$$= \frac{\text{OD of experimental sample}}{\text{OD of experimental control}} \times 100$$
(2)

2.8 Morphological investigation

Vero cells were allowed to multiply on coverslips (1,105 cells) and then incubated with AgNPs at various concentrations for 6–24 h before being fixed with ethanol/acetic acid (3:1; v/v) solution. For morphological observation, the cover slips were loosely placed to glass slides. Three monolayers of each experiment were photographed. Using a light microscope (Nikon, Japan) with a 20× magnification, the morphological changes of the treated cells were examined.

2.9 Parasite culture

Human erythrocytes from RP-mature C's layer of cells generated parasites: The RPMI culture was prepared by adding 25 mM HEPES, L-glutamine, and 16.2 g of powdered

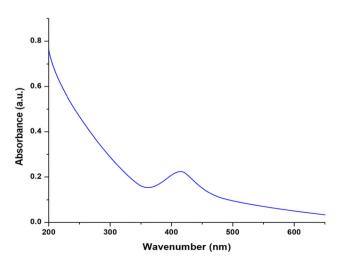


Figure 1: UV-Vis spectrum of M. longifolia-synthesized AgNPs.

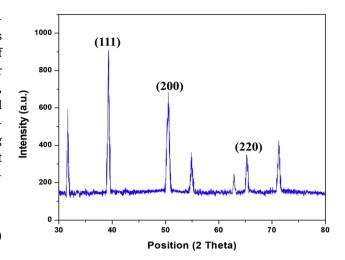


Figure 2: XRD pattern of M. longifolia-synthesized AgNPs.

RPMI 1640 without sodium bicarbonate. To create an initial parasitemia between 0.1% and 1.0%, freshly cleansed RBC was added to the parasites. In order to culture the expansion of new erythrocytes for every 4–5 days, the incubation environment was maintained with 1.5% O₂, 5% CO₂, and 95% nitrogen atmosphere [30]. The media were changed every 24 h.

2.10 In vitro antiplasmodial investigation

The control stock solution of chloroquine was prepared in DD water. The *M. longifolia* extract AgNP suspension was prepared in dimethyl sulfoxide. In all groups, with the exception of CQ, the last arrangement contained 0.4% DMSO. The antiplasmodial activity of the concentrate of the *M. longifolia* extract AgNP test was determined by

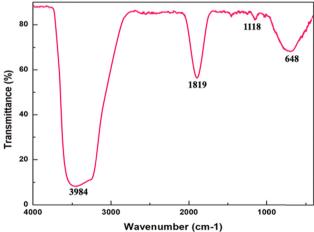


Figure 3: FTIR spectrum of M. longifolia-synthesized AgNPs.

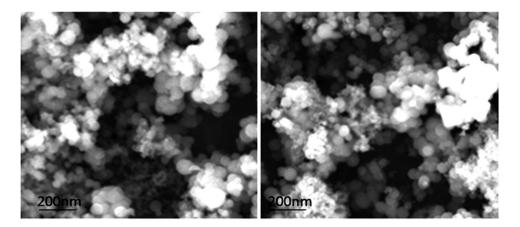


Figure 4: SEM micrograph showing the morphological characteristics of M. longifolia-synthesized AgNPs.

adding it to a 96-well plate as done by Rieckmann [31], with the modification shown by Carvalho et al. [32]. A solution of infected RBC containing most trophozoites was inoculated to the well to make a final volume of 100 mL. The positive control was CQ, and uninfected and contaminated RBC was incorporated with a negative standard. The well plates were kept at 37°C, followed by 24 h incubation with and without freshly containing AgNPs. After brooding for 24 h, Giemsa-stained blood films were set up in every well of the plates, and the level of restraint of parasite development was determined using a visualizing instrument by examining the number of sporozoites with at least minimum three independent test experiments with that of control wells.

2.11 Statistical analysis

SPSS programming version 16.0 variant was used for all experiments. Data from mosquitocidal tests were analyzed by probit investigation, containing lethal concentrations 50 and 90 [33]. In anti-plasmodial examination, values were determined as rate development hindrance;

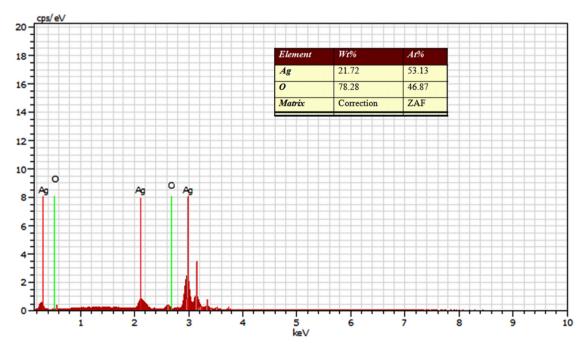


Figure 5: EDX spectrum of M. longifolia-synthesized AgNPs.

Table 1: Acute toxicity of M. longifolia aqueous extracts against larvae and pupae of the malaria vector A. stephensi

Target	LC ₅₀ (LC ₉₀) (mg·L ⁻¹)	95% confidence limit $LC_{50} \; (LC_{90}) \; (mg \cdot L^{-1})$		Regression equation	x²
		l instar	41.460 (121.404)	31.577 (106.579)	48.993 (145.744)
II instar	53.005 (145.398)	43.657 (124.530)	61.181 (182.580)	y = -0.735 + 0.014x	0.205n.s
III instar	72.285 (176.627)	63.084 (146.882)	84.228 (234.545)	y = -0.888 + 0.012x	0.054n.s
IV instar	86.002 (199.629)	74.895 (162.083)	104.335 (278.465)	y = -0.970 + 0.011x	0.316n.s
Pupae	151.951 (304.488)	119.258 (220.588)	252.400 (573.433)	y = -1.277 + 0.008x	0.056n.s

Control = no mortality; LC_{50} = lethal concentration killing 50% of the insects; LC_{90} = lethal concentration killing 90% of the insects; χ^2 = chi-square; n.s. = not significant (α = 0.05).

Table 2: Acute toxicity of M. longifolia-synthesized AgNPs against larvae and pupae of the malaria vector A. stephensi

Target	$LC_{50} (LC_{90}) (mg \cdot L^{-1})$	95% confidence Limit LC ₅₀ (LC ₉₀) (mg·L ⁻¹)		Regression equation	x²
		l instar	1.302 (6.660)	0.025 (5.975)	2.146 (7.619)
II instar	2.035 (10.058)	0.442 (8.829)	3.046 (12.092)	y = -0.325 + 0.160x	5.053n.s
III instar	2.181 (12.017)	0.185 (10.250)	3.348 (15.328)	y = -0.284 + 0.130x	2.150n.s
IV instar	3.876 (16.435)	1.991 (13.306)	5.028 (23.444)	y = -0.396 + 0.102x	0.007*
Pupae	6.102 (21.077)	4.664 (16.194)	7.592 (34.142)	y = -0.522 + 0.086x	0.072n.s

Control = no mortality; LC_{50} = lethal concentration killing 50% of the insects; LC_{90} = lethal concentration killing 90% of the insects; χ^2 = chi-square; n.s. = not significant (α = 0.05).

the focus causing half restraint of parasite development (IC_{50}) was determined from the medication fixation reaction curves.

3 Results and discussion

3.1 Characterization of *M. longifolia*-synthesized AgNPs

Humanity benefits from the development of affordable, dependable, and environmentally friendly approaches to increase the synthesis and effectiveness of nanoparticles [34]. Because of the impact of surface plasmon resonance, silver nitrate was reduced when plant filtrates were added, which was followed by a slight increase in the shading from clear to yellowish earthy color (SPR). UV-Vis spectroscopy, one of the most often used techniques, was used to observe the auxiliary portrayal of AgNPs. The light earthy shading was produced by increasing the hatching time.

The pale earthy-colored shading of silver colloids inclusion range showed a clear peak at 425 nm (Figure 1).

In concurrence with our outcomes, a peak with most extreme retention at 410 nm described the biosynthesis of *Aloe vera*-synthesized AgNPs [22]. Essentially, the

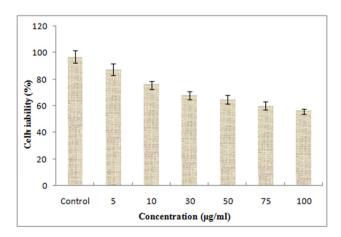


Figure 6: Cytotoxic effect of the synthesized AgNPs on Vero cells.

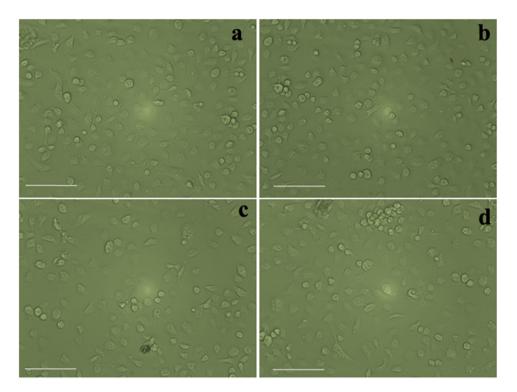


Figure 7: Morphological observation of synthesized AgNP-treated Vero cells: (a) control, (b) 25 μg·mL⁻¹, (c) 50 μg·mL⁻¹, and (d) 100 μg·mL⁻¹.

Delphinium denudatum AgNPs synthesized showed a peak with most extreme retention at 416 nm. Additionally, the position and condition of AgNP plasmon ingestion are greatly influenced by the molecule size, surface-adsorbed species, and the dielectric medium [35].

Four extreme peaks in the two-value range, spanning values of 25–60, are visible in the XRD spectrum. In the current experiment, the AgNPs were framed at two estimates of 38.32°, 52.45°, and 66.65° in comparison to planes for silver, individually (Figure 2). The XRD image clearly demonstrates that AgNPs were crystalline in character and were formed by the reduction of AgNO₃ particles by *M. longifolia*. For AgNPs made from Acalypha indica, similar results have been explained [36].

FTIR analysis was done to distinguish conceivable biomolecules on the *M. longifolia* leaf extract that might be liable for biosynthesis and adjustment of AgNPs. An FTIR spectrum for the synthesis of AgNPs is shown in Figure 3. The significant peaks in the FTIR range of AgNPs have been seen at 648, 1,216, 1,819, and 3,984 cm⁻¹. Additionally, for the FTIR range of *Delphinium denudatum* root extricate, the peaks were seen at 3,354, 2,952, 2,063, 1,651, 1,419, 1,383, 1,354, 1,171, 1,093, 780, 672, and 605 cm⁻¹. The FTIR had featured the nearness of various utilitarian gatherings, including alkane, alkene, methylene, amine, and carboxylic groups, which are known to be present in the biosynthesized AgNPs [37].

SEM micrographs demonstrated that *M. longifolia*-integrated AgNPs were primarily spherical in shape, ranging from 20 to 40 nm (Figure 4). *Ulva lactuca*-synthesized AgNPs were found to be in appropriation of sizes, mostly ranging from 20 to 35 nm [38]. The EDX graph uncovered a solid sign in the Ag area, affirming the nearness of natural Ag, demonstrating a sharp peak at 3 keV, for the synthesized AgNPs (Figure 5). Essentially, metallic Ag nanocrystals demonstrated an average optical retention peak roughly at 3 keV because of SPR [39,40]. EDX likewise indicated the nearness of oxygen, indicating that AgNPs were topped by the natural parts present in the seaweeds, as additionally featured by the FTIR investigation [41,42].

3.2 Mosquitocidal action

In the laboratory analysis, the *M. longifolia* filtrate was found to be poisonous against hatchlings and pupae of *A. stephensi*, regardless of whether tried at low dosages. LC₅₀ values were found to be 31.577 (I), 43.657 (II), 63.084 (III), 74.895 (IV), and 119.258 ppm (pupal population stages) (Table 1) at different instars, respectively. Likewise, a subsidiary impact was found as recently published by Benelli et al. [8]; Murugan et al. [43] had shown for other plant-borne mosquitocidals. Further, the

8 — Abdullah F. Shater *et al*. DE GRUYTER

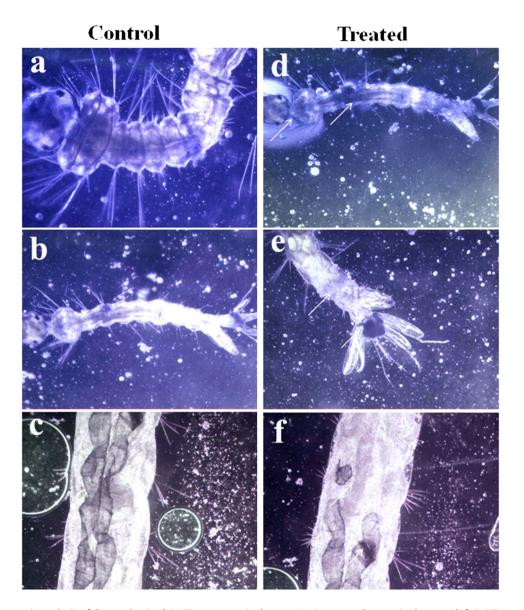


Figure 8: Microscopic analysis of the synthesized AgNPs on mosquito larvae: (a-c) untreated mosquito larvae; (d-f) AgNP-treated mosquito larvae.

research center analysis had shown *M. longifolia* interceded AgNPs to have profoundly expanded larval and pupal mortality against *A. stephensi*. The LC₅₀ values were found to be 1.302 (I), 2.035 (II), 2.181 (III), 3.876 (IV), and 6.102 ppm (pupal population stages) (shown in Table 2). In contrast to *A. stephensi*, Arokiyaraj et al. [44] considered the larvicidal activity of AgNPs included by botanical concentrate of *C. indicum*. It should be noted that additional AgNPs produced by *Aloe vera* were extremely viable at lower concentrations than those used in the current study [22]. Recently, Mondal et al. [45] reported that *Colocasia esculenta* stemsynthesized AgNP filtrate concentrate was biotoxic to *Culex quinquefasciatus* hatchlings, with greater mortality at extremely low concentrations.

3.3 Cytotoxic assay

The MTT test was used to determine how biosynthesized AgNPs affected the mortality of Vero cells. The *in vitro* cytotoxic activity of AgNPs (concentrations: 10–100 g·mL⁻¹) against the selected cells is shown in Figure 6. The test findings demonstrated that the particles did not completely prevent cell division. Instead, the fact that the majority of the cells (60%) were in a healthy state suggested that the substance was ideal for use in the selected cells. The results clearly showed that the IC₅₀ cell reasonability is what caused the cells to be so similar to one another. According to a previous research, AgNP particles incubated with Vero cells for 72 h showed reduced or no toxicity to mammalian cells [46].

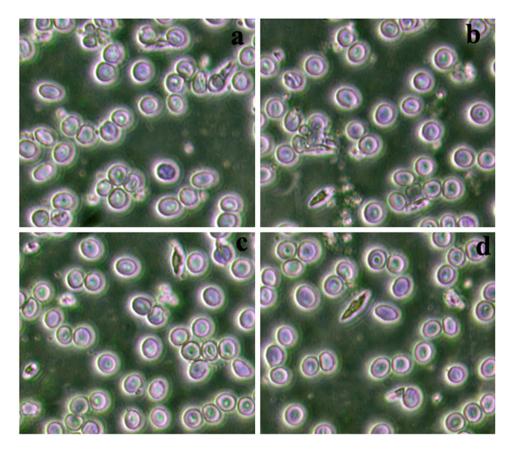


Figure 9: Antiplasmodial activity of the synthesized AgNPs against P. falciparum: (a) control, (b) 50 ppm, (c) 75 ppm, and (d) 100 ppm.

3.4 Cell morphology investigation

The morphological changes of chosen cells in the presence and absence of AgNPs at different concentrations are shown in Figure 7. It can be seen from Figure 7 that the control cells did not show any exceptional changes on their morphology. Nevertheless, AgNPs were not shown to have any unfavorable impact to the Vero cells. The cytological examinations evidenced that the synthesized AgNPs are profoundly biocompatible to the framework and explicitly harmful to the parasites.

3.5 Larval deformity

As shown in Figure 8, introduction of AgNPs caused neglect of improvement on the ordinary morphology, the regularly contortions including shrinkage mid-region and tracheal gills stomach extremities in mosquito hatchlings. The locomotory movement of hatchlings at 6 dpf time point (after the AgNP presentation period) was broken down irrespective of if the blended particles introduction could have a relentless consequence for larval conduct.

We found that the rewarded hatchlings decreased locomotory conduct and butt-centric papillae gnawing conduct. Farida et al. [47] had revealed the harmfulness of the metabolites of *B. bassiana* on the fourth instar hatchlings of *Cx. pipiens* and had observed numerous histological alterations and contortion in the rewarded larval tissues and body, particularly in fat cells and fingernail skin. Other findings had detailed that the midgut of tried mosquitoes'

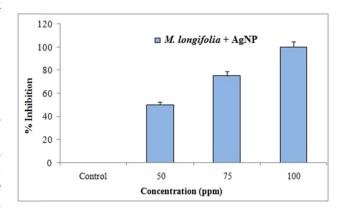


Figure 10: *In vitro* antiplasmodial activity of AgNPs synthesized using *M. longifolia* against the malaria parasite *P. falciparum*.

hatchlings had increased in the gut lumen and decreased the intercellular substance and deterioration of cores, when *P. daleae* mycelium were removed [48].

3.6 Antiplasmodial tests

Figures 9 and 10 show that the efficacy of AgNPs orchestrated with M. longifolia at various focuses on the restraint pace of the malarial parasites, which was seen subsequent to the plant extract treatment. Extreme inhibition ranges were seen subsequent to the treatment with synthesized AgNPs. The lowest inhibition range of parasitemia (44.5%) was seen in parasites at 50 ppm concentration of M. longifolia-synthesized AgNPs. Further, the parasitemia inhibition ranges were extremely high, about 44.5%, 66.2%, and 80.4% for 50, 75, and 100 ppm, respectively, for M. longifolia-synthesized AgNPs. Similarly, Mimosa pudica leaf extracts and AgNPs were shown to have a good antiparasitic activity [49]. Also, Murugan et al. [16] found that the antiplasmodial activities of O. basilicum and S. occidentalis were not in favor of CQ (s and r) strains of P. falciparum. Further, Murugan et al. [38] revealed that the antiplasmodial action of *U. lactuca*-seaweed-synthesized AgNPs were not in favor of CQ (s and r) strains of P. falciparum, while in *U. lactuca*-synthesized AgNPs, the IC₅₀ values were 76.33 (CQ-s) and $79.13 \,\mu\text{g}\cdot\text{mL}^{-1}$ (CQ-r). This information on the antiplasmodial activity of M. longifolia-synthesized AgNPs implies that IC₅₀ values would be useful in understanding their antimalarial properties.

4 Conclusion

The goal of this study is to study eco-friendly green-blended AgNPs against the *A. stephensi* malaria vector. The quick and inexpensive synthesis of AgNPs was confirmed by IR, SEM, XRD, UV-Vis spectroscopy. This study demonstrated that *M. longifolia*-intervened AgNPs can be used at small dosages to effectively reduce populations of the jungle fever vector. Up to $100 \, \mathrm{g \cdot mL^{-1}}$, the cytotoxic effect of the biosynthesized nanomaterial on African green monkey kidney cells was considered to be of negligible toxicity. The combined effects of the inserted AgNPs led to *A. stephensi* larval deformation during the instar phase, which prompted larval passage. In conclusion, *M. longifolia*-intervened AgNPs has the potential to impede the growth of the *P. falciparum* malaria parasite. This study demonstrated that the biosynthesized AgNPs

can be employed to build safer and more recent specialists for the control of intestinal illness in the near future.

Acknowledgements: The authors thank the Department of Zoology, Bharathiar University, for providing the laboratory facilities.

Funding information: The authors state no funding involved.

Author contributions: Chellasamy Panneerselvam: conducted the experiments and data compilation; Abdullah F. Shater, Fayez M. Saleh, Zuhair M. Mohammedsaleh: wrote the draft and discussed the results; Hattan Gattan, Bassam M. Al-Ahmadi, Nizar H. Saeedi: contributed to formal analysis and data curation; Mohammed M. Jalal, Chellasamy Panneerselvam: reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The authors confirm that the data supporting the findings of this study are available in the article.

References

- [1] Hotez PJ, Remme JH, Buss P, George G, Morel C, Breman JG. Combating tropical infectious diseases: report of the disease control priorities in developing Countries project. Clin Infect Dis. 2004;38(6):871–8.
- [2] Burfield T, Reekie SL. Mosquitoes, malaria and essential oils. Inter J Aroma. 2005;15(1):30–41.
- [3] Mittal PK, Adak T, Subbarao SK. Inheritance of resistance to Bacillus sphaericus toxins in a laboratory selected strain of Anopheles stephensi (Diptera Culicidae) and its response to Bacillus thuringiensis var. israelensis. Curr Sci. 2005;89(3):442-3.
- [4] Guerra CA, Snow RW, Hay SI. A global assessment of closed forests, deforestation and malaria risk. Ann Trop Med Parasitol. 2006;100(3):189.
- [5] Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post-harvest fungal infestation of food commodities. Innov Food Sci Emerg Tech. 2008;9(4):575–80.
- [6] Duval L, Nerrienet E, Rousset D, Sadeuh Mba SA, Houze S, Fourment M, et al. Chimpanzee malaria parasites related to *Plasmodium ovale* in Africa. PLoS One. 2009;4(5):5520.
- [7] Mulla MS, Thavara U, Tawatsin A, Chomposri J, Su T. Emergence of resistance and resistance management in field populations of tropical *Culex quinquefasciatus* to the microbial

- control agent Bacillus sphaericus. J Amer Mos Cont Assoc. 2003:19(1):39-46.
- [8] Benelli G, Bedini S, Cosci F, Toniolo C, Conti B, Nicoletti M. Larvicidal and ovideterrent properties of neem oil and fractions against the filariasis vector Aedes albopictus (Diptera: Culicidae): a bioactivity survey across production sites. Parasitol Res. 2015a;114(1):227-36.
- Azizullah A, Rehman ZU, Ali I, Murad W, Muhammad N, Ullah W, et al. Chlorophyll derivatives can be an efficient weapon in the fight against dengue. Parasitol Res. 2014;113(12):4321-6.
- [10] Benelli G, Bedini S, Flamini G, Cosci F, Cioni PL, Amira S, et al. Mediterranean essential oils as effective weapons against the West Nile vector Culex pipiens and the Echinostoma intermediate host *Physella acuta*: what happens around? An acute toxicity survey on non-target mayflies. Parasitol Res. 2015b;114(3):1011-21.
- [11] Alkofahi A, Rupprecht JK, Anderson JE, Mclaughlin JL, Mikolajczak KL, Scott BA. Search for new pesticides from higher plants. Insect Plan Orig. 1989;387:25-43.
- [12] Semmler M, Abdel-Ghaffar F, Al-Rasheid K, Mehlhorn H. Nature helps: from research to products against blood-sucking arthropods. Parasitol Res. 2009;105(6):1483-7.
- [13] Panneerselvam C, Murugan K, Kovendan K, Kumar PM, Subramaniam J. Mosquito larvicidal and pupicidal activity of Euphorbia hirta Linn. (Family: Euphorbiaceae) and Bacillus sphaericus against Anopheles stephensi Liston. (Diptera: Culicidae). Asia Pac J Trop Med. 2013;6(2):102-9.
- [14] Murugan K, Benelli G, Ayyappan S, Dinesh D, Panneerselvam C, Nicoletti M, et al. Toxicity of seaweedsynthesized silver nanoparticles against the filariasis vector Culex quinquefasciatus and its impact on predation efficiency of the cyclopoid crustacean Mesocyclops longisetus. Parasitol Res. 2015a;114(6):2243-53.
- [15] Murugan K, Benelli G, Panneerselvam C, Subramaniam J, Jeyalalitha T, Dinesh D, et al. Cymbopogon citratus-synthesized gold nanoparticles boost the predation efficiency of copepod Mesocyclops aspericornis against malaria and dengue mosquitoes. Exp Parasitol. 2015b;153:129-38.
- [16] Murugan K, Aarthi N, Kovendan K, Panneerselvam C, Chandramohan B, Kumar PM, et al. Mosquitocidal and antiplasmodial activity of Senna occidentalis (Cassiae) and Ocimum basilicum (Lamiaceae) from Maruthamalai hills against Anopheles stephensi and Plasmodium falciparum. Parasitol Res. 2015c;114(10):3657-64.
- [17] Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnol. 2007;18(10):105104.
- [18] Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol Int Res Process Env Clean Tech. 2009;84(2):151-7.
- [19] Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P. 'Green'synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotech. 2018:16(1):1-24.
- [20] Benelli G. Research in mosquito control: current challenges for a brighter future. Parasitol Res. 2015;114(8):2801-5.
- [21] Amerasan D, Nataraj T, Murugan K, Panneerselvam C, Madhiyazhagan P, Nicoletti M, et al. Myco-synthesis of silver nanoparticles using Metarhizium anisopliae against the rural

- malaria vector Anopheles culicifacies Giles (Diptera: Culicidae). J Pest Sci. 2016;89(1):249-56.
- Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Mahesh Kumar P, Nicoletti M, et al. Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from Aloe vera extracts: towards an effective tool against the malaria vector Anopheles stephensi? Parasitol Res. 2015;114(4):1519-29.
- [23] Khare P. Kishore K. Sharma DK. Medicinal uses. Phytochemistry and Pharmacological profile of Madhuca longifolia. Asia J Pharm Pharmacol. 2018;4(5):570-81.
- Yoshikawa K, Tanaka M, Arihara S, Pal BC, Roy SK, Matsumura E, et al. New Oleanene Triterpenoid Saponins from Madhuca longifolia. J Nat Prod. 2000;63(12):1679-81.
- [25] Bhatnagar SC, Awasthi YC, Mitra CR, Steroidal and other constituents of Madhuca latifolia leaves. Phyto chem. 1972;11(1):465-7.
- [26] Nimbekar T, Bais Y, Katolkar P, Wanjari B, Chaudhari S. Antibacterial activity of the dried inner bark of Madhuca indica JF GMEL. Bull Env Pharm Life Sci. 2012;1(2):26-9.
- [27] Bhaumik A, Kumar MU, Khan KA, Srinivas CH. The bioactive compounds obtained from the fruit-seeds of Madhuca longifolia (L) act as potential anticancer agents. Scholar J Appl Med Sci. 2014;2(4A):1235-8.
- Rajaganesh R, Murugan K, Panneerselvam C, Jayashanthini S, [28] Roni M, Suresh U, et al. Fern-synthesized silver nanocrystals: towards a new class of mosquito oviposition deterrents? Res Veter Sci. 2016;109:40-51.
- [29] Kovendan K, Murugan K, Vincent S, Barnard DR. Studies on larvicidal and pupicidal activity of Leucas aspera Willd. (Lamiaceae) and bacterial insecticide, Bacillus sphaericus, against malarial vector, Anopheles stephensi Liston.(Diptera: Culicidae). Parasitol Res. 2012;110(1):195-203.
- [30] Trager W, Jenson JB. Cultivation of malarial parasites. Nat. 1978;273(5664):621-2.
- [31] Rieckmann K. Visual in-vitro test for determining the drug sensitivity of Plasmodium falciparum. Lancet. 1982;319(8285):1333-5.
- [32] Carvalho LH, Brandao MG, Santos-Filho D, Lopes JL, Krettli AU. Antimalarial activity of crude extracts from Brazilian plants studied in vivo in Plasmodium berghei-infected mice and in vitro against Plasmodium falciparum in culture. Braz J Med Biol Res. 1991;24(11):1113-23.
- [33] Finney DJ. Probit analysis. Cambridge, UK: Cambridge University Press; 1971. p. 333.
- [34] Bhattacharya D, Gupta RK. Nanotechnology and potential of microorganisms. Crit Rev Biotech. 2005;25(4):199-204.
- Suresh G, Gunasekar PH, Kokila D, Prabhu D, Dinesh D, Ravichandran N, et al. Green synthesis of silver nanoparticles using Delphinium denudatum root extract exhibits antibacterial and mosquito larvicidal activities. Spectro Acta Part A Mol Biomol Spect. 2014;127:61-6.
- Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, [36] Kalaichelvan PT, Mohan NJCSBB. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Coll Surf B Biointer. 2010;76(1):50-6.
- [37] Cho KH, Park JE, Osaka T, Park SG. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electro Act. 2005;51(5):956-60.
- Murugan K, Samidoss CM, Panneerselvam C, Higuchi A, [38] Roni M, Suresh U, et al. Seaweed-synthesized silver

- nanoparticles: an eco-friendly tool in the fight against *Plasmodium falciparum* and its vector *Anopheles stephensi*? Parasitol Res. 2015d;114(11):4087–97.
- [39] Magudapathy P, Gangopadhyay P, Panigrahi BK, Nair KGM, Dhara S. Electrical transport studies of Ag nanoclusters embedded in glass matrix. Phy B Con Matt. 2001;299(1–2):142–6.
- [40] Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. Spectrochim Acta Part A Mol Biomol Spect. 2011;79(3):594–8.
- [41] Patil SV, Borase HP, Patil CD, Salunke BK. Biosynthesis of silver nanoparticles using latex from few euphorbian plants and their antimicrobial potential. App Biochem Biotech. 2012;167(4):776–90.
- [42] Roni M, Murugan K, Panneerselvam C, Subramaniam J, Nicoletti M, Madhiyazhagan P, et al. Characterization and biotoxicity of *Hypnea musciformis*-synthesized silver nanoparticles as potential eco-friendly control tool against *Aedes* aegypti and *Plutella xylostella*. Ecotoxicol Env Saf. 2015;121:31–8.
- [43] Murugan K, Dinesh D, Nataraj D, Subramaniam J, Amuthavalli P, Madhavan J, et al. Iron and iron oxide nanoparticles are highly toxic to *Culex quinquefasciatus* with little non-target effects on larvivorous fishes. Env Sci Poll Res. 2018;25(11):10504–14.

- [44] Arokiyaraj S, Dinesh Kumar V, Elakya V, Kamala T, Park SK, Ragam M, et al. Biosynthesized silver nanoparticles using floral extract of *Chrysanthemum indicum* L. potential for malaria vector control. Env Sci Poll Res. 2015;22(13):9759-65.
- [45] Mondal A, Hajra A, Shaikh WA, Chakraborty S, Mondal NK. Synthesis of silver nanoparticle with Colocasia esculenta (L.) stem and its larvicidal activity against Culex quinquefasciatus and Chironomus sp. Asia Pac J Trop Biomed. 2019;9(12):510.
- [46] Sujitha V, Murugan K, Paulpandi M, Panneerselvam C, Suresh U, Roni M, et al. Green-synthesized silver nanoparticles as a novel control tool against dengue virus (DEN-2) and its primary vector *Aedes aegypti*. Parasitol Res. 2015:114(9):3315–25.
- [47] Farida B, Sonia H, Hakima MK, Fatma B, Fatma H. Histological changes in the larvae of the domestic mosquito *Culex pipiens* treated with the entomopathogenic fungus *Beauveria bassiana*. Sci Res Essay. 2018;13(1):1–10.
- [48] Ragavendran C, Natarajan D. Insecticidal potency of Aspergillus terreus against larvae and pupae of three mosquito species Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti. Env Sci Poll Res. 2015;22(21):17224-37.
- [49] Marimuthu S, Rahuman AA, Rajakumar G, Santhoshkumar T, Kirthi AV, Jayaseelan C, et al. Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res. 2011;108(6):1541-9.