



Arch. Bas. App. Med.13 (2025) 69-78

www.archivesbamui.com

www.ojshostng.com/index.php/abam

Research Article

Biosynthesis of *Moringa oleifera* Silver Nanoparticles and Bio-efficacy against *Anopheles gambiae*

*Ohia C.M.D^{1,2}, Ana Godson R.E.E¹, Oladosu I.A³, Odeseye A.O⁴, Popoola K.O.K.⁵

¹Department of Environmental Health Sciences, Faculty of Public Health, College of Medicine, University of Ibadan, Nigeria

²Nanobios Lab, Department of Biosciences and Bioengineering, Indian Institute of Technology (IIT), Bombay, 400076, India.

³Department of Chemistry, Faculty of Science, University of Ibadan, Nigeria

⁴Nigerian Institute of Science Laboratory Technology, Samonda, Ibadan, Nigeria

⁵Department of Zoology, Faculty of Science, University of Ibadan, Nigeria

Accepted: October 16, 2025

Abstract

Mosquito control is pivotal in managing malaria and other mosquito-borne diseases worldwide. However, reliance on synthetic larvicides is increasingly constrained by environmental toxicity and insecticide resistance, necessitating the exploration of eco-friendly alternatives. This study investigates the biosynthesis of *Moringa oleifera*-synthesised silver nanoparticles (MOS-AgNPs) using aqueous extracts of *Moringa oleifera* seeds (AEMOS), characterizes their physicochemical properties, and evaluates their bio-efficacy against immature stages of *Anopheles gambiae*. MOS-AgNPs were synthesized by reacting freshly prepared AEMOS with 1mM silver nitrate (AgNO₃) solution. The formation of MOS-AgNPs was initially confirmed through a color change from light golden yellow to colloidal yellowish-brown. Characterization techniques, including ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and transmission electron microscopy (TEM) were used to establish physicochemical properties of the nanoparticles formed. For the bioassays, the larvicidal activity of MOS-AgNPs and AEMOS was assessed against immature *A. gambiae* at various concentrations of solutions of AEMOS (50, 100, 150, 200, 250ppm) and MOS-AgNPs (5, 10, 15, 20, 25ppm) in five replicates with distilled water as a control. The setup was observed for moribund and dead larvae and pupae until 96 hours post-exposure. Data were subjected to descriptive and inferential analyses. Data from the characterization of the nanoparticle revealed that the nanoparticles exhibited a surface plasmon resonance (SPR) band at 384.30 nm, spherical morphology, and an average size of 25 ± 2 nm. FTIR analysis confirmed functionalization with biomolecules from *M. oleifera* seeds. Dose-dependent toxic effects were observed from the bioassays, achieving ≥60% mortality within 24 hours of exposure. MOS-AgNPs exhibited superior efficacy, with LC₅₀ values ranging from 9.27 ppm in larval stage 1 (L1) to 12.42 ppm in the pupal stages as compared to AEMOS, which ranged from 203.99 ppm (L1) to 106.43 ppm (pupae). Overall, the stages of development of the mosquitoes had significant correlation (p<0.05) with the % mortality recorded within the exposure time across treatment concentrations used. The findings demonstrate that *M. oleifera* -synthesised silver nanoparticles are highly effective larvicidal agents against *A. gambiae*, underscoring their potential for integration into green mosquito vector control strategies. This study highlights the promise of plant-based nanotechnology as a sustainable and potent alternative to conventional larvicides in combating mosquito-borne diseases.

Key Words: *Moringa oleifera*, green synthesis, *Anopheles gambiae*, mosquito vector control, nanoparticles, plant-based larvicides

INTRODUCTION

Mosquitoes are principal vectors of numerous infectious diseases, including malaria, which remains a major global health challenge. Current control measures, including long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and larvicides, have significantly reduced malaria morbidity and mortality in the tropical regions of the world including Sub-Saharan Africa (Nalinya *et al.*, 2022; Zhou *et al.*, 2022; Musoke *et al.*, 2023). Specifically, the control of aquatic stages of mosquitoes using treatments such as

synthetic larvicides has been used to complement other main malaria control strategies like use of LLINs and IRS in malaria-endemic communities across the globe. However, these synthetic larvicides face limitations such as environmental toxicity, non-target organism effects, and the development of resistance in mosquito populations (Benelli *et al.*, 2017).

Recent advances in nanotechnology have opened avenues for the green synthesis of nanoparticles using plant extracts for mosquito control (Benelli *et al.*, 2017; Priya & Santhi *et al.*, 2014). These green approaches utilize the plant extracts as

agents in the reduction and capping of the nanoparticles during synthesis (Landage & Wasif, 2012). In addition, these eco-friendly approaches offer target-specific mosquito control with minimal environmental impact. Among these, silver nanoparticles (AgNPs) have garnered attention due to their high bio-efficacy at low doses (Mondal *et al.*, 2014; Benelli *et al.*, 2016). The unique properties of AgNPs, including their size, surface plasmon resonance (SPR), and functionalization, enhance their potential for use in mosquito control. This study focuses on the biosynthesis of *Moringa oleifera* nanoparticles using aqueous extract of *Moringa oleifera* seed, characterizes their properties, and assesses their mosquitocidal activity against immature (larvae and pupae) stages of *Anopheles gambiae*.

MATERIALS AND METHODS

Preparation of aqueous extract of *Moringa oleifera* seed:

M. oleifera plant parts including seeds and leaves were freshly harvested. The leaves were taxonomically identified and deposited at the Department of Botany Herbarium, University of Ibadan, Nigeria. Subsequently, the air-dried seeds were pulverized and kept in air tight containers. For the aqueous extraction, 200 mg of the powder was mixed with 10 mL of distilled water, agitated for 60 minutes, and filtered through Whatman No. 1 filter paper (Gerdes, 1997, Ferreira *et al.*, 2009). The filtrate recovered is the crude AEMOS extract. This was stored in the fridge until used for the mosquito assays.

Biosynthesis of *Moringa oleifera*-synthesised nanoparticles (MOS-AgNPs):

Freshly generated 1mM aqueous solution of silver nitrate (AgNO₃) was used in the production of *Moringa oleifera*-silver nanoparticles. For the biosynthesis, using continuous agitation, 10 mL of freshly produced AEMOS extract was added dropwise into 90 mL of 1mM AgNO₃. This was observed for colour change within minutes of the reaction (Sujitha *et al.*, 2015; Bhat *et al.* 2011). The reaction mixture was incubated in the dark for 24 hours to ensure complete silver ion bio-reduction and prevent photoactivation of AgNO₃ (Bhat *et al.*, 2011). Color change from colorless to brownish colloid confirmed nanoparticle formation.

Physicochemical characterization of MOS-AGNPs:

a. **UV-Visible spectroscopy (UV-Vis) Analysis:** The optical property and quality of the *Moringa*-synthesized Ag-NPs was determined within the wavelength range of 320–900 nm using a Perkin-Elmer Lambda 35 spectrophotometer 24 hours after the reaction. The samples (2ml) were placed in the quartz cuvette, and the light absorption spectra were calculated using 2ml of 1mM-AgNO₃ as control (Creighton *et al.*, 1979; Fafal *et al.*, 2017). Measurement of the surface plasmon resonance (SPR) of biosynthesized *Moringa oleifera* emulsions over the wavelength range verified the production of Ag-NPs (Creighton *et al.*, 1979; Fafal *et al.*, 2017).

b. Fourier Transform Infrared Spectroscopy (FTIR)

analysis: Functional groups involved in nanoparticle capping and stabilization were identified using a Perkin-Elmer LS-55-Luminescence spectrometer coupled to a JASCO IRT-7000 Intron Infrared Microscope (JASCO Corporation, Tokyo, Japan) in transmittance mode with a resolution of 4 cm⁻¹ and

a scanning range of 350–4400 cm⁻¹. The spectra were captured and determination of the biochemical and biomolecular composition of the capping agents of the bio-reduced MOS-Ag-NPs generated in the reaction was carried out within the range.

c. Transmission Electron Microscopy (TEM)

analysis: Morphology and size distribution of the nanoparticles were examined. The micrograph images of the samples were obtained and recorded on a Transmission Electron Microscope (CM12, Philips, Eindhoven) equipped with a digital camera (Morada, Soft Imaging System, Münster, Germany) and image analysis software (iTEM), operating at an acceleration voltage of 80,000kV and varying magnifications (25,000-140,000).

Mosquito collection and maintenance:

Immature stages (larvae) of *A. gambiae* were obtained from previously identified breeding hotspots of *A. gambiae* in Ibadan, Nigeria. Mosquito larvae were collected using standard 350 mL mosquito dippers (WHO-recommended size) from temporary and permanent breeding habitats such as tire marks, water pools and drains. Each dipper sample was taken systematically along the habitat margins, with multiple dips pooled per site to improve representativeness (Service, 1976; WHO, 2005). Collected larvae were transferred into labelled containers with habitat water and transported to the laboratory for identification and bioassay experiments. These were kept within standardized conditions in the insectary (25–30°C and 60–70% RH), adhering to normal mosquito maintenance protocols (WHO, 1995) and modifying them as described by Adebayo *et al.*, 1999. Adult mosquitoes that emerged from the collection were identified morphologically using taxonomic keys (Gilles & Coetzee, 1987). Identification of these immature (larval and pupa) stages were conducted using standard morphological and size-related criteria under a dissecting microscope according to WHO guidelines and established entomological keys (Service, 1993; WHO, 2005; Silver, 2008). For the bioassays, immature stages of the mosquitoes were used and labelled as first instar (L₁), Second instar (L₂), Third instar (L₃) and Fourth instar (L₄) and Pupa.

Mosquito bioassays of MOS-AGNPs and AEMOS on *A. gambiae* s.s.:

Life stages of *Anopheles gambiae* (L₁–L₄ larvae and pupae) were exposed to different concentrations of the nanoparticles, MOS-AGNPs (5, 10, 15, 20 and 25 ppm) and AEMOS (50, 100, 150, 200 and 250 ppm) dispensed into containers with 100-ml distilled water. For each treatment, twenty individuals per life stages were exposed in five replicates and distilled water served as control. Mortality was recorded daily 96 hours post-exposure. For the analysis, the 24-h count which provides the standard acute toxicity measure of the MOS-AgNPs and AEMOS, and the 96-h count detects any slower or pupal-stage mortality, were used to give a comprehensive efficacy evaluation (WHOPES, 2005). WHO protocol also notes that slower-acting agents (such as biological or botanical larvicides) may require extended observation (typically up to 48 h). Hence for the dose-response evaluation, the 48-h data were used to assess the response of the different mosquito stages to the treatment doses (WHOPES, 2005). Lethal concentrations (L₅₀ and L₉₀) were calculated using log-probit (Finney, 1971) with the SPSS version 23. Multivariate Analysis of Variance (MANOVA) was used to determine relationships and correlation analyses.

RESULTS

Characterization of Moringa-synthesized Ag-NPs (MOS-AgNPs)

UV-Visible spectroscopy analysis of *M. oleifera* synthesized-AgNPs (MOS-AgNPs): The absorption spectra of the *M. oleifera* synthesized-AgNPs from UV-Visible spectroscopy within 24 hours of the biosynthesis revealed a distinct absorbance peak maximum (sharp band) at 384.30 nm. This confirms the MOS-AgNPs formation (Fig. 1).

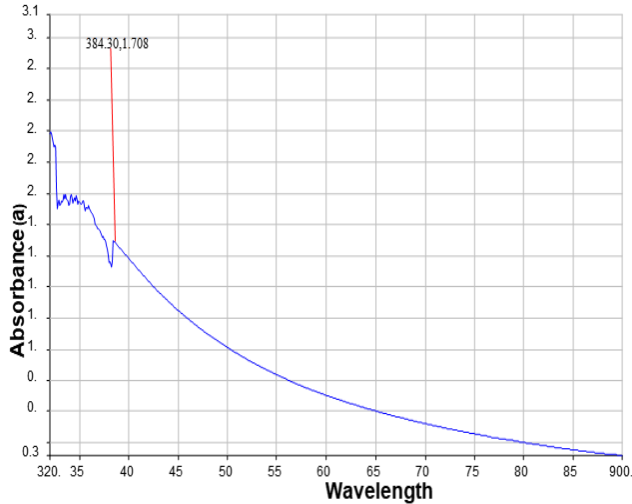


Fig. 1: UV-VIS spectra of *Moringa oleifera*-synthesised- silver nanoparticles (MOS-AgNPs).

Fourier transform infrared spectroscopy analysis of the MOS-AgNPs and AEMOS: The FTIR revealed that the Moringa synthesized-AgNPs had transmission peaks at 3447.00, 2938.00, 2357.23, 1649.38, 1541.93, 1388.30, 1240.33, 1115.46, 647.00, 596.00 and 465.46 cm^{-1} (Fig. 2a). The transmission peaks for the AEMOS were at 3446.00, 2930.18, 2353.82, 1652.05, 1541.36, 1449.28, 1251.00, 1115.00, 1065.41 and 651.00 cm^{-1} (Fig. 2b). While transmission peaks for the silver nitrate were seen at 3475.00, 1760.22, 1635.00, 1356.00, and 652.00 cm^{-1} (Fig. 2c). The FTIR showed that the 1760 and 1635 cm^{-1} signals in pure silver disappeared in the AgNPs with a corresponding presence of a novel signal peak at 1388.30 cm^{-1} . The peaks at 1388.30 cm^{-1} and others indicated successful nanoparticle stabilization by bioactive compounds from the *M. oleifera* extract.

Morphology of *M. oleifera* synthesized-AgNPs (MOS-AgNPs): The micrographs from the TEM provide insight into the morphology and size of the newly produced nanoparticles. The TEM micrograph showed poly-dispersed spherical particles with sizes ranging from 16 nm and 34 nm (Plate 3). The average size of the formed nanoparticle is <25nm.

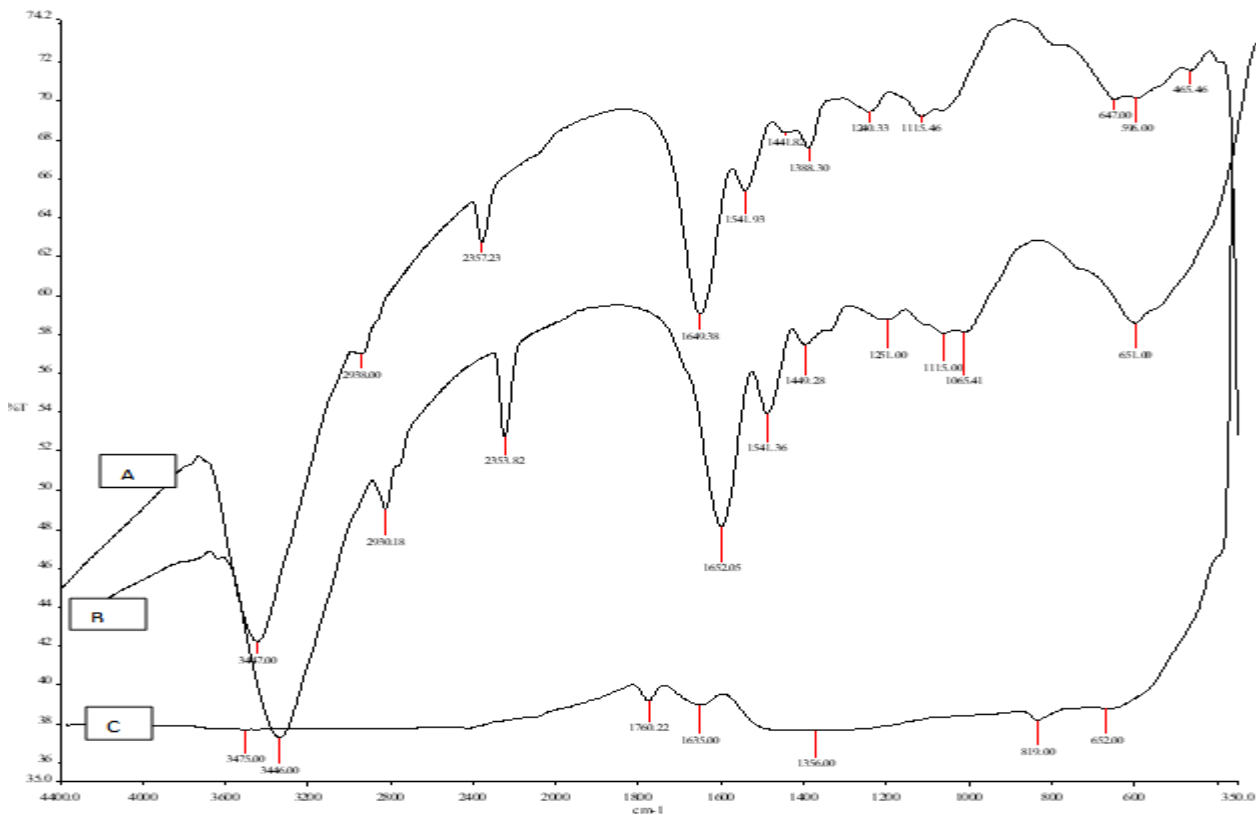


Fig. 2: FTIR spectra of powder of A) Moringa synthesised-AgNPs; B) AEMOS; and C) pure silver nitrate

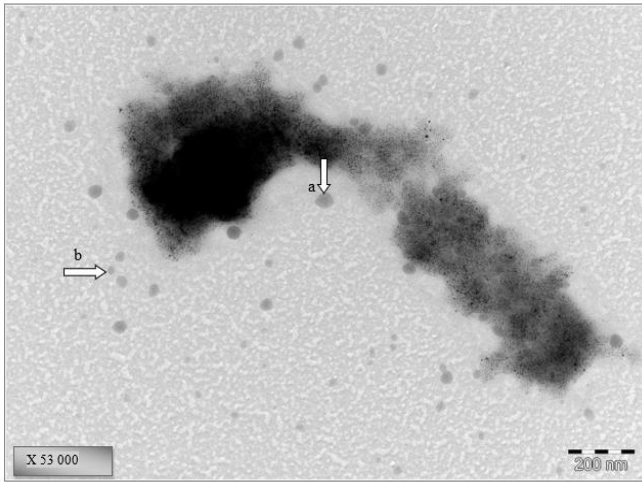
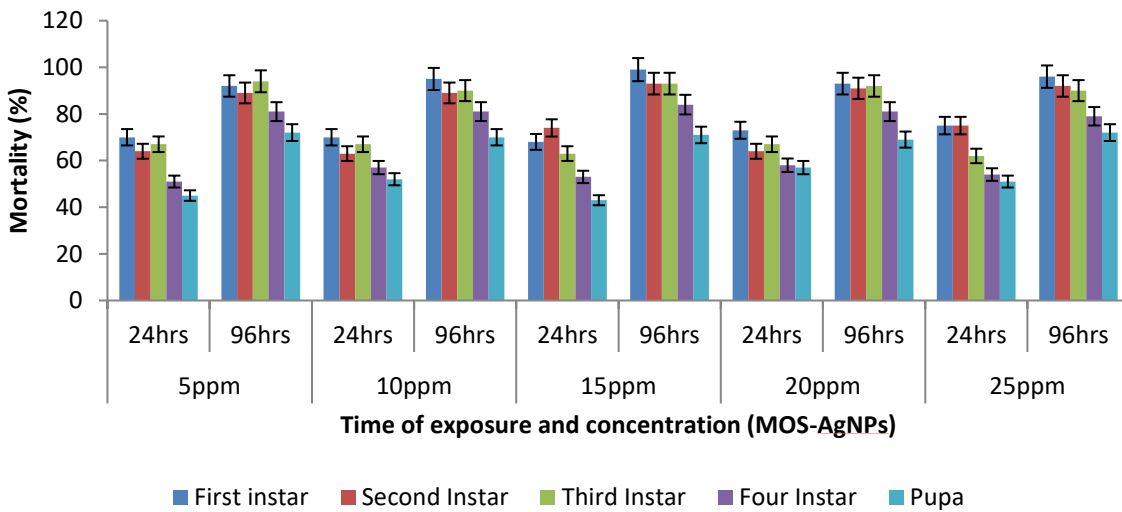


Plate 3: TEM micrograph showing the varying size of the NPs. a): a larger sized NP ~ 34nm; b) a smaller sized NP ~16nm

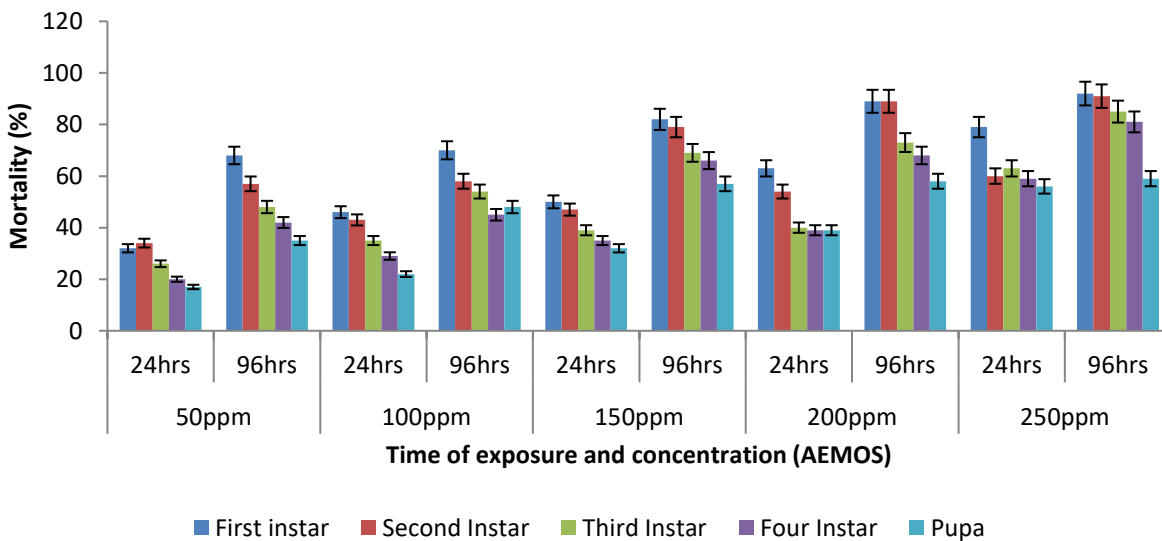
Larvicidal Activity of MOS-AgNPs and AEMOS

Susceptibility of the life stages of *Anopheles gambiae* s. s. to MOS-AgNPs and AEMOS: The MOS-AgNPs derived from *M. oleifera* seed extract showed high mosquitocidal efficacy against *A. gambiae*. All the mosquito stages (L₁, L₂, L₃, L₄, and Pupa) were susceptible to the nanoparticles (Fig. 4). The L₁ and L₃ stages were most susceptible to the nanoparticles and higher concentrations led to >50% mortality in the pupal stage. Mortality ranges of the L₂ and L₃ exposed to MOS-AgNPs was between >60% - 75% at 24 hours post-exposure (Fig. 4). The aqueous extract of *M. oleifera* seed (AEMOS) was similarly toxic to the mosquito stages L₁, L₂, L₃, L₄, and Pupa (Fig. 5). Across all the bioassays, control groups exhibited <5% mortality at both 24- and 96-hours post-exposure. As control mortality remained well within the WHO (2005) and OECD (1992) validity thresholds (≤10%), these data are not presented in the charts and tables.



Control data are not displayed as control groups showed <5% mortality at all timepoints and within validity thresholds (WHO, 2005; OECD, 1992)

Fig. 4: Effect of MOS-AgNPs on life stages of *Anopheles gambiae* at 24- and 96-hours post-exposure



Control data are not displayed as control groups showed <5% mortality at all timepoints and within validity thresholds (WHO, 2005; OECD, 1992)

Fig 5: Effect of AEMOS on life stages of *Anopheles gambiae* at 24- and 96 hours post-exposure

Dose-response relationship of the MOS-AgNPs and AEMOS treatments on *Anopheles gambiae*: The mortality rate across all the *Anopheles* stages was dose-dependent with an increase in mortality rates observed as exposure time and concentrations of each of the MOS-AgNPs and AEMOS increased. Control groups showed <5% mortality at all time points across the study. For the MOS-AgNPs, higher mortality rates of the mosquito stages were recorded with an increase in a concentration ranging from ≤60% at 5 ppm to 100% mortality at 25 ppm within 48 hours post-exposure (Fig. 6). Furthermore, the AEMOS produced increasing dose-dependent mortality effects on *A. gambiae* stages and the L₁ stage was the stage most affected by the increasing dose of the plant extract with >80% mortality within 48-hours post-exposure (Fig. 7).

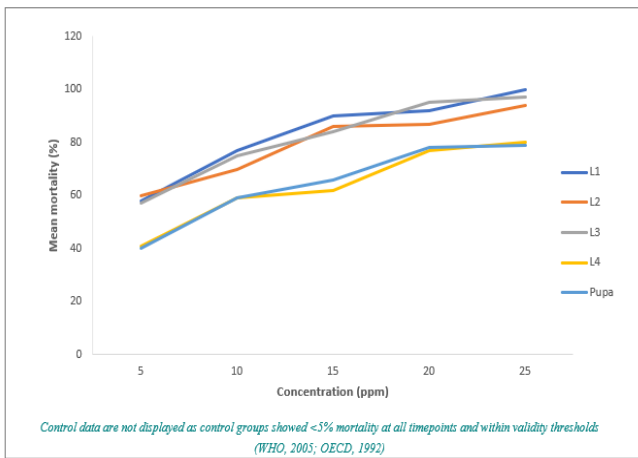


Fig. 6: The larvicidal effectiveness of different concentrations of MOS-AgNPs on *Anopheles gambiae* life stages at 48-hours post-exposure.

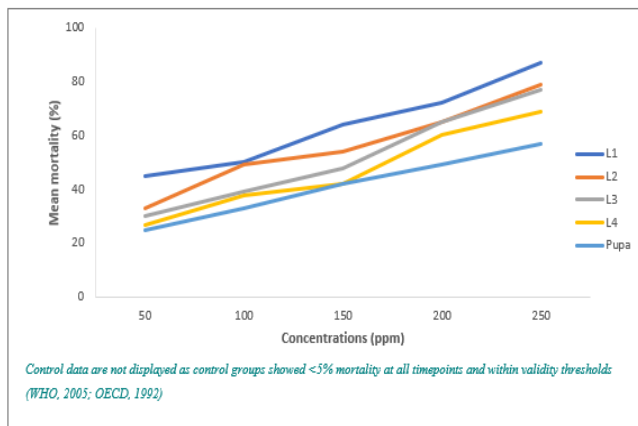


Fig. 7: The larvicidal effectiveness of different concentrations of AEMOS on *Anopheles gambiae* life stages at 48 hours post-exposure

Lethal Concentrations (LC₅₀ and LC₉₀) of MOS-AgNPs, AEMOS on *Anopheles gambiae* life stages: The MOS-AgNPs were very toxic at low doses on all the immature stages of the mosquito. The LC₅₀ values for MOS-AgNPs ranged from 9.27 ppm (L₁) to 12.42 ppm (pupae). The AEMOS treatment group revealed that the extract was effective as a bio-insecticide and its lethality increased as the mosquito aged as shown from the LC₅₀ value ranges from 203.99 ppm (L₁) to 106.43 ppm (pupae) (Table 1).

Table 1: LC₅₀ and LC₉₀ of MOS-AgNPs and AEMOS on *A. gambiae* life stages

STAGES	MOS-AgNPs		AEMOS	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
First instar (L ₁)	9.27	16.69	203.99	367.18
Second Instar (L ₂)	9.60	17.28	171.36	308.45
Third Instar (L ₃)	9.47	17.04	139.58	251.24
Fourth Instar (L ₄)	11.29	20.32	122.82	221.07
Pupa	12.42	22.35	106.43	191.58

¹LC values were determined by Probit Analysis (Finney, 1971)

²All concentrations in parts per million (ppm)

Relationship between treatment concentrations, mosquito stages and exposure time: With respect to the biosynthesised nanoparticle (MOS-AgNPs), the stages of development of the mosquitoes had significant correlation (p<0.05) with the % mortality recorded across the treatment concentrations (Table 2). This implies that the % mortality is associated with the developmental stages, although this associations were negative or inverse. In essence, the higher the stage of development of the mosquitoes, the lesser its susceptibility (in terms of % mortality) to exposure to the concentrations of the product. In addition, the correlation coefficient was < 0.5, implying that susceptibility of *A. gambiae* was moderately correlated with the concentration of AgNPs.

For the AEMOS, the stages of development, concentration and time of exposure of treatment are significantly associated to the recorded susceptibility (% mortality) of the mosquitoes (Table 2). Again, the stage of development was inversely related to mosquito susceptibility. However, in contrast to the MOS-AgNPs, treatment concentration and time of exposure had direct or positive relationship with the % mortality recorded throughout the study. This implies that the % mortality increased as the concentration was increased as well as the time of exposure. Similarly, as for MOS-AgNPs, the coefficient of correlation revealed that the significance of the correlation was moderate for stage and concentration.

Table 4.5: Relationship between treatment concentrations, *Anopheles* mosquito stages and exposure time

	MOS-AgNPs	AEMOS
Stages (L ₁ -L ₄ , pupa)	-0.32**	-0.35**
Concentration (ppm)	0.02	0.51
Time (Hr)	0.63**	0.68**

**Correlation is significant at the 0.01 level (1-tailed).

DISCUSSION

The crucial role of mosquito control in effectively combating vector-borne diseases like malaria remains well-established (Torto & Tchouassi, 2021; Anoopkumar & Aneesh, 2022).

Contemporary efforts focus on active identification, assessment and exploration of plant-based products using green nanotechnology as environmentally-friendly interventions to complement other control measures (Schäfer et al., 2019; Onen et al., 2023; Lokole et al., 2024; Dhir et al., 2024; Dass et al. 2024).

Green-synthesized AgNPs from plants have shown potentials as larvicides, positioning them as sustainable alternatives to chemical insecticides (Dass et al. 2024; Singh et al., 2025). In this study, the *Moringa-oleifera* synthesized AgNPs were confirmed through visual observation of colour change from colourless to yellowish and finally to colloidal brown indicating AgNP formation. These colour changes agree with previous studies (Sökmen et al., 2017; Shahid et al., (2018; George et al., 2023). The colour change is attributable to the arrangement of various macromolecules responsible for the catalytic formation and stabilization of the nanoparticles (Lateef et al., 2016; Restrepo & Villa, 2021).

UV-visible spectroscopy was used to confirm the development and stability of the newly formed nanoparticles after a 24-hour reaction time (Restrepo & Villa, 2021; Dass et al. 2024; Singh et al., 2025). The UV-visible absorption spectra showed a broad surface plasmon resonance (SPR) band with one peak at 384.30 nm indicating presence of a homogeneous distribution of hydrosol Ag-NPs. This distinctive UV-vis absorbance feature recorded is generally exhibited by AgNPs due to their surface plasmon resonance (SPR) (Creighton et al., 1979; Almatroudi, 2020; Kandwal et al., 2024). These SPR peaks are usually between 320 and 520 nm range and this is considered a crystalline spherical characteristic that is distinctly peculiar to AgNPs (Gopalakrishnan & Raghu, 2014; Fafal et al., 2017).

The TEM imaging from this study revealed spherically-shaped AgNPs with a mostly smooth crystalline surface. This aligns with previous researches that reported a size range of 10-100nm for most spherically shaped NPs (Kumar et al., 2018; Onen et al., 2023; Dhir et al., 2024; Dass et al. 2024; Singh et al., 2025). In addition, studies using *M. oleifera* plant also support our findings on the morphology of the MOS-AgNPs. Al-Rawag (*M. oleifera*)-synthesized and *A. arborescens*-mediated silver nanoparticles were reported to be spherically shaped using scanning electron microscopy (SEM) and HRTEM techniques (Al-Kalifawi, 2016; Kumar et al., 2018). In general, factors such as the capping agent, ultrasound radiation, reaction and crystallization time determine the morphological and size characteristics of nanoparticles (Restrepo & Villa, 2021; Hassan et al., 2022; Kandwal et al., 2024).

The Fourier Transform Infrared Spectroscopy (FTIR) was used to study the core-shell morphology of formed MOS-AgNPs. The analysis provided critical insights into the chemical composition and functional groups present in the nanoparticles (NPs), *Moringa* extract (AEMOS), and silver nitrate (AgNO_3), thus validating the existence of plant peptides in the formed nanoparticles compound used in this study. The FTIR spectra of the MOS-AgNPs displayed prominent transmission peaks at 3447.00, 2938.00, 2357.23, 1649.38, 1541.93, 1388.30, 1240.33, 1115.46, 647.00, 596.00, and 465.46 cm^{-1} , which confirm the successful formation of nanoparticles. In comparison, the AEMOS extract exhibited peaks at 3446.00, 2930.18, 2353.82, 1652.05, 1541.36, 1449.28, 1251.00, 1115.00, 1065.41, and 651.00 cm^{-1} , while silver nitrate showed peaks at 3475.00, 1760.22, 1635.00, 1356.00, 819.00, and 652.00 cm^{-1} . The emergence of new peaks and disappearance of certain signals in the MOS-AgNPs

spectrum underscore the transformations, processes and interactions that occurred during the nanoparticle synthesis.

The disappearance of the 1760.22 and 1635.00 cm^{-1} peaks from silver nitrate and the corresponding appearance of a new peak at 1388.30 cm^{-1} in the MOS-AgNPs spectrum underscores a point of contact between the aqueous extract and the silver nitrate. This suggests and indicate the reduction of silver ions (Ag^+) to silver nanoparticles (AgNPs). The peak at 1388.30 cm^{-1} is likely associated with vibrations from new functional groups introduced during the stabilization of the MOS-AgNPs by phytochemicals from the AEMOS extract, which act as reducing and capping agents. Our FTIR findings align with prior studies that reported plant-derived compounds such as phenols, flavonoids, and proteins play crucial roles in silver ion reduction and nanoparticle stabilization (Irvani et al., 2014; Ahmed et al., 2016; Moodley et al., 2018; Hamouda et al., 2019; Bindhu et al., 2020; Mehwish et al., 2021; Mohammed et al., 2022).

The strong broad peak at 3447.00 cm^{-1} in the NPs spectrum, similar to that of the MO extract, corresponds to O-H stretching vibrations, indicative of hydroxyl (O-H) group presence. These groups likely phenolic, alcoholic, and/or carboxylic acid compounds) alongside amine groups that might be overlapping with the O-H peak to give that visible peak (Moodley et al., 2018; Mehwish et al., 2021; Mohammed et al., 2022). It is important to note that these hydroxyl groups are abundant in AEMOS and are known to contribute to nanoparticle stabilization through hydrogen bonding and electrostatic interactions (Mittal et al., 2013; Mohammed et al., 2022).

The peak at 1649.38 cm^{-1} , corresponding to carbonyl (C=O) stretching, reflects potential involvement of carbonyl groups in the nanoparticle synthesis process. This was further corroborated by the presence of similar peaks in the MO extract spectrum, therefore, highlighting the active participation of *Moringa oleifera* phytochemicals representing carbonyl groups from polyphenols like catechin, theaflavin, ketones, aldehydes, or amides (especially amide II band), a prominent band in the infrared spectra of proteins in the nanoparticle formation (Sujitha et al., 2015). It is noteworthy that the peak between 1631-1650 cm^{-1} has previously been documented as involved in the protein stabilization of nanoparticles (Moodley et al., 2018; Guimarães et al., 2020; Mehwish et al., 2021; Mohammed et al., 2022).

Additional peaks in the NPs at 1240.33 and 596.00 cm^{-1} , absent or not prominent in the AEMOS spectrum, point to structural and compositional modifications post-synthesis. These signals likely correspond to unique vibrations of new bonds or interactions formed in the stabilized nanoparticles (Hamouda et al., 2019; Mohammed et al., 2022).

The findings from the IR data agree with earlier studies that documented that compounds capping AgNPs may be polyphenols having aromatic rings and bound amide functional groups (Sujitha et al., 2015; Selvam et al., 2017). From this data, evidence indicates that the protein within the *M. oleifera* extract played a major role in the coating and capping and biosynthesis of the silver nanoparticles (Guimarães et al., 2020) and these proteins provide covering to the AgNPs and prevents agglomeration of the nanoparticles compound formed. This is in consonance with earlier studies that have reported that free amide groups in the protein molecules can interact with AgNPs (Sarkar et al., 2007). Also, carbonyl groups and free amine in the protein residues of the compound have stronger potential to prevent agglomeration

by binding AgNPs and thereby promote the stabilization of AgNPs (Sangeetha et al., 2016; Eker et al., 2025).

These FTIR results validate the role of AEMOS extract in the bio-reduction and stabilization of the silver nanoparticles, demonstrating the efficacy of the green synthesis method used (Moodley et al., 2018; Hamouda et al., 2019; Bindhu et al., 2020; Mehwish et al., 2021; Mohammed et al., 2022). The observed chemical modifications support strong interactions between silver and *M. oleifera* phytochemicals, which can enhance the application of these NPs in mosquito vector control and allied areas. Future research could focus on detailed quantification of the specific functional groups, their influence and contribution to the unique properties of the synthesised nanoparticle properties to optimize their application potential.

Plant-based mosquitocides are composed of naturally occurring botanical compounds (phytochemicals) possessing a wide array of chemically active ingredients that disrupt most or all biological processes within the mosquito vector. In our study, the bioassays revealed that all the concentrations of the MOS-AgNPs were effective against all the immature (L₁-L₄ and pupa) stages of *A. gambiae* with LC₅₀ values ranging from 9.27 to 12.42 ppm. This aligns with recent findings that green-synthesized AgNPs have superior larvicidal and pupacidal efficacy and bioavailability across diverse mosquito species, including *An. stephensi* and *C. quinquefasciatus* (Murugan et al., 2015; Santhosh et al., 2015; Benelli, 2016; Kovendan et al., 2016; Ga'al et al., 2018; Suresh et al., 2018; Onen et al., 2023; Lokole et al., 2024; Dhir et al., 2024; Dass et al. 2024).

The mosquitocidal promise of the studied AgNPs at low doses is attributed to the presence of the nanoparticles facilitated through the capping of the biomolecules by the molecules of *M. oleifera* extract. While the mechanism of action could be related to structural distortion triggered by the movement of the nanoparticles through the membranous layer of the immature stages (larval, pupa) of the mosquitoes. These nanoparticles possibly attach to exposed protein-containing structures, or phosphorus-containing compounds like the DNA, thereby causing possible denaturation of organelles and enzymes in the digestive tract of the mosquito and the induction of reactive oxygen species within the mosquito (Rai et al., 2009; Das et al., 2021). The broad-spectrum activity of the nanoparticles is possibly due to the synergistic blend of the AgNPs and the plant-facilitated capping mediators, that act as promoters of the adherence to elemental silver in the reduction and stabilization process of AgNPs (Xu et al., 2020).

Taken together, the findings of this study demonstrate the potential of *M. oleifera* -synthesized AgNPs as eco-friendly larvicides. The observed color changes during synthesis and the distinct SPR peak corroborate previous reports on AgNP formation (Gopalakrishnan & Raghu, 2014). FTIR analysis revealed functional groups involved in the reduction and stabilization of nanoparticles, aligning with studies highlighting the role of plant-derived compounds in nanoparticle synthesis (Lateef et al., 2016). Furthermore, the larvicidal activity of MOS-AgNPs was superior to AEMOS, reflecting the enhanced bio-efficacy of nanoparticles. This aligns with findings that nanoscale delivery systems improve bioavailability and target specificity (Mondal et al., 2014; Onen et al., 2023; Dass et al. 2024).

CONCLUSION

75

This study highlights the potential of *M. oleifera* -synthesized AgNPs as an innovative, eco-friendly larvicidal tool for mosquito control. The green synthesis of nanoparticles using *M. oleifera* seed extract provides a cheaper alternative for the control of the malaria vector *Anopheles gambiae* with high larvicidal efficacy. Furthermore, the study reveals a simple, low-toxic and low-cost approach in the development of nano-based products for use in mosquito vector management and control. Future research should explore large-scale production and field application to combat vector-borne diseases sustainably.

Ethical approval

Not applicable.

Consent for publication

All the authors agreed to publish the data in this journal.

Consent to participate

Not applicable.

Credit authorship contribution statement

Ohia C.M.D: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Ana G.R.E.E: Supervision, Methodology, Investigation, Formal analysis, Data curation. Oladosu I. A: Visualization, Data curation, Validation, Writing – review & editing. Odeseye A.O: Visualization, Validation, Investigation, Formal analysis. Popoola K.O.K: Visualization, Validation, Investigation, Formal analysis.

Declaration of Competing Interest

All of the authors declare there is no conflict of interest.

Acknowledgements

CO was supported under the PhD Fellowship by the Consortium for Advanced Research Training in Africa (CARTA). CARTA is jointly led by the African Population and Health Research Center and the University of the Witwatersrand and funded by the Carnegie Corporation of New York (Grant No--B 8606.RO2), Sida (Grant No:54100029), the DELTAS Africa Initiative (Grant No: 107768/Z/15/Z). The DELTAS Africa initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (UK) and the UK government. The statements made and views expressed are solely the responsibility of the Fellow.

Electron microscopy sample preparation and imaging were performed with devices supported by the Microscopy Imaging Center (MIC) of the University of Bern, Switzerland.

REFERENCES

- Adebayo T.A., Gbolade A.A., and Olaifa J.I. 1999. Comparative study of toxicity of essential oils to larvae of three mosquito species. *Nigerian J. Nat. Prod. and Medicine*: 3: 74-76.
- Ahmed, S., Ahmad, M., Swami, B. L., and Ikram, S. 2016. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *Journal of radiation research and applied sciences*, 9(1): 1-7. <https://doi.org/10.1016/j.jrras.2015.06.006>
- Al-Kalifawi, E. J. 2016. Green Synthesis of Silver Nanoparticles Using Leaf Extract of Al-Rawag tree (*Moringa oleifera* Lamarck) Cultivated in Iraq and Efficacy

- the Antimicrobial activity. Mesop. *Environ. J. Special Issue A*: 9-48.
- Almatroudi, A. 2020. Silver nanoparticles: synthesis, characterisation and biomedical applications. *Open life sciences*. 15(1): 819-839.
- Anoopkumar, A. N., and Aneesh, E. M. 2022. A critical assessment of mosquito control and the influence of climate change on mosquito-borne disease epidemics. *Environment, Development and Sustainability* 24(6): 8900-8929.
- Benelli, G. 2016. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. *Parasitology research* 115(1): 23-34.
- Benelli, G., and Beier, J. C. 2017. Current vector control challenges in the fight against malaria. *Acta Tropica* 174: 91-96.
- Bhat, R., Deshpande, R., Ganachari, S. V., Huh, D. S., and Venkataraman, A. 2011. Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom *Pleurotus florida* and their antibacterial activity studies. *Bioinorganic Chemistry and Applications* 2011: 1-7.
- Bindhu, M. R., Umadevi, M., Esmail, G. A., Al-Dhabi, N. A., & Arasu, M. V. (2020). Green synthesis and characterization of silver nanoparticles from *Moringa oleifera* flower and assessment of antimicrobial and sensing properties. *Journal of Photochemistry and Photobiology B: Biology* 205: 111836.
- Creighton, J. A., Blatchford, C. G., and Albrecht, M. G. 1979. Plasma resonance enhancement of Raman scattering by pyridine adsorbed on silver or gold sol particles of size comparable to the excitation wavelength. *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics* 75: 790-798.
- Das, N. C., Roy, B., Patra, R., Choudhury, A., Ghosh, M., and Mukherjee, S. 2021. Surface-Modified Noble Metal Nanoparticles as Antimicrobial Agents: Biochemical, Molecular and Therapeutic Perspectives. *Nanotechnology for Advances in Medical Microbiology*: 165-205.
- Dass, K., Mariappan, P., Ramya, P., and Prakash, N. 2024. Green synthesis of silver nanoparticles for mosquito control: A review of larvicidal activity from plant extracts. *Uttar Pradesh Journal of Zoology* 45(22): 242-266.
- Dhir, R., Chauhan, S., Subham, P., Kumar, S., Sharma, P., Shidiki, A., and Kumar, G. 2024. Plant-mediated synthesis of silver nanoparticles: unlocking their pharmacological potential—a comprehensive review. *Frontiers in Bioengineering and Biotechnology* 11: 1324805.
- Eker, F., Akdaşçi, E., Duman, H., Bechelany, M., and Karav, S. 2025. Green Synthesis of Silver Nanoparticles Using Plant Extracts: A Comprehensive Review of Physicochemical Properties and Multifunctional Applications. *International Journal of Molecular Sciences* 26(13): 6222.
- Fafal T., Taştan P., Tüzün B.S., Ozyazici M., and Kivcak B. 2017. Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using *Asphodelus aestivus* Brot. aerial part extract. *South African Journal of Botany* 112: 346-353.
- Ferreira, P. M., Carvalho, A. F., Farias, D. F., Cariolano, N. G., Melo, V. M., Queiroz, M. G., Martins, A. and Machado-Neto, J. G. 2009. Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. *Anais da Academia Brasileira de Ciências* 81(2): 207-216.
- Finney, D. J. 1971. Probit analysis 3rd ed Cambridge Univ. Press. London, UK. 333pp.
- Ga'al, H., Fouad, H., Mao, G., Tian, J., and Jianchu, M. 2018. Larvicidal and pupicidal evaluation of silver nanoparticles synthesized using *Aquilaria sinensis* and *Pogostemon cablin* essential oils against dengue and zika viruses vector *Aedes albopictus* mosquito and its histopathological analysis. *Artificial cells, nanomedicine, and biotechnology* 46(6): 1171-1179.
- George, I. E., Cherian, T., Ragavendran, C., Mohanraju, R., Dailah, H. G., Hassani, R., ... and Mohan, S. 2023. One-pot green synthesis of silver nanoparticles using brittle star *Ophiocoma scolopendrina*: Assessing biological potentialities of antibacterial, antioxidant, anti-diabetic and catalytic degradation of organic dyes. *Heliyon* 9(3).
- Gerdes C. 1997 Como limpar e tratar agua suja com sementes de *Moringa oleifera*, Centro de Fortaleza. Centro de Pesquisa e Assessoria. 18p.
- Gillies, M. T., & Coetzee, M. 1987. A supplement to the Anophelinae of Africa South of the Sahara. *Publ S Afr Inst Med Res* 55: 1-143.
- Gopalakrishnan, R., and Raghu, K. 2014. Biosynthesis and characterization of gold and silver nanoparticles using milk thistle (*Silybum marianum*) seed extract. *Journal of Nanoscience*. 2014: 1- 9.
- Guimarães, M. L., da Silva, F. A. G., da Costa, M. M., and de Oliveira, H. P. 2020. Green synthesis of silver nanoparticles using *Ziziphus joazeiro* leaf extract for production of antibacterial agents. *Applied Nanoscience* 10: 1073-1081.
- Hamouda, R. A., Hussein, M. H., Abo-Elmagd, R. A., and Bawazir, S. S. 2019. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. *Scientific reports* 9(1): 13071.
- Hasan, K. F., Xiaoyi, L., Shaoqin, Z., Horváth, P. G., Bak, M., Bejó, L., ... and Alpár, T. 2022. Functional silver nanoparticles synthesis from sustainable point of view: 2000 to 2023—A review on game changing materials. *Heliyon*, 8(12).
- Iravani, S., Korbekandi, H., Mirmohammadi, S. V., and Zolfaghari, B. 2014. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in pharmaceutical sciences* 9(6): 385-406.
- Kandwal, A., Parveen, S., Bachheti, R. K., Bachheti, A., and Khajuria, A. K. 2024. Green synthesis of silver and silver oxide nanoparticles from plants and their characterization. In *Metal and metal-oxide based nanomaterials: synthesis, agricultural, biomedical and environmental interventions*. Singapore: *Springer Nature Singapore*. pp. 1-24.
- Kovendan, K., Chandramohan, B., Dinesh, D., Abirami, D., Vijayan, P., Govindarajan, M., Vincent, S. and Benelli, G. 2016. Green-synthesized silver nanoparticles using *Psychotria nilgiriensis*: toxicity against the dengue vector *Aedes aegypti* (Diptera: Culicidae) and impact on the predatory efficiency of the non-target organism *Poecilia sphenops* (Cyprinodontiformes: Poeciliidae). *Journal of Asia-Pacific Entomology* 19(4): 1001-1007.
- Kumar S.S.D, Houreld N.N., Kroukamp E.M., and Abrahamse H. 2018. Cellular imaging and bactericidal mechanism of green-synthesized silver nanoparticles against human pathogenic bacteria. *Journal of Photochemistry & Photobiology, B: Biology: J Photochem Photobiol B*.178:259-269.doi:10.1016/j.jphotobiol.2017.11.001
- Landage, S. M., and Wasif, A. I. 2012. Nanosilver—an effective antimicrobial agent for finishing of textiles.

- International Journal of Engineering Sciences & Emerging Technologies* 4(1): 66-78.
- Lateef, A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Azeez, L., Ajibade, S.E., Ojo, S.A., Gueguim-Kana, E.B. and Beukes, L. S. 2016. Biogenic synthesis of silver nanoparticles using a pod extract of *Cola nitida*: Antibacterial and antioxidant activities and application as a paint additive. *Journal of Taibah University for Science* 10(4):551-562.
- Lokole, P. B., Byamungu, G. G., Mutwale, P. K., Ngombe, N. K., Mudogo, C. N., Krause, R. W., and Nkanga, C. I. 2024. Plant-based nanoparticles targeting malaria management. *Frontiers in Pharmacology* 15: 1440116.
- Mehwish, H. M., Rajoka, M. S. R., Xiong, Y., Cai, H., Aadil, R. M., Mahmood, Q, He, Z and Zhu, Q. 2021. Green synthesis of a silver nanoparticle using *Moringa oleifera* seed and its applications for antimicrobial and sun-light mediated photocatalytic water detoxification. *Journal of Environmental Chemical Engineering* 9(4): 105290.
- Mittal, A. K., Chisti, Y., and Banerjee, U. C. 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology advances* 31(2): 346-356. <https://doi.org/10.1016/j.biotechadv.2013.01.003>
- Mohammed, A. A., Mohamed, A., El-Naggar, N. E. A., Mahrour, H., Nasr, G. M., Abdella, A., Ahmed, R.H., Irmak, S., Elsayed, M.S.A, Selim, S., Elkesh, A., Alkhalifah, D.H.M, Hozzein, W.N. and Ali, A. S. 2022. Antioxidant and antibacterial activities of silver nanoparticles biosynthesized by *Moringa oleifera* through response surface methodology. *Journal of Nanomaterials* 2022(1): 9984308.
- Mondal, N. K., Chowdhury, A., Dey, U., Mukhopadhyay, P., Chatterjee, S., Das, K., and Datta, J. K. 2014. Green synthesis of silver nanoparticles and its application for mosquito control. *Asian Pacific Journal of Tropical Disease* 4: S204-S210.
- Moodley, J. S., Krishna, S. B. N., Pillay, K., Sershen, F., and Govender, P. 2018. Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. *Advances in Natural Sciences: Nanoscience and Nanotechnology* 9(1): 015011.
- Murugan, K., Dinesh, D., Kumar, P. J., Panneerselvam, C., Subramaniam, J., Madhiyazhagan, P., and Benelli, G. 2015. *Datura metel*-synthesized silver nanoparticles magnify predation of dragonfly nymphs against the malaria vector *Anopheles stephensi*. *Parasitology research* 114(12): 4645-4654.
- Musoke, D., Atusingwize, E., Namata, C., Ndejjo, R., Wanyenze, R. K., and Kanya, M. R. 2023. Integrated malaria prevention in low-and middle-income countries: a systematic review. *Malaria Journal* 22(1): 79.
- Nalinya, S., Musoke, D., and Deane, K. 2022. Malaria prevention interventions beyond long-lasting insecticidal nets and indoor residual spraying in low-and middle-income countries: a scoping review. *Malaria journal* 21(1): 31.
- Onen, H., Luzala, M. M., Kigozi, S., Sikumbili, R. M., Muanga, C. J. K., Zola, E. N., ... and Memvanga, P. B. 2023. Mosquito-borne diseases and their control strategies: an overview focused on green synthesized plant-based metallic nanoparticles. *Insects* 14(3): 221.
- Priya S. and Santhi S. 2014. A Review on Nanoparticles in Mosquito Control – A Green Revolution in Future. *International Journal for Research in Applied Science & Engineering Technology* Volume 2 Issue XII, December, 2014.
- Rai, M., Bonde, S., Golinska, P., Trzcińska-Wencel, J., Gade, A., Abd-Elsalam, K., Shende, S., Gaikwad, S. and Ingle, A. 2021. *Fusarium* as a novel fungus for the synthesis of nanoparticles: mechanism and applications. *Journal of Fungi* 7(2): 139.
- Rai, M., Yadav, A., and Gade, A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances* 27(1): 76-83.
- Restrepo, C. V., and Villa, C. C. 2021. Synthesis of silver nanoparticles, influence of capping agents, and dependence on size and shape: A review. *Environmental Nanotechnology, Monitoring & Management* 15: 100428.
- Sangeetha, R., Pavithra Niranjana, P. N., and Dhanalakshmi, N. 2016. Characterization of silver nanoparticles synthesized using the extract of the leaves of *Tridax procumbens*. *Research Journal of Medicinal Plants* 10(2): 159–166.
- Santhosh, S. B., Yuvarajan, R., and Natarajan, D. 2015. *Annona muricata* leaf extract-mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector. *Parasitology research*. 114(8): 3087-3096.
- Sarkar, S., Jana, A. D., Samanta, S. K., and Mostafa, G. (2007). Facile synthesis of silver nano particles with highly efficient anti-microbial property. *Polyhedron* 26(15): 4419-4426.
- Schäfer, R.B., Liess, M., Altenburger, R., Filser, J., Hollert, H., Roß-Nickoll, M., Schäffer, A. and Scheringer, M. 2019. Future pesticide risk assessment: narrowing the gap between intention and reality. *Environmental Sciences Europe*. 31(1):21. <https://doi.org/10.1186/s12302-019-0203-3>
- Selvam, K., Sudhakar, C., Govarthanan, M., Thiyagarajan, P., Sengottaiyan, A., Senthilkumar, B., and Selvankumar, T. 2017. Eco-friendly biosynthesis and characterization of silver nanoparticles using *Tinospora cordifolia* (Thunb.) Miers and evaluate its antibacterial, antioxidant potential. *Journal of Radiation Research and Applied Sciences* 10(1): 6-12.
- Service, M. W. (1976). Mosquito ecology: field sampling methods.
- Service, M. W. (1993). Mosquitoes (Culicidae). In Medical insects and arachnids Dordrecht: Springer Netherlands. pp. 120-240.
- Shahid, M., Zhou, Y., Cheng, X. W., Zar, M. S., Chen, G., and Tang, R. C. 2018. Ferulic acid promoted in-situ generation of AgNPs@ silk as functional colorants. *Journal of Cleaner Production* 176: 736-744.
- Silver, J. B. 2008. Mosquito ecology: field sampling methods. Dordrecht: Springer Netherlands.
- Singh, B., Kumar, D., Soni, M., Sinha, J., Saroha, P., Sharma, K., ... and Singh, H. 2025. Larvicidal and anti-bacterial efficacy of silver nanoparticles derived from *Oxalis corniculata*. *Bioscience Nanotechnology*, 1(1), 2.
- Sökmen, M., Alomar, S. Y., Albay, C., and Serdar, G. 2017. Microwave assisted production of silver nanoparticles using green tea extracts. *Journal of Alloys and Compounds*, 725, 190-198.
- Sujitha, V., Murugan, K., Paulpandi, M., Panneerselvam, C., Suresh, U., Roni, M., Nicoletti, M., Higuchi, A., Madhiyazhagan, P., Subramaniam, J., Dinesh, D., Vadivalagan, C., Chandramohan, B., Alarfaj, A. A., Munusamy, M. A., Barnard, D. R., and Benelli, G. 2015. Green-synthesized silver nanoparticles as a novel control tool against dengue virus (DEN2) and its primary vector *Aedes aegypti*. *Parasitology research* 114(9): 3315–3325. <https://doi.org/10.1007/s00436-015-4556-2>

- Suresh, U., Murugan, K., Panneerselvam, C., Rajaganesh, R., Roni, M., Al-Aoh, H. A. N., Trivedi, S., Rehman, H., Kumar, S., Higuchi, A. and Canale, A. 2018. Suaeda maritima-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector *Aedes aegypti* and the tobacco cutworm *Spodoptera litura*. *Physiological and Molecular Plant Pathology* 101: 225-235.
- Torto, B., and Tchouassi, D. P. 2021. Grand challenges in vector-borne disease control targeting vectors. *Frontiers in Tropical Diseases* 1: 635356.
- World Health Organization (WHO). 1995. Manual on practical entomology in malaria. World Health Organization.
- World Health Organization (WHO). 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/13.
- World Health Organization (WHO). 2005. Guidelines for laboratory and fieldtesting mosquito larvicides. http://www.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_13.pdf.
- Xu, L., Wang, Y. Y., Huang, J., Chen, C. Y., Wang, Z. X., and Xie, H. 2020. Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics* 10(20): 8996–9031. <https://doi.org/10.7150/thno.45413>
- Zhou, Y., Zhang, W. X., Tembo, E., Xie, M. Z., Zhang, S. S., Wang, X. R., ... and Lu, Q. B. 2022. Effectiveness of indoor residual spraying on malaria control: a systematic review and meta-analysis. *Infectious Diseases of Poverty* 11(04): 29-42