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Larvicidal potential of two silver nanoparticles (*Moringa oleifera* and *Ficus exasperata*) against laboratory and field strains of *Anopheles gambiae* (Diptera: Culicidae) in Lagos, Nigeria

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Abstract

Background: The interest in larvicides of plant origin is generally renewed in vector control because of their safety compared to synthetic larvicides. However, there are concerns about the relative safety dose of these phytochemicals on non-target organisms which led to the development of plant derived nanoparticles. In this study, we examined the bioefficacy of low doses of two green synthesized nanoparticles on immature stages of *Anopheles* mosquitoes in Nigeria. Aqueous plants (*Moringa oleifera* and *Ficus exasperata*) extracts were used in the biosynthesis. The prepared Ag-NPs were characterizations using Fourier-transform infrared spectroscopy (FT-IR), UV-Vis spectroscopy, and scanning electron microscopy (SEM). Third and early fourth instars of known susceptible laboratory strains of *Anopheles gambiae s.s.* (KISUMU strains) and pyrethroid resistant field strain of *An. gambiae* were exposed to serial dilutions of 0.25, 0.5, 0.75, 1.0 and 2.5 ppm of each phyto nanoparticles. Moribund and dead larvae were observed after 24 and 48 h post exposure, and the results were analysed with descriptive statistics.

Results: With the laboratory mosquitoes, *Moringa oleifera* AgNP effected high mortalities of 88–100% ($LC_{50} = 0.39$ ppm; $LC_{95} = 0.62$ ppm) at 24 h post exposure except at the lowest concentration, while *Ficus exasperate* AgNP induced a 32–100% mortality ($LC_{50} = 0.51$ ppm; $LC_{95} = 1.15$ ppm) except at the lowest concentration. In the field populations, mortality in *Moringa oleifera* and *Ficus exasperata* was 23–93% ($LC_{50} = 0.65$ ppm; $LC_{95} = 2.28$ ppm) and 37–50% ($LC_{50} = 1.51$ ppm; $LC_{95} = 391.64$ ppm) respectively. There was no significant difference in mortality values between the laboratory and field strains ($P < 0.05$) at both 24 and 48 h post exposure times.

Conclusions: Overall, the study demonstrates the bioefficacy and potential use of green synthesized nanoparticles, at very low concentrations for the control of *Anopheles* larvae even in areas where resistance to the current chemical insecticides have been reported.

Keywords: Silver nano-particle larvicides, Laboratory and field strains, *Anopheles gambiae*, Nigeria

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Background

The relative importance of mosquitoes in disease transmission has made them the target of several life cycle control activities including chemical, non-chemical and/or biological control (WHO, 2017a).

The four classes of chemical-based control are Pyrethroids, Organophosphates, Organochlorines and Carbamates (Mazzarri & Georghiou, 1995) which are quite impactful when deployed (WHO, 2017a); however, their drawback is the emerging development of resistance in mosquito species (Liu, 2015). Since 2010, a total of 60 countries have reported resistance to at least one class of insecticide, with 49 of those countries reporting *Anopheles* resistance to two or more classes (WHO, 2017a). Larval control is a complement to major vector control interventions such as the use of long-lasting insecticide-treated nets (LLINs) and indoor residual spray (IRS) (Killeen, Fillinger, & Knols, 2002; WHO, 2017b). The concerns over chemical larvicides (physiological resistance by vectors, adverse environmental effects, high operational cost and community acceptance) necessitate sourcing for improved eco-friendly agents. Furthermore, malaria vector resistance to permethrin and deltamethrin has been reported in various parts of Lagos State (Awolola et al., 2018; Fagbohun, Oyeniyi, Idowu, Otubanjo, & Awolola, 2019; Oduola et al., 2012). On this note, plant extracts have in some cases found interesting use as mosquito repellents (Samidurai, Jebanesan, Saravanakumar, Govindarajan, & Pushpanathan, 2009), and for the management of larvae or adult mosquitoes (Ghosh, Chowdhury, & Chandra, 2012; Interior Health, 2009). While the confinement provided by larval sites contributes to the potency of phytochemicals, concerns however exist on the dosage and relative toxicity for programmatic control. To improve the efficacy of the larvicides, nanoparticles are incorporates as delivery agents, particularly with sizes of 10–1000 nm, thereby making the larvicides more powerful even at low doses (Mondal et al., 2014).

Several biosynthetic pathways to AgNPs using biomass extracts of *Citrus limon* (Mohapatra, Kaintura, Singh, Kuriakose, & Mohapatra, 2015), *Parthenium hysterophorus* (Mondal et al., 2014), *Thevetia peruviana* (Oluwaniyi et al., 2016), *Ocimum sanctum* (Singhal, Bhavesh, Kasariya, Sharma, & Singh, 2011) and pineapple leaf (Elemike et al., 2014) have been reported. Meanwhile, larvicidal activities of AgNPs using different plant extracts such as *Agaricus bisporus* (Dhanasekaran & Thangaraj, 2013), *Agave sisalana* (Nunes et al., 2015), *Azadirachta indica* (Neem) (Soni & Prakash, 2014), *Delphinium denudatum* (Suresh et al., 2014), *Feronia elephantum* (Veerakumar, Govindarajan, Rajeswary, & Muthukumaran, 2014), *Ficus racemosa* (Velayutham, Rahuman, Rajakumar, et al., 2013), *Morinda tinctoria* (Kumar, Nattuthurai, Gopinath, & Mariappan, 2014) and *Sterculia foetida* L. (Rajasekharreddy & Rani, 2014) have been reported against different larvae stages of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Hitherto there has been no report on the biosynthesis of AgNPs using *Ficus exasperata*. In the present study, we report the biosynthesis of silver nanoparticles using the aqueous extracts of *Moringa oleifera* and *Ficus exasperate* and their larvicidal potential explored against laboratory and suspected resistant field strains of *Anopheles gambiae* mosquitoes in Lagos Nigeria.

Methods

Preparation of plant extracts

The taxonomic identification of freshly collected leaves of *Moringa oleifera* family Moringaceae and *Ficus exasperata* family Moraceae were done at the Herbarium, Department of Botany, University of Lagos, Lagos, Nigeria. Thereafter, after thoroughly washing the leaves with distilled water and drying with paper absorbents, 40 g of leaves was soaked in 400-ml distilled water for 5 min before boiling for 15 min, allowed to cool and filtered through Whatman-1 filter paper. The filtrate was kept refrigerated until further use (Nilanjuna, Samrat, & Piyali, 2014).

Synthesis of silver nanoparticles ($AgNO_3$)

Three hundred millilitres of distilled water was added to 2.54 g of silver nitrate and stirred to give (0.05 M) silver nitrate solution. The solution was stirred with a magnetic stirrer for 5 min during the addition of 20 ml of the plant extract and observed for colour change. The initial pale yellow colour of the mixture changed to dark brown after 72 h indicating the reduction from Ag^+ to Ag^0. The resulting mixture was centrifuged at 4000 rpm for 20 min. The supernatant was discarded and the residue was oven dried at 70 °C to afford the silver nanoparticles as a brownish powder (Umoren, Obot, & Gasem, 2014).

Characterization of silver nanoparticles ($AgNO_3$)

Evaluation of the chemical composition of the surface of the silver nanoparticles and the molecular environment of the capping agents were performed using Fourier transform infrared spectroscopy (FTIR) with attenuated total reflectance (Bruker- tensor 27) operating in the wavenumber range of 4000–500 cm^{-1}. The microstructure of the samples were evaluated in a scanning electron microscope (SEM) (Quanta 200 FEG model) equipped with an electron probe microanalyzer system. The samples were attached to a sample holder via carbon adhesive before being observed at an accelerating voltage of 15 kV. Absorbance values were measured by a UV-Vis spectrophotometer (Varian cary 50 Bio UV-VIS spectrophotometer).

Collection of field Anopheline mosquitoes

Laboratory bred *Anopheles gambiae s.s.* (KISUMU strains) were taken from insectary at Nigerian Institute of Medical Research, Yaba, Lagos. This mosquito colony has been maintained at the insectary for over 10 years and known to be susceptible to all classes of insecticide. Field *Anopheles* mosquitoes, however, were collected from areas where resistant Anopheline populations have been reported in and around Lagos State (Adeogun, Popoola, Oduola, Olakiigbe, & Awolola, 2017; Awolola, Oduola, Obansa, Chukwurah, & Unyimadu, 2007; Oduola et al., 2012) and kept under standard insectary condition (25–29 °C; 78–82% relative humidity). All the field Anopheline samples collected were identified morphologically using the standard taxonomic key (Gillies & Coetzee, 1987).

Determination of larvicidal activities of the silver nanoparticles of *Moringa oleifera* and *Ficus exasperata* against *Anopheles gambiae* complex

Twenty-five late third instars of *Anopheles* mosquito larva were released into 100-ml distilled water containing a mixture of nanoparticles at different doses 0.25 ppm, 0.5 ppm, 0.75 ppm, 1.0 ppm and 2.5 ppm, each in four replicates. A total of 100 larvae in four replicates were kept in 100-ml water without phyto-nanoparticles (used as control). Larval mortality was observed every 10 min for 1 h for immediate mortality and then observed for 24 h and 48 h post exposure. Lethal concentration that killed 50% and 90% of the mosquito larva (LC_{50} and LC_{90}) was determined using Probit regression analysis with the statistical software package (SPSS).

Result

Fourier transform infrared spectroscopy analysis of the AgNPs

Surface characterisation and functionalities of the AgNPs were evaluated using Fourier transform infrared spectroscopy. The available functionalities provide insight into the viability of the suspected capping agents to achieve reduction of the silver ions to afford the silver nanoparticles. The FTIR spectra of silver nanoparticles displayed diagnostic peaks in Fig. 1, initially as a poorly resolved peak centered around 1010 cm^{-1} assigned to C-N stretching of the amine, at 1542 cm^{-1} N-H bending of amide, the broad band at 3500–2900 cm^{-1} is due to bounded hydroxyl (–OH) or amine group (–NH) and aliphatic (–CH). Also, the peak centered around 1626 cm^{-1} was assigned to carboxyl group (–C=O) stretching vibration, while bands at 1000 cm^{-1} may be attributed to C-O stretching vibrations.

In Fig. 2, the broad absorption central around 3500 to 2600 cm^{-1} may be attributed to O-H stretching vibration, at 1277 cm^{-1} O-H bending vibrations, while bands at 1752 and 1641 cm^{-1} are characteristic of carboxyl group (–C=O) stretching vibrations, at 1551 cm^{-1} N-H bending of amide, similarly bands indicating the presence of hydroxyl groups at 950 to 1030 cm^{-1} may be attributed to C-O stretching vibrations (Badri & Natarajan, 2010; Maria et al., 2007; Thirumurgan, Tomy, Jai Ganesh, & Gobikrishnan, 2010).

UV-Vis spectroscopy analysis of AgNPs

The absorption spectra of silver nanoparticles synthesized from *Moringa oleifera* and *Ficus exasperata* extracts are shown in Fig. 3, and furthermore providing information on the sizing characteristics. The UV-Vis absorption maxima were obtained at 430 and 440 nm for *Moringa oleifera* and *Ficus exasperata* respectively. The bands are attributed to the surface plasmon resonance of AgNPs formed by reduction of aqueous Ag ions (Ali et al., 2015).

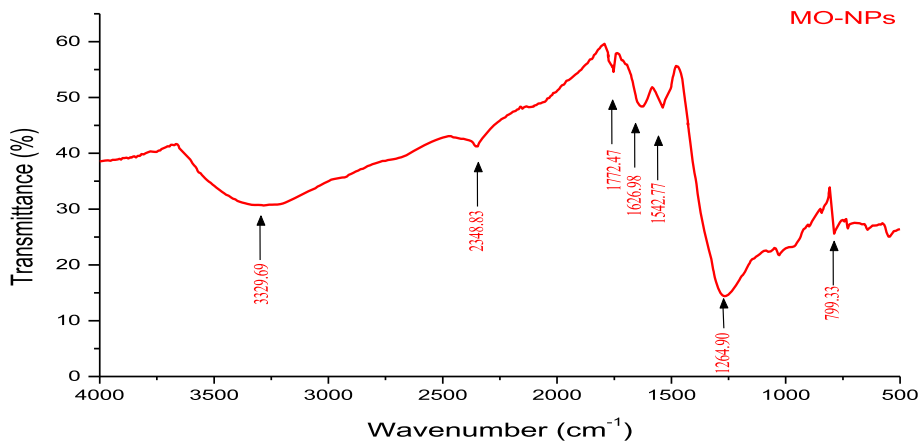

Fig. 1 Fourier transform infrared spectroscopy (FTIR) spectra of silver nanoparticles synthesized from *M. oleifera* leaf extract

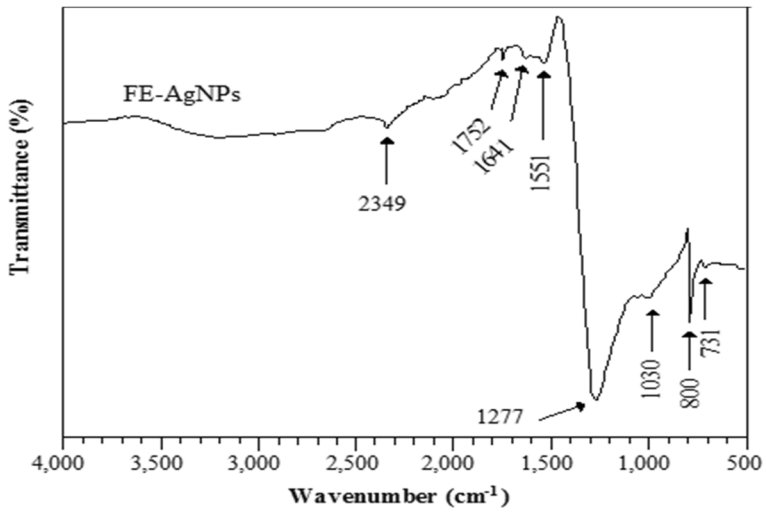

Fig. 2 Fourier transform infrared spectroscopy (FTIR) spectra of silver nanoparticles synthesized from *F. exasperata* extract

Morphology of synthesised AgNPs

The SEM micrographs of the silver nanoparticles which are presented in Fig. 4a and b provides information on the morphological pattern of the *as* prepared MO-AgNPs and FE-AgNPs respectively. Analysis of the micrographs shows that the morphologies are characterized by closely packed particles that are well distributed to give homogeneous surfaces of small pore sizes. The unit sizes for both MO-AgNPs and FE-AgNPs were as small as 500 nm which are shown in the red circle on the SEM and in the particle size distribution chart in Fig. 5. It is evident that the smaller particles formed larger aggregates of about 500 nm during the biosynthesis. The results obtained confirm that plant extracts can effectively control the shape and size of the AgNPs.

Figure 6 shows the 3-D plots of the AgNPs surface morphology. The aggregation characteristics of the NPs show a more even distribution in the MO-NPs compared to the FE-NPs. The surface of the FE-NPs shows larger relative particle sizes and thus provides larger pore spaces.

Larvicidal activity

The laboratory strains of *Anopheles*, KISUMU strains, used for this work are known to be susceptible to all classes of insecticides and were all *Anopheles gambiae s.s*. A total of 1400 field samples were used in the study were all identified as members of the *Anopheles gambiae s.l.* The results of the larvicidal activities of *Moringa oleifera* and *Ficus exasperata* phyto-nanoparticles are shown in Fig. 7. Mortality rate appears to be dose dependent with the percentage mortality increasing with dose and time of exposure. Laboratory mosquitoes were more sensitive to the effect of the nanoparticles than the field

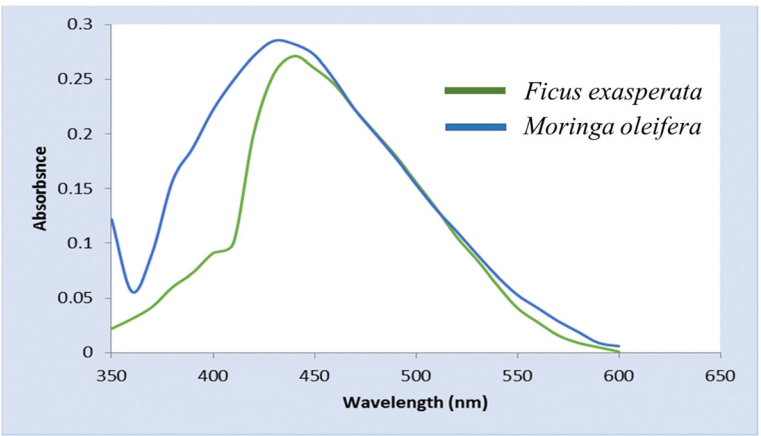

Fig. 3 UV-Vis absorption spectra of silver nanoparticles synthesized by *Moringa oleifera* (blue line) and *Ficus exasperata* (green line)

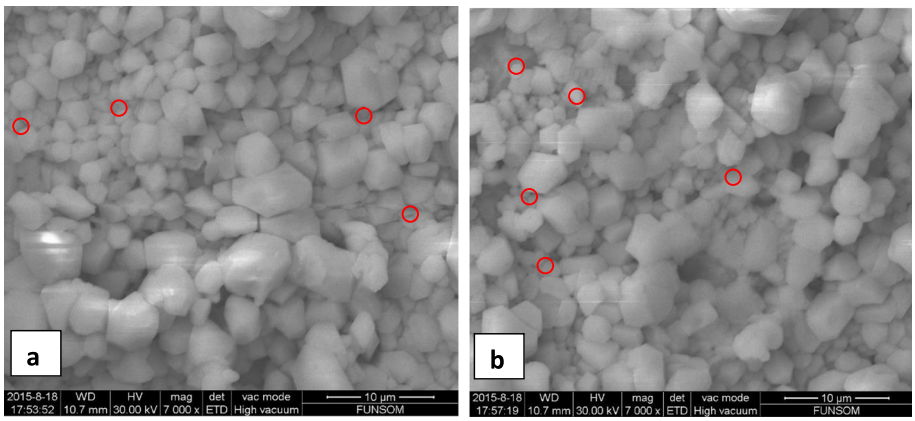

Fig. 4 SEM micrographs of the synthesized silver nanoparticles using plant extracts of **a** *Moringa oleifera* and **b** *Ficus exasperata*

strains (Fig. 7). In the laboratory mosquitoes, higher mortality (88–100%) was observed from 0.5 ppm for *Moringa oleifera* while for *Ficus exasperate*, mortality was (32–100%) was observed at a slightly higher concentration of 0.75 ppm at the 24 h and 48 h post exposure. However, with the field collected mosquitoes, *Moringa oleifera*, 23–93% mortality; *Ficus exasperata*, 37–50% at the same concentrations (Fig. 7).

The LC_{50} and LC_{95} values of the synthesized nanoparticles followed the same trend as the mortality values. At the 24 h post exposure period, 0.39 and 0.6 ppm of *Moringa oleifera* were required to kill 50% and 95% of the laboratory mosquitoes while relatively higher concentrations of 0.65 and 2.28 ppm were required to kill the field collected mosquitoes respectively. As for *Ficus exasperata*, the LC_{50} and LC_{95} concentrations were 0.51 and 1.15 ppm and 1.51 and 391.64 ppm for laboratory and field collected mosquitoes respectively (Table 1). In terms of bioactivity, it appears that *Moringa oleifera* tends to be more effective at lower concentrations as compared with *Ficus exasperata*. A comparison of the mortality values of the laboratory and field collected mosquitoes indicates no significant difference between the two at both 24 h and 48 h post exposure period ($P < 0.05$).

Discussion

The FTIR spectra of silver nanoparticles displayed diagnostic peak characteristics of the available functionalities that facilitated the reduction of the silver ions to afford the silver nanoparticles. Characteristic functionalities include amide, amine, hydroxyl, carbonyl of amide and aliphatics. The result of the surface functionality study is indicative of the presence of functional groups that are capable of reducing the silver ions as well as stabilization of the silver nanoparticles obtained in the form of capping agents. The UV-Vis spectroscopy provides details

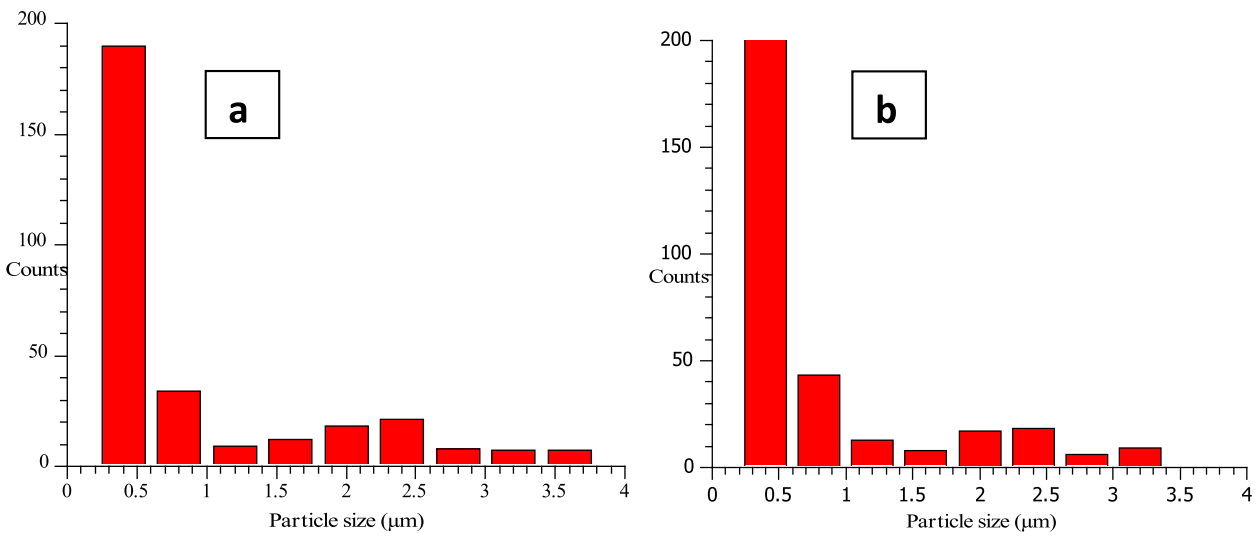

Fig. 5 Particle size distribution for the synthesized silver nanoparticles using plant extracts of **a** *Moringa oleifera* and **b** *Ficus exasperata*

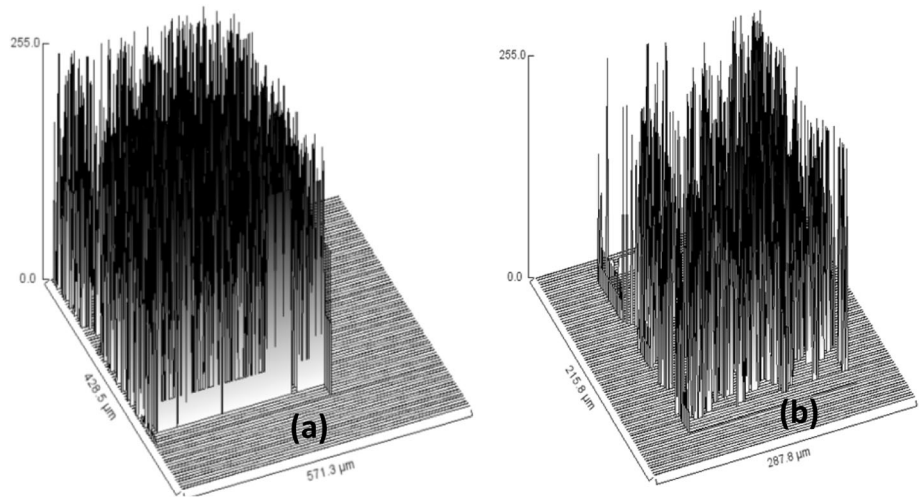

Fig. 6 3-D plots showing arrangement of particles on the surface of *Moringa oleifera* (**a**) and *Ficus exasperata* (**b**). Surface of the FE-AgNPs presents evidence of larger pore spaces

of the absorption maxima while the pattern can be used to make informed inference on the characteristics of the particle sizing. In this study, the absorption maxima were observed at 430 and 440 nm for *Moringa oleifera* and *Ficus exasperata* respectively. Apparently, they were not broad, thereby indicating a relatively narrow size range for the nanoparticles (Ali et al., 2015).

The average particle sizes were further elucidated using the software ImageJ. While the morphology of the particles are characterized by closed packing which are well distributed to give homogeneous surfaces of small pore sizes. The observed pattern is however influenced by a number of factors such as precursor and extract concentrations, incubation time and reaction temperature. It is also evident that nucleation of the smaller particles afforded the larger aggregates observed with size around 500 nm. The MO-NPs were observed to be more evenly distributed relative to the FE-NPs.

Efforts at exploiting the potentials of plant derived substitute for chemical larvicides have yielded several phyto-chemicals with bioactivities against mosquitoes (Edriss, Satti, & Alabjar, 2012; Kamaraj et al., 2011; Rathy, Sajith, & Harilal, 2015). As compared with other larvicides, *Moringa oleifera* and *Ficus exersperata* have exhibited activity against *Aedes aegypti*, *Culex quiquinfasciatus* and *Anopheles stephesis* mosquitoes. In this regards, extracts of *Moringa oleifera* without AgNPs exhibited (Prabhu, Murugan, Nareshkumar, Ramasubramanian, & Bragadeeswaran, 2011) LC_{50} and LC_{90} values ranging between 57.79 and 143.20 ppm. This may be partly due to the presence of Quercetin and kaempferol which have antioxidant properties with potential therapeutic uses (Pace-Asiak, Hahn, Diamandis, Soleas, & Goldberg, 1995). Interestingly, a much reduced concentration of the phytochemicals with the introduction of AgNPs has been reported with a concentration range of 10.24–21.17 ppm for L1-Pupal samples of *Aedes aegypti* (Sijutha et al., 2015).

In this study, the efficacy of *Moringa oleifera* and *Ficus exersperata* at low concentrations could be adduced to the presence of the AgNPs. The mechanism by which this occurred could be the ability of the AgNPs to penetrate through larval membranes by binding to sulphur-containing proteins or phosphorous containing compounds like DNA, leading to the denaturation of some organelles and enzymes (Rai, Yadav, & Gade, 2009). Additionally, MO-AgNPs and FE-AgNPs killed most of the laboratory strains of *Anopheles gambiae s.s.* (KISUMU strains) at concentrations between 0.75 and 2.5 ppm with a higher range of 0.75 and 2.5 ppm for MO-AgNps and 2.5 ppm for FE-AgNPs respectively in the field strains. This is comparable with reports which also showed extremely low concentrations that fall within 1–30 mg/l (Dinesh et al., 2015; Rai et al., 2009; Rawani, Ghosh, & Chandra, 2013). The result of this study can also be favourably compared with that of wild Indian almond tree, *Sterculia foetida* which showed LC_{50} values lower than 4.5 ppm against *Anopheles stephensi*, *Aedes aegypti,* and *Culex quinquinfasciatus* (Rajasekharreddy & Rani, 2014).

The reason for the differences between the laboratory mosquitoes and the field strains data is still not yet ascertained, but it is quite plausible to be the result of the insecticidal resistance of the field strains. Reports from this study area have showed that mosquito population are resistant to all classes of insecticide available for use in public health (Adeogun et al., 2017; Oduola et al., 2010, 2012). Though information on the use of

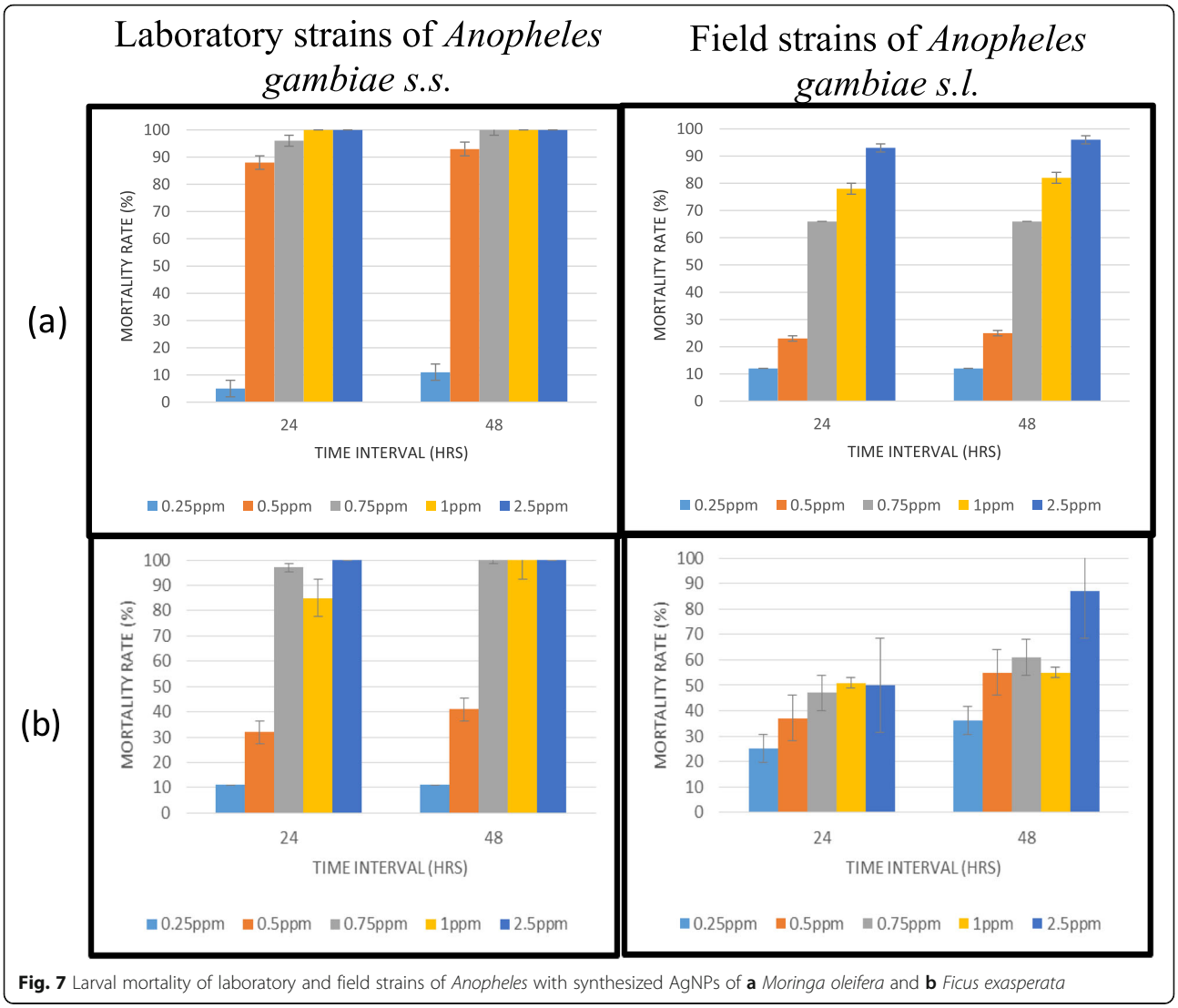

Fig. 7 Larval mortality of laboratory and field strains of *Anopheles* with synthesized AgNPs of **a** *Moringa oleifera* and **b** *Ficus exasperata*

phytochemicals for larval control from this area is not available, it is believed that the high level of insecticide resistance that has been reported in the populations of mosquitoes collected from the area might have had effect on the sensitivity of the mosquito populations at the initial stage of 24 h post exposure which decreased over time. Phytochemicals are known to have extended efficacy and usually have more than the "contact and kill" effect on insects; they show multiple effects which can reduce the longevity of the mosquitoes and expose them

Table 1 Larvicidal toxicity of synthesized (AgNPs) against field and laboratory strains of *Anopheles gambiae*

Extract+AgNP	24 h			48 h		
	LC 50 (95%C.L.)	LC 50 (95%C.L.)	Slope ± SE	LC 50 (95%C.L.)	LC95 (95%C.L.)	Slope ± SE
Moringa+AgNP(LS)	0.39 (0.30–0.45)	0.62 (0.50–1.00)	3.26 ± 0.27	0.34 (0.32–0.36)	0.52 (0.48–0.58)	4.23 ± 0.3
Moringa+AgNP(FS	0.65 (0.40–1.01)	2.28 (1.33–15.37)	0.56 ± 0.08	0.62 (0.43–0.87)	1.93 (1.23–7.07)	0.69 ± 0.08
χ^2 ***(P value)***	*15.0(0.24)*			*10.04(0.27)*		
Ficus+AgNP (LS)	0.51 (0.09–0.99)	1.15 (0.71–2.61)	1.36 ± 0.12	0.45 (0.18–0.75)	0.82 (0.56–19.61)	2.21 ± 0.18
Ficus+AgNP (FS)	1.51 (1.01–3.53)	391.64 (47.89–500.29)	− 0.12 ± 0.60	0.49 (0.13–0.83)	8.58 (2.79–8.99)	0.41 ± 0.07
χ^2 ***(P value)***	*20.0 (0.22)*			*6.67 (0.35)*		

P is significant at $P < 0.05$
LS laboratory strain, *FS* field strain

to adverse conditions and predation, and can also make resistance unlikely (Vatandoost & Vaziri, 2004). This was believed to have been exhibited in these populations as the significant difference in mortality values between the laboratory and field collected mosquitoes decreased over time. Though we did not observe the population beyond 48 h, it is possible for the values to decrease further and may later become insignificant indicating that insecticide resistance may not be a barrier to the efficacy of the nanoparticles.

Conclusion

We have reported herein the biosynthesis of AgNPs using aqueous extracts of *Moringa oleifera* and *Ficus exersperata.* The efficacy of the *Moringa oleifera* nanoparticles was higher at lower concentrations than that of *Ficus exasperate* on both the laboratory and field collected mosquitoes. Factors such as insecticide resistance in the field mosquito population do not appear to have effect on the efficacy of the two extracts as mortality increased with increase in time of exposure. This shows potentials of the nanoparticles for use as part of integrated approach towards the fight against mosquito vectors.

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Declarations

The content of this article is the sole responsibility of the author(s) and does not necessarily reflect the views or policies of anyone or organization.

Authors' contributions

IET, AOA, LAA, MAY, OWS and OAK carried out the field and laboratory studies; IET, OOA, LAA, AOA, JAB and ATS design and supervised the study; AOA, MAY, OWS and FIK carried out the data analysis and wrote the first manuscript draft; IET, JAB, OAT and OOA critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data generated during this study are included in this published article and also available upon request at the Vector research laboratory of Nigeria Institute of Medical Research (NIMR), Lagos.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared that there are no competing interests.

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