

Nanoinsecticidal Efficacy of Ag/Ni Bimetallic Nanoparticles (BMNPs) on Lymphatic Filariasis Vector

Danbature Wilson Lamayi¹, Ezra Abba^{2*}, Zaccheus Shehu¹
and Muhammad Mustapha Adam¹

¹Department of Chemistry, Faculty of Science, Gombe State University, Nigeria.

²Department of Zoology, Faculty of Science, Gombe State University, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DWL and ZS designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors EA and MMA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Nanoparticles are gradually gaining wide scientific interest due to their various applications in catalysis, magnetism, medicine, optics, as antibacterial and nanolarvicidal agents. This research aimed at evaluating the larvicidal activity of green synthesized Ag/Ni BMNPs from the aqueous root extract of *Borassus aethiopum* as the stabilizing agent as well as their spectroscopic investigation using UV-Visible and FT-IR spectroscopy.

Place and Duration of Study: The study was conducted in Gombe State University between August and December, 2019.

Methodology: In this study, Ag/Ni hybrid bimetallic nanoparticles was synthesized using an eco-friendly method from the secondary metabolites of *Borassus aethiopum* acting as the reducing agent.

*Corresponding author: E-mail: ezra.abba@gmail.com;

Results: Optical measurements using UV-Vis showed the maximum absorption wavelength at 410nm while the FT-IR result for the root extract showed peaks at 3443.26cm^{-1} , 2929.48cm^{-1} , 1651.28cm^{-1} , and 1080.12cm^{-1} corresponding to OH stretch, sp^3 C-H stretch, C=C stretch and C-O-C stretching respectively. These were replaced in the spectra of the BMNPs with the absence and appearance of some others indicating that they were involved in the capping process. The lethal concentration (LC_{50}) was found to be 5.730, 13.585 and 15.735 mg/L for 1st, 2nd and 3rd/4th instars respectively. Also, the lethal concentration (LC_{90}) was found to be 88.444, 195.689 and 236.889 mg/L for 1st, 2nd and 3rd/4th instars respectively.

Conclusion: The larvicidal bioassay result showed a dose-dependent mortality rates against *Culex quinquefasciatus* larvae which suggest they can be developed to control the insect population.

Keywords: Ag/Ni BMNPs; *Culex quinquefasciatus*; larvicidal activity; lethal concentration; lymphatic filariasis.

1. INTRODUCTION

Mosquito vectors are solely responsible for transmitting many diseases such as malaria, dengue fever, chikungunya, Japanese encephalitis, lymphatic filariasis (LF), yellow fever, West Nile Virus Infection, etc. [1,2]. The diseases classified as Neglected Tropical Diseases (NTD) are those diseases that have a history of being overlooked and neglected at international, national and even community levels [3-5]. Lymphatic filariasis, also referred to as Bancroftian filariasis or elephantiasis is one of the devastating NTD globally and is caused mainly by the nematode *Wuchereria bancrofti*. The other two species are *Brugia malayi* and *Brugia timori* [6-7]. It is a mosquito-borne disease which is majorly endemic in the tropical regions. *Anopheles* spp, *Culex* and *Mansonia* are responsible vectors of LF [8]. The constant use of synthetic mosquito repellent has resulted in resistance in mosquitoes. Insecticide resistance and lack of adequate attention to the application instructions of topical pediculicides are the major reasons for failure in treatment. In the integrated mosquito management, emphasis is laid on the application of especially bio-friendly alternative strategies in mosquito control [1-2]. Essential oils or plant extracts are good and safe alternatives due to their low toxicity to mammals and biodegradability [9]. Therefore, the development of a reliable and eco-friendly process for the synthesis of nano-dimensional materials is an utmost important aspect of nanotechnology [10].

Nanoparticles are widely used in many commercial applications including health, nutrition, environmental health and agriculture [1,11]. It plays a major role in various applications. Most researchers focused their study on bimetallic nanoparticles due to their

several modes or mechanisms of synthesis such as chemical, physical, and biological methods [12]. However, of the various method of nanoparticles synthesis, biological method is slow but can be persistent, inexpensive, and brings no harm to living organisms and ecosystem; it does not eliminate pathogens or disease, rather creates a natural balance [13]. Due to the promising nature of the research area many metals, metal oxides and hybrid bimetallics were synthesized from different origins and through different forms. The catalytic property of metal nano materials is also of great interest because of their particle size [14]. Synthesized triangular shaped Ni NPs were tested for *in-vitro* anti-inflammatory and larvicidal activity which resulted in enhancement while compared to crude leaf extracts of *Aegle marmelos* Correa [15]. Nano sized silver particles have the ability to penetrate through the cell membrane [16]. Silver nanoparticles have many applications in catalysis, surface enhanced Raman scattering, medicine, drug and gene delivery and environmental sensing [17]. Nickel nanoparticles could be used for catalytic dehydrogenation of 2-butanol to 2-butanone [18].

Also, [19] reported the synthesis Silver/Nickel allied bimetallic nanoparticles using the leaf extract of *Canna indica* acted as reducing agent. The finding suggests antibacterial application of Ag/Ni against *S. pyogenes* and *E. coli* and antifungal agent against *C. albicans*. Bimetallic Cu-Ni NPs showed a bacteriostatic effect against *S. aureus*, *E. coli* and *S. mutan* [20]. According to [21], the insecticidal activity of Ag nanoparticles against the *A. neri* was investigated. The result suggests the possibility of using Ag nanoparticles to eradicate pests and can be used as valuable tools in pest management programs of *A. neri*.

This research aimed at evaluating the larvicidal activity of green synthesized Silver/Nickel Bimetallic Nanoparticles (Ag/Ni BMNPs) from the aqueous root extract of *Borassus aethiopum* as the stabilizing agent as well as their spectroscopic investigation using UV-Visible and FT-IR spectroscopy. This work is very crucial as it meets the demand to search for bio-friendly mosquito control measure.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Aqueous Root Extract

Fresh and healthy root extracts of *Borassus aethiopum* were collected and transported from Kaltungo Local Government area, Gombe State. They were washed thoroughly first with running tap water and then rinsed with de-ionized water and grinded into small particles. The plant extract was prepared by suspending 30 g of the fine particles of the roots in 200 mL of de-ionized water and boiled on a hot plate at 80°C for 30 minutes. The extract was allowed to cool and filtered using Whatman number 1 filter paper. The filtrate was used immediately for the synthesis.

2.2 Preparation of Metal Precursors

A 10mM AgNO₃ and 10mM NiCl₂.6H₂O solutions were prepared each by suspending 0.43 g and 0.59 g of the metal salt in 250 ml de-ionized water in standard Erlenmeyer flasks respectively.

2.3 Synthesis of Silver-Nickel Bimetallic Nanoparticles

A 400mL hybrid solution containing 200 ml each equimillimolar AgNO₃ and NiCl₂.6H₂O solutions was added step wise to 80 ml of the prepared root extract while boiled on a hot plate with occasional stirring for 30 minutes at 80°C. A visual change of colour was observed. The nanoparticles obtained thus were decanted and used for further studies.

2.4 UV-Vis Spectrophotometric Analysis

Ultra Violet-Visible spectrophotometer model 6705 was used for optical measurement between the ranges of 250-800 nm wavelength by placing aliquot liquid obtained after the synthesis against de-ionized water as the reference solvent in quartz 1 x 1 cm cuvettes operated at a resolution of 1 nm. A spectrum was plotted for absorbance against the corresponding wavelengths (nm).

2.5 FT-IR Analysis

A sample of the root extract as well as the nanoparticles obtained after evaporating the supernatant liquid after the synthesis and dried in an oven were used for functional group analysis using Fourier Transform Infrared Spectrophotometer(PerkinElmer Spectrum Version 10.03.09) to identify the interaction between them.

2.6 Larvicidal Bioassay

The *Cx quinquefasciatus* larvae were collected using the [22] guidelines. Twenty larvae (1st, 2nd and 3rd/4th instars) were placed in a beaker to which 5, 10, 20, 25 and 50 ppm aliquot each of the synthesized Ag/Ni bimetallic were added and de-ionized water was added to make the solution 100 ml. Test of each concentration against each instar was replicated twice. In each case, the control comprised of 20 larvae in 100ml de-ionized water. The mortality data was recorded after 24 hours.

2.7 Statistical Analysis

Probit analysis was done to evaluate the LC₅₀ and LC₉₀ values using statistical software SPSS 2016. Also, the correlation and chi square values were computed.

3. RESULTS AND DISCUSSION

3.1 Visual Formation of Ag/Ni BMNPs and UV-Visible Spectroscopy

The change of colour in the reaction mixture, Fig. 1D from the bimetallic precursors Fig. 1 B and C as well as the green solution, Fig. 1 A to the formation of Ag/Ni BMNPs, Fig. 1 E are shown in Fig. 1. The formation of Ag/Ni Bimetallic nanoparticles was first noticed by color change from light green to dark green within 15 minutes as a result of the surface Plasmon absorption [23]. The supernatant liquid was decanted and used for UV-Visible Spectrophotometric analysis. In the bimetallic Ag/Ni NPs, there were narrow absorption spectra which increased in peak intensity without any shift in wavelength (410 nm). Similarly, Ag/Ni BMNPs was synthesized using aqueous leaf extract of *Canna indica* and the surface Plasmon resonance was found at 421 nm [19]. Hence, this signified presence of spherically shaped nanoparticles. More to that, the surface of the hybrid nanoparticles in Fig. 1 E

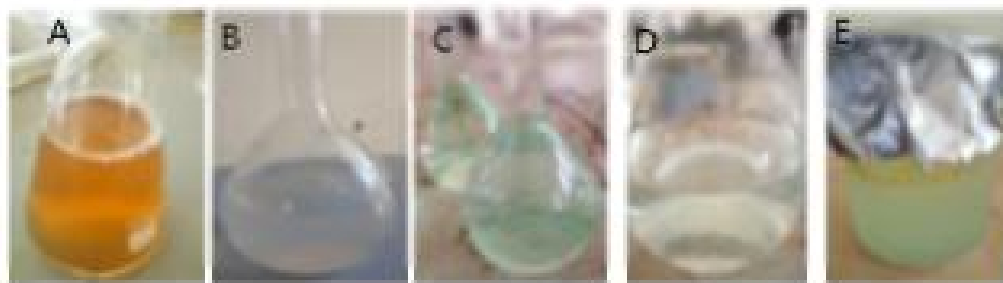


Fig. 1. A; Root extract of Palmyra, B; Silver nitrate solution, C; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ solution, D; (mixture of B and C), E; Ag-Ni BMNPs (mixture of D + A)

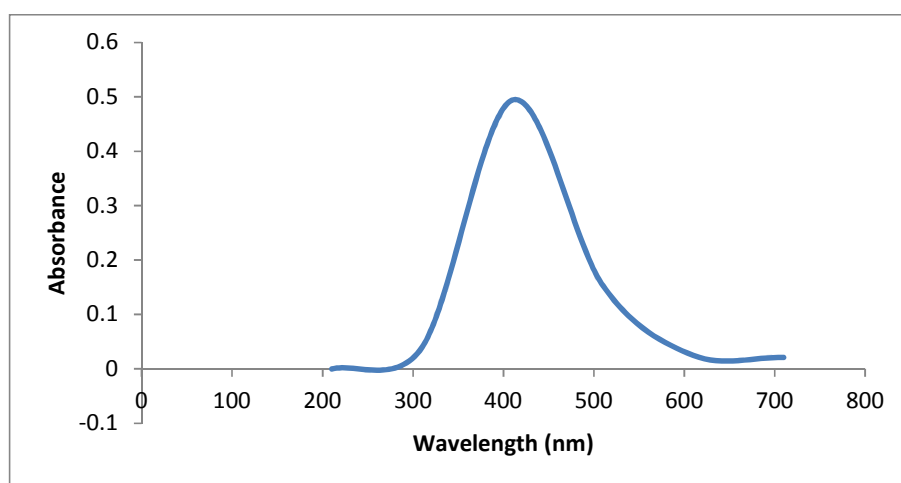


Fig. 2. UV-Visible spectrum for Ag/Ni BMNPs

is proposed to be enriched with silver, optically enhanced by nickel which is in line with previous work [24]. The UV-Visible Spectrum is depicted in Fig. 2.

3.2 FT-IR Result

Fourier Transform-Infra Red Spectroscopy was applied to investigate the interactions between the aqueous root extract of *Borassus aethiopum* and the aqueous solution of the mixture of Silver-Nickel salt. The FT-IR spectra of the root extract and for the biosynthesized Ag/Ni BNPs are displayed in Figs. 3 and 4 respectively. The FT-IR spectra for the root extract, Fig. 3 displayed peaks at 3443.26 cm^{-1} , 2929.48 cm^{-1} , 1651.28 cm^{-1} , 1384.14 cm^{-1} and 1080.12 cm^{-1} . These were replaced in the spectra of the Ag/Ni BMNPs, Fig. 4 with bands due to O-H stretching vibration mode at 3391.48 cm^{-1} which overlapped with the N-H stretching in terpenoid found within this region, a medium sharp peak for C-H absorption at 2924.52 cm^{-1} , C=C stretching

at 1626.76 cm^{-1} , C-O deformation at 1046.97 cm^{-1} and peak at 1384.57 cm^{-1} . Most notably is the appearance of a prominent peak at 532.12 cm^{-1} for Ag/Ni BMNPs (Fig. 4) stretching and the disappearance of the peaks at 1162.66 cm^{-1} , 986.29 cm^{-1} , 861.19 cm^{-1} and 525.69 cm^{-1} in root extract (Fig. 3). These results indicate that the aqueous root extract of *Borassus* served as reducing and capping agents for Ag/Ni BMNPs.

3.3 Larvicidal Bioassay Result

The mortality of *Culex quinquefasciatus* larvae when exposed to different concentrations of the Ag/Ni bimetallic nanoparticles represents the larvicidal activity, Fig. 5. The mortality rates were determined after 24 hours and are given in Table 1. The larvicidal activity was found to be concentration dependent. Moreover, development stage is also a factor because the Ag/Ni BMNPs showed better activity against 1st instars, followed by 2nd instars and 3rd/4th instars. Table 1 represents the percentage (%) mortality

rates at concentrations of 5, 10, 20, 25 and 50 mg/L. The outcome of the result showed effective larvicidal effect for all the 3 instars with LC_{50} and LC_{90} values obtained as: 3rd/4th instars (LC_{50} =15.735, LC_{90} =49.240), 2nd instar (LC_{50} =13.585, LC_{90} =195.689) and 1st instar (LC_{50} =5.730, LC_{90} =105.542) mg/L. These results are comparable with the results reported by [25] and [26] for the larvicidal activity of Ag NPs and Ag-Co BMNPs against *Culex quinquefasciatus* respectively. The LC_{50} and LC_{90} values also have some correlation with the result obtained by [27], whose LC_{50} =10.59, 11.10, 11.90, 12.71 ppm; LC_{90} = 32.11, 35.12, 37.48, 42.17 ppm, for 1st, 2nd, 3rd and 4th instars respectively. Larvicidal activity of Au NPs synthesized using *Hybanthus*

enneaspermus against 4th instar larvae of *Culex tritaeniorhynchus* was studied and the LC_{50} and LC_{90} were found to be 26.05 and 52.40 mg/L respectively [28]. The LC_{50} value of 26.05 mg/L is higher than the current study for 3rd/4th instar with LC_{50} values of 15.735 and it indicates that the larvicidal activity of Ag/Ni BMNPs is high. Also, [29] reported the larvicidal activity (LC_{50}) of Cu/Ni BMNPs against *Culex quinquefasciatus* larvae to be 14.746, 18.251 and 18.496 mg/L for 1st, 2nd, and 3rd/4th instars respectively. Thus, the current showed higher larvicidal activity as compared with literatures reports. The chi square values were significant at $P<0.05$ with values 3.301, 3.939 and 4.818 for first, second and third/fourth instars respectively, Table 1.

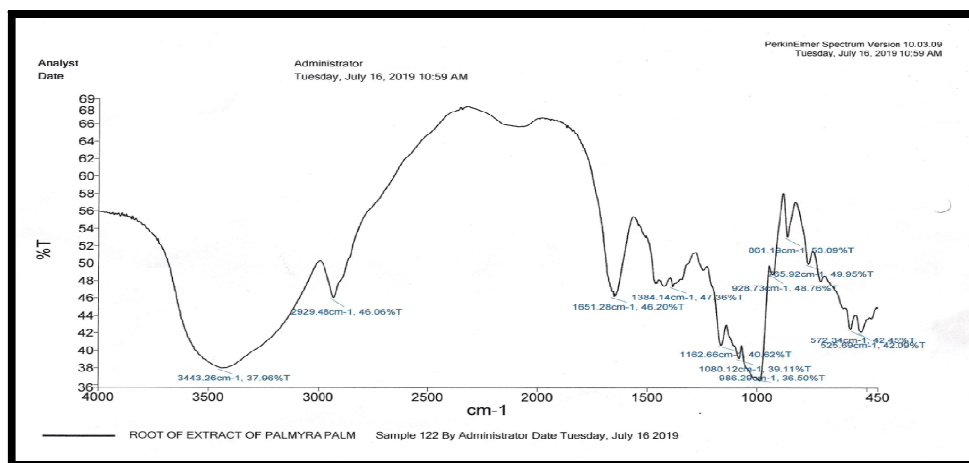


Fig. 3. FT-IR spectra for root extract of *Borassus aethiopum*

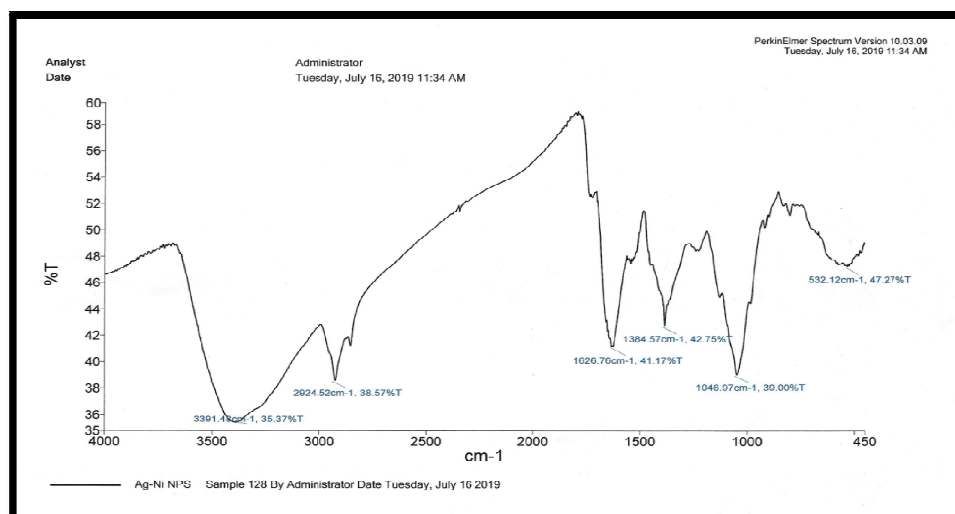
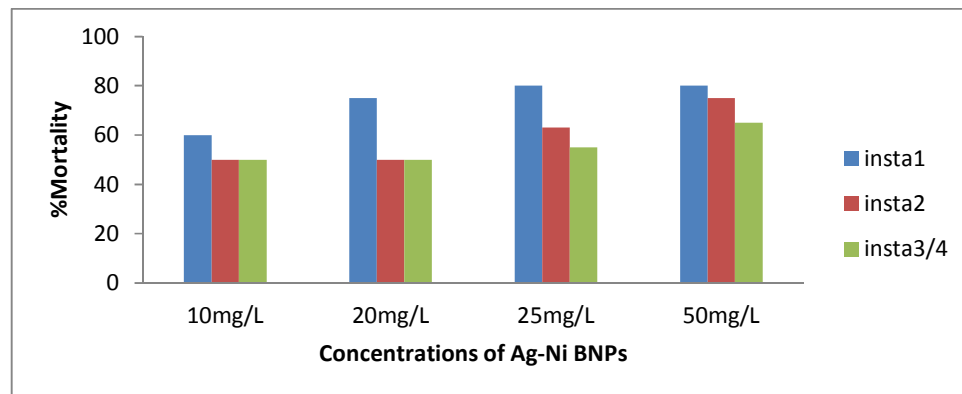


Fig. 4. FT-IR spectra for Ag/Ni BMNPs

Table 1. Larvicidal activity of Ag/Ni BMNPs against *Culex quinquefasciatus*

Larvae stage	Concentration (mg/L)	Mortality (%)	LC ₅₀ (mg/L)	LC ₉₀ (mg/L)	95% Confidence		χ^2	r
1 st instar	5	20	5.730	88.444	3.316-	54.623-	3.301	0.790
	10	50			7.930	214.488		
	20	63						
	25	65						
	50	75						
2 nd instar	5	30	13.585	195.689	10.335-	105.714-	3.939	0.916
	10	60			17.192	602.549		
	20	65						
	25	75						
	50	78						
3 rd /4 th instar	5	45	15.735	236.889	12.164-	122.243-	4.818	0.865
	10	70			20.111	806.102		
	20	75						
	25	85						
	50	88						

LC₅₀: lethal concentration that kills 50% of larvae, LC₉₀: lethal concentration that kills 90% of larvae, r: correlation coefficient; χ^2 : chi square

**Fig. 5. Effect of Ag/Ni BMNPs on *Culex quinquefasciatus***

4. CONCLUSION

Ag/Ni BMNPs were synthesized using the root extract of *Borassus aethiopum* and characterized using UV-Visible and FT-IR spectroscopy. Their insecticidal efficacy was assessed against 1st, 2nd, and 3rd/4th larval instars of *Culex quinquefasciatus*. The lethal concentration (LC₅₀) was found to be 5.730, 13.585 and 15.735 mg/L for 1st, 2nd and 3rd/4th instars respectively. The outcome of this research provides a promising step in search of a bio-friendly mosquito larval source management. The results of this work implies that Ag/Ni BMNPs could be a potential mosquito larvicide. This, therefore calls for the need to further this research to produce efficient nanoparticles to control the vector population.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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