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Nanolarvicidal Effect of Green Synthesized Ag-Co Bimetallic Nanoparticles on *Culex quinquefasciatus* Mosquito

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Abstract

Synthesis of bimetallic nanoparticles has employed many applications especially as larvicidal agents, these bimetallic nanoparticles therefore need to be produced via a cost-effective and eco-friendly route. Here, green synthesis of Ag-Co hybrid nanoparticles obtained from aqueous root extract of palmyra palm was reported. The hybrid nanoparticles formation was noticed by a colour change from light pink to light brown and further studied using UV-Vis and FT-IR spectrophotometers. The maximum absorption wavelength, λ_{max} as determined by the UV-Visible Spectrophotometer was found to be 420 nm. The FT-IR showed the formation and stabilization of the BMNPs. The nanolarvicidal potency was evaluated by the application of varying concentration ranging from 5 to 50 mg/L against first to fourth instars of larvae and recording the percentage mortality after 24 hours. Probit analysis showed the LC_{50} and LC_{90} for 1st instar to be 5.237 mg/L and 49.240 mg/L, 9.310 mg/L and 94.969 mg/L for 2nd instar, 13.626 mg/L and 105.542 mg/L for 3rd/4th instars respectively. This result therefore suggests that the nanoparticles can be used as potential control for larval population growth.

Keywords

Nanolarvicide, Green Synthesis, Ag-Co Bimetallic Nanoparticles, *Culex quinquefasciatus* Mosquito

1. Introduction

Diseases caused by mosquito are of a serious threat to modern world in many aspects such as mortality [1]. Mosquitoes are the vectors responsible for many diseases that include most commonly malaria and lymphatic filariasis. *Culex quinquefasciatus* species bite man persistently and transmit a neglected disease known as filariasis in the tropical regions. The *Anopheles* species are of more interest because they are responsible for transmitting malaria [2]. Larviciding is the act of sinking mosquito densities in their propagation places before they grow into adults [3]. *Culex quinquefasciatus* is domestic mosquito specie found in the vicinity of human habitat. Biological control can be achieved by the application of nanoparticles obtained through plant-mediated synthesis which is less toxic and eco-friendly [4].

The phyto-mediated synthesized nanoparticles can be a rapid, simple, cost effective and environmentally safer biopesticide for controlling the malarial vector [5]. Green chemistry is generally the use of methods and techniques to eliminate or reduce the generation or use of feedstock, byproduct, product, reagents, and solvents, etc. that are detrimental to human health or to the environment [6]. Much literature has reported the green plant-mediated synthesis of nanoparticles which is more favored by researchers due to its eco-friendliness over photochemical reduction, heat evaporation, electrochemical reduction, and chemical reduction. Some of these reducing agents reported for larvicidal activity include actinobacterium, *Streptomyces* sp. [7], *Streptomyces* sp. [8], *Aganosma cymosa* leaf extract [9], bud extract of *Polianthus tuberose* [4], bark extract of T*erminalia arjuna* [[5], petal extracts of *Tagetes* sp. and *Rosa* sp. [10].

Palmyra palm with the scientific name *Borassus aethiopum* is one of the trees usually referred to as the Palms. It is a member of the family Arecaceae and economically and medicinally useful. For example it is used as vegetable [11] and commonly in West Africa [12]. This study reports the green synthesis of Ag-Co hybrid bimetallic nanoparticles using the aqueous root extract of palmyra palm, their partial characterization using UV-Visible and FT-IR spectrophotometers as well as their larvicidal effect on first, second and third/fourth instars of *Culex quinquefasciatus* larva.

2. Materials and Methods

2.1. Palmyra Root Sample Collection and Preparation

Palmyra root samples were dug from Kalorgu in Kaltungo Local Government Area of Gombe State. They were transported to Chemistry Laboratory of Gombe State University in polythene bag. The root samples were washed several times with water and distilled water to removed impurities. About 100 g was crushed using pestle and mortar and transferred to a 250 ml beaker. It was placed on a magnetic stirrer and 100 ml of distilled water was added. The mixture was warmed for 1 h with continuous stirring at 60˚C to extract the phytochemicals. It was then filtered and kept for the synthesis of silver—cobalt nanocomposite.

2.2. Test Larvae Collection

The test larvae (*Culex quinquefasciatus*) were obtained from stagnant open water bodies in Gombe town.

2.3. Green Synthesis of Silver-Cobalt Bimetallic Nanoparticles

One hundred milliliters of the prepared palmyra palm root extract was mixed with a solution of 500 ml containing 250 ml each of 0.01mol/dm^3 $AgNO_3$ and $CoCl_2$ (1:5 v/v) gradually on a hot plate at 80˚C while stirring for 30 minutes in a 600 ml beaker [13]. Change in color of the reaction mixture from light pink to light brown was visually noticed. The solution was stored for 24 hours after which the nanoparticles obtained were evaporated and dried in an oven at 105˚C.

2.4. Ultraviolet-Visible Spectroscopic Investigation

Optical measurement was carried out using UV-Visible Spectrophotometer model 6705 for the wavelength between 250 to 800 nm by placing 1 mL sample of the supernatant used for the synthesis in 1 × 1 cm cuvettes operated at a resolution of 1 nm and de-ionized water as the blank solvent.

2.5. Fourier Transform Infrared Spectrophotometry Analysis

The dried synthesized Silver-Cobalt bimetallic nanoparticles and root extract of Palmyra palm were characterized using Fourier Transform Infrared Spectroscopy. This was done to determine which functional groups were involved in the bio-reduction process. PerkinElmer Spectrum Version 10.03.09 was used.

2.6. Larvicidal Bioassay

Twenty larvae (first, second and third/fourth instar) each were placed in a beaker to which 5 ppm of the synthesized Ag/Co bimetallic nanoparticles diluted with de-ionized water was added to make the solution 100 ml. Test of this concentration against each instar was replicated twice. In each case, a control comprising of 20 larvae in 100 ml de-ionized water was used as reference. The test was carried out for further concentration of 10, 20, 25 and 50 ppm. The mortality data was collected after 24 hours [10] and the percentage mortality was calculated as follow:

$$\text{Percentage mortality} = \frac{\text{Number of dead Larvae}}{\text{Number Lavae introduced}} \times 100$$

2.7. Statistical Analysis

All data were analyzed using SPSS 16.0. Probit analyses for LC_{50}, LC_{90}, chi square as well as correlation analysis were evaluated.

3. Results and Discussions

3.1. Optical Measurement Using UV-Visible Spectrophotometer

The formation of the Ag-Co bimetallic nanoparticles was first noticed by change of color of the mixture of Ag-Co salt and the extract from milky, **Figure 1(D)** to light brown, **Figure 1(E)** within 15 minutes as a result of the surface Plasmon resonance which is due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation [13].

Figure 1. 0.01 M $CoCl_2$ (A), 0.01 M $Ag(NO_3)$ (B), Aqueous root extract Palmyra palm (C), Mixture of Ag-Co immediately after addition (D), and Ag-Co BMNPs formation (E).

The supernatant liquid was used for UV-Vis spectroscopic analysis which is frequently used to characterize synthesized metal nanoparticles. The maximum absorption peak was found at 420 nm (**Figure 2**).

3.2. FTIR Analysis

FT-IR spectroscopy was used to investigate the functional groups involved in the reducing and capping process. The FT-IR spectra of the root extract and that of the biosynthesized Ag-Co BNPs are shown in **Figure 3** and **Figure 4** respectively. The FT-IR spectra of the Ag-Co displayed peaks due to O-H stretching frequency at 3389.93 cm^{-1}, medium sharp peak for C-H absorption at 2924.28 cm^{-1}, C=C stretching at 1633.48 cm^{-1}, C=N stretching, C-C stretching and the C-O at 1541.53 cm^{-1}, 1384.59 cm-1 and 1046.88 cm^{-1} bands respectively. Similar result was reported by [14]. These have replaced those observed in the spectrum of the root extract which were peaks at 3443.26 cm^{-1}, 2929.48 cm^{-1}, 1651.28 cm^{-1}, 1384.15 and 1080.12 cm^{-1} respectively. Most notably is the appearance of a prominent peak at 1541.53 cm^{-1} due to C=N stretching which was absent in the root extra spectra and the disappearance of the peaks at 1162.66 cm^{-1}, 986.29 cm^{-1}, 861.19 cm^{-1} and 525.69 cm^{-1}. This variation is due to various metabolites such as tannins and saponins that may be present because no existing literature reported the photochemical. The active metabolites are responsible for the bioreduction [15].

3.3. Larvicidal Results

The larvicidal activity was evaluated by recording the mortality of *Culex quinquefasciatus* larvae when exposed to different concentrations of the Ag-Co bimetallic nanoparticles represents after 24 hours. It was found be concentration dependent. Moreover, development stage is also a factor because the Ag-Co BMNPs showed better activity against 1st instars, followed by 2nd instars and 3rd or 4th instars. **Table 1** and **Figure 5** represent the % mortality rates at concentrations of 5, 10, 20, 25 and 50 ppm. The outcome of the result showed effective larvicidal effect for all the 3 instars with LC_{50} and LC_{90} values obtained as: 1st instar (LC_{50} = 5.237 ppm, LC = 49.240 ppm), 2nd instar (LC_{50} = 9.310 ppm, LC_{90} = 94.969 ppm) and 3rd or 4th instar (LC_{50} = 13.626 ppm, LC_{90} = 105.542 ppm). These results are comparable with that obtained by Elijah *et al.* (2016) for the larvicidal activity of Ag NPs where the LC_{50} and LC_{90} values after 24 h exposure were 4.43 ppm and

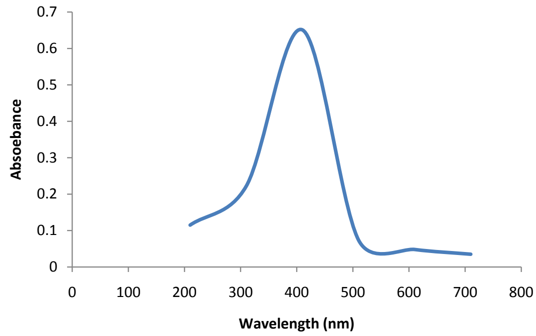

Figure 2. UV-visible spectrum for Ag-Co BMNPs.

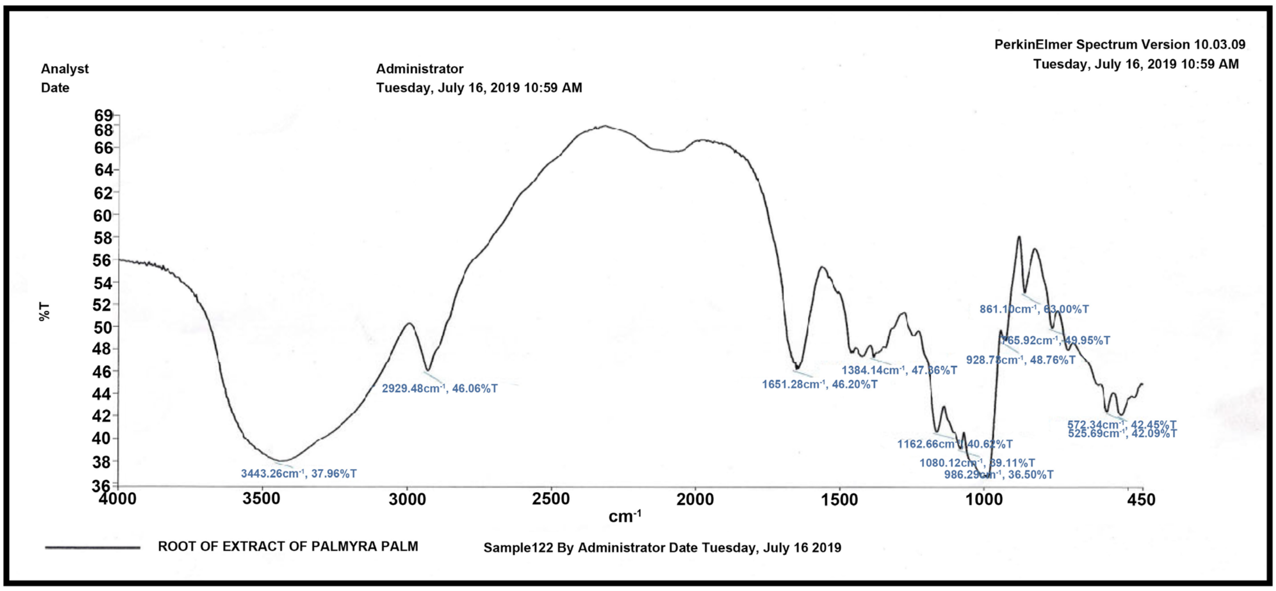

Figure 3. FT-IR spectrum for root extract of *Borassus aethiopum*.

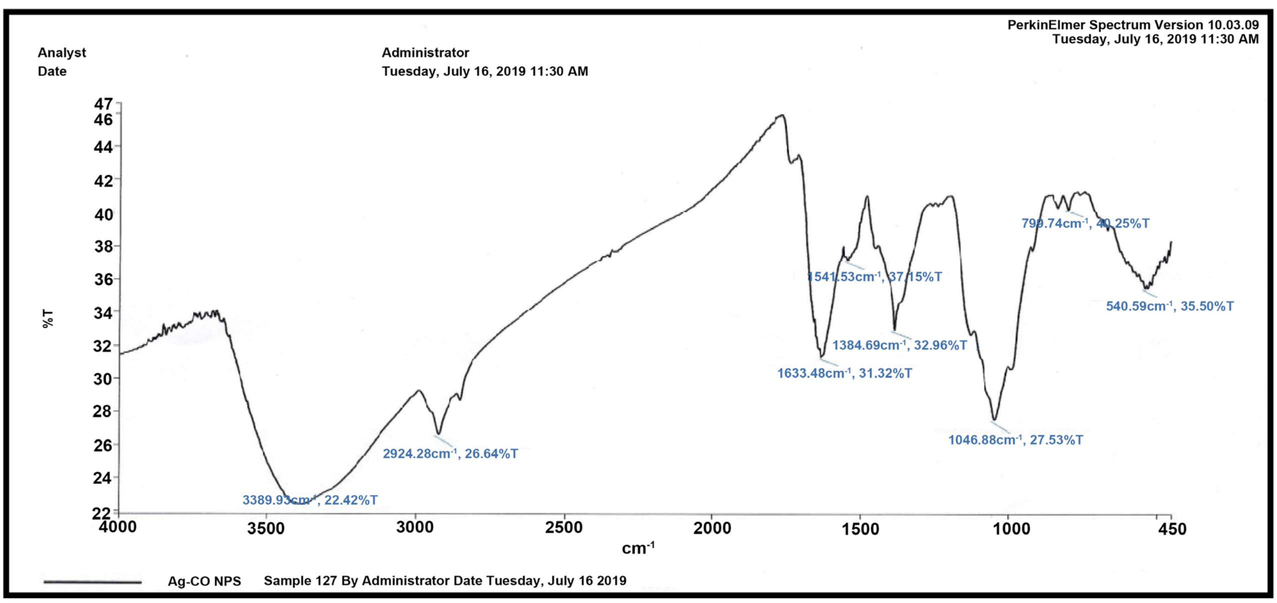

Figure 4. FT-IR spectrum for Ag-Co BMNPs.

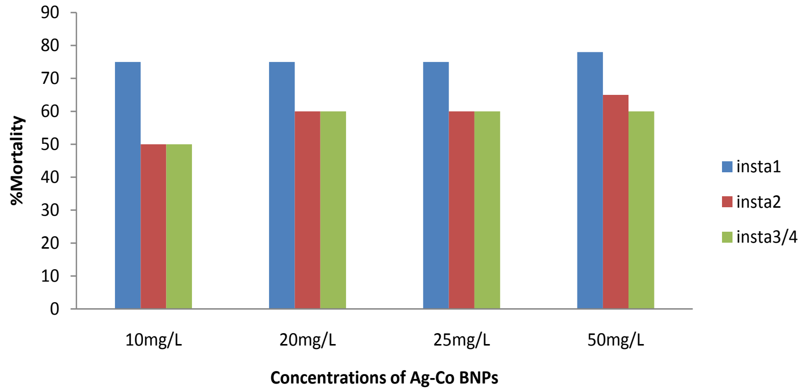

Figure 5. Ag-Co BMNPs larvicidal activity on *Culex quinquefasciatus* larvae.

Table 1. Effect of different concentrations of Ag-Co BMNPs on *Culex quinquefasciatus* larvae.

Laevae stage	Concentration (mg/l)	Mortality (%)	LC_{50}	LC_{90}	95% Confidence		χ^2	r
					LC_{50}	LC_{90}		
1^{st} Instar	5	25	5.237	49.240	3.316 - 7.003	36.377 - 84.233	3.804	0.805
	10	50						
	20	50						
	25	60						
	50	70						
2^{nd} instar	5	30	9.310	94.969	2.375 - 15.629	41.314 - 4167.317	6.958	0.782
	10	50						
	20	50						
	25	63						
	50	75						
$3^{rd}/4^{th}$ instar	5	45	13.626	105.542	7.276 - 21.458	49.564 - 1270.669	6.183	0.823
	10	60						
	20	75						
	25	80						
	50	80						

LC_{50} and LC_{90} are the lethal concentrations that would kill 50 and 90% of the exposed larvae respectively; r is the regression co-efficient and χ^2 Chi square.

8.37 ppm against 3rd and 4th instars. The LC_{50} and LC_{90} values also have some correlation with the result obtained by Kanayairam and Ravichandran (2016) 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. A similar result was also observed by Shanmugasundaram and Balagurunathan [7] with LC_{50} = 48.98 mg/L, r = 0.956 and χ^2 value of 14.307).

4. Conclusion

Secondary metabolites of the root extract of Palmyra palm were used to synthesize Ag-Co bimetallic nanoparticles using $AgNO_3$ and $CoCl_2$ metal salts. Their formation was visually noticed by a color change and further characterized using UV-Visible and FT-IR spectrophotometers. These plant-mediated nanoparticles were active against *Culex quinquefasciatus* mosquito larvae.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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