GREEN SYNTHESIS AND NANOTOXICITY ASSAY OF COPPER-COBALT BIMETALLIC NANOPARTICLES AS A NOVEL NANOLARVICIDE FOR MOSQUITO LARVAE MANAGEMENT

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ABSTRACT

Plant mediated nanoparticles have been investigated to possess many applications in many fields such as pharmaceuticals, therapeutics and other commercial products. In this study, Copper/Cobalt bimetallic nanoparticles were synthesized by an eco-friendly and cost effective method using Palmyra palm fruit extract and characterized using various techniques such as UV-visible spectrophotometry and Fourier transform infrared spectrometry. Green synthesis method was used in obtaining the nanoparticles and the agar well diffusion method was used in evaluating the larvicidal activity. The resulting nanoparticles were evaluated to find possible application as nanolarvicide against first, second and third instar of Culex quinquefasciatus larvae in terms of percentage mortality. The lethal concentration values were LC50=12.036, LC90=143.316; LC50=14.774, LC90=263.456; LC50=16.076, LC90=296.758 ppm for the first, second, and third instars respectively implying moderate activity of this copper/cobalt nanoparticles. It could be concluded that copper-cobalt bimetallic nanoparticles synthesized using fruit extract of palmyra palm could be a potential nanolarvicide for mosquito larvae management.

1. INTRODUCTION

Nanotechnology is among the most reliable fields in science nowadays. Nobel metal nanoparticles are synthesized using plant extracts due to the advantage of eco-friendliness achieved in this route (Reddy et al., 2014; Tooba, Vikas, & Rajesh, 2017). Advanced nano-biotechnology coupling biotechnology and nanotechnology has led to provide commendable development of various techniques used for the nanoparticles synthesis leading to the pinnacle of their application in the areas of life-sciences (Ibrahim et al., 2008) and increased merit for it is eco-friendly, nontoxic, and safe option, since plant extract-mediated biosynthesis of nanoparticles is economically advantageous and offers natural capping agents in the form of proteins (Mahesh & Shivayogeeshwar, 2018).
Nanoparticles are materials that are in nanoscale size (1-100 nm), they are very small particles with improved thermal conductivity, catalytic reactivity, nonlinear optical performance, antibacterial activity and chemical stability due to their large surface area-to-volume ratio (Abdullahi & Mustapha, 2018; Rajakumar & Rahuman, 2011). Nanoparticles can be classified based on their origin, dimension and structure. They are either obtained from nature or made artificially with respect to their origin (Magallhães et al., 2010). On the basis of dimension, nanomaterials can be zero dimensional, one-dimensional, two-dimensional and three-dimensional. Structural configuration and morphology of nanoparticles were said to be amalgamated and dispersions (Agarwal, Kumar, & Rajeshkumar, 2017). Many researchers recently are diverting themselves from using synthetic methods because plants produce more stable nanoparticles compared to chemical methods, it is also very straightforward to scale up and the risk of contamination is also low (Bianca et al., 2018).

Mosquitoes are important vectors for spreading most notorious diseases such as malaria, yellow fever, dengue fever, and filariasis (Veerakumar, Govindarajan, & Rajeswary, 2013). In Nigeria, mosquito borne diseases constitute the major health problem. They also account for low productivity, economic loss and social disgrace (Rawani, Ghosh, & Chandra, 2010). The extensive use of synthetic mosquito repellent has resulted in resistance in mosquitoes (Amita, Dutta, & Mondal, 2016).

In this study, the nanotoxicity assay of green synthesized Copper-Cobalt bimetallic nanoparticles was evaluated on Culex quinquefasciatus larvae.

2. MATERIALS AND METHODS

2.1. Plant Sample and Test Larvae Collection and Preparation

Fresh fruits of Palmyra palm obtained from Kaltungo Local Government Area, Gombe State were transported to Gombe State University via road. The fruits were washed with running tap water and rinsed with de-ionized water. The samples were cut into small units and ground with the aid of a crucible. About 40 g of it was weighed and mixed with 800 mL of de-ionized water and boiled at 80 °C on a hot plate with frequent stirring for 15 mins. The extract was cooled and filtered through Whatman no. 1 filter paper. The test larvae (Culex quinquefasciatus) were obtained from stagnant open water bodies in Gombe town.

2.2. Green Synthesis of Copper-Cobalt Bimetallic Nanoparticles

A solution containing 500 ml each of 0.01mol/dm³ CuSO₄.6H₂O and CoCl₂.6H₂O were gradually mixed with two hundred milliliters of the prepared Palmyra palm fruit extract (1:5 v/v) on a hot plate at 80 °C while stirring for 30 minutes in a 2000 ml beaker. There was a noticeable change in color of the reaction mixture from light pink to light blue. The mixture was stored for 24 hours after which the nanoparticles settled, evaporated and centrifuged in an oven at 105 °C.

2.3. Ultraviolet-Visible Spectroscopic Investigation

UV-Visible Spectrophotometer Agilent technology model 6705 was used for the investigation of the optical properties of the bio-synthesized Copper-Cobalt bimetallic nanoparticles by determining the absorbance at wavelength of between 200 to 800 nm by placing 1mL sample of the NPs synthesized in 1 x 1 cm cuvettes operated at a resolution of 1 nm and de-ionized water as the reference solvent.

2.4. Fourier Transform Infrared Spectrophotometry Analysis

The dried synthesized Copper-Cobalt bimetallic nanoparticles were characterized using Fourier Transform Infrared Spectroscopy. This was carried out to account for the functional groups involved in the bio-reduction and capping process from 450 to 4000 cm⁻¹. PerkinElmer Spectrum Version 10.03.09 was used.
2.5. Nanotoxicity Assay

Twenty larvae (first, second and third instar) each were placed in cups to which different concentrations (5, 10, 20, 25 and 50 ppm) of the synthesized Copper-Cobalt bimetallic were subjected to the larvae to evaluate the dose response bioassay. Test of these concentrations against each instar was replicated twice and presented by the average of the two. In each case, a control comprising of 20 larvae in 100 ml de-ionized water was used as reference. The mortality data was collected after 24 hours. The Probit result was evaluated using statistical software SPSS 2016. Percentage mortalities were calculated by dividing the number of dead larvae and the total larvae introduced multiplied by 100 (Elijah et al., 2016).

3. RESULTS AND DISCUSSION

3.1. Formation and UV-Visible Spectrophotometric Analysis

The formation of Copper-Cobalt Bimetallic nanoparticles was first noticed by color change from light blue to dark blue within 15 minutes as a result of the surface Plasmon absorption. UV-Visible Spectroscopic analysis was carried out here which is one of the techniques frequently used for optical measurement. The maximum absorption peak was shown at 0.717 absorbance with corresponding wavelength ($\lambda_{\text{max}}$) at 500 nm. However, the $\lambda_{\text{max}}$ is consistent with 510 nm reported by Ramasamy, Lee, and Lee (2016) for Au/Ag BMNPs. A graph of absorbance against wavelength was plotted and is shown in Figure 1.

![Figure 1. Absorbance against wavelength for Cu/Co NPs.](image)

3.2. Fourier Transform Infrared Spectroscopy

FT-IR spectroscopy was applied to determine the functional groups involved in the bio-reduction of Copper-Cobalt bimetallic nanoparticles. The various functional groups present in the BNPS were identified. It displayed bands due to O-H stretching vibration mode at 3410.39 cm$^{-1}$ and band at 3660.62 for N-H stretching in terpenoid found within this region. Also observed, were a sharp peak for C-H absorption at 2924.37 cm$^{-1}$, C=C stretching at 1632.89.76 cm$^{-1}$, C-O deformation at 1112.62 cm$^{-1}$ and peak at finger print region for the metal-bonds. This is fairly the same with the result obtained by Akinsiku et al. (2018). The FT-IR spectra are depicted in Figure 2.

3.3. Nanotoxicity Assay Results

Larvicidal effects of different concentrations of Copper-Cobalt bimetallic particles exposed to instar I, II and III larvae of Culex quinquefasciatus was studied by recording the mortality rates after a day. The mortality rate increased with increasing concentration and attenuated with increasing larval stage. The Probit result was evaluated using statistical software for 5, 10, 20, 25 and 50mg/L dose exposure is depicted in Table 1 and represented in form of a histogram for better comparative understanding, Figure 3. The lethal concentrations obtained were (LC$_{50}$=14.75, LC$_{90}$=83.96) for instar I, (LC$_{50}$=18.25, LC$_{90}$=258.83) for instar II and (LC$_{50}$=18.5, LC$_{90}$=331.5) for third instars respectively (Kanayairam & Ramanibai, 2016). The Larvicidal activity of synthesized silver nanoparticles using isoamyl acetate identified in Annona squamosa leaves against Culex quinquefasciatus showed similar trend.
Recently, Cu-Zn and Cu-Ag bimetallic NPs were synthesized using leaf extract of *Oscimum sanctum* (Linn) and its larvicidal activities were evaluated on the 3rd instar of Anopheles stephesis. For, Cu-Zn BMNPs, the LC$_{50}$ and LC$_{90}$ were 444.734 and 1077.953 mg/L respectively. And for the Cu-Ag BMNPs, the LC$_{50}$ and LC$_{90}$ were 888.792 and 192.93 mg/L respectively (Minal & Prakash, 2016).

It is quite obvious to say that the toxicity of both Cu-Zn and Cu-Ag BMNPs on larvae of anopheles stephesis is low as compared to Copper-Cobalt bimetallic nanoparticles in the current study. However, in our previous report, Ag-Co bimetallic nanoparticle was synthesized using root extract of Palmyra plant and the larvicidal activity was studied on *Culex quinquefasciatus* larvae. The LC$_{50}$ and LC$_{90}$ were 5.237 and 49.240 mg/L for first instar, 9.310 and 94.969 mg/L for second instar as well as 13.626 and 105.542 mg/L for third/fourth instar respectively (Danbature, Shehu, Yoro, & Adam, 2020). Though within the same range, our previous report yielded good activity than the current study.

![Figure-2. FT-IR spectra of bio-synthesized Copper-Cobalt NPs.](image)

| Larva stage | Conc. (mg/L) | % mortality | LC$_{50}$ | LC$_{90}$ | 95%confidence | X$^2$ | r |
|-------------|--------------|-------------|---------|---------|----------------|------|------|
| 1st Instar  | 5            | 45          | 12.036  | 296.758 | 9.242 - 12.696 | 2.522 | 0.940 |
|             | 10           | 45          |         |         | 5.526 - 1992.496 |      |      |
|             | 20           | 63          |         |         | 12.696         |      |      |
|             | 25           | 63          |         |         | 125.724 - 1992.496 |      |      |
|             | 50           | 75          |         |         | 9.242 - 12.696 |      |      |
| 2nd Instar  | 5            | 35          | 14.774  | 263.456 | 13.653 - 17.719 | 1.483 | 0.907 |
|             | 10           | 40          |         |         | 10.047 - 1096.818 |      |      |
|             | 20           | 58          |         |         | 17.719         |      |      |
|             | 25           | 63          |         |         | 126.906 - 1096.818 |      |      |
|             | 50           | 70          |         |         | 12.696         |      |      |
| 3rd Instar  | 5            | 15          | 16.076  | 143.316 | 18.667 - 91.908 | 5.123 | 0.846 |
|             | 10           | 40          |         |         | 15.522 - 291.078 |      |      |
|             | 20           | 55          |         |         | 22.663         |      |      |
|             | 25           | 60          |         |         | 91.908 - 291.078 |      |      |
|             | 50           | 68          |         |         | 22.663         |      |      |
4. CONCLUSION

The nanotoxicity assay of the green synthesized Copper-Cobalt bimetallic nanoparticles was studied against *Culex quinquefasciatus* larvae. The study showed better result as compared with previous studies on larvicidal potential of bimetallic nanoparticles by other researchers. Therefore, Copper-Cobalt bimetallic nanoparticles synthesized using fruit extract of palmyra palm could be a potential nanolarvicide for mosquito larvae management if other studies are properly conducted such field test as well as the effect of the nanoparticles on the non-target organism.

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