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Bio-Fabrication of ZnO-CuO Nanoporous Composite and Its Application as Nanolarvicidal Agent for Malaria Vectors

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Authors' contributions

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

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ABSTRACT

Bio-fabrication of ZnO-CuO nanoporous composite was successfully synthesized using Gum Arabic. The morphology, elemental composition, surface plasmon resonance and functional groups bonds of the ZnO-CuO nanoporous composite were confirmed by SEM, EDX, UV-Visible and FTIR techniques respectively. Nanolarvicidal activity of ZnO-CuO nanoporous composite was tested against 1st, 2nd, 3rd, and 4th instars of malaria vectors for 24 hours using different concentrations. The lethal concentrations (LC_{50}) of ZnO-CuO nanoporous composite against 1st, 2nd, 3rd, and 4th instars were found to be 8.841, 8.734, 8.963 and 10.557 mg/L respectively. Whereas, the LC_{90} of ZnO-CuO nanoporous composite against 1st, 2nd, 3rd, and 4th instars were found to be 19.062, 28.063, 40.888 and 79.567 mg/L respectively. For all instars, the correlation coefficients were found

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in the range of 0.945 to 0.999 and it indicates concentration dependent larvicidal activity. It was observed that the lower the instar, the higher the larvicidal activity and conversely. Therefore, ZnO-CuO nanoporous composite could be used as nanolarvicidal agent especially for malaria vectors.

Keywords: Bio-fabrication; ZnO-CuO nanoporous composite; nanolarvicidal; malaria Vector; gum arabic.

1. INTRODUCTION

Nanocomposite is a combination of two or more substances with atleast one within nanoparticles range (1-100nm) [1]. Several techniques are used in preparation of nanocomposite which include; microwave solvothermal, solution combustion, microwave irradiation, deposition process,micro-emulsion, solvo-thermal, sol-gel(gelatin media), wet chemical, microwave hydrothermal, hydrothermal, microwave decomposition, simple precipitation, physical vapor, mechano-chemical and green synthesis [2]. Green synthesis is simple, cost effective and environmentally friendly than other chemical and physical syntheses. This is due to the fact that green synthesis involves the use of plant parts (leaves, barks, flowers as well as extrude like Gum Arabic), bacteria and fungi whereas chemical methods uses toxic substances [3]. Some characterization techniques for structural analysis, shape, size, charge, surface area etc. of nanocomposite are Fourier transform infrared spectroscopy(FTIR), Scanning electron microscope (SEM), Transmission electron microscope (TEM), X-ray diffraction(XRD), Selected area electron diffraction(SED), UV-Visible spectroscopy, Atomic force microscopy(AFM), Thermogravimetric analysis, Dynamic light scattering(DLS), Fluorescence spectroscopy and Braunauer-Emmet-Teller method etc. [1,2,4,5]. Zinc oxide and Copper oxide as well as their nanocomposites are used in various applications. For example ZnO/CuO for photodegradation of toxic textile dye [6-10], ZnO-CuO for volatile organic compounds (VOCs) sensor [11], ZnO-CuO for dye sentisized solar cells [12], ZnO/CuO for photovoltaic application [13] as well as ZnO-CuO for antimicrobial application [14,15].

New developments in nanotechnology have led to utilization of nanoparticles and nanocomposites as a novel nanolarvicide for mosquito larvae control. As such ZnO nanoparticles have been reported to have higher larvicidal activity against mosquito larvae of different species [16-22]. Not only ZnO nanoparticles, but also CuO nanoparticles have been reported to possess nanolarvicidal potency on mosquito larvae [23-25]. However, bimetallic (metal/metal), metal/metal oxide or metal oxide/metal oxide nanoparticles are known to have greater larvicidal effect on mosquito larvae than the individual nanoparticles due to synergistic effects [3]. Thus, Elango et al. [26] have synthesized Ni-Pd bimetallic NPs using Coir dust extract which was found to have effect on *Aedes aegypti* fourth instar larvae(LC_{50}; 288.88 mg/L and LC_{90}; 483.06 mg/L). Ag-Co bimetallic NPs synthesized with the root extract of Palmyra palm (*Borassus aethiopum*) had proved to be effective against *Culex quinquefasciatus* larvae [3]. Elsewhere, leaf extract of *Oscimum sanctum* (Linn) was used in synthesizing Cu-Zn and Ag-Cu bimetallic NPs and their larvicidal activities were investigated on third larvae of *Anopheles stephensi*. The LC_{50} and LC_{90} of Cu-Zn bimetallic NPs were 444.734 and 1077.953 mg/L respectively. And the LC_{50} and LC_{90} of Ag-Cu bimetallic NPs were 888.792 and 192.93 mg/L respectively [27]. Efficacy Cu-Zn bimetallic nanoparticles synthesized using *Azardirachta indica* (Neem) leaf extract was conducted on 3rd instar larvae of filariasis vector *Culex quinquefasciatus* (Say). For the monmetallic Copper nanoparticles the LC_{50} for 3rd instar larvae after 24h was recorded at 7% and it was not effective for the monmetallic Zn nanoparticles. For the Cu-Zn bimetallic nanoparticles the LC_{50} for 3rd instar larvae after 24h was recorded at 7% which showed good improvement than individual monometallic nanoparticles [28]. Also, some metal/metal oxide nanocomposites such as Al_2O_3/Zn [22], Ag/Ag_2O [29] and Ag-TiO_2 [30] showed high efficacy against different mosquito larvae.

In this study, ZnO-CuO nanoporous composite was fabricated with Gum Arabic and characterized using FTIR, UV-Visible spectroscopy, Scanning electron microscope (SEM) and energy dispersed x-ray (EDX). The nanolarvicidal activity was conducted on the larvae of malaria vectors.

2. MATERIALS AND METHODS

2.1 Experimental Site

The work was conducted in Chemistry Laboratory of the Chemistry department and Insectary of the department of Biological Sciences, Gombe State University. The mosquito larvae used were obtained from different breeding sites in Gombe town.

2.2 Collection of Gum Arabic

Gum Arabic (an extrude from *Acacia senegalensis*) was collected from Billiri, Gombe State. The choice and collection of Gum Arabic was on the basis of cost effectiveness, medicinal property and ease of availability. After collection, the Gum Arabic was allowed to dry properly under the sun for 24 hours. Then, was crushed to powder using pistil and mortar.

2.3 Synthesis of ZnO-CuO Nanoporous Composite

The method of [14] was followed for the synthesis of ZnO-CuO nanoporous composite. One gram (1g) of Gum Arabic (GA) was placed in a beaker, then 40 ml of distilled water was added. It was then placed on a magnetic stirrer hotplate for dissolution at 90°C for 10 minutes. Subsequently, 2 g of Zn $(NO_3)_2.6H_2O$ (DBH product) and 2g of $Cu(NO_3)_2.6H_2O$ (DBH product) were then added and stirred continuously for 2 hours. A blue-green resin was formed. The resin was transferred into a crucible and covered with a paper foil and then placed in the laboratory furnace for 2 hours at 450 ^{0}C.

2.4 Characterization of ZnO-CuO Nanoporous Composite

Spectral analyses were used to characterize ZnO-CuO nanoporous composite. UV-VIS spectrophotometer (Perkin Elmer Lamda Spectrophotometer) was used for monitoring the formation of the nanoparticles with absorbance in the range of 200–800 nm. While Fourier transform infrared (FTIR) analysis of the sample was measured using Perkin Elmer Spectrum version 10.03, the FTIR spectra taken were analyzed and discussed for the possible functional groups for the formation of the composite. To study the morphology of composite, scanning electron microscopy (SEM; JEOL, Model JFC-1600) was employed. Energy dispersed x-ray (EDX) technique was used to study the chemical composition of the nanocomposite.

2.5 Mosquito Collection

The breeding sites (unpolluted water, potholes, rice fields, temporary rain pools. etc.) in Gombe metropolis were scouted for the *Anopheles* larvae. The larvae were collected, reared and maintained in the Insectary for larvicidal bioassay. The collection was done based on the method of [15] with little modifications. The larvae were identified in the following the standard procedure reported in [19].

2.6 Larvicidal Bioassay

A stock solution of 100 mg/L of the composite was prepared. From the stock suspension, different concentrations; 10, 20, and 25 mg/L prepared subsequently by serial dilution. Then 100 mL of these concentrations were placed together with twenty larvae each of first, second, third and fourth instars in plastic cups. Test of each concentration against each instar is repeated two times. The mortality of the larvae were recorded after 24 hours exposure. Each concentration against each instar was tested in 4 replicates and the control comprised 25 larvae in 200 ml of distilled water for each set according to [16]. Percentage mortality was obtained thus:

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae or pupae}}{\text{Number of larvae or pupae introduced}} \times 100$$

2.7 Data Analysis

The mean percent larval mortality data were subjected to analysis of variance (ANOVA). And the LC_{50}; LC_{90} were estimated from 24 hour concentration with mortality data using probit analysis (SPSS version 2016).

3. RESULTS AND DISCUSSION

3.1 Characterization of ZnO-CuO Nanoporous Composite

Fig. 1 represents the absorption spectrum of synthesized ZnO-CuO nanoporous composite at different wavelengths ranging from 280 to 800 nm using UV-Vis spectrophotometer (Perkin Elmer Lamda Spectrophotometer). The maximum absorption wavelength observed at 280 nm revealed the formation of the nanocomposite. Similarly, it was earlier reported that ZnO-CuO nanocomposite generally consists of a characteristic peak that range between 270 and 400 nm [31]. Also, low transmittance below 380 nm indicate strong absorbance for ZnO-CuO nanocomposite was previously reported [11].

The FTIR spectrum of ZnO-CuO nanoporous composite as shown in Fig. 2, exhibited prominent peaks at 3428.88, 2923.33, 1621.11, 1384.49, 1093.29 and 482.92 cm^{-1}. These distinct peaks is noted to represent the multifunctional groups indicating –OH stretching, C-H (due to aldehyde), –OH bending of water molecules, and C-C for 3428.88, 2923.33, 1621.11, 1384.49 92 cm^{-1} present in the nanocomposite. Findings showed that due to inter-atomic vibrations, the absorption of metal oxides falls below 1000 cm^{-1}. Thus absorptions peaks at 1093.29 and 482.92 cm^{-1} are responsible for Zn-O/ Cu-O interactions. This concur with FTIR analysis of ZnO-CuO nanocomposite as reported by the findings of [6,7,9,14,31].

Scanning Electron Microscopy (SEM) determinations of the sample showed the formation of nanocomposite and ZnO-CuO nanoporous composite was clearly distinguishable. The SEM analysis showed that it is nanoporous and possess irregular shape (Fig. 3). Nonetheless, the morphological structure of ZnO-CuO was found to be Nanoflakes, Flower-like, Networked flakes, Nanorod, Nanowire, and Spherinoid by [5,6,9,11,12,31] respectively.

Fig. 4 represents the elemental composition of the ZnO-CuO nanoporous composite. The findings showed that Copper, Zinc and Oxygen has the weight concentrations of 39.40, 37.46 and 2.39 % respectively (Table 1). This also confirmed the formation of ZnO-CuO nanocomposite in good proportion. However, the other elements present were confirmed to be from the Gum Arabic used for the synthesis as reported by [32].

3.2 Larvicidal Assay

In this study, the larvae of malaria vectors were exposed to different concentrations of ZnO-CuO nanoporous composite for 24 hours. The result is presented in Table 2. Larval average percentage mortality for 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instars were found to increase with increase in concentrations of ZnO-CuO nanoporous composite. The lethal effect for the 1^{st} – 4^{th} larval instars of malaria vectors showed the values of LC_{50}=8.841, 8.734, 8.963 and 10.577 mg/L respectively and LC_{90}=19.062, 28.063, 40.888 and 79.567 mg/L respectively. Similarly, concentrations dependent mortality has been reported [10,16,27-31]. Findings of larvicidal activity of ZnO nanoparticles revealed that the LC_{50} and LC_{90} were found to be 0.72 and 27.29 mg/L respectively on third instar of *Culex quinquefasciatus* [29]. These activities are more than the current study. However, larvicidal activities of Ag [27], Al_2O_3-ZnO [29], Ag [31], Ag-Co [10], and Cu/Ni [16] nanoparticles on the third larvae of *Culex quinquefasciatus* showed the LC_{50} to be 48.98 mg/L, 7.93 mg/L, 21.84 mg/ml, 13.63mg/L, and 18.50 mg/L respectively. Thus, these larvicidal activities in literatures are less when compared with the current investigations and this indicates the applicability of ZnO-CuO nanoporous composite as a potential nanolarvicide.

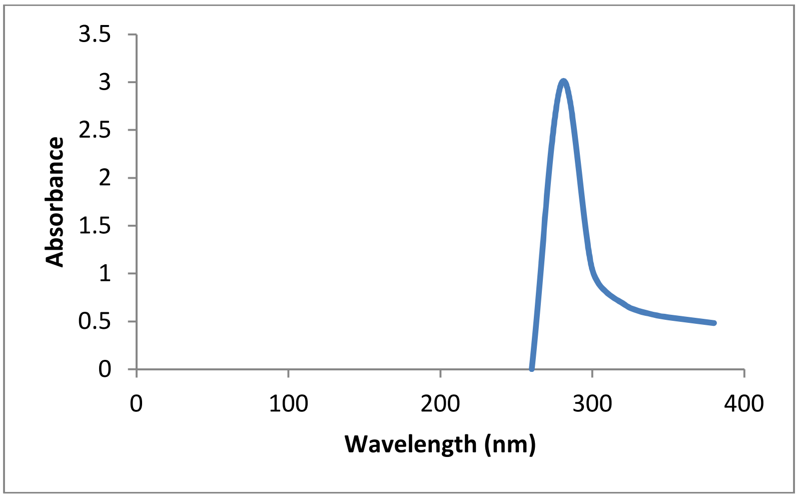

Fig. 1. UV-Visible spectrum of ZnO-CuO nanoporous composite

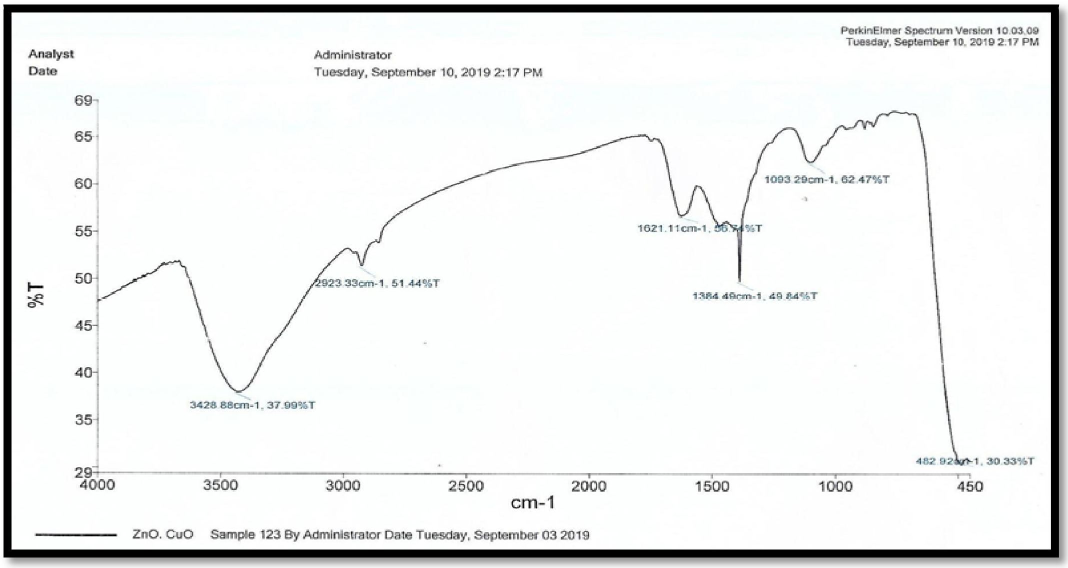

Fig. 2. FTIR spectrum of ZnO-CuO nanoporous composite

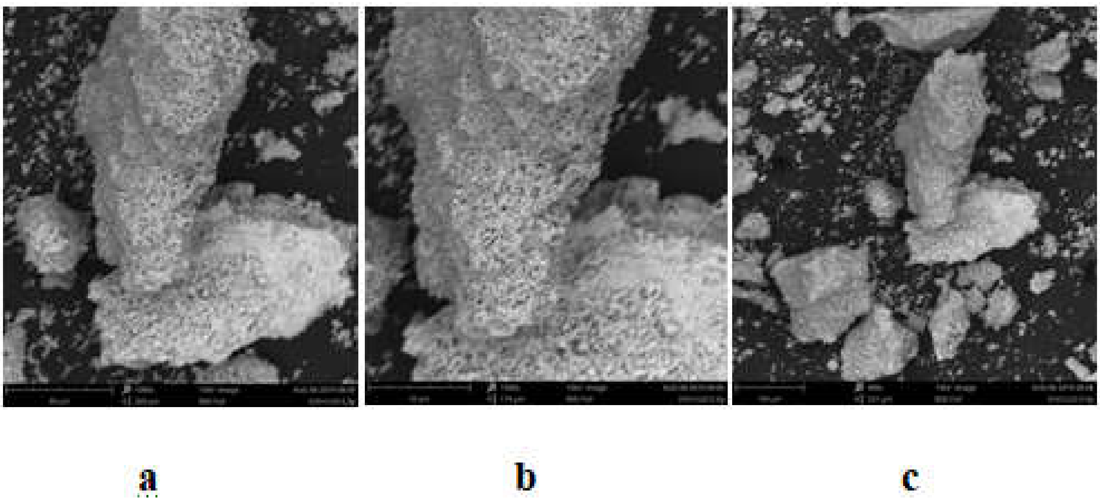

Fig. 3. SEM image of synthesized ZnO-CuO nanoporous composite A(100µm), B(50µm, 1500×) and C(80µm, 1000×)

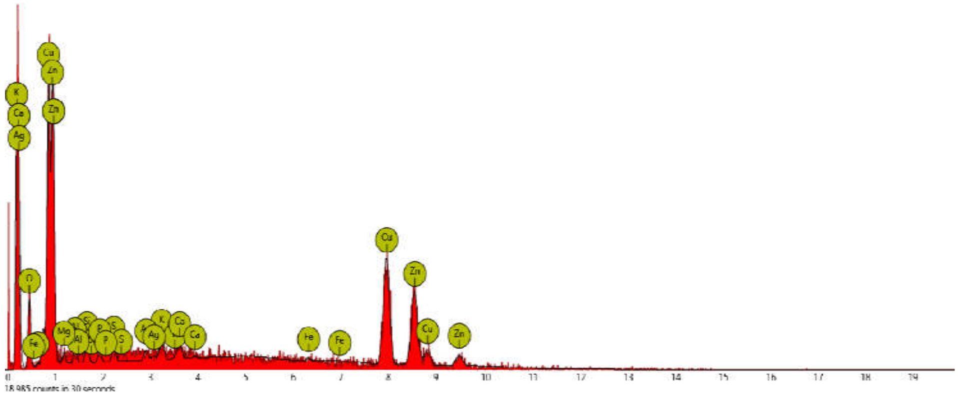

Fig. 4. EDX spectrum of ZnO-CuO nanoporous composite

Table 1. Elemental composition of ZnO-CuO nanoporous composite

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
29	Cu	Copper	28.76	39.40
30	Zn	Zinc	26.57	37.46
11	Na	Sodium	31.23	15.48
8	O	Oxygen	6.92	2.39
47	Ag	Silver	0.48	1.11
19	K	Potassium	0.84	0.71
14	Si	Silicon	1.10	0.67
20	Ca	Calcium	0.76	0.66
13	Al	Aluminium	0.92	0.53
12	Mg	Magnesium	0.94	0.49
16	S	Sulfur	0.65	0.45
15	P	Phosphorus	0.61	0.41
26	Fe	Iron	0.20	0.24

Table 2. Effect of synthesized ZnO-CuO nanocomposite on larvae malaria vector

Larval Instar	Conc. (Mg/L)	Mortality (%)	SD (±)	LC_{50} mg/L	LC_{90} mg/L	χ^2	r
1st	10	60	5.2	8.841	19.062	2.306	0.999
	20	85	7.3				
	25	100	2.3				
2nd	10	65	6.5	8.734	28.063	0.328	0.945
	20	85	3.3				
	25	85	5.2				
3rd	10	55	6.5	8.963	40.888		
	20	70	3.3			0.588	0.984
	25	85	5.2				
4th	10	50	0	10.577	79.567	0.6	0.945
	20	60	0				
	25	75	3.3				

LC_{50}, lethal concentration (mg/L) that kills 50% of larvae; LC_{90}, lethal concentration that kills 90% of larvae; SD, standard deviation, Mean value of five replicates; r, correlation coefficient, χ^2, chi square

4. CONCLUSION

The synthesis of nanocomposite using green path, especially plant derivatives is widely explored in recent times. The plant metabolites generate metallic nanoparticles that are ecofriendly. The focus of this study was on green synthesis of ZnO-CuO nanoporous composite using Gum Arabic. The synthesized nanocomposite was characterized using relevant techniques. Consequently, the ZnO-CuO nanocomposite was applied on the larvae of malaria vectors. This is in search of a substitute for conventional chemical based insecticides which the insects were reported to have developed resistance against them. The ZnO-CuO nanoporous composite showed high efficiency against the larvae of malaria vectors and has the advantage of less risk of developing resistance by the mosquitoes after a prolonged exposure.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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