LARVICIDAL ACTIVITY OF AMARANTHUS HYBRIDUS EXTRACT AND BIO-SYNTHESIZED COPPER NANOPARTICLES AGAINST MOSQUITO VECTORS

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Larvicidal Activity of Amaranthus Hybridus Extract and Bio-synthesized Copper Nanoparticles against Mosquito Vectors

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Abstract— This research covers the synthesis and characterization of Cu nanoparticles using leaf extract of Amaranthus hybridus and the larvicidal evaluation of Amaranthus hybridus and Cu nanoparticles. Nanoparticles are an important area in biotechnology that has been attracting interest recently. The bottom-up method of synthesizing nanoparticles using plant extract (green synthesis) was adopted for this study. In this study, copper nanoparticles were successfully synthesized using the leaf extract of African spinach and characterized using various instruments. The FTIR analysis reveals the presence of various functional groups, such as the O-H (hydroxy) group, which was observed at 3363 cm^{-1}; the O=C (carbonyl) group, which appears at 1633 cm^{-1}; the sp^3 C-H peak, which appears at 2924 cm^{-1}; C-C at 1014 cm^{-1}; and the Cu-O peak, which was observed at 676 cm^{-1}. A similar peak was not observed on the FTIR of Amaranthus hybridus. The SEM analysis shows crystalline irregular morphology. While the XRD analysis shows a face-centered cubic crystalline (FCC) shape, the size of the Cu nanoparticles was calculated to be 18.61 nm. The larvicidal activity of Amaranthus hybridus shows lethal concentration (LC) against Culex larvae; for the first instar, LC50 was found to be 40.42 mg/L, and LC90 was 82.08 mg/L. Second instar LC50 was 43.75 mg/L, and LC90 was 85.42 mg/L. For third/fourth instar, LC50 was 42.40 mg/L, while LC90 was 82.40 mg/L. Lethal dose/lethal concentration against Anopheles (LC50) for all instars was 32.86, 47.20, and 43.20 mg/L, respectively. LC90 was found to be (70.35, 87.20, 83.20) mg/L. The larvicidal activity of Cu NPs was calculated, and LC50 against Culex was found to be 22.20, 26.05, and 30.97 mg/L, respectively, and LC90 was found to be 42.20, 49.30, and 63.23 mg/L. While the LC50 of Anopheles larvae was found to be (28.84, 36.07, 35.40) mg/L and the LC90 was (52.09, 71.79, 64.60) mg/L, the correlation for both Culex and Anopheles' larvae was significant for all instars.

Keywords— Amaranthus Hybridus, Nanoparticles, Anopheles Larvae.

I. Introduction

Amaranthus hybridus falls under the Amaranthaceae family, encompassing 60-70 species. The Amaranthus genus is widespread across temperate and tropical zones worldwide, boasting about 70 species and 4,000 varieties [1]. In tropical African regions, its leaves and tender shoots are harvested and prepared by cooking, frying, or steaming, serving as a key food source during droughts [2]. Known as "amaranth" or "pigweed," Amaranthus hybridus is an annual herbaceous plant reaching heights of 1-6 feet. It contains phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenols, phytic acids, and hydrocyanic acid, alongside nutrients like β-carotene, thiamine, riboflavin, niacin, pyridoxine, ascorbic acid, tocopherols, and amino acids [3]. Also, according to [4], Amaranthus hybridus contains alkaloids, flavonoids, saponins, and tannins, while glycosides, anthraquinones, and phlobatannins were not detected. Medicinally, the plant addresses conditions like eye, ear, and stomach issues; dysentery; diarrhea; and serves as a diuretic, lactation aid, and treatment for hemorrhoids, menstrual issues, venereal diseases, paralysis, epilepsy, convulsions, and spasms [1].

The global nanoparticle market has expanded with growing interest in nanotechnology. "Nanoparticle" derives from the Greek "nano," meaning "dwarf," referring to particles with a size of one-billionth of a meter in diameter [5]. These particles, ranging from 1 to 100 nanometers, have drawn significant focus over the last decade for their diverse applications, especially in medicine [6].

Materials sized 1-100 nm are universally recognized as nanoparticles or nanomaterials [7]. Their small scale enhances versatility, aiding cellular uptake and supporting in vitro research and industrial applications [8, 9]. Metal nanoparticles, formed from metal salts or precursors, are valued for their localized surface plasmon resonance (LSPR) traits, with alkali and noble metals like copper, silver, and gold showing broad absorption in the visible solar spectrum [10].

Mosquitoes are categorized under phylum Arthropoda, class Insecta, and order Diptera [11], with three subfamilies, namely Toxorhynchitinae, Anophelinae, and Culicinae [12]. Over 3,530 mosquito species exist, organized into 43 genera within the Culicidae family [13]. Among them, Anopheles, Aedes, and Culex stand out for transmitting diseases to humans, birds, and mammals [14]. The Anopheles genus, named by Johann Wilhelm Meigen in 1818, includes "nail" or "marsh" mosquitoes [15], primarily known for carrying malaria via the Plasmodium parasite, a major health concern in tropical and subtropical areas [16]. Of over 450 Anopheles species, roughly 40 efficiently spread malaria [17]. In Africa, dominant vectors like Anopheles fenestus and Anopheles gambiae span central regions, joined by Anopheles arabiensis in sub-Saharan West Africa, while Anopheles darlingi leads in South America, and Asia features species like Anopheles minimus, Anopheles punctulatus, and Anopheles dirus, complicating vector studies [18].

The Culex genus thrives in tropical and temperate regions, with around 770 species, some acting as disease vectors for birds, humans, and animals [19, 20]. Notable species like Culex pipiens and Culex quinquefasciatus are critical for transmitting diseases, aided by their ability to feed on both humans and animals, spreading West Nile virus, Japanese encephalitis, St. Louis encephalitis, filariasis, and avian malaria [21]. Culex mosquitoes are vital in epidemiology, carrying pathogens like the filariasis parasite and arboviruses, driving morbidity and mortality in subtropical and tropical zones [22].

A. Mechanism of Action of Metal Nanoparticles on Larvae

- Oxidative stress: metal nanoparticles induce the production of reactive oxygen species (ROS) that lead to oxidative damage in mosquito larvae [23]. Metal nanoparticles trigger oxidative stress by producing ROS such as superoxide (O_2-), hydroxyl radicals (OH.), and hydrogen peroxide (H_2O_2). In mosquito larvae, excessive ROS disrupt cellular balance by damaging lipids, proteins, and DNA.
- Membrane disruption: studies on Cu NPs have shown that they cause rupture in the midgut epithelium of larvae, causing leakage of cellular contents and impaired osmoregulation physical damage due to interaction of metal nanoparticles and cellular membrane [24] [25]. Similarly, the metal nanoparticles destabilize cell membranes through electrostatic interactions or direct physical damage. The positive charge of the metal nanoparticles enables them to bind to the negatively charged membrane components, altering membrane permeability.
- Enzyme inhibition: metal nanoparticles can interfere with enzyme activities that are crucial for larval survival and development [26]. Cu^{2+} ions from Cu nanoparticles suppress acetylcholinesterase, a key enzyme for function, resulting in paralysis and death. Also, metal nanoparticles impair proteases and amylases in the larval gut, hindering nutrient digestion and absorption, which prevents molting or pupation [27].
- Physical contact and cuticle penetration: Due to their nano size, metal nanoparticles interact directly with the larval cuticle (the protective outer layer). Metal nanoparticles also breach the cuticle via spiracles and the digestive tract, inflicting mechanical harm to epithelial cells [26].

B. Materials

Beaker, weighing balance, spatula, cuvette, stirring rod, syringe, hot plate, Whatman No. 1 filter paper, measuring cylinder, hot air oven, mortar and pestle, plastic cups, FTIR machine (PerkinElmer), spectrum version 10 03 09, UV-vis spectrophotometry machine (Jenway 6705), SEM machine (Hitachi 4160), and XRD spectrophotometer (X-pert plus). CuSO4. 6H2O, distilled water, KBr.

II. METHODOLOGY

A. Preparation of 0.1 M Copper Sulfate Hexahydrate (CuSO4. 6H20)

We weighed 6.69 g (0.025 mol.) of $CuSO_4{\cdot}6H_2O$ and transferred it into a 250 cm^3 volumetric flask. We added a little quantity of distilled water and then shook it until it completely dissolved, and then we filled it to the mark using distilled water.

B. Synthesis of Copper Nanoparticles

We poured 250 cm^3 of 0.1 M copper sulfate solution into a 500 cm^3 beaker. Using a syringe, we slowly added 62 cm^3 of aqueous Amaranthus hybridus leaf extract dropwise to the copper sulfate solution with continuous stirring. We maintained the temperature at 60°C. We observed a color change and noticed the formation of copper nanoparticles as precipitate formed. We allowed the reaction mixture to stand for 24 hours to let the particles settle. Then, we decanted the mixture, washed the residue with deionized water, and dried it at 110°C in a hot air oven for 6 hours to obtain fine Cu NPs. We adopted the method of [28] with little modification.

C. Characterization

We carried out various analyses and employed them for the characterization of the synthesized Cu nanoparticles. We employed various characterization techniques, which include UV-visible spectrophotometry, Fourier transform infrared (FTIR) spectrophotometry, scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX), and X-ray diffraction (XRD).

D. UV-Visible Spectrophotometry

We collected a small aliquot of Cu nanoparticles and poured it into a clean cuvette, then placed it into the UV-Vis spectrophotometer (Jenway 6705) and scanned from 200 nm to 800 nm to find the wavelength of maximum absorbance (λmax). Then we varied the wavelength and ran it to find the absorbance. We used distilled water as a blank. We employed the method of [29]. With little modification.

E. Fourier Transform Infrared (FTIR)

We took 50 mg of Cu nanoparticles and mixed them with 250 mg of KBr. Using a hydraulic press, we made the mixture into a pellet and then pressed it into a disc. We used this disc to acquire the spectra within the range 4000–200 cm^{-1}.. We carried out the analysis using an FTIR spectrophotometer (PerkinElmer).

F. Scanning Electron Microscopy (SEM)

We conducted SEM analysis using SEM (Hitachi 4160) in order to determine the morphology, size, and composition of the element present in the synthesized nanoparticles.

G. X-Ray Diffraction (XRD) Analysis

We conducted XRD analysis using X-pert plus in order to find the average crystalline size. The Debye-Scherrer equation was used to calculate the average crystalline size. The Debye-Scherrer equation is as follows:

$$D = K\lambda/\beta COS\theta$$

Where D = particle size
K = Constant Volume
λ = X-ray wavelength (0.154nm)
θ = Bragg's angles (in degrees).
P = Line broadening at half the maximum intensity

H. Larvicidal Bioassay

We collected Culex and Anopheles' larvae from stagnant water in Gombe metropolis and sorted them into three groups based on their growth stages. We combined the third and fourth instars into one group due to the difficulty in obtaining a uniform population. Meanwhile, we prepared a 100 mg/L Cu NPs stock solution and used it to produce other subsequent concentrations through serial dilution. In a plastic cup, we filled it with 100 ml of distilled water to serve as the control. Next, we prepared 100 ml of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L Cu Np concentrations, and then we placed 25 larvae into each concentration and assessed them for larvicidal activity. We recorded the larval mortality after 24 hours of exposure.

I. Statistical Analysis

We calculated the percentage mortality using Abbott's formula. Then, we carried out probit analysis to determine lethal concentration at 50% (LC50) and lethal concentration at 90% (LC90), correlation, and chi-square distribution using statistical software SPSS 2016.

III. RESULT AND DISCUSSION

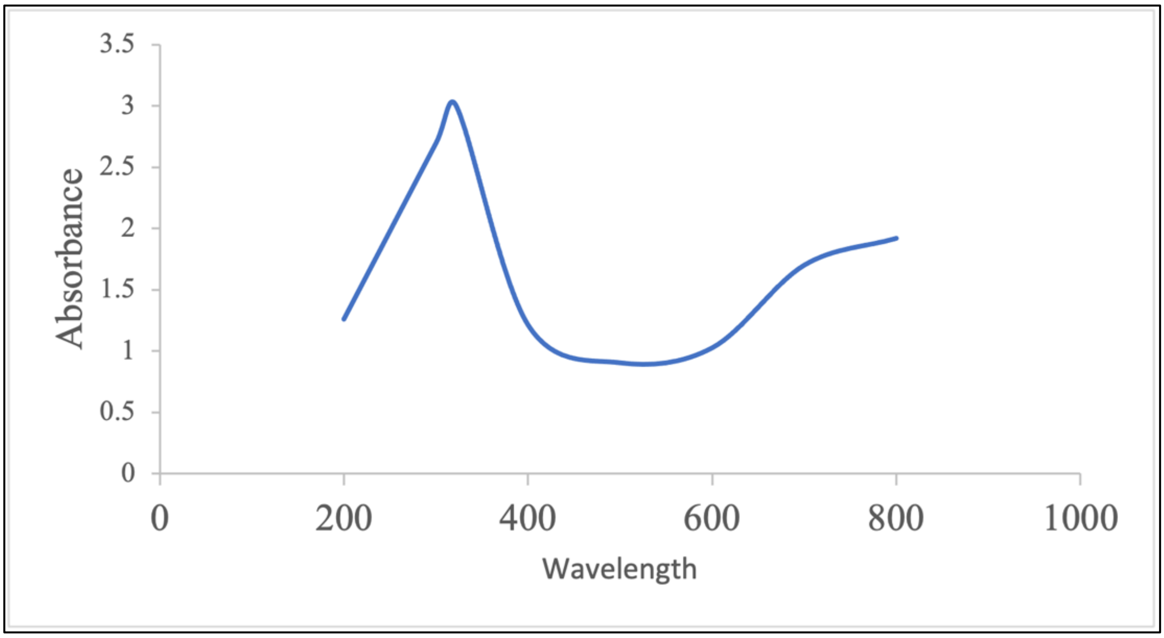

Fig. 1. Showing the result of UV-vis analysis of Cu NPs

The wavelength of maximum absorbance was found at 322 nm, which is near the region of absorbance of violet color, which corresponds to the change of color of the solution from light blue to dark blue. A similar λmax was found by [30], which shows the maximum absorbance at 340nm. The absorbance was ascribed to surface plasmon resonance. The wavelength of maximum absorbance λmax is affected by many factors; hence, the λmax of Cu NPs is not peculiar [31].

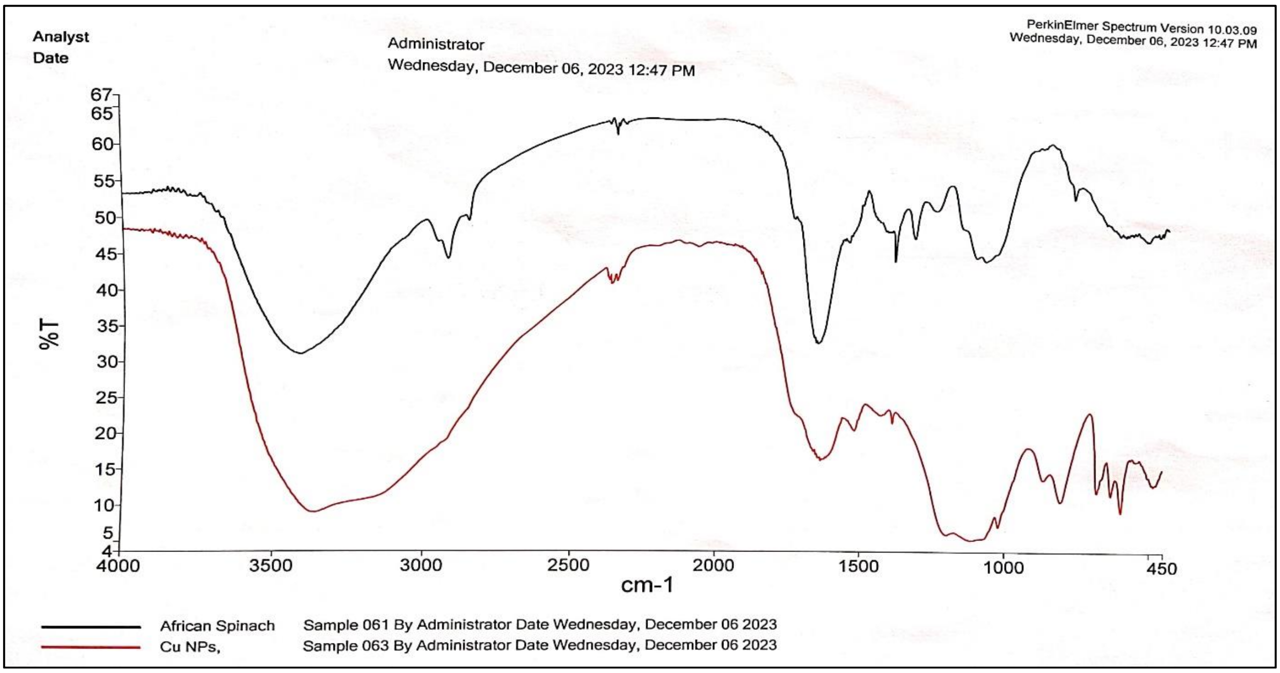

Fig. 2. Superimposed FTIR of African spinach and Cu NPs

Major peaks were observed at 3418 cm-1, 3363 cm-1, 2924 cm-1, 1644 cm-1, 1633 cm-1, 1384 cm-1, 1384 cm-1, 1072 cm-1 1014 cm-1, 676 cm-1 for the African spinach and Cu NPs which are due to vibration of bonds (stretching or bending) of molecules.

Table 1: Showing IR absorption (cm-1) frequencies of African spinach and Cu NPs

African spinach (Cm^{-1})	**Cu NPs (Cm^{-1})**	**Literature (Cm^{-1})**	**Assignment**	**Probable source**
3418 Br (S)	3363 Br (S)	3200-3500	OH	Alcohol, phenol, carboxylic acid, tannin, alkaloid, flavonoid
2924 Sh (M)	N. O	2800-3000	Sp^3 C-H	Alkane
1644 Sh (S)	1633 Sh (S)	1620-1680	C=O, C=C, C=N	Carbonyl, alkene, alkaloid, Aldehyde, ketone.
1314 Sh (M)	1384 Sh (S)	1360-1390	C-H, C-N	Amine, alkane, alkene, alkyl,
1072 Br (M)	1014 Br (S)	1150-1250	C-C, C-O	Carboxylic acid, alkane, ether, esther, alkoxy
N. O	676 Sh (S)	450-600	Cu-O	$CuSO_4$. $7H_2O$, Phytochemicals

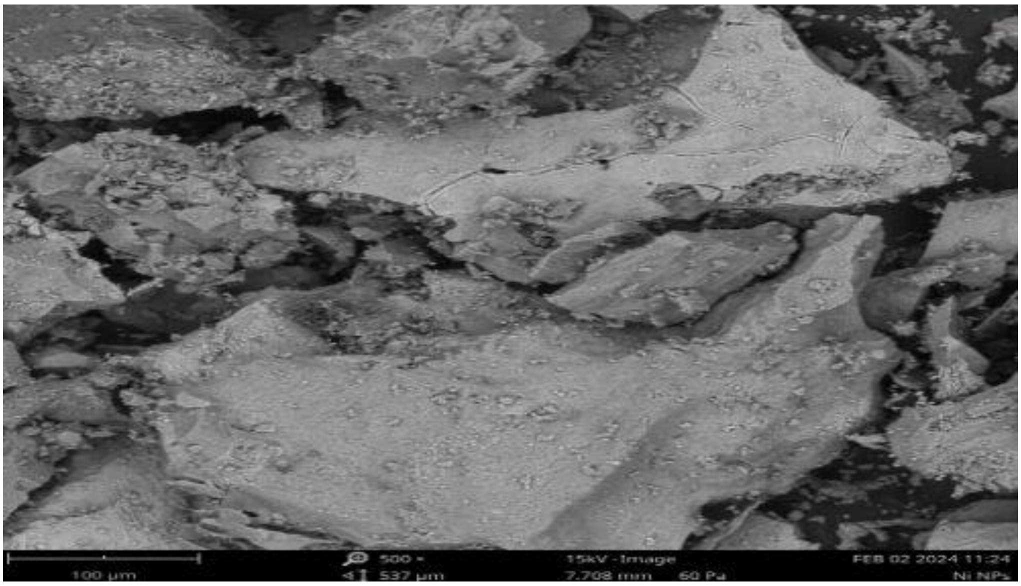

Fig. 3. Showing the SEM result for Cu NPs

The SEM result of the Cu nanoparticles under ×500 magnification, as shown in figure 4, shows irregular surface morphology, mostly crystalline with triangular-like shapes of different sizes, which might be due to aggregation of the nanoparticles

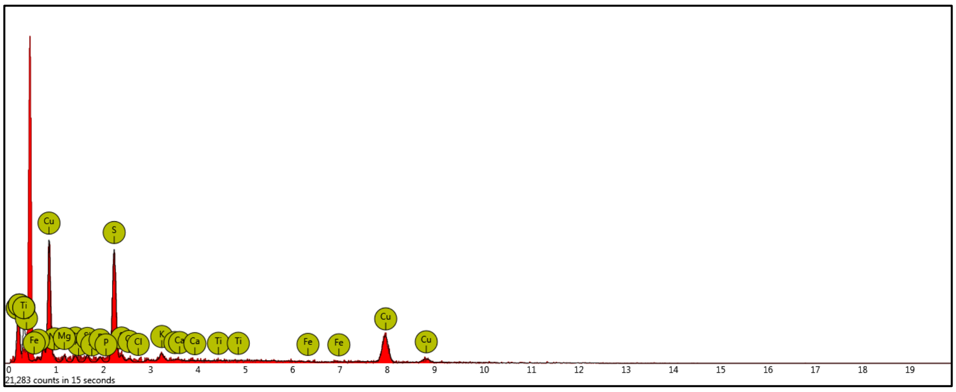

Fig. 4. The EDX of Cu NPs

In the copper NPs Cu and sulfur have the higher percentage of 60.54% and 23.85% while iron and titanium have the least percentage of 0.34% and 0.24% respectively. Other elements are in trace amounts.

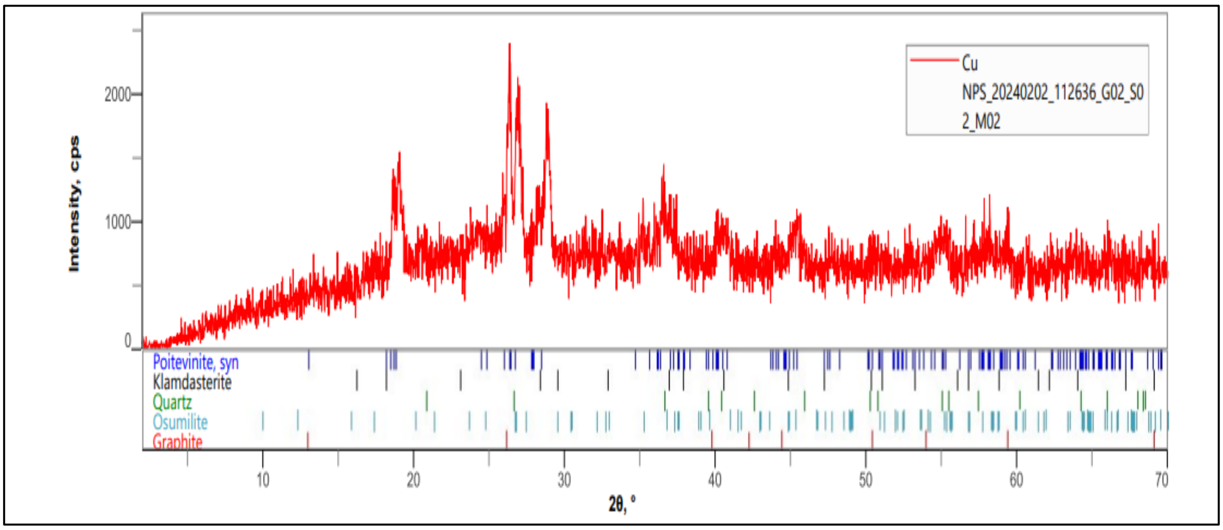

Fig. 5. Showing the XRD result of Cu NPs

The XRD analysis of the synthesized Cu nanoparticles shows eight (8) prominent peaks at 2θ = 19.02, 26.33, 26.87, 28.87, 36.51, 40.49, 45.27, and 55.20, which correspond to the planes of (200), (202), (220), (300), (321), (303), (323), and (404). Respectively, this shows a face-centered cubic (FCC) structure, which is in agreement with the crystalline structure of Cu. The sizes were calculated to be 12.5nm, 55.51nm, 16.92nm, 19.55nm, 8.62nm, 10.67nm, 13.61 nm, and 11.47 nm, respectively, and the average crystalline size was 18.61nm. This is similar to the report by [32] that found the average crystal size to be 11±1 nm.

Table 2: Showing the larvicidal activity of African spinach on culex larvae

CULEX	S/N	CONC (mg/L)	% MOTALITY	LC50 (mg/L)	LC90 (mg/L)	R	X^2
FIRST INSTAR	1	10	20				
	2	20	28				
	3	30	44	40.42	82.08	0.980	
	4	40	52				0.714
	5	50	56				
SECOND INSTAR	1	10	16				
	2	20	24				
	3	30	44	43.75	85.42	0.958	
	4	40	48				0.787
	5	50	52				
THIRD/ FORTH INSTAR	1	10	16				
	2	20	20				
	3	30	52	42.40	82.40	0.877	
	4	40	48				0.430
	5	50	52				

Table 2 above shows the result of larvicidal activity of plant extract on Culex larvae; the highest mortality was found to be 56%, 52%, and 52% at 50 mg/L for all instars, respectively. The lethal concentrations (LC) at 50% and 90% were calculated, and the results show good correlation.

Table 3: Showing the larvicidal activity of African spinach on anopheles' larvae

ANOPHELES	S/N	CONC (mg/L)	% MOTA LITY	LC50 (mg/L)	LC90 (mg/L)	R	X^2
FIRST INSTAR	1	10	20	32.86	70.35	0.984	0.853
	2	20	28				
	3	30	48				
	4	40	56				
	5	50	64				
SECOND INSTAR	1	10	16	47.20	87.20	0.967	0.810
	2	20	20				
	3	30	28				
	4	40	48				
	5	50	52				
THIRD/ FORTH INSTAR	1	10	12	43.20	83.20	0.953	0.830
	2	20	28				
	3	30	44				
	4	40	48				
	5	50	52				

Table 3 above shows the result of larvicidal activity of African spinach on Anopheles' larvae; the highest mortality was found to be 62%, 52%, and 52% at 50 mg/L for all instars, respectively. The lethal concentrations (LC) at 50% and 90% were calculated, and the results show a good correlation above 0.9 for all instars. The mortality is attributed to the presence of phytochemicals in the leaves of the plant extract. This work can be compared to the work of [33], which found similar results.

Table 4: Showing the larvicidal activity of Cu nanoparticles on culex larvae

CULEX	S/N	CONC Mg/L	% MOTA LITY	LC50 mg/L	LC90 mg/L	R	X^2
FIRST INSTAR	1	10	24	22.20	42.20	0.990	0.880
	2	20	44				
	3	30	68				
	4	40	92				
	5	50	100				
SECOND INSTAR	1	10	20	26.05	49.30	0.995	0.049
	2	20	44				
	3	30	56				
	4	40	72				
	5	50	92				
THIRD/ FORTH INSTAR	1	10	20	30.97	63.23	0.967	0.104
	2	20	36				
	3	30	56				
	4	40	64				
	5	50	68				

Table 4 above shows the result of larvicidal activity of Cu nanoparticles on Culex larvae; the highest mortality was found to be 100%, 92%, and 68% at 50 mg/L, while the lowest mortality (24%, 20%, and 20) % was recorded at 10 mg/L for all instars, respectively. The lethal concentrations (LC) at 50% and 90% were calculated, and the results show good correlation above 0.9 for all instars. Similar findings were reported by [34].

Table 5. Showing the larvicidal activity of Cu NPs on anopheles' larvae

ANOPHELES	S/N	CONC Mg/L	% MOTA LITY	LC50 mg/L	LC90 mg/L	R	X^2
FIRST INSTAR	1	10	24	28.84	52.09	0.976	0.099
	2	20	32				
	3	30	44				
	4	40	68				
	5	50	92				
SECOND INSTAR	1	10	24	36.07	71.79	0.973	0.110
	2	20	28				
	3	30	40				
	4	40	60				
	5	50	64				
THIRD/ FORTH INSTAR	1	10	16	35.4	64.60	0.990	0.635
	2	20	32				
	3	30	40				
	4	40	60				
	5	50	68				

Table 5 above shows the larvicidal activity of Cu NPs on Anopheles' larvae. For the first instar, the LC50 was found to be 2.84 mg/L, 52.09 mg/L for LC90, and the correlation was 0.976. For the second instar, LC50 was 36.07 mg/L and LC90 was 71.79 mg/L, and the correlation was 0.973, while for the third/fourth instar, LC50 was 35.4 mg/L and LC90 was 64.60 mg/L with a correlation of 0.990. The highest mortality was recorded at 50 mg/L, while the lowest mortality was recorded at 10 mg/L. The correlation was significant; this work relates to the research done by [35].

IV. Conclusion

Cu NPs were synthesized using aqueous African spinach extract and the nanoparticles were obtained as a fine powder. The nanoparticles were subjected to series of characterization as follows, the UV analysis of the nanoparticles, the wavelength of maximum absorbance of was found at 322 nm for and Cu NPs. The FTIR analysis reveals the various functional groups that are present in the plant extract and Cu NPs the functional groups include hydroxy group (O-H), carbonyl (=O), Sp3 C-H, Sp2 C=H and metal-oxygen (M-O). The SEM analysis of Cu nanoparticles shows crystalline structure with irregular morphology. The XRD analysis of Cu nanoparticles shows FCC crystalline shape with size 18.61nm for Cu NPs.

The larvicidal activity of plant extract and Cu nanoparticles against culex and anopheles' larvae was carried out. The highest mortality was found at 50 Mg/L the lethal dose or lethal concentration (LC) was calculated for 50% and 90% mortality also the correlation shows higher significance with minimum of 0.861 and maximum of 0.995. Cu NPs was the most effective with lowest mortality of 20% and highest mortality of 100% for culex larvae and 16% lowest and 92% highest mortality for anopheles' larvae.

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