

Evaluating the Potential of Green Synthesized Zinc Oxide Nanoparticles from the Fruit Extracts of *Balanites Aegyptiaca* as an Antimicrobial and Larvicidal Agent

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ABSTRACT

This study evaluates the potential of green-synthesized zinc oxide nanoparticles (ZnO-NPs) derived from the fruit extract of *Balanites aegyptiaca* as an antimicrobial and larvicidal agent. The synthesised nanoparticles were characterized using a UV-Visible spectrophotometer, FTIR, XRD and SEM analysis. The antimicrobial potentials of the ZnO-NPs were evaluated against two Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*), two Gram-negative bacteria (*Salmonella typhi*, *Klebsiella pneumoniae*), and two fungal species (*Candida albicans*, *Aspergillus niger*). The ZnO-NPs have demonstrated moderate activity across Gram-positive, Gram-negative and fungal strains at the highest concentration (30 µg/ml). The larvicidal efficacy of the synthesized ZnO-NPs was tested against *Anopheles* mosquito larvae at concentrations of 40, 50, and 60 mg/L. The ZnO-NPs caused 100% mortality in third and fourth instar larvae, with LC₅₀ values ranging from 35.30 to 59.47 mg/L across instars. These findings underscore the potent larvicidal and antimicrobial properties of ZnO-NPs synthesized from the fruit extract of *Balanites aegyptiaca*, with dose-dependent effects and notable efficiency against disease vectors and pathogens. The study supports the development of eco-friendly nanobiopesticides and therapeutics from medicinal plants.

Keywords: Anopheles Larvae, Antimicrobial, *Balanites aegyptiaca* (Fruit), Green Synthesis, Larvicidal, Mortality.

INTRODUCTION

Nanoparticles are defined as particles with at least one dimension measuring less than 100 nanometers, exhibiting various unique properties such as a high surface area-to-volume ratio, specific crystal structures, tunable pore sizes, and the ability to influence cellular and molecular activities in living organisms [1]. Nanoparticles (NPs) are particles engineered or manipulated at the atomic scale, typically ranging from 1 to 100 nanometers. Due to their nanoscale dimensions, they exhibit unique properties that differ significantly from those of bulk materials. Their small size also results in a relatively larger surface area compared to their bulk counterparts [2]. Nanoparticles (NPs) involve the characterization, design, and engineering of both biological and non-biological structures smaller than 100 nanometers, which exhibit unique and novel functional properties [3]. Research on nanoparticles (NPs) has gained significant attention due to their enhanced electrochemical reactivity, thermal conductivity, and nonlinear optical properties, which enable a wide range of unique applications [4]. Metal nanoparticles have recently garnered considerable interest due to their remarkable properties, including a large surface area, high stability, ease of chemical modification, effectiveness as fillers for enhanced permeability, and versatility in synthesis. These nanoparticles can be produced in sizes ranging from the micrometric to the nanometric scale using either top-down (destructive) or bottom-up (constructive) approaches. Zinc oxide (ZnO) is considered a biologically safe material that exhibits photo-oxidizing and photocatalytic effects on both chemical and biological entities [2]. Nano-sized zinc oxide (ZnO) exhibits various morphologies and has demonstrated significant antibacterial activity against a wide range of bacterial species, as reported by numerous

researchers [5]. Zinc oxide (ZnO) is currently being studied as an antibacterial agent in both microscale and nanoscale forms. When reduced to the nanometer scale, ZnO exhibits enhanced antimicrobial properties, enabling nano-sized particles to interact more effectively with bacterial surfaces and potentially penetrate the bacterial cell. These interactions result in unique bactericidal mechanisms. The primary mode of action is toxic interaction with bacterial cells, making nano-sized ZnO particularly valuable for antimicrobial applications, especially in the food industry [6]. Zinc oxide nanoparticles (ZnO-NPs) exhibit notable antibacterial properties, primarily due to their increased specific surface area resulting from reduced particle size, which enhances their surface reactivity [2]. Zinc oxide nanoparticles (ZnO-NPs) have attracted significant global research interest due to their broad biological activity. They are relatively non-toxic to human cells, biodegradable, and have the potential to significantly enhance the bioactivity of pharmacophores. Compared to their bulk counterparts, ZnO-NPs demonstrate superior antibacterial performance, attributed to quantum confinement and size-dependent effects. Their ability to inhibit bacterial growth through multiple mechanisms makes them highly effective in combating bacterial contamination-related diseases, particularly as conventional antibiotics face increasing challenges due to rising bacterial resistance [7]. The biosynthesis of zinc oxide nanoparticles (ZnO-NPs) using extracts from therapeutic plants, fungi, bacteria, and algae enhances their stability and biocompatibility across various biological contexts. This bio-fabrication approach also alters their physicochemical properties, contributing to their enhanced biological efficacy. Furthermore, ZnO-NPs function as effective nanocarriers for conventional drugs, owing to their cost-effectiveness, biodegradability, and biocompatibility [8]. Zinc oxide nanoparticles (ZnO-NPs) have been explored for a wide range of applications, including drug delivery systems, biosensing, gene delivery, nanomedicine, biological imaging, coatings for medical implants, electronic sensing devices, wastewater treatment, and communication technologies (9).

Balanites aegyptiaca (L.) Del., commonly known as the 'desert date,' is an evergreen, woody, and spiny flowering tree that can grow up to 10 meters tall. A member of the Balanitaceae family, it is widely distributed across arid regions of Africa and Southern Asia. This plant is a rich source of therapeutic compounds, including saponins, flavonoids, alkaloids, lipids, proteins, carbohydrates, and organic acids. Various parts of the tree have been traditionally used in medicine to treat a wide range of ailments [10]. It is well noted for its various medicinal properties, including antidiabetic, anthelmintic, antibacterial, and antiviral activities. Different parts of the plant, such as the bark, unripe fruits, and leaves, have been reported to exhibit anthelmintic, antifertility, purgative, and antidysentery properties [11].

Balanites aegyptiaca is a widely cultivated desert plant known for its versatile applications. It primarily thrives in arid and semi-arid regions across Africa, the Middle East, and South Asia. [12]. Traditionally, various parts of *Balanites aegyptiaca* have been valued for their medicinal applications in the treatment of conditions such as skin boils, leukoderma, malaria, wounds, colds, syphilis, and liver and spleen disorders. The fruit, commonly known as the desert date, is the most notable part of the tree. This drupe is pubescent when unripe and becomes yellowish and smooth upon ripening. It comprises four distinct layers: the outer skin (epicarp), the fleshy pulp (mesocarp), the woody shell (endocarp), and the inner seed (kernel). All four layers have potential uses in both industrial and pharmaceutical contexts. The edible portions—particularly the pulp and kernel—are known to yield oil. Nutritionally, the pulp is especially rich in carbohydrates (62.63%) and protein (9.19%), with lower contents of fat (2.58%) and dietary fibre (2.93%) [13]. Local inhabitants utilise the fruits of *Balanites aegyptiaca* for the treatment of various ailments. [14]. The fruit is also used in the treatment of jaundice, while the oil extracted from the seeds serves as a laxative and is traditionally employed to treat a variety of ailments, including hemorrhoids, stomach aches, jaundice, yellow fever, syphilis, and epilepsy [11].

MATERIALS AND METHODS

Collection of Plant Materials

Fruits of *Balanites aegyptiaca* were collected from the field within the premises of the University of Maiduguri, Borno State, Nigeria, and taken to the Department of Plant Science for identification and authentication. The samples were gathered during the dry season, then sorted and stored in clean polythene bags for further analysis.

Anopheles mosquito larvae were collected from various identified breeding sites within Gombe metropolis, including sewage and sullage water bodies such as cesspools, cesspits, drains, and septic tanks. A ladle and a

collection bottle were used for the sampling. The ladle was positioned at approximately a 45-degree angle and gently lowered into the water until one side was just beneath the surface, ensuring minimal disturbance to prevent the larvae from diving. The collected larvae were subsequently maintained and reared in the laboratory for larvicidal bioassay experiments. The collection was done based on Abba *et al.* [9].

Sample Preparation

Fruits of *Balanites aegyptiaca* were collected, thoroughly washed with distilled water, and air-dried in the shade to prevent phytochemical degradation. The mesocarp was then carefully separated from the shell

Preparation of *Balanites aegyptiaca* Fruit Extract

The aqueous extraction of *Balanites aegyptiaca* fruit was adapted from Al-Senani (2020) [15], with minor modifications. Ten grams (10 g) of the sample were dissolved in 200 mL of distilled water in a 250 mL conical flask. The mixture was heated at 60 °C for 3 hours in a water bath with constant stirring. After cooling to room temperature, the solution was filtered using Whatman No. 1 filter paper. The freshly prepared aqueous extract was then used for the synthesis of zinc oxide nanoparticles.

Synthesis of Zinc Oxide Nanoparticles.

Ten millilitres (10 mL) of *Balanites aegyptiaca* fruit extract were added to 50 mL of 0.1 M ZnCl₂ and 5 mL of 0.5 M NaOH. The mixture was stirred using a magnetic stirrer hot plate at 50 °C for 30 minutes, during which a noticeable colour change indicated the formation of zinc oxide nanoparticles. The nanoparticles were then separated by filtration, dried, and stored for later use [15].

Characterization of Zinc Oxide Nanoparticles and Fruit Extract

The optical properties of the synthesized zinc oxide nanoparticles and the fruit extract were analyzed using UV-Visible spectrophotometry within a wavelength range of 200–800 nm. Fourier-Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups present in the samples. X-ray Diffraction (XRD) was used to determine the crystal structure of the nanoparticles, while Scanning Electron Microscopy (SEM) was utilized to examine their morphology and particle size.

Antimicrobial Activity

The antimicrobial activity of the synthesized zinc oxide nanoparticles was evaluated against selected bacterial and fungal pathogens using the agar well diffusion method. This widely used laboratory technique assesses the ability of a substance to inhibit microbial growth, and is commonly applied in testing the antimicrobial efficacy of plant- and microbe-derived extracts. To conduct the assay, a bacterial suspension was evenly spread onto the surface of an agar plate. Wells were then created in the agar using a sterile borer, and the nanoparticle solution was introduced into each well. The plates were incubated, and subsequently observed for clear zones of inhibition surrounding the wells. The diameters of these zones were measured to quantify antimicrobial activity. An increase in the size of the inhibition zone with rising nanoparticle concentration indicated enhanced antimicrobial effectiveness.

Larvicidal Activity

The larvicidal activity was assessed following the method described by Abba *et al.* [9], with slight modifications. A 0.1 g sample of zinc oxide nanoparticles was dissolved in distilled water using a 1000 mL volumetric flask to prepare a stock solution with a concentration of 100 mg/L. The bioassay was conducted by introducing different larval instars (1st to 4th) into 200 mL plastic cups, with four replicates and a control group for each instar, each containing twenty-five larvae. To each replicate, 100 mL of dechlorinated water was added. Zinc oxide nanoparticles were then introduced at concentrations of 40 mg/L, 50 mg/L, and 60 mg/L. Larval mortality was recorded, and the percentage mortality was calculated using the following formula: Percentage Mortality =

$$\frac{\text{Number of Dead Larvae}}{\text{Number of Introduced Larvae}} \times 100$$

RESULTS AND DISCUSSIONS

Electronic Spectra Results of Zinc Oxide Nanoparticles and Fruit Extract

The UV–Visible spectra for fruit extract and ZnO nanoparticles are presented in Figures 1A and 1B. ZnO nanoparticle displayed an absorbance band at 348 nm, and the fruit extract shows an absorbance band at 308 nm. The movement of the absorbance band to a higher wavelength suggests a reduction in size from bulk molecules to the nanoscale. This is due to the surface Plasmon vibration and excitation of bio-reduction and capping or stabilising agents present in the fruit extract [16]. This also corresponds to the work done by Naranjan *et al.* [17] and Preeti [18], which suggested the possibility of finding the Plasmon band of zinc oxide nanoparticles in the range of 300-400nm.

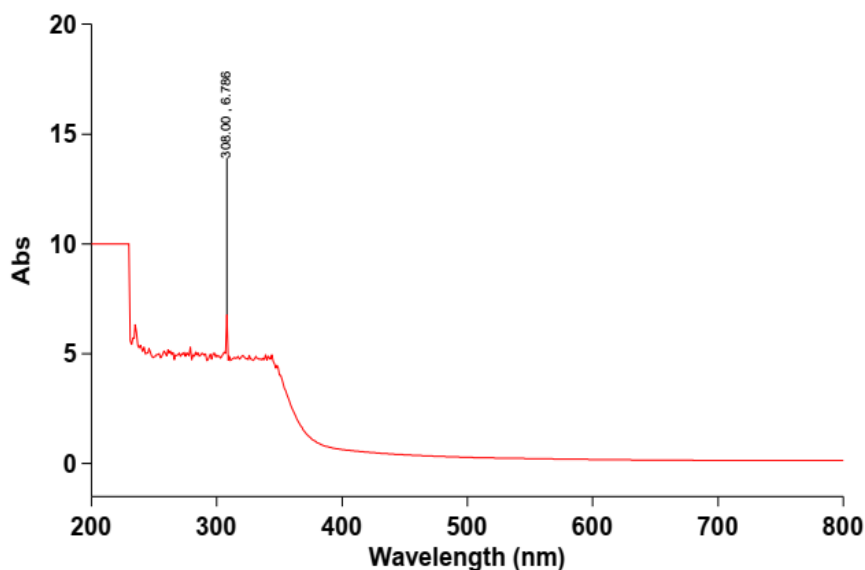


Figure 1A: Electronic Spectrum of *Balanites aegyptiaca* Fruit Extract

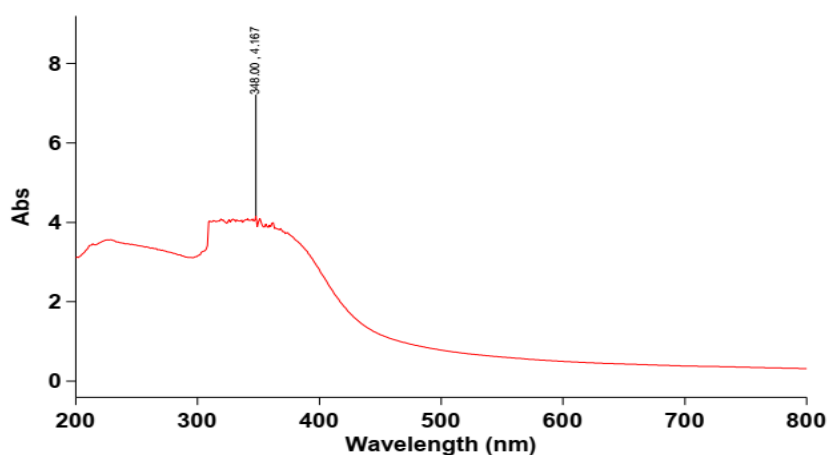


Figure 1B: Electronic Spectrum of ZnO Nanoparticles Synthesized Using Fruit Extract

Fourier Transform Infrared (FTIR) Results of Fruit Extract and Zinc Oxide Nanoparticles

The FTIR spectrum of *Balanites aegyptiaca* fruit extract and ZnO nanoparticles are presented in Figures 2A and 2B. The absorption bands observed for fruit extract were 3268 cm^{-1} , 2120 cm^{-1} and 1638 cm^{-1} indicating OH stretching, C=C stretching and C=O stretching respectively. While for the ZnO nanoparticles synthesized from the fruit extract, the following bands were observed at 3563 cm^{-1} , 3436 cm^{-1} , 2922 cm^{-1} , 2108 cm^{-1} , 1606 cm^{-1} , 1423 cm^{-1} , 1263 cm^{-1} , 1028 cm^{-1} , 818 cm^{-1} and 701 cm^{-1} . These bands are absent in the spectra of the extract, signifying the formation of nanoparticles (19). The band at 3563 cm^{-1} , 3436 cm^{-1} are due to OH alcohol/ phenols,

2922 cm^{-1} is for C=O stretching for alkenes, 2108 cm^{-1} is for C-O alcohol, 1606 cm^{-1} for C=C alkenes, 1423 cm^{-1} for CH_3 bending and C-C is for ring aromatic, 1263 cm^{-1} is for C-N aromatic amines, 1028 cm^{-1} is for C-O stretching due to alcohol, and 818 cm^{-1} and 701 cm^{-1} are due to C-Br alkyl halides.

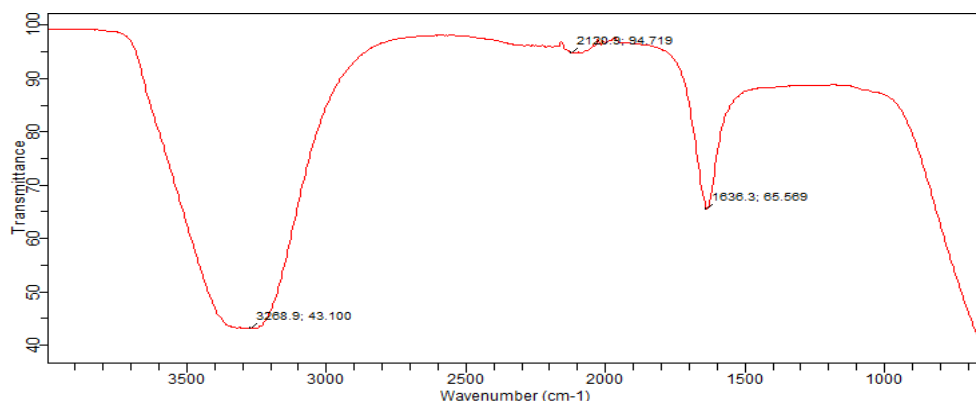


Figure 2A: FTIR Spectrum of Balanites aegyptiaca Fruit Extract

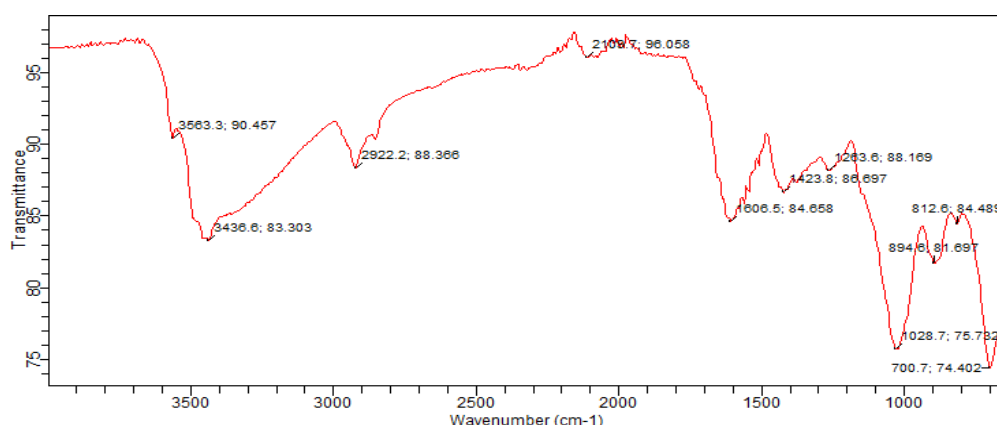


Figure 2B: FTIR Spectrum of ZnO Nanoparticles Synthesized Using Fruit Extract

XRD Results of ZnO Nanoparticle Synthesized from The Fruit

The XRD result for the ZnO nanoparticle synthesized from the fruit is presented in Figure 3. It was observed that five prominent peaks were observed at $2\theta=12.89^\circ$, 21.67° , 33.46° , 43.47° and 52.51° with respect to the plane of (111), (201), (211), (221) and (311) respectively. It shows Face Centered Cubic (FCC) structure and the average crystalline size of 46.77 nm and it corresponds to the literature reported by Yangma and Jinhou (2020) [20].

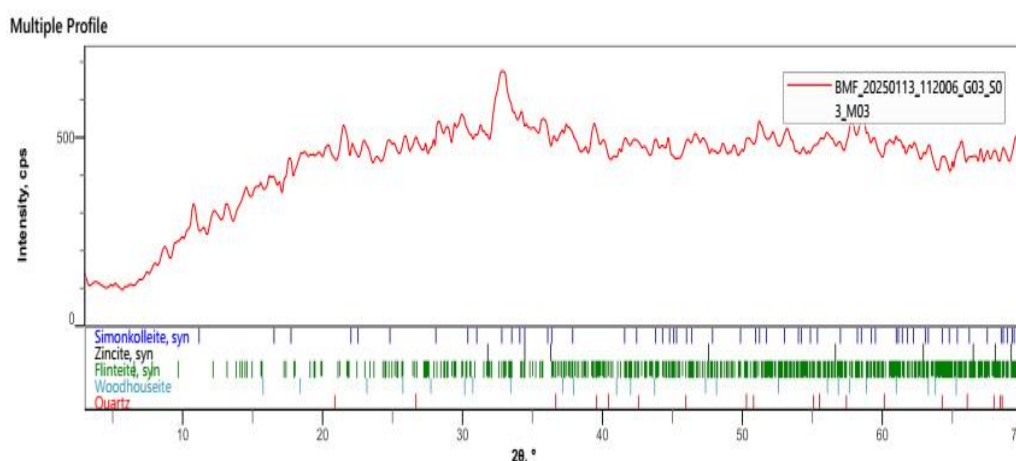


Figure 3: XRD Result of ZnO Nanoparticle Synthesized Using Fruit Extract

SEM Analysis of Zinc Oxide Nanoparticle Synthesized from The Fruit

The SEM result for the ZnO nanoparticles synthesized from the fruits are presented in Figure 4. The morphology of the green synthesized ZnO nanoparticles shows that the particles have monodispersed granular and partially agglomerated morphology. The result obtained is in agreement with the previous works done by Dominic *et al.* (2020) [21] and Hassan *et al.* (2019) [22].

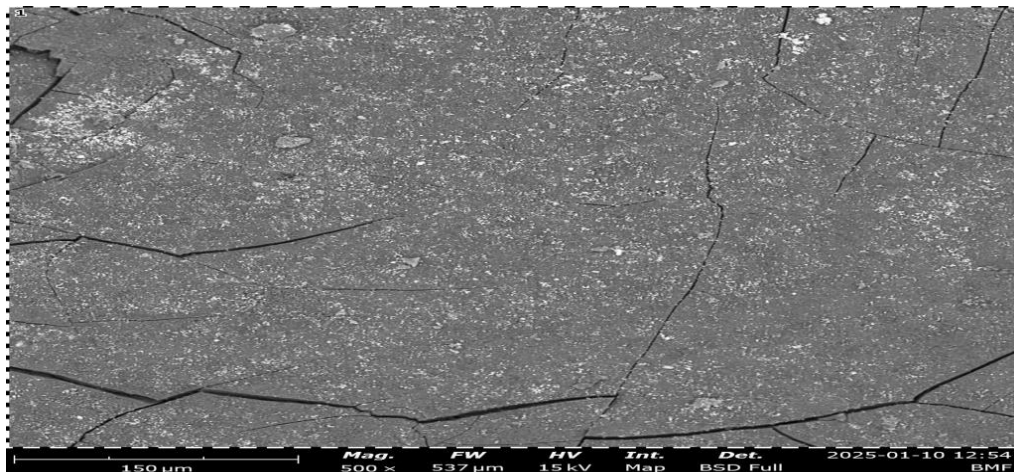


Figure 4: SEM Result of ZnO Nanoparticle Synthesized Using Fruit Extract

Antimicrobial Activity Results

The antimicrobial activity test result for zinc oxide nanoparticle synthesized from the fruit of *Balanites aegyptiaca* are presented in Table 1. The results have shown dose dependent effect across the micro-organisms studied. From the results, the Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) have showed highest sensitivity with 18.00 ± 0.00 mm and 17.00 ± 0.00 mm zone of inhibition respectively as compared to standard drug Augmentin (23.00 ± 0.00 mm and 24.00 ± 0.00 mm) at the highest concentration of (30 µg/ml). The Gram – negative bacteria (*Salmonella typhi*) has shown moderate sensitivity with 15.00 ± 0.00 mm zone of inhibition as compared to standard drug Augmentin (22.00 ± 0.00) at the highest concentration of (30 µg/ml). The fungal strain (*Candida albicans*) has showed a remarkable susceptibility effect with (16.00 ± 0.00 mm) zone of inhibition compared to the standard drug (fulcin) with 20.00 ± 0.00 mm zone of inhibition. The fungal strain (*Aspergillus niger*) has shown resistance across the zinc oxide nanoparticle treated. This corresponds to that obtained by Zacchaeus *et al.* (2018) [23] which shows an activity of 29, 18 and 12 mm at concentration 100, 150 and 250 mg/L respectively.

Table 4.3: Antimicrobial Susceptibility Test of ZnO-NP of *Balanites aegyptiaca* Fruit

S/N	Organism	Concentration (mg/ml)/ Mean Zone of Inhibition in diameter (mm)				
		30	20	10	Aug. (30µg/ml)	Ful. (50µg/ml)
1	Staphylococcus aureus	17.00 ± 0.00^a	12.00 ± 0.00^b	8.00 ± 0.00^c	24.00 ± 0.00^d	-
2	Streptococcus pyogenes	18.00 ± 0.00^a	12.33 ± 0.33^b	8.00 ± 0.00^c	23.00 ± 0.00^d	-
5	Klebsiella pneumonia	11.00 ± 0.00^a	7.00 ± 0.00^b	0.00 ± 0.00^c	20.00 ± 0.00^d	-
6	Salmonella typhi	15.00 ± 0.00^a	10.00 ± 0.00^b	00.00 ± 0.00^c	22.00 ± 0.00^d	-
7	Aspergillus niger	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-	21.00 ± 0.00^b
8	Candida albicans	16.00 ± 0.00^a	10.00 ± 0.00^b	0.00 ± 0.00^c	-	20.00 ± 0.00^d

Key: Aug = Augmentin, Ful = Fulcin

Larvicidal Test Activity of ZnO Nanoparticle Synthesized from The Fruit

Table 2 shows the larvicidal activity of ZnO nanoparticle synthesized from the fruit of *Balanites aegyptiaca* on Anopheles' larvae. The results shows that the percentage mortality of the first instar larvae at 40, 50 and 60 mg/L concentrations of ZnO nanoparticle are 100, 100 and 100% respectively. The second instar showed 92, 100 and 100% mortality when tested with 40, 50 and 60 mg/L concentrations of the nanoparticle respectively. For the third instar larvae, the percentage mortality for the 40, 50 and 60 mg/L concentrations were 74, 100 and 100% respectively. While for the fourth instar, the mortality at 40, 50 and 60 mg/L concentrations were 64, 84 and 100% respectively. This agrees with the findings of Bello *et al.* [24] and Shehu *et al.* [25], where they reported a high toxicity of Cu Nanoparticles and ZnO – CuO nanoporous composite against the larvae of anopheles and malaria vector after exposure for 24 hours. Overall, the highest mortality was observed in the first, instar (100%) at the concentration of 40, 50 and 60 mg/L, second instar (100%) at concentrations 50 and 60 mg/L, third instar (100%) at concentrations 50 and 60 mg/L, fourth instar (100%) at concentration of 60 mg/L, while the lowest was found in the fourth instar (64%) at 40 mg/L concentration. Wilson *et al.* [26] and Danbature *et al* [27] reported that the first instar larvae are more susceptible to nanoparticles and the susceptibility decreases with the growing larval instars subjected to the same concentration of nanoparticles. In a similar work reported by Sharon *et al.* [28], the first instar larva of *Aedes aegypti* showed high susceptible to copper nanoparticles than the 2nd, 3rd and 4th larval instars. This revealed that the more matured the larva, the less susceptible they are to nanoparticles. This is due to the fact that most juvenile larvae are more delicate and therefore easily intoxicated by nanoparticles [9]. The calculated LC₅₀ values were 59.47, 52.74, 37.15 and 35.30 mg/L for the first to fourth instar larvae respectively. The findings of Bello *et al.* (2015) [24] revealed that the larvicidal activity of bio – synthesized Copper nanoparticles of African spinach on Anopheles' larvae (LC₅₀ = 47.20 mg/L), a lower LC₅₀ compared to the findings of the present work. On the other hand, Rawani *et al.* [29] reported the larvicidal activity of silver Nanoparticles which showed LC₅₀ value of 1.59 ppm for berries dry leaves and fresh leaves against the larvae of *Anopheles stephensi*. These variations may be attributed to several factors such as the reducing agent (species of plant or parts) used in the synthesis, the type of metal and the mode of action [9]. The mortality rate recorded in this work showed that the activity of the ZnO nanoparticle synthesized from the fruit of *Balanites aegyptiaca* on the Anopheles Mosquito larvae is dose-dependent as reported by other works [9], [25], [26], [27], [30], [31], and [32].

Table 2: Larvicidal Activity of ZnO Nanoparticle Synthesized from The Fruit Against first to fourth Instars Larvae of Anopheles Mosquito

Anopheles	S/N	Conc. (mg/L)	% Mortality At 48 Hours	LC ₅₀ (mg/L)	R ²
First	1	40	100		
Instar	2	50	100	59.47	0.970
	3	60	100		
Second	1	40	92		
Instar	2	50	100	52.74	0.981
	3	60	100		
Third	1	40	74		
Instar	2	50	100	37.15	0.960
	3	60	100		
Fourth	1	40	64		

Instar	2	50	84	35.30	0.943
	3	60	100		

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