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Original Research Article

Green Synthesized Zinc Oxide Nanoparticles from *Coffea arabica*: Bioprospecting and Functional Potential as an Antioxidant and Larvicidal Agent against *Aedes aegypti*

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ABSTRACT

The ZnO-NPs have been successfully produced and characterized using *Coffea arabica* extract using the green method. The successful formation of nanoparticles was indicated by a color change brown. Phytochemical characterization revealed that *Coffea arabica* extract is rich in bioactive compounds, including alkaloids, flavonoids, and phenolics, which have significant antioxidant potential. The results of the antioxidant activity tests showed that increasing concentrations of *Coffea arabica* extract correlated with enhanced free radical scavenging activity, with a relatively low IC_{50} value, indicating this coffee extract can serve as an effective source of antioxidants. Histological assessment revealed damage to cell structures due to exposure to ZnO-NPs, including membrane dilation, epithelial layer damage, and signs of apoptosis and necrosis. The ZnO-NPs synthesized result from *Coffea arabica* extract showed possess larvicidal potential and beneficial antioxidant properties, however attention is needed regarding potential toxicity at certain concentrations.

Keywords: *Zinc oxide nanoparticles*, Coffea arabica, Green synthesis, Antioxidant activity, Larvicidal agent, *Aedes aegypti*

Introduction

Green synthesized is a methods utilized for synthesizing nanoparticles using environmentally friendly.[1,2,3] This process of using natural or biological materials such as plants, microorganisms, or enzymes as reducing and stabilizing agents,[4,5] without toxic chemicals or extreme reaction conditions.[6,7] Green synthesis has made it easier, faster, and more cost-effective to create metallic nanoparticles such as copper, silver, gold, and selenium in recent years.[8,9,10,11] The Mosquitoes play a significant role in the spread of various diseases in both urban and rural areas. The most common mosquito-borne diseases such as dengue, Zika, and Chikungunya.[13,14,15] To address this issue, researchers recommend the application of nanotechnology as a solution for insect-borne diseases.[16,17,18] Potential of green synthesized plant-based metallic nanoparticles have attratted great to controlling insect vectors, including mosquitoes.[19,20,21] The research have been shown that nanoparticles made from copper, gold, palladium, silver, zinc, carbon, and silica effectively reduce mosquito populations.[22,23,24,25] These nanoparticles disrupt various biological processes in mosquitoes, thus helping to control the spread of the diseases they carry.[26, 27] Zinc oxide (ZnO) is another essential element living organisms require for various physiological and structural functions.[28,29,30,31]

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ZnO nanoparticles (ZnO NPs) are gaining popularity in various scientific fields due to their low toxicity, high stability, and unique properties.[32,33,34,35] The research has shown that biologically synthesized ZnO NPs are safer, more environmentally friendly,[36,37] and more cost-effective than those produced using chemical or physical methods. Recent studies indicate that ZnO NPs are safe, compatible with living organisms, and free from harmful substances.[38,39,40,41] Additionally, various scientific methods are available for the biological synthesis of ZnO NPs from plant extracts such as dried leaves, seeds, and bark. Given these advantages, it is increasingly important to research and synthesize ZnO nanoparticles to leverage their benefits in practical applications.[42,43,44,45] *Coffea arabica* belongs to the genus Coffea in the Rubiaceae family and presents a high concentration of phenolic compounds with strong antioxidant properties that can inhibit infections, including those caused by severe acute respiratory syndrome coronavirus type 2.[46,47,48] Research has identified chlorogenic acid as a major component of *Coffea arabica* extract, along with small amounts of caffeic acid.[49 50] This extract has been proven to possess antioxidant, anti-photoaging, and anti-inflammatory effects, demonstrating its benefits on human skin fibroblasts and genetically unmodified mice. Additionally, it efficiently mitigates reactive oxygen species (ROS) induced by ultraviolet (UV) radiation. Recent studies have emphasized the remarkable properties of *Coffea arabica* extract. One investigation revealed its strong larvicidal activity against mosquito larvae, particularly *Aedes aegypti*, a species responsible for spreading diseases such as dengue fever, Zika virus, and chikungunya. Similarly, research by Kaitana et al. (2023) demonstrated that Pangi leaf extract effectively eliminates mosquito larvae and acts as a natural insect repellent.[51] Building on these findings, this study focuses on evaluating the biotoxic potential of ZnO nanoparticles synthesized from *Coffea arabica* extract against *Aedes aegypti* larvae, assessing their pathological markers, and comparing the results with those from green and roasted *Coffea arabica* extracts. The text discusses mosquito-borne diseases like dengue, Zika, and Chikungunya, highlighting their increasing global health impact and the challenges of controlling them with conventional methods such

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as chemical insecticides. It emphasizes the need for innovative, environmentally friendly solutions like nanotechnology, specifically green synthesis of nanoparticles. This approach is safer and more sustainable, avoiding toxic chemicals and extreme reaction conditions, making it cost-effective and efficient.[52] Metal nanoparticles such as silver (Ag), gold (Au), and zinc oxide (ZnO) have shown significant potential as antiviral and larvicidal agents. They can inhibit dengue virus replication and disrupt the biological structure of vector mosquitoes. The biological synthesis of nanoparticles using plant extracts is highlighted as a safer and more eco-friendly alternative to chemical or physical methods. ZnO nanoparticles produced via biological synthesis have high stability, low toxicity, and good biocompatibility, suggesting their potential in medical applications and vector control.[53,54] Plant extracts like *Coffea arabica* have demonstrated antioxidant, anti-inflammatory, and significant larvicidal effects against *Aedes aegypti*, the main vector for diseases like dengue and Zika. Nanoparticle synthesis's utilization of these extracts could be crucial in developing effective and eco-friendly vector control strategies. The urgency of this research lies in the need for more environmentally friendly and effective nanotechnology-based solutions to combat the threat of insect-borne diseases. In this study, we successfully synthesized ZnO nanoparticles from *Coffea arabica* extract using green synthesis methods. The ZnO nanoparticles showed significant larvicidal activity against *Aedes aegypti* larvae, alongside notable antioxidant properties. However, histological analysis revealed potential tissue damage at certain concentrations, indicating both promise and caution for future applications in vector control.

Materials and Methods

Chemicals and Tools

Coffea arabica beans from Toraja, South Sulawesi-Indonesia, ZnO, Methanol, as well as reagents such as Benedict's, Biuret's, Phenols, Dragendorff's, Braemer's, Shinoda's, Liebermann's, and Frothing's, were used alongside phosphate buffer, sulfuric acid, NaOH, and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Sodium acetate anhydrous (Smart Lab) and acetic acid pro analysis (Merck) were included, while folin ciocalteu reagent (Merck), aluminum chloride pro analysis (Merck), and Na_2CO_3 were sourced from WINLAB laboratory chemicals reagent, UK. The research utilized a spectrophotometer (T60-UV-Vis PG-Instruments, UK) and a centrifuge (Benchmark Scientific Z326E [Z326-E] Hermle Universal Centrifuge).

Rearing of Target Insects

Aedes aegypti mosquito eggs were collected from colonies maintained in the Parasitology Laboratory, Faculty of Medicine, Hasanuddin University. This step ensures that the mosquito species used is *Aedes aegypti*, which is relevant for the study as it is known to be a vector for diseases such as dengue fever and Zika virus. The eggs were then allowed to hatch into larvae under controlled laboratory conditions, including a room temperature of 28°C, appropriate humidity, and natural lighting. These conditions were designed to mimic their natural environment to ensure optimal hatching. The hatched larvae were kept in dechlorinated tap water to avoid toxic effects that could interfere with the research outcomes. Additionally, the larvae were fed aquarium fish food, a standard nutritional source to support their growth until they reached the stage required for the study. This method was designed to ensure that the larvae were reared in a uniform environment, free from external factors that could potentially affect the research results.

Plant Extract and Synthesis of ZnO NPs

20 grams of green *Coffea arabica* powder and 20 grams of roasted *Coffea arabica* powder were weighed separately to ensure accuracy. Each powder type was then dissolved in 100 mL of distilled water, resulting in two distinct solutions. The mixtures were heated to a temperature of 80°C using a magnetic stirrer to ensure proper dissolution and optimal extraction of compounds. The heating process was maintained for 1 hour to maximize extraction efficiency, and the resulting solutions were filtered using ashless filter paper (No. 11) to remove solid residues. In parallel, a 40 mM zinc oxide (ZnO) solution was prepared. The ZnO solution was then mixed with each *Coffea arabica* extract in a 1:1 ratio, ensuring uniform mixing. The formation of ZnO nanoparticles (ZnO NPs) was visually confirmed by a color change in the mixture from brown to light green, indicating successful nanoparticle synthesis. The ZnO NPs were collected in powder form by washing the solution three times with distilled water to remove impurities. The washed mixture was then centrifuged at 12,000 rpm for 15 minutes using a centrifuge (HERMLE Z 366 K1) to separate the nanoparticles from the liquid phase. Finally, the isolated ZnO NPs were dried in an oven at 80°C for 16 hours to remove any remaining moisture, resulting in a fine nanoparticle powder ready for further analysis or applications.

Characterization of Phytochemicals of Coffea arabica

Alkaloid Test

Two mL of the plant extract is placed into a test tube to test for the presence of alkaloids in the sample. Two mL of dilute hydrochloric acid (HCl) and stirred thoroughly. Afterward, a few drops of Wagner's reagent are added to the mixture. The presence of alkaloids will be indicated by forming a reddish-brown precipitate, signaling a positive reaction for alkaloids.[54, 57]

Flavonoid Test

The flavonoid test is performed by adding 2 mL of the plant extract into a test tube. A few drops of dilute sodium hydroxide (NaOH) solution are added to the extract. If the mixture turns yellow, it indicates the presence of flavonoids. A few drops of dilute hydrochloric acid (HCl) are added to the mixture to confirm the result. The disappearance of the yellow color after adding HCl will confirm the presence of flavonoids in the sample.[55, 57]

Terpenoid Test (Salkowski Test)

2 mL of the plant extract is mixed with 2 mL of chloroform in a test tube and shaken well. After that, 2 mL of concentrated sulfuric acid (H_2SO_4) is carefully added along the side of the tube to form a layer. If terpenoids are present, a reddish-brown color will appear at the interface between the chloroform and sulfuric acid layers, indicating a positive reaction.[55, 57]

Saponin Test (Foam Test)

To test for the presence of saponins, 2 mL of the plant extract is added to a test tube along with 5 mL of distilled water. The mixture is shaken vigorously for about 15 minutes and left to stand to observe foam formation. The presence of saponins is confirmed if a stable and persistent froth forms on the surface of the mixture.[55, 57]

Phenolic Test (Ferric Chloride Test)

The phenolic test is conducted by adding a few drops of 1% ferric chloride ($FeCl_3$) solution to 2 mL of the plant extract in a test tube. The mixture is then stirred and observed for a color change. The presence of phenolic compounds will be indicated by a change in color to bluish-green or dark, signaling a positive reaction for phenols.[55, 57]

Antioxidant Potential of Coffea arabica

The antioxidant potential was evaluated through the DPPH assay. Solutions for the control, sample, and blank were prepared, and their absorbance was recorded at 517 nm after a 30-minute incubation period. The antioxidant activity was calculated based on the radical scavenging activity (RSA) formula (1):

$$\%RSA = [\frac{(A\ control - A\ test)}{A\ control}] \times 100\% \quad (1)$$

Characterization of ZnO NPs

The spectrum of ZnO nanoparticles (ZnO NPs) was analyzed using a visible/UV spectrophotometer, covering a wavelength range of 300 to 800 nm. (The research utilized a spectrophotometer (T60-UV-Vis PG-Instruments, UK). To further characterize the surface morphology and structural properties of ZnO NPs synthesized from *Coffea arabica*, analysis was performed using a scanning electron microscope (SEM) (Tabletop Microscope, HITACHI TM3000, Japan). Fourier-transform

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infrared spectroscopy with an infrared spectrometer (IR Prestige-21, Shimadzu) was used to identify the functional groups contributing to the reduction and stability of ZnO NPs. Scanning Electron Microscope (SEM) Tabletop Microscope.

Larvicide Bioassay
The larvicide bioassay was performed to assess the effectiveness of ZnO nanoparticles (ZnO NPs) and *Coffea arabica* extracts in controlling *Aedes aegypti* parasites. The bioassay was conducted using transparent 250 mL cups, with each assay repeated three times to ensure the reliability and accuracy of the statistical results. Each cup was filled with 20 freshly collected larvae and various concentrations of ZnO NPs (10, 20, 30, 40, and 50 ppm), as well as roasted and green Coffea arabica extracts (10, 20, 30, 40, and 50 ppm), were tested. The lethal concentration (LC_{50}) values after 24 hours of treatment were calculated using SPSS software, with the significance threshold set at $p \leq 0.05$, determined through probability analysis.

Histopathological Study
Aedes aegypti larvae were treated with ZnO NPs derived from *Coffea arabica* to observe its effects on the morphology and structure of the larvae. As part of the control procedure, some larvae were immersed in 10 percent formalin buffer solution for 24 hours to kill and preserve the tissue. After treatment, the larvae preserved in formalin underwent a dehydration process using graded alcohols, followed by clearing with xylene to remove any remaining alcohol. The larvae were then sectioned into thin slices, approximately 5 to 7 microns thick, using a microtome. These sections were subsequently stained with hematoxylin-eosin, a standard staining technique used to assess cellular structures based on procedures described in previous studies. This staining process allowed the researchers to observe any changes in the larval tissue treated with ZnO NPs compared to the formalin-preserved control group.

Morphological Study
The morphological change on dead Aedes aegypti larvae treated with ZnO NPs was carefully examined under a microscope (Nikon Microsystems, Japan). The outer body parts of larvae, such as siphon, setae, eyes, antennae, anal gills, abdomen, thorax, and head, were closely checked. Record and compare any visible deviations or discolorations in the treated larvae to a control group.

Ecotoxicology Study
This study aimed to investigate the effects of green ZnO nanoparticles (ZnO NPs) on the survival rate of *Artemia salina*. To begin the experiment, *Artemia salina* eggs were incubated until they hatched, resulting in nauplii being used as the study subjects. The newly hatched nauplii were exposed to various concentrations of ZnO NPs, namely 10, 20, 30, 40, and 50 ppm, to observe the effects of different concentrations on their survival rate. After 24 hours of exposure, the survival rate was calculated using the mortality rate formula (2):
Mortality Rate (%) = (Number of Deaths) / (Number of Lives) x 100 (2)
In this calculation, the number of deaths was determined by counting the nauplii that died after exposure. In contrast, the number of lives was based on the nauplii that survived after the 24-hour period. This approach allows for the analysis of how ZnO nanoparticles affect the survival of Artemia salina at different concentrations.

Results and Discussion

Production and Characterization of ZnO-NPs
Zinc Oxide Nanoparticles (ZnO-NPs) were produced, indicated by a visible color change from brown. The nanoparticles were then characterized using UV-Vis spectroscopy and FTIR methods. Further information on this characterization can be found in our previous research.[55]

Phytochemical Profile of Coffea arabica Samples
Table 1 shows the results of phytochemical screening on *Coffea arabica* samples aimed at detecting the presence of various bioactive compounds in the *Coffea arabica* extract.

Table 1: Results of phytochemical testing on *Coffee arabica* samples

Phytochemical test	Result	[54]
Alkaloid	Negative	Positive
Flavonoid	Positive	Positive
Terpenoid	Negative	Negative
Saponin	Negative	Negative
Phenolic	Positive	Positive

The result obtained from phytochemical screening:
Alkaloids (+):
A positive result (+) indicates that the *Coffea arabica* sample contains alkaloids. Alkaloids are organic compounds that generally have bioactive properties and are commonly found in various plants. Alkaloids like caffeine are the main components that contribute to the stimulant properties of *coffea*.
Flavonoids (+):
The presence of flavonoids in the sample is also marked by a positive result (+). Flavonoids are a group of phenolic compounds with strong antioxidant properties, which can help fight free radicals and offer health benefits such as reducing the risk of heart disease.
Terpenoids (Steroids) (-):
A negative result (-) indicates that terpenoids or steroids were not detected in this *Coffea arabica* sample. Terpenoids and steroids are compounds that typically play a role in biological activities such as anti-inflammatory and hormone regulation. Their absence may suggest that this *Coffea arabica* is richer in phenolic and alkaloid compounds than terpenoids.
Saponins (-):
A negative result (-) for saponins indicates that these compounds are absent in the *Coffea arabica* sample. Saponins are known for their antimicrobial properties and ability to form foam in water. Their absence may imply that this *Coffee arabica* does not exhibit characteristics commonly associated with saponins.
Phenolics (+):
A positive result (+) indicates the presence of phenolic compounds. Phenolic compounds play an essential role as antioxidants, helping to prevent oxidative damage to cells and tissues. The presence of phenolics in *Coffee arabica* suggests significant potential health benefits, particularly in protecting against degenerative diseases.
Overall, these phytochemical test results indicate that the tested *Coffee arabica* sample is rich in bioactive compounds such as alkaloids, flavonoids, and phenolics, all of which have high potential health benefits, especially related to antioxidant activity. However, the absence of terpenoids and saponins suggests that other bioactive compounds may be more dominant in this *coffea*.

The Effect of ZnO NPs Mediated by Coffea arabica on Antioxidant Potential
Based on the results of antioxidant tests on roasted *Coffea arabica* samples, it can be seen that there is an increase in antioxidant activity as the sample concentration increases. At a concentration of 10 µg/mL, antioxidant activity reached 21.00% and continued to increase, reaching 62.63% at 160 µg/mL (Table 2). The higher the concentration of *Coffea arabica* extract, the greater its ability to scavenge free radicals, as evidenced by the increased percentage of antioxidant activity. The IC_{50} value of the roasted *Coffea arabica* was 108.0229 µg/mL, indicating moderate antioxidant activity. This relatively low IC_{50} value implies potential as a source of antioxidants, which can be utilized in health products.

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Table 2: Antioxidant activity roasted *Coffee arabica* samples

Concentration (µg/mL)	Antioxidant activity (%)	IC_{50} Value (µg/mL)	IC_{50} Value (µg/mL) [56]
10	21.00		
20	23.75		
40	32.38	108.02	126.60
80	45.88		
160	62.63		

Characterization of ZnO NPs from Toraja Coffea arabica
UV-Vis Spectroscopy
The UV-Vis spectrophotometer characterization graph of zinc oxide nanoparticles (ZnO NPs) from *Coffea arabica* samples shows a significant absorbance peak at around 382 nm with an absorbance value close to 10. This peak reflects the characteristic feature of ZnO NPs, which typically exhibit maximum absorption in the wavelength range of 370-400 nm. High absorption at this wavelength indicates the successful synthesis of ZnO NPs in the sample (Figure 1). In addition to the main peak, the graph shows several additional absorbance peaks around 371 nm and 338 nm, which may reflect particle size variations or additional features within the material.
After the main peak, there is a sharp decrease in absorbance at both lower and higher wavelengths, indicating that the material has optimal absorption around 382 nm. Overall, this graph confirms the typical optical characteristics of ZnO NPs in the *Coffea arabica* sample, which may open up applications in various fields, such as photocatalysis or the development of ZnO-based sensors.

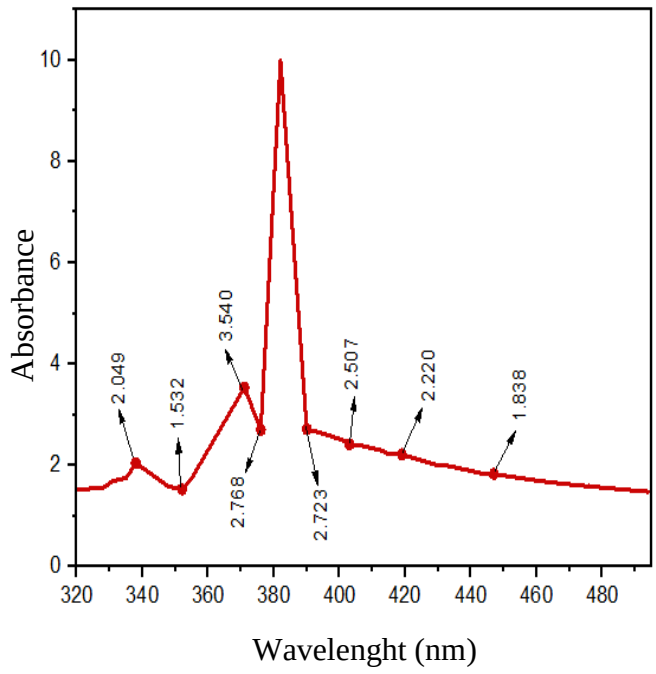

Figure 1: The UV-Vis spectrophotometer characterization graph of zinc oxide nanoparticles (ZnO NPs)

SEM (Scanning Electron Microscope) Analysis
ZnO NPs' analysis of aggregated surface morphology and a porous structure are studied using scanning electron microscopy (SEM) (Figure 2). This is a common characteristic of ZnO nanoparticles, which often experience agglomeration due to their high surface energy, causing particles to cluster together. This aggregation can influence the optical and catalytic properties of ZnO, enhancing the surface area beneficial for certain applications. At various magnifications (1.5, 3.0, and 4.0k), the particles display a rough, irregular granular structure, with sizes ranging from several micrometers to nanometers, indicating that the synthesis method produces multi-scale ZnO particles. The observed porosity on the particle surface suggests the presence of spaces between particles, likely due to natural organic compounds from *Coffee arabica* acting as reducing and stabilizing agents for the nanoparticles. This porosity can provide advantages in applications like photocatalysis and adsorption by increasing surface interaction. The SEM images were captured at 15 kV, a common setting used to balance resolution and sample penetration, especially for nanoparticles. With scale bars of 10 µm and 30 µm shown in the images, it can be inferred that the particle sizes vary, including larger agglomerates and finer particles within each cluster. These findings indicate that ZnO nanoparticles derived from *Coffee arabica* extract exhibit a porous structure with aggregation and surface roughness, potentially enhancing their functional properties in applications such as catalysis, adsorption, or antimicrobial agents.

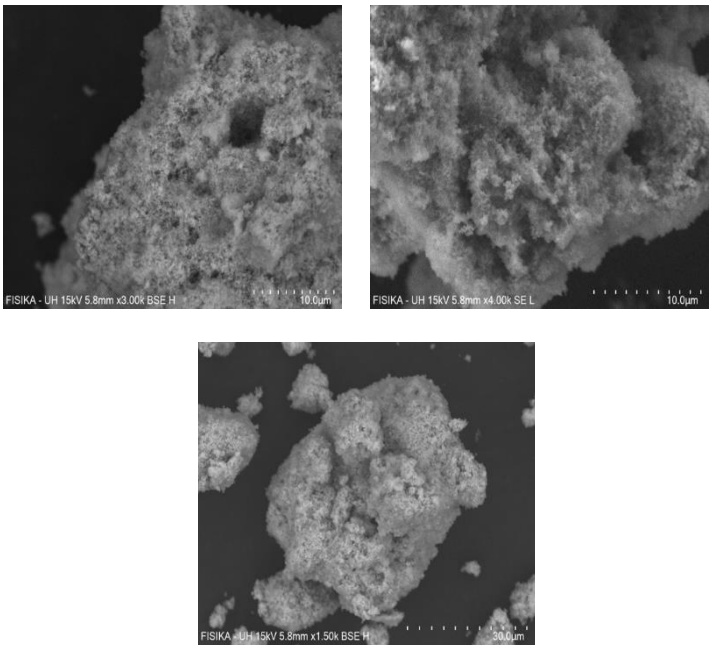

Figure 2: SEM (Scanning Electron Microscope) analysis of ZnO nanoparticles synthesized from *Coffee arabica* extracts

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FTIR Analysis Results

Fourier determined the functional groups- transform infrared spectroscopy (FTIR) to reveal several characteristic absorption peaks (Figure 3). Functional groups observed include carbonyl (C=O), which typically appears in the wavelength range of around 1650-1750 cm^{-1}, amide (C-N), which appears in the absorption region of approximately 1200-1350 cm^{-1}, N-H (stretching or bending of nitrogen) usually appears around 3200-3500 cm^{-1}, and C-N amide which also appears in the 1200-1350 cm^{-1} range. These results suggest that the green synthesis process yielded caffeine or the main compounds found in coffee. Meanwhile, Zn-O typically shows characteristic vibrations in the 400-600 cm^{-1} region in the FTIR spectrum.

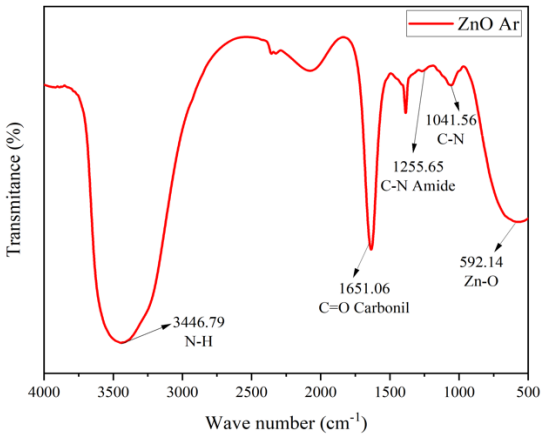

Figure 3: FTIR analysis ZnO-NPs by *Coffea arabica*

Histological Evaluation of Tissue Damage Due to Exposure to ZnO-Arabica Nanoparticles

Histological analysis is a scientific technique that observes the structure and condition of tissues at the microscopic level, typically with special staining that highlights cellular details and tissue components. This method allows researchers or doctors to detect structural changes or tissue damage caused by diseases, treatments, or certain interventions, such as nanoparticle exposure.

In this study, the results of the histological tests indicate damage to the Lumen Space (LS) and dilation of the Perithttopic Membrane (PM), suggesting that ZnO nanoparticles may induce oxidative stress in the tissue, impacting cellular structure and leading to swelling or damage to the membrane. ZnO is known as an antimicrobial agent, but at certain concentrations, it can be cytotoxic to normal cells (Figure 4). Furthermore, the rupture of the Epithelial Layer (EL) indicates that ZnO nanoparticles, possibly in conjunction with Arabica, disrupt the cellular structure that protects the surfaces of organs or tissues. This disruption may occur due to changes in the microstructure of the tissue caused by the interaction of nanoparticles with the cell membrane. Additionally, hypertrophy of cells (H) may occur as a response to the stress experienced by cells due to exposure to ZnO nanoparticles, where cells attempt to adapt to an unusual environment but instead undergo pathological enlargement. Finally, the presence of karyorexis (Kr) and karyolysis (Kl), which involve fragmentation and degradation of the cell nucleus, indicates apoptosis or necrosis due to ZnO nanoparticle toxicity. Excessive production of reactive oxygen species (ROS) often triggers this process, which disrupts mitochondrial function and leads to nuclear damage.

Morphological Study

The physical changes observed in the larvae include discoloration of the body, cellular damage, or deformation, particularly in major organs such as the thorax and abdomen, which can be observed microscopically and can be seen in Figure 5. In addition, the larvae experienced a cessation of movement, indicating signs of damage or death. The chemical effects due to the accumulation of nanoparticles were also evident, characterized by areas of the larvae's body appearing brighter or more reflective, as observed in the images before and after the treatment (Figure 4).

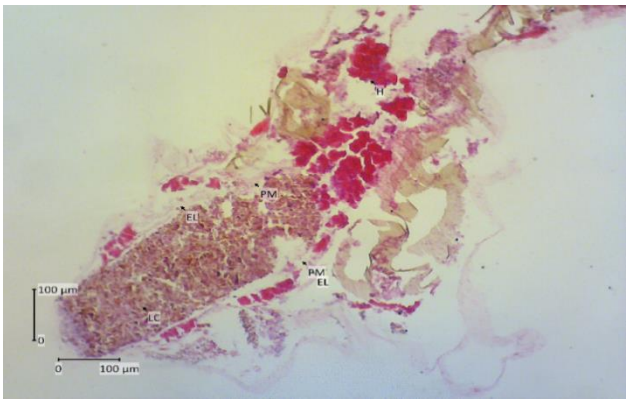

Figure 4: Histological Evaluation of Tissue Damage Due to Exposure to ZnO-Arabica Nanoparticles on *Aedes aegypti* Larvae

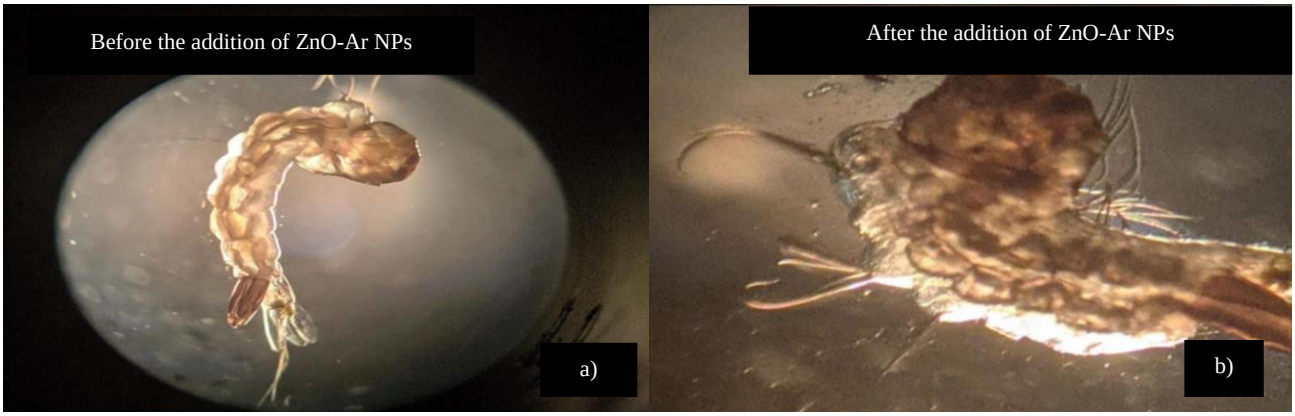

Figure 5: Effect of ZnO-Ar NPs Addition on *Aedes aegypti* Larvae a). before the addition of ZnO-Ar NPs): *Aedes aegypti* larvae in intact condition without treatment with ZnO-Ar NPs nanoparticles, b). after the addition of ZnO-Ar NPs: The larvae show significant morphological changes, including tissue damage due to exposure to ZnO-Ar NPs nanoparticles.

Conclusion

In this study, green synthesis of ZnO-NPs was successfully synthesized from *Coffea arabica* extract. Current results show that *Coffea arabica* extract is rich in bioactive compounds, including alkaloids, flavonoids, and phenolics. Antioxidant activity assays performed using the DPPH, with a relatively low IC_{50} value, indicate that this coffee extract can be an effective source of antioxidants. Histological assessment revealed damage to cell structures due to exposure to ZnO-NPs, including membrane dilation, epithelial layer damage, and signs of apoptosis and necrosis. This indicates that ZnO-NPs can induce oxidative stress and may be toxic to tissues.

Conflict of Interest

The authors declare no conflict of interest

Author's Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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