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A Biotechnology-Based Approach to Reducing the Infestation Potential of Pediculus humanus capitis Using Eco-Friendly ZnO Nanoparticles

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Abstract

Pediculus humanus capitis was a prominent and widespread problem, especially among children, and its prevalence has been documented even in developed countries. This study aimed to investigate the antiparasitic activity of human head lice. The study used an aqueous extract of Abelmoschus esculentus, commercial nano zinc oxide with a particle size ranging from 90 to 200 nm, and bio-prepared nano zinc oxide with a particle size of 35 nm. After studying their physical properties and proving their existence, a study was conducted to find their effectiveness against this parasite. The water extract of Abelmoschus esculentus was tested at different concentrations, and it was determined that its LC50 was 71 mg/L Furthermore, when using commercial nano zinc oxide at several concentrations, increasing the concentration increased the killing rate, with its LC50 found to be 19.8 mg/L. As for the prepared nano-bio-zinc oxide, it can be observed that the antiparasitic activity value generally increases with increasing concentration of the substance, with its LC50 reaching 15 mg/L. Results demonstrate a significant difference, with the prepared zinc oxide exhibiting higher biological activity. Nanoparticle preparation using biological systems represents a promising alternative for developing a large-scale strategy for eliminating parasites.

Keywords: Pediculus humanus capitis, Abelmoschus esculentus, and Zinc Oxide NPs.

1. Introduction

Pediculus humanus capitis has been an obligate parasite on humans for thousands of years [1]. Across the world, continued spread of this parasite and its ongoing infestations [2]. The prevalence of this parasite has been found at all levels of society [3]. Schoolchildren are most susceptible to this parasite [4]. The parasite spreads through several factors, including close contact in crowded communities with low economic conditions [5]. The parasite is transmitted through direct physical contact, i.e., touching contaminated clothing or bedding. Head lice bite up to five times daily compared to body lice [6].

The diagnosis of lice infestation depends on the presence of itching or scratching, which is caused by lice or nits associated with bacterial infections [7, 8]. One old method of treating head lice is manually removing them with a lice comb and shaving the scalp [9, 10]. P. humanus capitis, also known as human head lice, is an ectoparasite. The spread of P. humanus capitis among schoolchildren results are from unsanitary conditions, which negatively impact society and are a public health concern [11]. It is essential to identify alternatives to synthetic products for treating lice that are environmentally friendly and low in toxicity to the host.

Green synthesis is being used due to its cost-effectiveness, environmental friendliness, and ease of scaling up for large-scale synthesis [12]. The study reports synthesis of ZnO NPs using zinc nitrate and an aqueous extract of Abelmoschus esculentus. Biosynthesized nanoparticles from Abelmoschus esculentus were found to exhibit high activity against lice and larvae. Therefore, the study focuses on the synthesized ZnO NPs and their anti-lice properties.

2. Materials and Methods

2. 1 Materials

Abelmoschus esculentus was collected from a farm in Wasit Province, Iraq, where it was washed several times to remove dust, dried, ground into a powder, and stored in a plastic container. Zinc nitrate $Zn(NO_3)_2.6H_2O$ and ZnO NPs with particle sizes ranging from (90-200) nm were supplied by the Indian companies Central Drug House (CDH).

2.2 Preparation of Extract and Bio-Nanomaterial

The preparation of bio-nano zinc oxide included plant extract using the aqueous extract method, where deionized water is used at a rate of 10 mL of water for every 1 gram of dried plant. Then, place the prepared plant extract in an esterified solution at 70 °C for 30 minutes. After that, the extract was filtered, and the result kept in a sealed container at 4 °C [13].

The second stage is to dissolve zinc nitrate hexahydrate in 100 mL of deionized water to obtain a concentration of 1 Molar solution. Then, 25 mL of the plant extract is added dropwise using a burette, with 98%

purity of (NH_4OH). The mixture stirred using a magnetic stirrer at a temperature of 70-80 °C. The preparation time does not exceed one hour. The final stage is centrifugation and washing the sample with deionized water until it reaches a pH of 7 [14]. Then, the precipitate, after cleansing and removing impurities, is placed in a crucible and put in an oven at a temperature of 400 °C for two hours [15, 16]. This produces a brown-colored powder, which is a bio-nano zinc oxide [16].

2.3 Pediculus humanus capitis Parasite Sample Collection

P. humanus capitis parasite was identified in the laboratories of the College of Science, University of Wasit. Obtained from children aged from 3 to 12 years old with parental consent. The affected individuals had not been treated with any pediculicidal treatment for at least the previous month. Samples were collected using a metal lice comb. Adult head lice were carefully removed from the comb's teeth and placed in clean plastic boxes. After collection, the head lice were transported to our laboratory [17].

2.4 Pediculicidal activity

After preparing the bio-nano zinc oxide, it was suspended in distilled water at different concentrations (5, 10, 15, 20, 25, and 30 mg/L), with three replicates. Each louse was then transferred to a glass shower petri dish, and 0.05 mL of the previously prepared substance was placed on its dorsal side using a micropipette. After 15 seconds of contact with the agent, the louse was transferred to a Petri dish lined with filter paper and observed under a microscope until death or complete death.

All Petri dishes were placed aside in a dark room at a temperature of 26 ± 0.5 °C and a humidity of 70 ± 1 %. The time elapsed for each test agent was recorded as the "drop" time. Louse death was confirmed when the appendages stopped moving or shook when touched with the needle. Five lice were used for each determination [18].

3. Characterization of bio-ZnO_2NPs

UV–Vis spectroscopy is an important method for determining the absorption peak of produced ZnO nanoparticles, which exhibit an absorption peak in the UV spectrum at a wavelength of 291 nm, as depicted in figure 1. The result is consistent with several research studies [19].

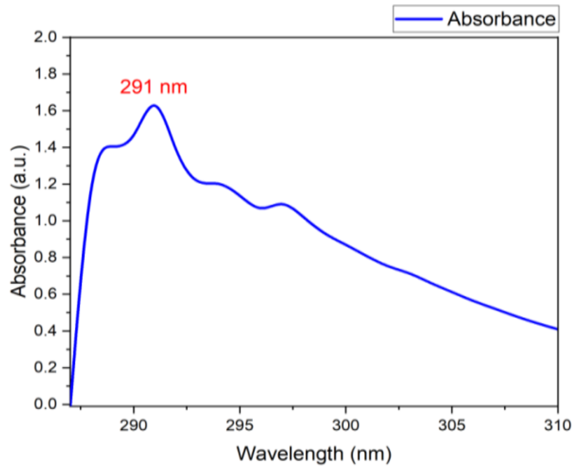

Figure 1: UV absorption of ZnO NPs.

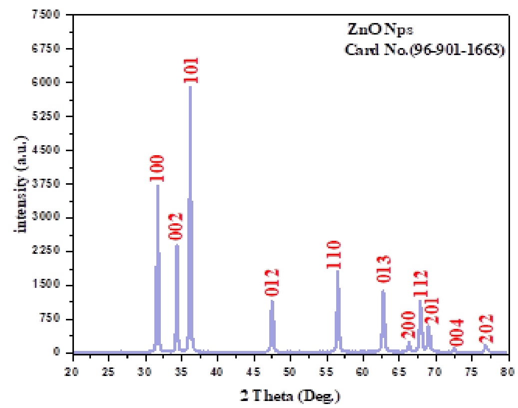

Figure 2: XRD spectra of ZnO NPs.

However, figure 2 shows X-ray diffraction (XRD) patterns of zinc oxide powder. Patterns indicated that the prepared sample was polycrystalline with a hexagonal wurtzite structure. Figure 2 shows the structural and geometric parameters. The XRD pattern of ZnO nanoparticles has peaks at 2θ = 31.76°, 34.47°, 36.23°, 47.45°, 56.61°, 62.85°, 66.39°, 67.96°, 69.1°, 72.56°, and 77.01°, which correspond to the (100), (002), (101), (012), (110), (013), (200), (112), (201), (004) and (202) planes, respectively, which are in accordance with the JCPDS card data: (96-901-1663).

FTIR analysis was used to find the possible functional groups (phytomolecules) in the plant extracts to create ZnO NPs. Several peaks were observed in the FTIR analysis of abelmoschus esculentus extract in figure 3 reflecting the complex nature of plant metabolites. The peaks at around 3561cm^{-1} represent the hydroxyl (O-H) stretching vibrations that may be from phenolic compounds [20].

The C-O stretching in the vibration range (1851, 1732) cm^{-1} may be due to carboxylic acids or natural esters [21]. The vibrations at (1446, 1334) cm^{-1} indicate C-H stretching due to the presence of carbohydrates [22]. The vibrations at 1229 cm^{-1} indicate a C-O vibration due to the polyhydroxyl group in okra fruit [23]. The characteristic peaks at 461 and 698 cm^{-1} indicate the presence of Zn-O, proving a successful preparation [16].

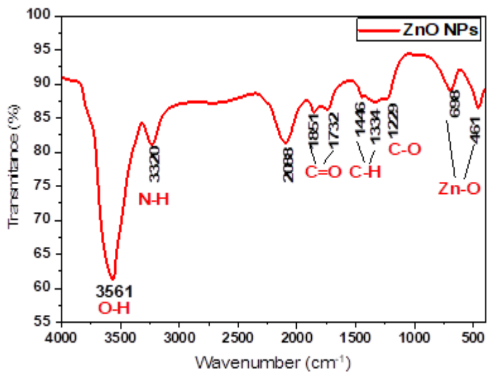

Figure 3: FTIR analysis of ZnO NPs.

The morphological properties of the nanoparticles were studied using scanning electron microscopy. ZnO nanoparticles appeared to be spherical, with various other shapes, and had a size of up to 35 nm, as shown in figure 4.

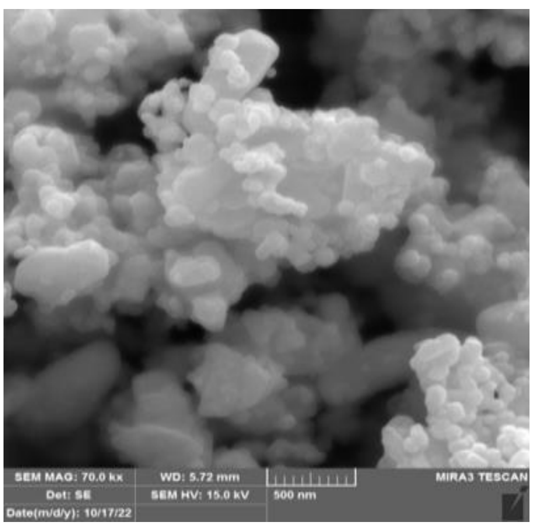

Figure 4: SEM micrograph of the Bio- ZnO NPs.

4. Results and Discussion

The antiparasitic activity evaluation against human head lice (P. humanus capitis) demonstrated that bio-synthesized zinc oxide nanoparticles (ZnO NPs), commercial ZnO NPs, and the aqueous extract of Abelmoschus esculentus (okra) all exhibited significant pediculicidal effects, with the bio-synthesized formulation showing superior performance. The bio-synthesized ZnO NPs achieved an LC50 value of 15 mg/L with 97% mortality at 30 mg/L, representing a 24% improvement over commercial ZnO NPs (LC50 = 19.8 mg/L, 80% mortality at 30 mg/L).

While, the okra extracts alone required higher concentrations (LC50 = 71 mg/L) but demonstrated concentration dependent efficacy from 17% mortality at 40 mg/L to 85% at 100 mg/L. These results are consistent with previous studies where Rajakumar and Rahuman (2011) reported similar pediculicidal activity of ZnO NPs against P. humanus capitis with comparable LC50 values, while Murugan et al. (2017) demonstrated enhanced antiparasitic activity of plant-mediated ZnO NPs using Momordica charantia extract, supporting the synergistic effect of plant-nanoparticle combinations [24, 25].

The superior efficacy of bio-synthesized NPs can be attributed to the optimized 35 nm spherical morphology facilitating enhanced penetration through lice exoskeleton, combined with the presence of bioactive phytochemicals from Abelmoschus esculentus including flavonoids, terpenoids, and phenolic compounds that act as both reducing and capping agents, as demonstrated by recent studies on okra-mediated nanoparticle synthesis showing enhanced antimicrobial properties [26, 27]. The statistical reliability of results ($r^2 > 0.945$ for all formulations) confirms strong dose-response relationships, while the mechanism of action likely involves penetration through spiracles and intersegmental membranes, generation of reactive oxygen species causing cellular damage, and membrane disruption enhanced by plant-derived capping agents [28].

This biosynthesis approach offers significant advantages over traditional chemical methods through reduced manufacturing costs, enhanced biocompatibility, scalable production using readily available plant materials, and potential for reduced resistance development. It positions it as a promising, eco-friendly alternative for large-scale parasite elimination strategies with clinical translation potential.

Table 1: Antiparasitic activity of Pediculus humanus capitis using in vitro prepared zinc oxide bionanoparticles, aqueous extract of Abelmoschus esculentus and commercial zinc oxide nanoparticles.

Material used	**Species**	**Concentration (mg/L)**	**Mortality % (mg/L)**	**LC_{50} (mg/L)**	**r^2**
extract of *Abelmoschus esculentus*	*Pediculus humanus* capitis	100	85±0.12	71	0.981
		80	57±0.04		
		60	30±0.02		
		40	17±0.11		
		20	80±0.24		
ZnO Nps Synthesized		5	24±0.22	15	0.952
		10	34±0.17		
		15	50±0.09		
		20	65±0.54		
		25	86±0.23		
		30	97±0.17		
ZnO Nps		5	15±0.59	19.8	0.945
		10	24±0.05		
		15	33±0.01		
		20	43±0.14		
		25	62±0.21		
		30	80±0.09		
Control H_2O		-	-	-	-

5. Conclusion

This study concluded that the laboratory-prepared zinc oxide nanomaterial is nontoxic, environmentally friendly, and effective against the parasite P. humanus capitis. Both the aqueous extract of Abelmoschus esculentus and commercial zinc oxide were effective in eliminating P. humanus capitis and reducing its effects. Future research should focus on in vivo safety assessment, optimization of synthesis parameters, and evaluation of long-term stability to facilitate clinical translation.

6. References

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