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Table Of Contents

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Vol. 2 No. 3 (2025): Desember
DOI: 10.21070/ijhsm.v2i3.358

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Nano Zinc Oxide and Prosopis farcta Against Cotton Seed Bug: Nano Oksida Seng dan Prosopis farcta terhadap Hama Biji Kapas

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Abstract

General Background Nanotechnology has gained increasing attention as an alternative strategy for insect pest management. **Specific Background** Zinc oxide nanoparticles and plant-derived extracts have been reported as promising bio-based agents against agricultural pests. **Knowledge Gap** However, limited studies have evaluated green-synthesized zinc oxide nanoparticles derived from Prosopis farcta fruits against cotton seed bug populations under local Iraqi conditions. **Aims** This study aimed to synthesize zinc oxide nanoparticles using an alcoholic extract of Prosopis farcta fruits and assess their biological activity against immature and adult stages of Oxycarenus hyalinipennis under laboratory conditions. **Results** The alcoholic fruit extract showed the highest mortality, reaching 90.00% within 24 hours at 2000 ppm, followed by biosynthesized zinc oxide nanoparticles, while pure zinc oxide nanoparticles produced lower mortality rates. Characterization confirmed successful nanoparticle synthesis with nanoscale dimensions and distinct morphological features. **Novelty** This research demonstrates the use of Prosopis farcta fruit extract as a dual reducing and stabilizing agent for zinc oxide nanoparticle synthesis with insecticidal properties. **Implications** The findings support the potential application of plant-based nanomaterials as alternative tools for sustainable pest management programs.

Keywords: Zinc Oxide Nanoparticles, Prosopis Farcta, Green Synthesis, Oxycarenus Hyalinipennis, Pest Control

Key Findings Highlights:

1. Alcoholic fruit extract produced the highest mortality within 24 hours.
2. Biosynthesized particles showed stronger activity than commercial material.
3. Early developmental stages were more susceptible than later stages.

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Vol. 2 No. 3 (2025): Desember
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Introduction

The cotton seed bug, Oxycarenus hyalinipennis, is a species of Hemiptera and is considered one of the most important pests affecting cotton crops in Iraq. It leads to the destruction of the cotton season and causes a loss of seed germination capability. Males can reach a length of approximately 3.8 mm, while females reach about 4.3 mm [1]

Cotton plants are attacked by numerous insect species more than 700 species have been identified. The losses caused by insect attacks on crops in general can range from insignificant levels to more than 30%, and the entire crop can be destroyed if a defined pest management program is not followed.

This insect is one of the primary pests of cotton and also infests several plants from the Malvaceae family in addition to cotton. The adults and nymphs feed by sucking the sap from the seeds of plants in the Malvaceae family. As a result, seed weight is reduced, seed color changes, the seeds shrink in size, and the amount of extracted oil decreases, as is the case with infected cotton seeds. [1]. Currently, nanotechnology is preparing to enter the commercial field and holds great promise in agricultural biotechnology. It includes several agricultural applications, including pest control and detection [2]. Nanotechnology deals with materials or particles on the nanoscale that is, 10^{-9} of a unit of measurement, or one-billionth of a meter and has become one of the most important technologies used in pest control today. [3] Several nanomaterials, such as silver and gold nanoparticles, have been used in insect control, such as for mosquitoes [4]. Additionally, sulfur and silver nanoparticles have been used to control the fruit fly Drosophila melanogaster [5].

Based on previous studies related to the use of nanoparticles in insect and pest control, and due to the lack of such studies in Iraq, zinc oxide nanoparticles were selected to study their effects on certain biological aspects of the cotton seed bug Oxycarenus hyalinipennis and to assess the potential of using this material for pest control.

Therefore, the objectives of the study were:

1. Prepare alcoholic extract of Prosopis farcta fruits.

2. Biologically synthesize zinc oxide nanoparticles using alcoholic extract.

3. Treat the adult and immature stages of the insect with pure zinc oxide nanoparticles, plant extract and biologically synthesized zinc oxide nanoparticles, and determine the mortality rates and morphological deformities resulting from the treatments.

Prosopis farcta (Leguminosae)

Prosopis farcta is a fast-growing, evergreen wild shrub filled with thorns. It grows in many regions and belongs to the family Mimosaceae, within the legume order Leguminales (Fabales). The genus Prosopis includes many species, of which two are widespread in Iraq: Prosopis farcta and Prosopis juliflora, especially in wild and uncultivated areas [6]. P. farcta is considered one of the perennial medicinal plants, although it has not received much attention from researchers, except for a few local and international studies. Some studies have confirmed its importance — for example, its chemical extracts have been studied for their effects on high blood sugar levels, while other studies have shown that Prosopis farcta has inhibitory effects against certain pathogenic bacteria, and also demonstrated efficacy in reducing prostate enlargement [7]. It has also been found that the glycoside content is high in the flowers and leaves of Prosopis farcta, but lower in the seed coat and completely absent in the roots. Glycosides are the result of specific chemical processes occurring within plant cells , the roots of Prosopis farcta contain a high percentage of caffeic acid, while the flowers and leaves are rich in rutin, which is absent in the roots.

Other studies focused on measuring the nutrient content of this plant, showing that it is rich in magnesium, iron, and manganese, which constitute the majority of the mineral elements found in its seeds [7].

Prosopis farcta is also traditionally used to treat kidney stones, and a decoction of its ripe fruits is used to treat acute and chronic diarrhea, dysentery, and other digestive system issues such as colonic spasms and intestinal inflammation. Its fruits and leaves contain various polyphenolic flavonoids such as quercetin, vitexin, isovitexin, rutin, kaempferol, apigenin, vicenin, and luteolin — all of which are known for their antioxidant activity [8][9].

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Figure 1.

Insect collection and identification

Samples of the cotton seed bug O. hyalinipennis were collected from infested cotton plants during the months of March and April in the Sumer sub-district of Al-Diwaniyah City, one of the agricultural areas in Iraq. The insects were placed in containers and transported to the laboratory. They were identified as O. hyalinipennis (Order: Hemiptera) based on an insect taxonomic key. The identified specimens were used to establish new laboratory colonies for use in the study. A number of adult males and females were placed in a glass container with an open top, covered with muslin cloth and secured with a rubber band. The container also contained a quantity of healthy cotton seeds to serve as a food source.

The container was then placed in an incubator at a temperature of 28°C with 70% relative humidity, which was maintained by placing a dish containing distilled water inside the incubator. The humidity was monitored using a hygrometer placed inside the incubator, in order to ensure appropriate conditions for rearing a sufficient number of insects for the experiments.

Figure 2.

Preparation of Pure Zinc Oxide Nanoparticle Concentrations

A solution of pure zinc oxide nanoparticles was prepared at concentrations of 500, 1000, 1500, and 2000 ppm (parts per million). Each concentration was prepared separately by weighing specific amounts of the material (50, 100, 150, and 200

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 3 (2025): Desember
DOI: 10.21070/ijhsm.v2i3.358

)mg, respectively and dissolving them in 2 mL of ethyl alcohol. The volume was then brought up to 100 mL using deionized water, based on the following equation:

Concentration (ppm) = weight (mg) / size (L)

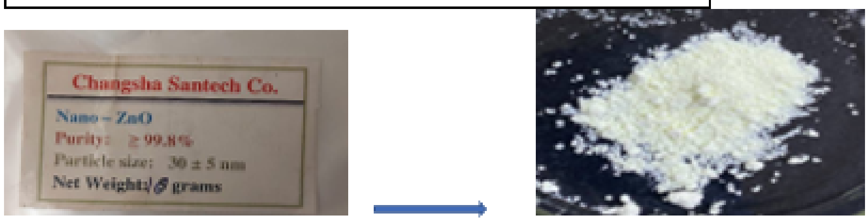

Figure 3.

Preparation of Biosynthesized Zinc Oxide Nanoparticle Concentrations

A solution of biosynthesized zinc oxide nanoparticles was prepared at concentrations of 2000, 1500, 1000, and 500 ppm (parts per million). Each concentration was prepared separately by weighing specific amounts of the material 200, 150, 100, and 50 mg, respectively and dissolving them in 100 mL of deionized water, according to the following equation:

Concentration (ppm) = weight (mg) / size (L)

Material and methods

Preparation of the plant alcoholic extract

Fruits of the P. farcta plant were collected from an agricultural area in Al-Diwaniyah city. The fruits were washed with water to remove dirt and then left to dry completely at room temperature. After drying, they were ground into a fine powder using a blender. A 20 g sample of the powder was weighed and placed in a 1000 mL glass flask containing 100 mL of 96% ethyl alcohol. The mixture was heated using a hot plate stirrer at 45°C for 24 hours with continuous stirring. Afterward, the extract was filtered through filter paper and left to cool to room temperature inside glass dishes. Finally, the extract was stored in a refrigerator. [10]

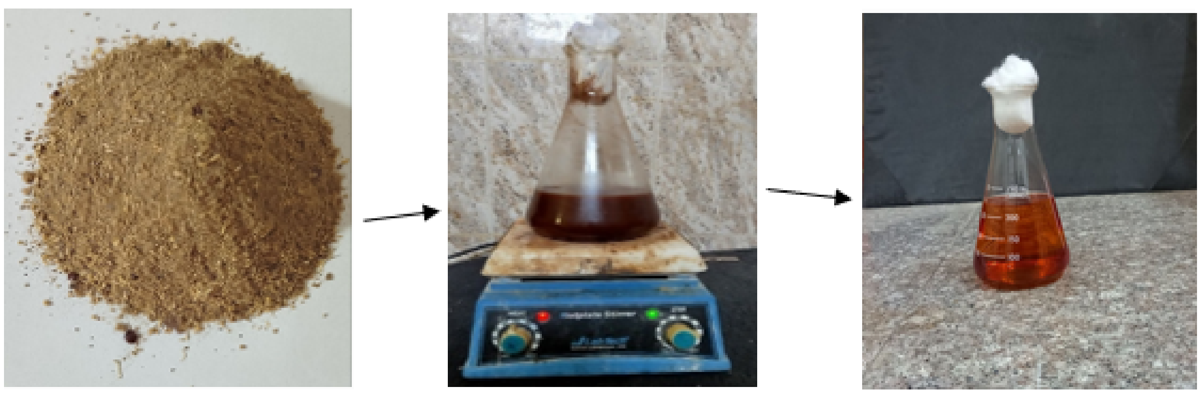

Figure 4.

Biosynthesis of Zinc Oxide Nanoparticles

1 millimolar (mM) solution of zinc oxide was prepared as a standard solution by dissolving 0.008138 grams of zinc oxide (ZnO) in 100 mL of deionized water. The molecular weight of zinc oxide was calculated by summing the atomic weights of zinc and oxygen, as follows:

Molecular weight of ZnO = Atomic weight of zinc (65.38) + Atomic weight of oxygen (16 × 1) = 81.38 g/mol

Then, 7 mL of the alcoholic extract of P. farcta fruits was added to 100 mL of the pure zinc oxide nanoparticle solution under

Vol. 2 No. 3 (2025): Desember
DOI: 10.21070/ijhsm.v2i3.358

dark conditions and at room temperature, and the mixture was left for 10 minutes to allow the color change from white to pale yellow.

Effect of Zinc Oxide Nanoparticles on the Mortality of Early and Late Nymphal Stages

A total of 20 nymphs (early and late stages) were used per replicate, with three replicates for each concentration. The nymphs were placed in 30 mL containers lined with filter paper. Each group of nymphs was sprayed with 3 mL of a given concentration of the zinc oxide nanoparticle solution. A control group was treated with distilled water, with three replicates for each. After treatment, the nymphs were transferred to an incubator .The mortality rates were recorded after 24 hours and corrected according [11].

Effect of Zinc Oxide Nanoparticles on Adult Mortality

A total of 20 adult insects were used per replicate, with three replicates for each concentration. The adults were placed in 30 mL containers lined with filter paper. Each group was sprayed with 3 mL of the respective concentration of the nanoparticle solution. A control group was also included, treated with distilled water, with three replicates for each concentration. After treatment, the adults were transferred to an incubator, and mortality rates were recorded after 24 hours. Mortality percentages were corrected according to the method described in [11].

Statistical Analysis

The experiments were designed according to factorial experiments (two- and three-factor), based on a completely randomized design (C.R.D). The corrected mortality percentages (as per [11]) were first calculated, and then these percentages were subjected to arcsine transformation to obtain angular values. The data were then statistically analyzed using (L.S.D) test at a significance level of $p > 0.05$. In addition, Probit analysis was calculate the LC_{50} values for the larval stages exposed to the tested material (zinc oxide), using the Statistical Package for the Social Sciences (SPSS) software.

Results and Discussion

Characterization of Nanoparticles

Color Transformation

After adding the plant extract to the zinc oxide solution, a color change from white to pale yellow after approximately 3 minutes. This indicates the reduction of zinc ions in the solution into zinc nanoparticles (Figure 3-1).

This result agrees with what was reported in [12], where a similar observation was made using oleander leaf extract. However, the resulting colors in similar reactions varied in other studies depending on the plant extract used. For example, the reaction turned black when using Centella asiatica extract, and light brown when using extracts from Mimusops elengi and Plumbago auriculata [13].

The size of the formed nanoparticles determines the resulting solution color in the reaction. The closer color is red, the smaller size of the formed nanoparticles [14].

Figure 5. A B C Figure (3-1): Color transformation of the zinc oxide solution due to the effect of the plant extract. (A) P. farcta fruit extract solution. (B) Zinc oxide solution before adding the extract. (C) Zinc oxide solution after adding the extract.

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Vol. 2 No. 3 (2025): Desember
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Material type	Concentration	Nymph role		Material effect	Concentration effect
		Early Nymph Average±S.D	Advanced Nymph Average±S.D	Average±S.D	Average±S.D
Nano pure	control	0.00	0.00	13.44^{a**}	Control 0.00^{a}
	500	12.41	8.13		500 17.66^{b}
	1000	13.29	10.4		
	1500	20.22	12.41		
	2000	31.5	26.03		
Plant extract	control	0.00	0.00	28.17^{b}	1000 20.93^{c}
	500	21.4	19.65		1500 27.73^{d}
	1000	25.55	22.5		
	1500	32.35	29.76		
	2000	61.27	69.24		
Nano synthesis	Control	0.00	0.00	34.99^{c}	2000 61.34^{e}
	500	22.44	21.94		
	1000	29.28	24.59		
	1500	38.04	33.61		
	2000	90.00	90.00		
Phase effect		26.52^{a}	24.55^{b}	Overall mortality rate= 25.53	
material LSD ، 1.13 =concentration LSD ، 0.72=phase LSD ، 0.88 =material LSD 2.78 =concentration +phase+interfere					

Figure 6. Table (3-1): The effect of zinc oxide nanoparticles on the mortality percentage of the immature stages of O. hyalinipennis

* S.D = standard deviation

** the averages with the same letter have no significant difference

It can be observed from Table (3-1) that all concentrations of the alcoholic extract of carob fruits had a greater effect on the mortality of both early and late nymphal stages of O. hyalinipennis compared to the other treatments. The highest mortality rate was recorded at the highest concentration of 2000 ppm, reaching 90.00% within 24 hours, compared to 0.00% in the control group.

The biologically synthesized zinc oxide nanoparticles also showed a stronger effect on the mortality of early and late nymphs compared to pure zinc oxide nanoparticles at all tested concentrations. The mortality rates ranged from 21.405% to 19.657% for the early nymphs and 12.418% to 8.130% for the late nymphs at the lowest concentration of 5000 ppm. The fruit extract caused mortality, attributed to the high concentration of active compounds, which is significantly influenced by the harvesting season. Also contain very high levels of tannins and caffeic acid [9].

The primary active materials in P. farcta L. fruit are a range of bioactive compounds, including phenolic compounds like gallic and vanillic acids, flavonoids such as C-glycosyl flavones, and tannins. The fruit also contains other phytochemicals and essential substances, such as alkaloids, quinones, triterpenoids, and specific fatty acids like palmitic acid, which contribute to its medicinal properties and antioxidant activity [9].

Biogenic silver has been shown to cause damage to the digestive tract and weaken it. It can also induce swelling in the outer layer of the integumentary system as a result of body dehydration or blockage of spiracles and tracheal tubes. Additionally, it damages the protective wax layer present in the cuticle [12], [16].

The higher mortality rate observed in early nymphal stages compared to other stages across all treatments can be explained by several factors. These include the delicate nature of the early nymphs' skin during the initial stage of development or the

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Indonesian Journal on Health Science and Medicine
Vol. 2 No. 3 (2025): Desember
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fact that newly hatched nymphs require large amounts of food for growth. This results in the ingestion of large quantities of food (or treated material), which may lead to digestive poisoning. Another possible reason is that the nanoparticles may have directly affected the nymphs, rendering them weak and causing their rapid death. Moreover, the bioactive compounds in the plant extracts may inhibit feeding, ultimately leading to nymphal death due to starvation [10], [17].

It was also observed that the late nymphal stage was the least affected by the extracts. Resistance appears to increase with the age of the nymphs, possibly due to their ability to metabolize and detoxify the toxic compounds present in the various plant extracts into non-toxic forms [10].

The effectiveness of various nanomaterials has been demonstrated against certain pathogens. For example, zinc oxide nanoparticles (ZnO) have been shown to reduce populations of different bacteria and fungi [18].

Additionally, [10] reported that treating the external surface of eggs and larval stages of the Musca domestica (housefly) with certain plant extracts can disrupt embryonic development, leading to unhatched eggs and larval mortality. This suggests the possible presence of hormone-like compounds in the plant extracts that mimic insect hormones and interfere with normal development. The TiO2 NPs were found highly effective against the larvae of Anopheles stephensi (100% mortality in the first and fourth instar, 50–90% mortality in second instar, and 40–80% mortality in third instar larvae) than the ZnO NPs and aqueous stem extract [19].

Material / concentration	Nanopure Average ±S.D*	Plant extract Average ±S.D	Nanosynthesis Average ±S.D	Consentration Average Average ±S.D
Control	0.00	0.00	0.00	0.00
500	**8.13**	**20.83**	**22.50**	**17.15[b]**
1000	**11.28**	**21.4**	**24.08**	**18.92[c]**
1500	**17.01**	**27.92**	**29.24**	**24.73[d]**
2000	**26.56**	**68.16**	**90.00**	**61.57[e]**
Material average	**12.59[a]**	**27.66[b]**	**33.16[c]**	**General average** **24.47**

Figure 7. Table (3-2): Biological efficacy of the tested nanoparticle concentrations on the mortality of the adult stage of O. hyalinipennis

* S.D = standard deviation

**The average with the same letter have no significant difference

It is evident from Table (3-2) that the tested concentrations had a significant effect on the survival of O. hyalinipennis adults. A mortality rate of 90.00% was recorded at the highest concentration when using the P. farcta fruit extract, within 24 hours of treatment. The biologically synthesized zinc oxide nanoparticles showed higher mortality rates than pure zinc oxide nanoparticles at all tested concentrations, reaching mortality rates ranging from 68.163% to 26.565% at the highest concentration, respectively. The toxic effects of biogenic zinc oxide nanoparticles on adult insects are attributed to several mechanisms, including the generation of reactive oxygen species (ROS), oxidative stress induction, disruption of cellular membranes, and protein structure damage by unfolding, in addition to affecting the immune system [20].

[21] reported that nano-silica particles caused significant mortality in adult insects, with an average kill 71.6%, which increased with longer exposure periods.Furthermore, silver nanoparticles had a greater impact than other treatments on adult mortality of Tribolium castaneum, with mortality 54.3063% and LC_{50} 2.341 ppm for biogenic silver nanoparticles, compared 49.6390% and an LC_{50} 2.426 ppm for pure silver nanoparticles [17].

Several deformities were observed as a result treatment with biosynthesis zinc oxide nanoparticles, affecting both nymphs and adults. Deformed adults emerged, exhibiting abnormalities such as abdominal deformities, loss of cuticle pigmentation, or inability to close their wings. These deformities significantly affected the insect's ability to complete its life cycle.

The inability of the insect to fold its wings may be due to several factors caused by the nanoparticles, including structural deformities in the exoskeleton (cuticle),disruption in the formation of the cuticle or wing malformations, damage to the muscles responsible for wing movement and genetic defects arising during developmental stages [12], [22].

Additionally, several deformities were noted in cotton seeds due to feeding by adult insects and nymphs, which extract sap from plants of the Malvaceae family. As a result seed weight is reduced by approximately 15–20%, seeds become discolored and shriveled, oil content is decreased, cotton lint may show spotting and the percentage of infested seeds may reach 50–95% before the second harvest.

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Indonesian Journal on Health Science and Medicine
Vol. 2 No. 3 (2025): Desember
DOI: 10.21070/ijhsm.v2i3.358

Figure 8.

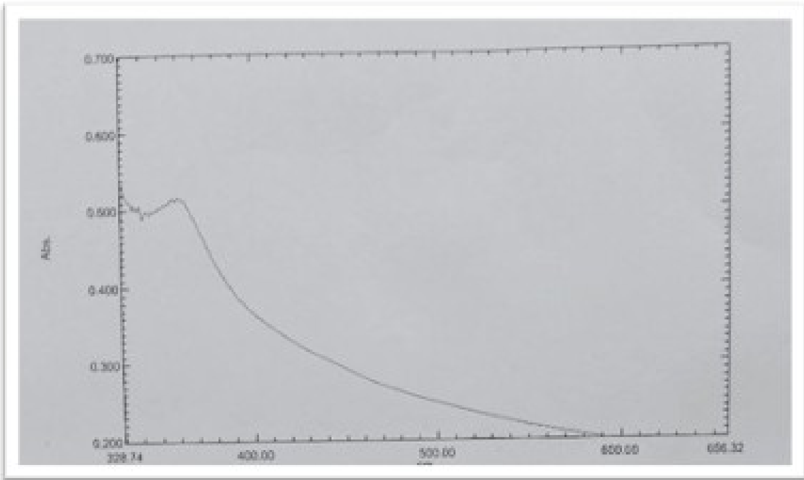

Figure 9. Figure 2: UV spectrum of biosynthesized ZnO nanoparticles.

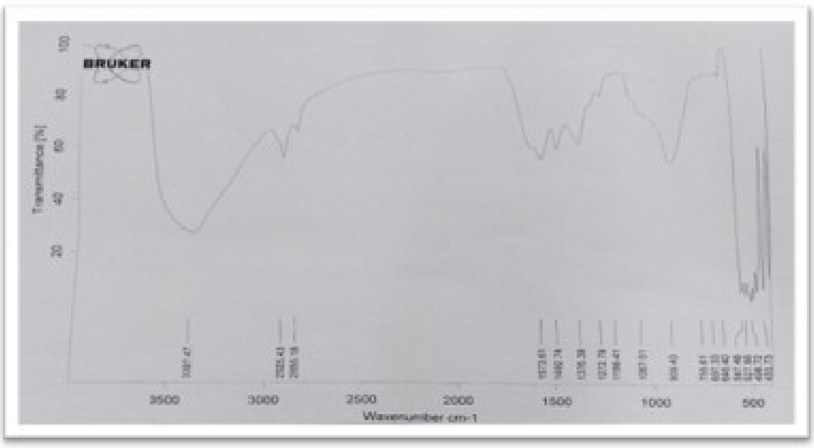

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Figure 10. Figure 3 : FTIR spectrum of ZnO NPs.

Zinc Oxide Nanoparticle Spectrum (ZnO NPs)

A broad O–H stretching peak is observed in 3397.47, though slightly weaker than in the pure extract. This indicates the presence of residual functional groups from the plant extract on the nanoparticle surface, suggesting their role in capping or stabilizing the ZnO nanoparticles. Signals between 2925.43 and 2855.18 refers to these peaks correspond to C–H stretching vibrations, similar to those in the plant extract but with reduced intensity, indicating traces of organic residues from the extract still present on the ZnO surface. FTIR spectroscopy revealed key functional groups presented in ZnO NPs , peaks in 1625.81 and 1422.74 cm^{-1} may be attributed to N–H bending or C=C stretching vibrations, likely originating from phenolic or amine compounds involved in reducing and stabilizing the ZnO nanoparticles.Reduction in peaks between 1200–1000 cm^{-1} and decrease in peak number and intensity in this region, suggests the consumption of certain active components especially C–O groups during the ZnO formation process. Distinct peaks at ~496.40 cm^{-1} and 434.72 cm^{-1} , Absorption bands of ZnO stretching, providing clear spectral evidence for the successful synthesis of zinc oxide nanoparticles.

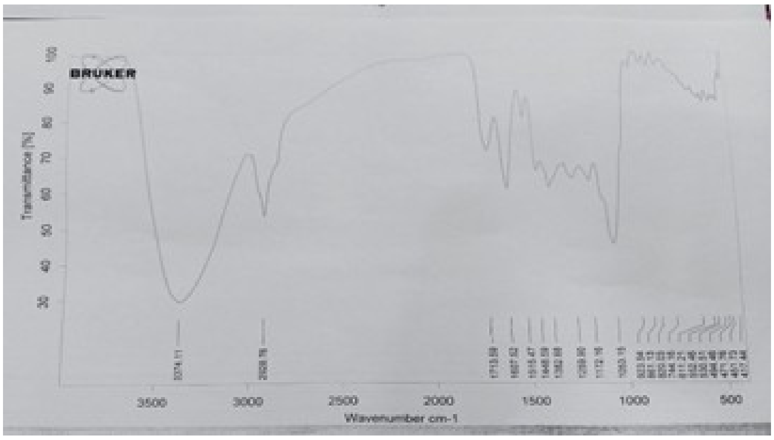

Figure 11. (Figure 3-4) : FTIR spectrum of Alcoholic extract

Plant Extract FTIR Spectrum Interpretation

In (3374.11 cm^{-1}) band corresponding O–H stretching, indicative of hydroxyl groups from alcohols and phenolic compounds, also presence of bioactive polyphenols with reducing and antioxidant properties in peak 2938.76 cm^{-1} represents C–H stretching vibrations of aliphatic (alkanes) compounds , presence of organic molecules that may include fatty acids or other hydrocarbon chains in the extract. A strong, sharp peak C=O stretching in ketones, carboxylic acids, or esters, may play roles in metal ion chelation or reduction during nanoparticle synthesis this appear in 1713.59 cm^{-1}. In 1625–1013 cm^{-1} (Multiple Peaks), a combination of peaks representing functional groups, including:

C=C stretching in aromatic rings, the presence of flavonoids or other aromatic phenolics.

C–O stretching vibrations from alcohols or esters, confirming oxygenated functional groups in the extract.

C–N stretching, pointing to amines or amide compounds, possibly from alkaloids or proteins that may assist in nanoparticle stabilization or capping.

The FTIR spectrum of the plant extract reveals a rich presence of hydroxyl, carbonyl, aromatic, and nitrogen-containing functional groups, which are typically involved in the reduction of metal ions and stabilization of nanoparticles.

Electron Microscope (SEM) Analysis

SEM examination revealed that (ZnO NPs) were spherical and the particle diameter ranged from 32.42 to 45.86 nanometers, with particle size of 38.29 ± 6.88 nm. This morphology indicating successful synthesis of ZnONPs using the plant extract.

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Indonesian Journal on Health Science and Medicine
Vol. 2 No. 3 (2025): Desember
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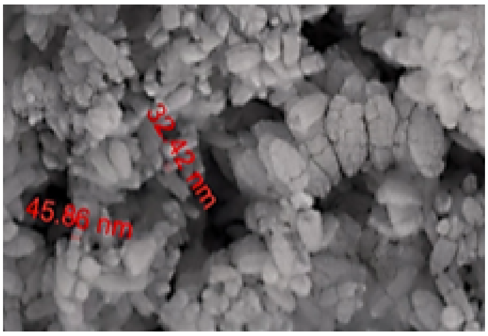
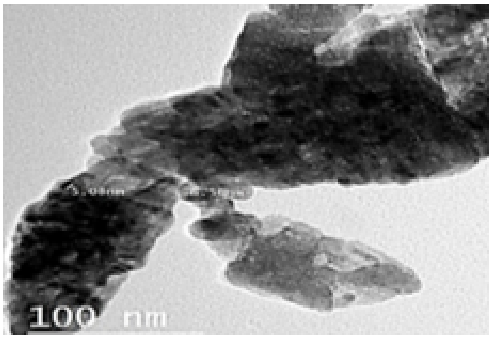


SEM analysis of ZnO NPs



TEM analysis of ZnO NPs

Figure 12.

Transmission Electron Microscope (TEM) Analysis

Conducted at low energy and high resolution, confirmed the successful synthesis of hexagonal-shaped zinc oxide nanoparticles (ZnO NPs). The particle sizes (5.08 - 6.56) nanometers, with an average particle size of 5.82 ± 0.74 nm. This high-resolution imaging reveals that the nanoparticles are uniformly shaped and ultra-small, indicating effective control over the synthesis process. The hexagonal morphology is crystalline form of ZnO, which may contribute to enhanced surface area and reactivity, making the nanoparticles highly suitable for biological applications.

Conclusion

The current study demonstrated the effectiveness of both an extract of the fruit of the Prosopis var. var. and biosynthesized zinc oxide nanoparticles as effective insecticides against various life stages of the insect O. hyalinipenis under laboratory conditions. Among all treatments, the alcoholic fruit extract exhibited the highest mortality rates, followed by the biosynthesized zinc oxide nanoparticles, while the purified zinc oxide nanoparticles were the least effective. The enhanced effectiveness of the biosynthesized zinc oxide nanoparticles is attributed to their small size, high surface activity, and the presence of bioactive phytochemicals that act as reducing and stabilizing agents. These results highlight the potential of biosynthetic nanomaterials and plant extracts as safe and effective alternatives to conventional chemical pesticides in integrated pest management programs.

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