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Effects of zinc oxide nanoparticles (ZnO NPs) synthesized from different plant leaf extracts on mealworm larvae *Tenebrio molitor* L.,1758 (Tenebrionidae: Coleopetera)

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ABSTRACT

Synthesize of zinc nanoparticles adopted through a simple, and eco-friendly biosynthesis process, utilizing the eucalyptus (*Eucalyptus camaldulensis*), river oak (*Casuarina cunninghamiana*) and dill (*Anethum graveolens*) leaves as sources and evaluate the insecticidal effects of the produced zinc oxide nanoparticles against the mealworms *Tenebrio molitor* L.,1758.The produced ZnO nanoparticles were characterized by X-ray diffraction (XRD), UV–visible, and transmission electron microscopy (TEM). The laboratory research was carried out with feeding method (the leaf immersion in ZnO NPs solution with different concentrations). The mortality effects of all the three synthesized ZnO nanoparticles against the studied larval stage was recorded in various period of time. the results of the statistical analysis showed that there were significant differences in the average mortality rate according to plant consisting of zinc nanoparticle, in which the highest average of the larval mortality was obtained (61.83%) for river oak, with an average percentage of adult emergence (27.50 %). Similarly the LC50 values of the ZnO NPs derived from the used plants was showed a varying effect on the larvae of mealworm with feeding method and that this effect varied according to the plant species in which for the River oak was (396.27 ppm), Eucalyptus plant (3630.78 ppm)and Dill plant was(6280.58 ppm). This result concluded that Zinc Oxide Nanoparticles from plant sources has larvicidal properties but the most effective one was from River oak plant and they serve as eco-friendly an alternative to synthetic insecticides for controlling insect stages. Hence the biogenic Zinc Oxide Nanoparticles can be used as potential insecticidal agent for the studied insect.

1.Introduction

Nanotechnology has gained a significant importance. Particularly ,in the realm of nano-biotechnology applications, which plays a crucial role in enhancing the quality of life (Singh *et al*., 2013). The effectiveness of micro-particle in well activity relies on their characteristics including size, shape and wettability (Abdel-Azeem and Osman, 2021). Nanomaterials have been defined as materials that their size ranging from 1 to 1000 nm in at least one; nevertheless, they are generally well-defined as having a diameter within the range of 1 - 100 nm. The produced zinc nanoparticles with different chemical methods might pose a concern to the environment (Lin *et al*., 2004). Currently most of the scholars are focusing on a way that is environmental-friendly to tolerate the universe (Stolarczyk *et al*., 2014). As a result, there is a growing demand to develop eco-friendly methods for NP production that is not relying on harmful ingredients. Various techniques have been utilized to prepare nanoparticles, encompassing chemical, physical, and biological methods microorganisms, enzymes, and extracts of plants have been proposed as an alternative to chemicals in the different ways have been used for nanoparticle preparation. The biosynthesis of nanoparticles from plants offers several benefits when it comes to producing metal or metal oxide nanoparticles on a large scale. This approach eliminates the requirement for lethal or toxic chemicals (Mohanpuria *et al*., 2008).Biological synthesis by plant species is helpful in the large-scale production of metal or metal oxide nanoparticles without usage of any toxic, hazardous chemicals (Devi and Gayathri, 2014). Recently, plant parts: leaf (Karam and Abdulrahman,2022),peel (Sukri *et al*., 2019),bark (Ansari *et al*., 2020), flower (Dobrucka and Długaszewska, 2016), seed (Sharma *et al*., 2010), tuber (Safawo et al., 2018) extract mediated biological process for the synthesis of zinc oxide nanoparticles. Zinc oxide nanoparticles have been investigated as potential insecticides for controlling pest populations in agriculture and other settings (Jameel *et al.*, 2020). However, it is important to note that the use of ZnO NPs for insect control raises concerns about their potential impact on non-target organisms and the environment (Côa *et al*., 2020). Overall, studies demonstrate that ZnO can have a range of effects on different insect species, including toxicological, behavioral, and physiological effects. These findings have important implications for the use of ZnO in agriculture and pest control strategies. Mealworms *Tenebrio molitor* L. Tenebrionidae are commonly used as a food source for pets such as birds, reptiles, and small mammals. They are also consumed by humans in some cultures, and are considered a good source of protein. Therefore this study aimed to biosynthesize ZnO nanoparticles derived from three sources of plants and to determine the efficacy of the produced ZnO nanoparticles towards Mealworm larva under laboratory conditions and clarify its toxicity .

2.MATERIALS AND METHODS

Experiments were conducted in both material laboratory (Physics department) and advanced insect laboratory (Biology department) at the college of Education, Salahaddin University-Erbil, Iraqi Kurdistan region.

Materials

The plants extract used for the synthesis of Zinc Oxide nanoparticles were Eucalyptus , River oak and Dill leaves collected from plants in Erbil province, Kurdistan-region, Iraq and identified by a botanist from the biology department, while distilled water, deionized water, zinc acetate dehydrate Zn(CH3CO2)2·2H2O. and sodium hydroxide (NaOH) were purchased from sigma-Aldrich.. Mealworms larvae, has been used for the purpose of bioassay tests.

2.1.Collecting plant leaves and Rearing of insects

The collected plant leaves have been identified within the herbarium of biology department college of Education as Eucalyptus (*Eucalyptus camaldulensis*), River oak (*Casuarina cunninghamiana*) and Dill (*Anethum graveolens*), While the mealworms larva were obtained from insect farming poultries .The insect specimens have been sent to Iraqi Natural History Research Center and Museum No.455 on 9/5/2022 and identified as (*Tenebrio molitor* L.,1758)The larvae were reared under laboratory conditions in containers comprising oat grain and lettuce and deposited in incubators 25±3°C with 65 ±5% relative humidity and constant darkness (Eberle *et al.*, 2022 ; Ribeiro *et al*., 2018),

2.2. Plant extracts preparation

The wet obtained leaves were thoroughly washed with distilled water and left on dark place for 2 weeks to be dried. The dried leaves were crushed and converted into fine powder by using ceramic grinding bowl and sieved through a mesh .After the process of shading, drying, and grinding (6) g of dried leaves were mixed with 100 ml of distilled water, the mixture was boiled for 1 hour at 60 °C by using Magnetic Stirrer with hot plate. Then the mixture was placed under reflux conditions to be extracted. After that aqueous extract of the leaves were gained by mixture filtering using Whatmann No. 1 filter paper. Then the aqueous plant extract had been ready to be used in further testing (Sundrarajan *et al.*, 2015).

2.2.1Biosynthesis of Zinc oxide NPs

Zinc oxide was synthesized by using green synthesis method according to (Sundrarajan *et al.,* 2015). Thirty ml aqueous plant extracts were mixed with 3 g of zinc acetate dehydrate and boiled over with magnetic Stirrer with a hot plate at 90 °C – 400 rpm (round per minute) stirring, then drops of NaOH solution were added until the color changed from dark yellow to yellowish-white color. After completion of reaction 50 ml of distilled water was added to the paste and settled for 24 hours in order to the NPs will be precipitated, then it will be dried on the heater (at 90 °C) to be only NPs remain. The paste was transferred into a crucible, which was kept on furnace at 400℃ for 2 hours until the color of the precipitates changed to white color fig.(1). Then precipitates were ground and stored for further analysis (Suresh *et al.*, 2018).

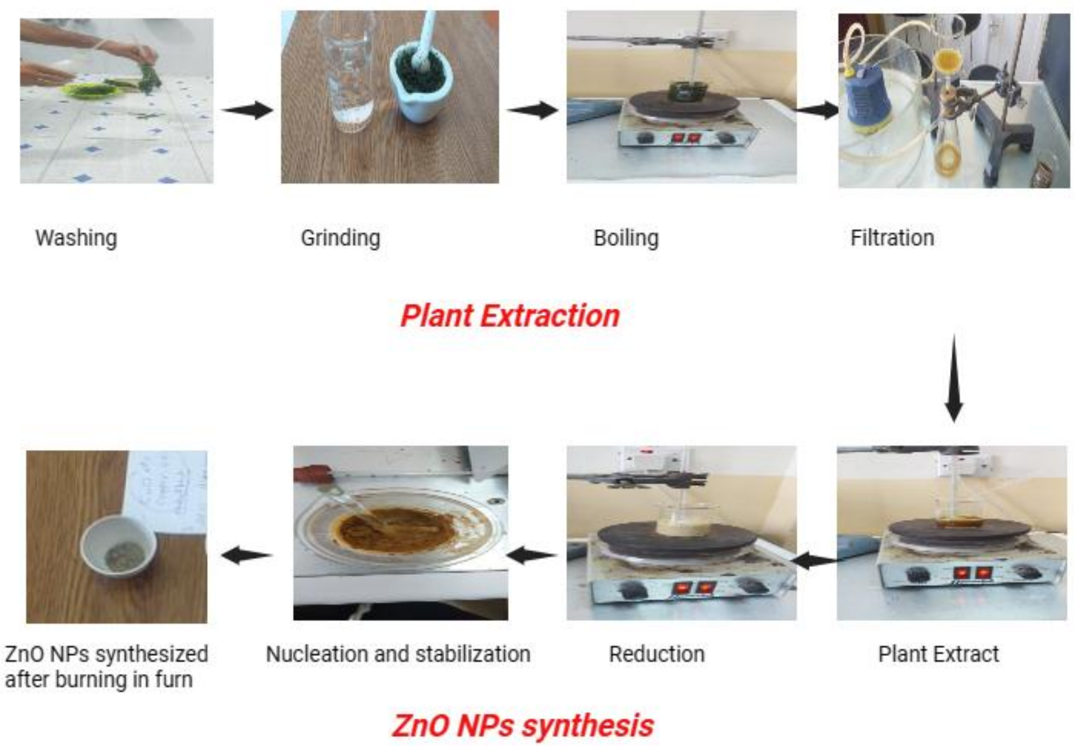

Figure 1: The process of Zinc Oxide nanoparticle synthesis

2.3.Zinc oxide NP characterization

2.3.1UV-spectrophotometer:

A Shimadzu UV-Visible double beam spectrophotometer (model UV-1800, Japan) with a fixed 1nm bandwidth and 1 cm quartz cell was utilized for spectrophotometric measurement, and the computer was connected to a double beam spectrophotometer in order to record zero order spectra.

2.3.2Transmission Electron Microscope (TEM):

The size and morphology of the ZnO nanoparticles were investigated by transmission electron microscope (JEOL electron microscope JEM-100 CX).

2.3.3X-ray Diffraction Method (XRD) analysis:

The X-ray diffraction (XRD) distribution of ZnO nanoparticles was acquired using RigakuX-RAY Diffractometer (Rigaku, Japan) that generated Cu Kα radiation (with an angular resolution of 1.5418angstrom). It is being employed to evaluate the crystalline particle sizes that have been manufactured. For the purposes of characterization, a tiny quantity of powder sample was used. At room temperature, X-ray generators were running at a voltage of 40 kV and applying a current of 30 mA to the target (Degefa *et al*., 2021).

2.4.Preparation of desired ZnO NPs concentrations

Various concentrations were prepared by diluting the original ZnO NPs nanoparticles using deionized distilled water to achieve 250ppm, 1000ppm, 2500ppm and 5000ppm, plus control which contained just deionized distilled water(Gopalakrishnan *et al.*, 2021).

2.4.1 Bioassay Test

Different concentrations (control, 250, 1000, 2500 and 5000 ppm) of ZnO NPs solution were examined on mealworm larvae For the bioassay tests three replications were utilized each with 10 larvae in small plastic containers (25 ml) for multiple times of exposure through feeding (1) g of lettuce leaflets immersed in ZnO NPs solution concentrations .The percentage of insect mortality was corrected according to Abbot's formula. LC50 values were determined by using probit analysis statistical method of Finney (1971).

Abbot's formula: Corrected Mortality (%)

$$=\left(\frac{\%\ \mathrm{MT}-\%\mathrm{MC}}{100-\mathrm{MC}} * 100\right)$$

Where, T = No. of dead larvae in treated replicates.

C = No. of dead larvae in control replicates.

Toxicity index for

$$\text{LC50}=\frac{\text{LC50 of the most effective compound}}{\text{LC 50 of the least effective compound}} \times 100$$

2.5.Statistical analysis

SPSS version (26) was used to examine data of the bioassay tests. All data were presented as Mean ± SD and the Mean was compared using Student's t-test at the 5% probability level. Furthermore, one-way analysis of variance (ANOVA) Duncan s test was used to do statistical comparisons of the studied plants and concentrations. In all situations analyzed, P values less than 0.05 were considered significant. , Also probit analysis by Finney 1971 was used to estimate LC50 for the plants which been selected for the green synthesis of ZnO NPs.

3.RESULTS AND DISCUSSION

Zinc oxide NPs characterization

UV-spectrophotometer of Dill plant

Zinc oxide nanoparticles (ZnONPs) synthesized using *Anethum graveolens* aqueous extract were studied. Formation of zinc oxide nanoparticles were confirmed by a UV-vis spectrophotometer .UV–vis absorption spectrum of synthesized zinc oxide nanoparticles was recorded for the sample in the range of 200–800 nm. The spectrum showed that the absorbance peak was reached 320 nm as shown in (Fig.2) corresponding to the characteristic band of zinc oxide nanoparticles, our results were close to the absorbance peak of *(Azizi et al.*, 2014) results which was 334 nm.

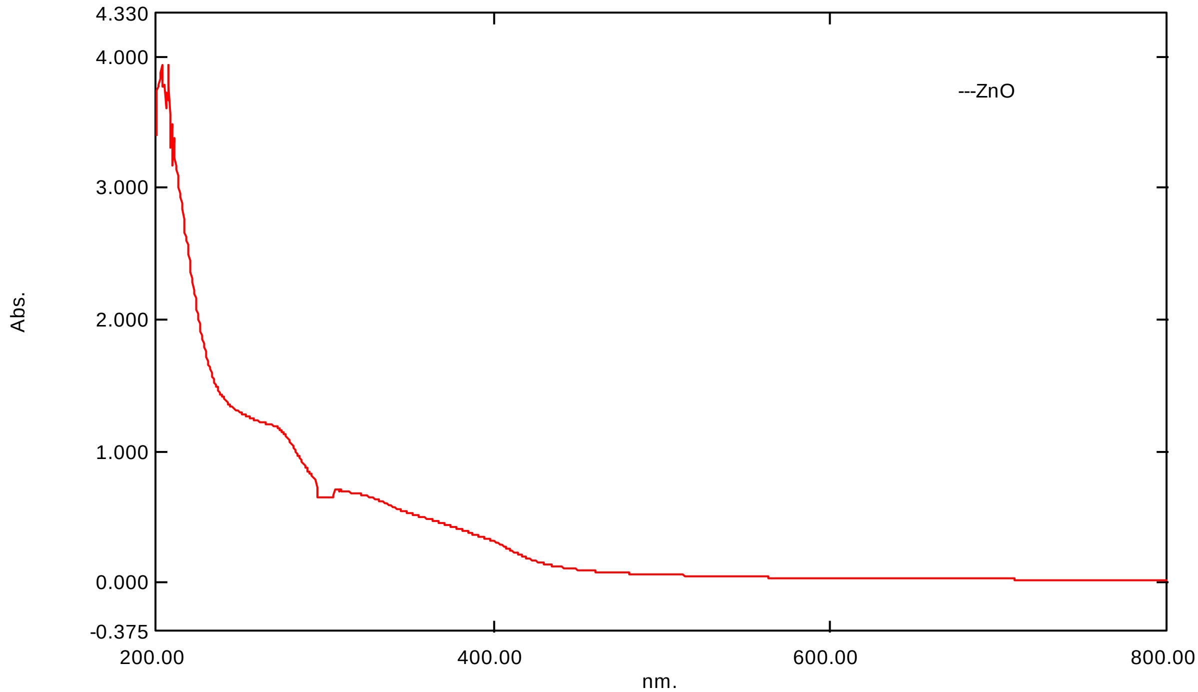

Figure 2: UV-vis spectrum of ZnONPs synthesized using Dill leaves

UV-spectrophotometer of Eucalyptus plant

Zinc oxide nanoparticles (ZnONPs) synthesized using *Eucalyptus camaldulensis* aqueous extract were studied. Formation of zinc oxide nanoparticles were confirmed by a UV-vis spectrophotometer. UV–vis absorption spectrum of synthesized zinc oxide nanoparticles was recorded for the sample in the range of 200–800 nm. The spectrum showed that the absorbance peak was reached 305 nm as shown in (Fig.3) corresponding to the characteristic band of zinc oxide nanoparticles. our results was close to the results obtained by (Anjali *et al*., 2021) which was 310 nm.

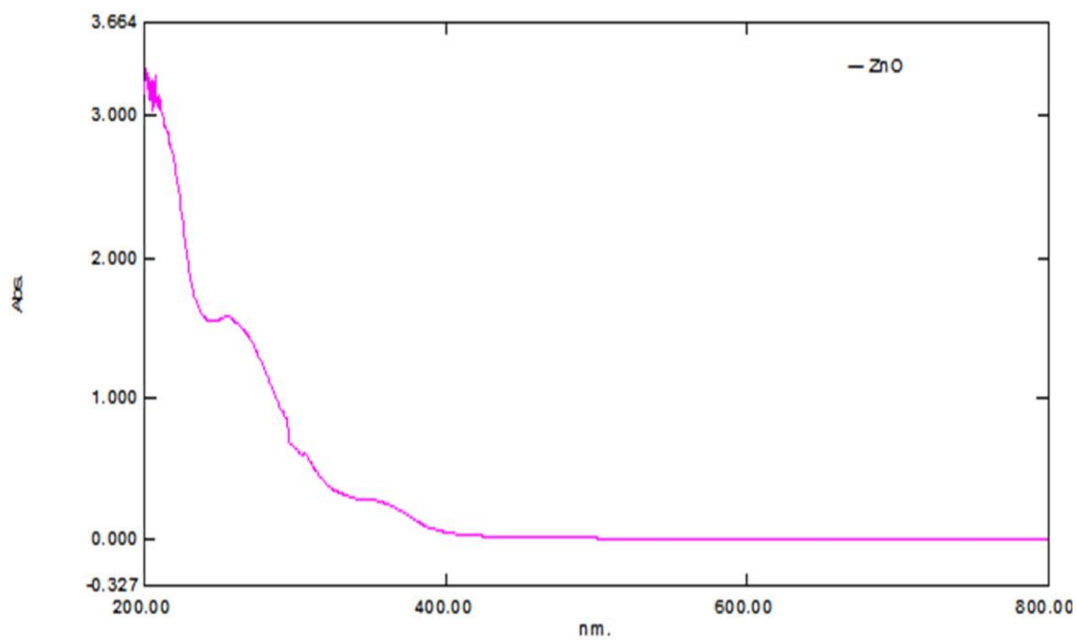

Figure 3: UV-vis spectrum of ZnONPs synthesized using Eucalyptus leaves

UV-spectrophotometer of River oak plant

The investigation focused on studying zinc oxide nanoparticles that were produced by utilizing an aqueous extract of *Casuarina cunninghamiana*. The sample's UV-visible absorption spectrum was measured for synthesized zinc oxide nanoparticles, covering a wavelength range from 200 to 800 nm. The spectrum indicated that the absorbance peak, corresponding to the characteristic band of zinc oxide nanoparticles, occurred at 305 nm, as depicted in Figure (4). our results was close to the results of (Anjali *et al*., 2021) which was 310 nm.

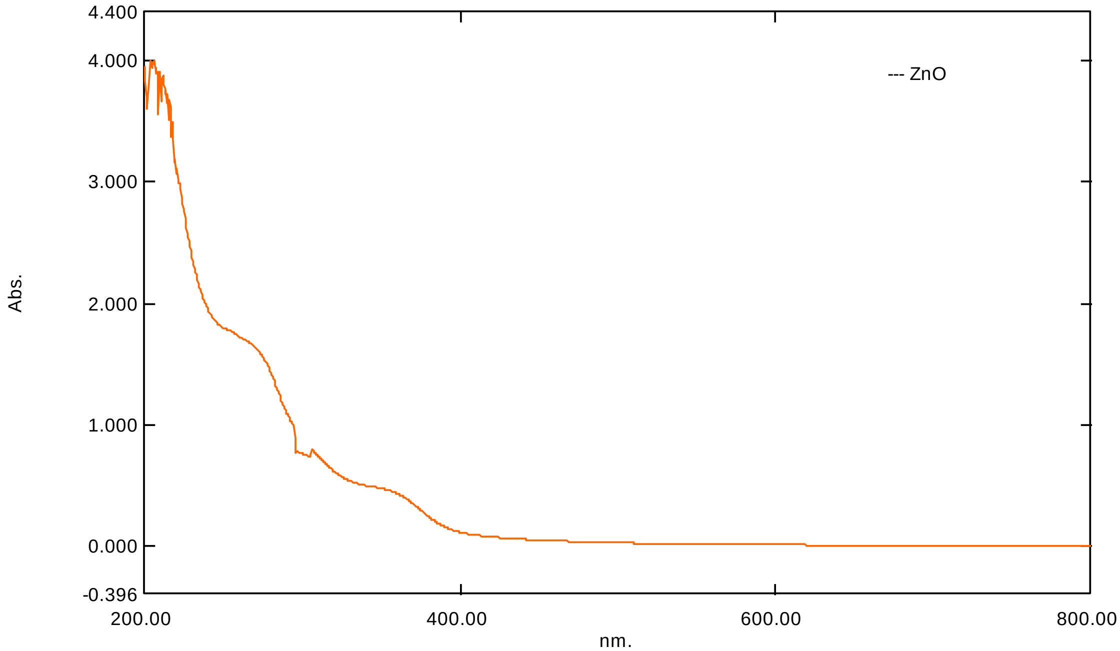

Figure 4: UV-vis spectrum of ZnONPs synthesized using River oak leaves

Transmission Electron Microscope (TEM): Transmission Electron Microscope of ZnO NPs obtained from Dill plant

The zinc oxide nanoparticles created were examined using transmission electron microscopy, as depicted in Figure (5), at a scale of 100 nanometers. The analysis revealed that the ZnONPs possess a spherical structure and exhibit an average size varying from 13 to 19 nanometers.

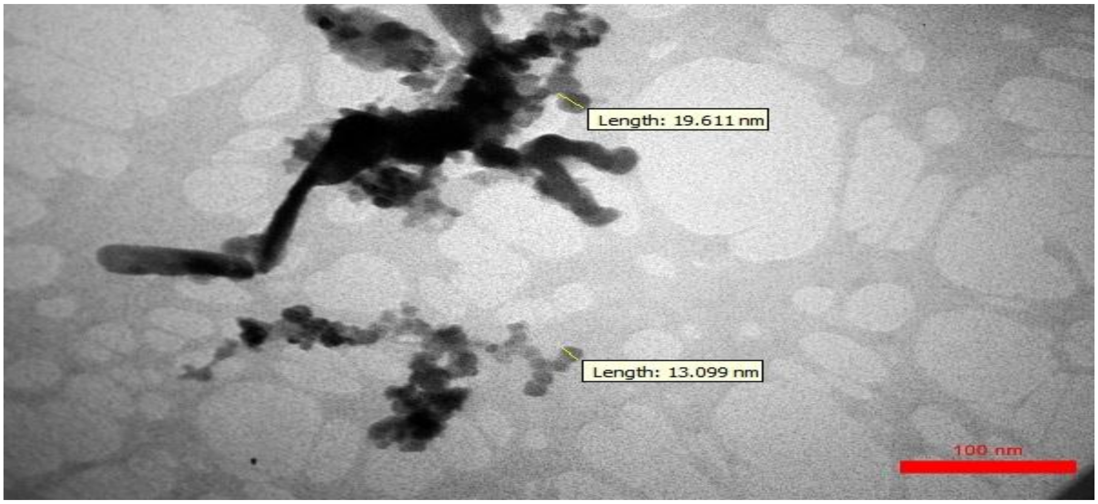

Figure 5: TEM image of green synthesized ZnO NPs from Dill plant

Transmission Electron Microscope of ZnO NPs obtained from Eucalyptus plant

The zinc oxide nanoparticles that were synthesized were examined using transmission electron microscopy, as shown in Figure (6), at a scale of 50 nanometers. The observation revealed that the ZnONPs exhibit a spherical morphology and have an average size of approximately 18 nanometers.

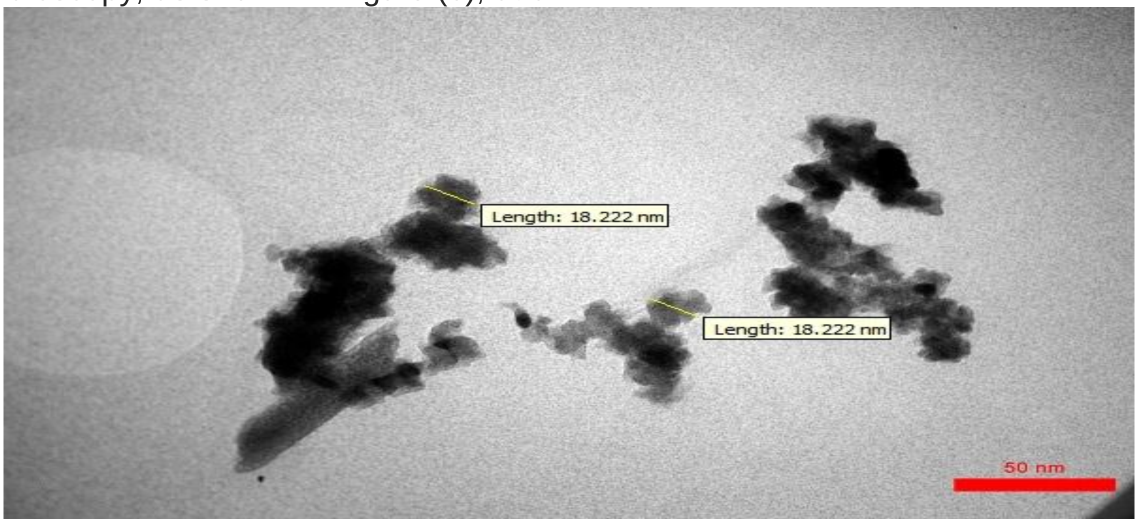

Figure 6: TEM image of green synthesized ZnO NPs from Eucalyptus plant

Transmission Electron Microscope of ZnO NPs obtained from River oak plant

The transmission electron microscopy analysis of the synthesized zinc oxide nanoparticles is presented in Figure (7), with a scale of 100 nanometers. The observation indicates that the ZnONPs display a spherical morphology with an average size ranging from 10 to 13 nanometers. Additionally, there were some larger particles observed, referred to as bulks, with sizes reaching up to 119 nanometers.

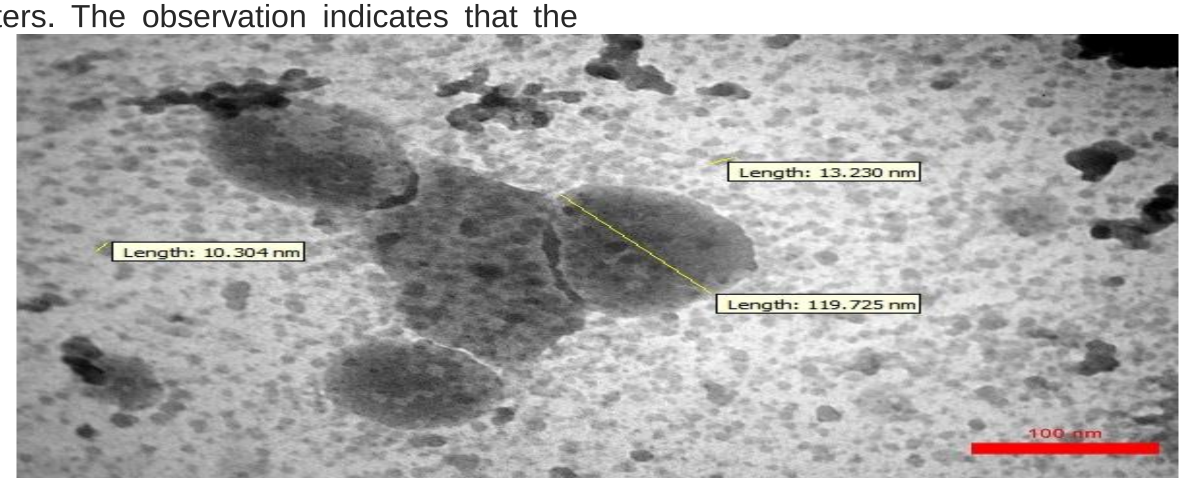

Figure 7: TEM image of green synthesized ZnO NPs from River oak plant

X-ray Diffraction Method (XRD) analysis of ZnO NPs:

X-ray Diffraction Method (XRD) analysis for ZnO NPs obtained from (Dill plant)

The X-ray Diffraction Method analysis of ZnO nanoparticles obtained from the River Oak plant is depicted in Figure 8. The synthesized ZnO nanoparticles displayed three prominent crystalline peaks at 2θ values of 31.8, 34.4, and 36.25 degrees. These peaks can be indexed to the crystal planes 100, 002, and 101, respectively, there were eight additional low peaks observed in the X-ray Diffraction Method (XRD) analysis at 2θ values of 47.51, 56.67, 62.86, 66.40, 67.91, 69.09, 72.56, and 76.92 degrees. These peaks can be attributed to the crystal planes 102, 110, 103, 200, 112, 201, 004, and 202, respectively. The existence of these crystalline peaks validates the occurrence of the hexagonal wurtzite phase in the synthesized ZnO nanoparticles. These findings are consistent in line with (Vijayakumar *et al*., 2016, Umavathi *et al.*, 2021) results of plant-mediated ZnO NP synthesis.

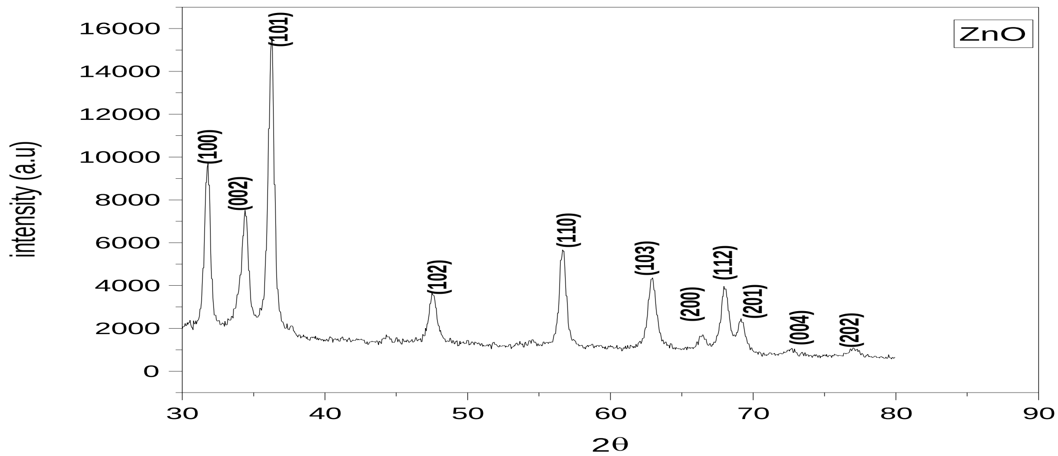

Figure 8: XRD pattern of ZnONPs synthesized using dill plant

X-ray Diffraction Method (XRD) analysis of ZnO NPs obtained from (Eucalyptus plant)

The X-ray Diffraction Method analysis of ZnO nanoparticles obtained from the River Oak plant has been depicted in Figure (9). The XRD pattern of the green-synthesized ZnO nanoparticles revealed three prominent crystalline peaks at 2θ values of 31.8, 34.45, and 36.3 degrees. These peaks can be indexed to the crystal planes 100, 002, and 101, respectively, In addition to the prominent crystalline peaks mentioned earlier, the X-ray Diffraction Method (XRD) analysis also revealed eight low peaks at 2θ values of 47.6, 56.67, 62.86, 66.40, 67.91, 69.09, 72.56, and 76.92 degrees. These peaks can be attributed to the crystal planes 102, 110, 103, 200, 112, 201, 004, and 202, respectively. The presence of these crystalline peaks confirms the formation of the hexagonal wurtzite phase. our result was in agreement with (Vijayakumar *et al*., 2016, Umavathi *et al*., 2021) results of plant-mediated ZnO NP synthesis.

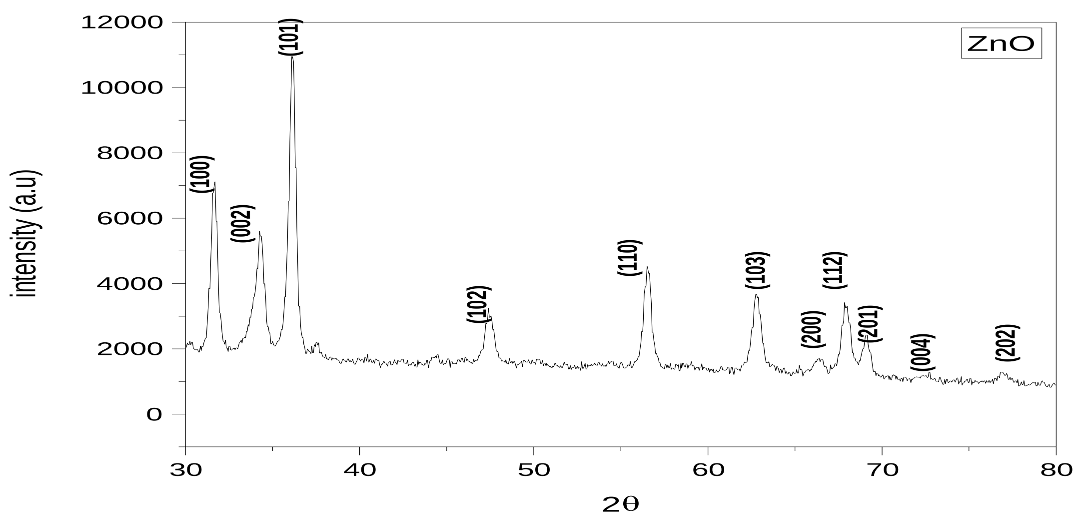

Figure 9: XRD pattern of ZnONPs synthesized using Eucalypts plant

X-ray Diffraction Method (XRD) analysis of ZnO NPs obtained from (River oak plant)

The X-ray Diffraction Method analysis of ZnO nanoparticles obtained from the River Oak plant is presented in Figure (10). The XRD pattern of the green-synthesized ZnO nanoparticles exhibits three predominant crystalline peaks at 2θ values of 31.74, 34.2, and 36.17 degrees. These peaks can be indexed to the crystal planes 100, 002, and 101, respectively. Additionally, there are eight low peaks observed at 2θ values of 47.55, 56.67, 62.86, 66.40, 67.91, 69.09, 72.56, and 76.92 degrees. These peaks are attributed to the crystal planes 102, 110, 103, 200, 112, 201, 004, and 202, respectively. The presence of these crystalline peaks confirms the formation of the hexagonal wurtzite phase in the synthesized ZnO nanoparticles.These crystalline peaks confirm the formation of the hexagonal wurtzite phase, our result was in agreement with (*Vijayakumar et al.*, 2016, Umavathi *et al*., 2021) results of plant-mediated ZnO NP synthesis.

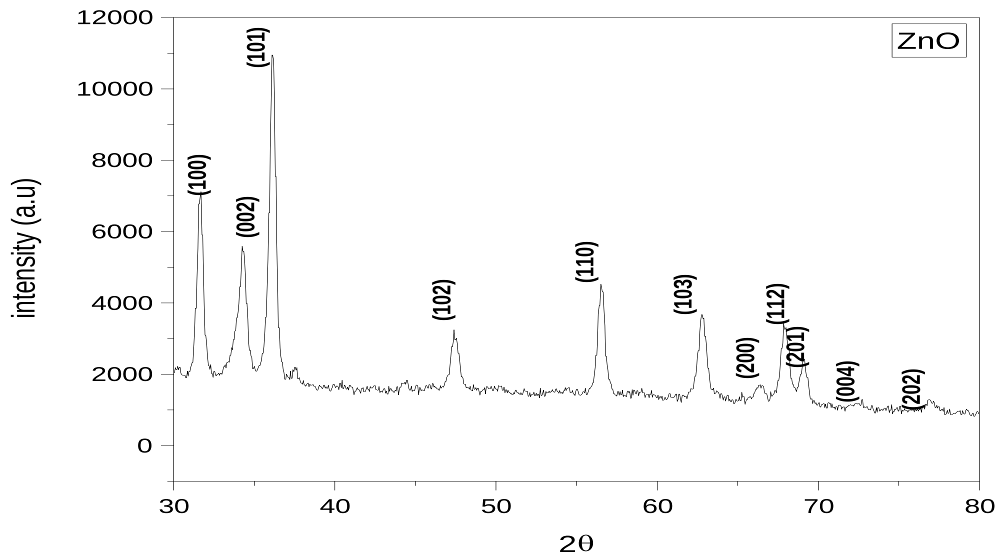

Figure 10: XRD pattern of ZnONPs synthesized using River oak plant

Bioassay of ZnO NPs synthesized from different plant sources against larvae of mealworm with feeding method

Larval stage

It is clear from the table (1) that ZnO NPs which has been formed from plants (Dill, Eucalyptus and River oak) showed a different effect in the average of larval mortality rate according to the plant and that the average of larvae killing ranged between (35.76%) for dill plant and (61.83%) for river oak after five weeks of treatment. in which the highest average of the killing rate was for oak (61.83%), followed by the treatment with dill and eucalyptus plant, where they reached (35.76%) and (37.60%) respectively, with no significant differences between them. However, statistical analysis showed significant differences between the plants and the control treatment. As for the effect of the ZnO NPs treatment on the adult emerge as shown in table(2), the results showed that zinc nanoparticles formed from oak had the greatest effect on the emergence rates of the studied insect, where the average percentage of the oak plant was (27.50%) while it was (41.68 and 42.53%) for each of the dill and eucalyptus plants respectively with significant differences between the studied plants and the control which was (53.30%), Several studies have investigated the effects of ZnO NPs on the mortality and growth of different insect larvae that is concentrations and species dependent and had a similar conclusion, for instance, a study by Elmasry, (2021) investigated the effects of ZnO NPs on the larvae of *Spodoptera litura*, a major pest of many crops, he found that the ZnO NPs significantly reduced the survival rate of the larvae and caused growth inhibition, which mortality percentage increases after treating. Moreover Jameel et al., (2020) presented the adult emergence of *S. litura*. after treating with different concentrations of ZnO−thiamethoxam and thiamethoxam increases in a dose-dependent manner, he observed that the treatments with the different concentrations of thiamethoxam alone and ZnO−thiamethoxam, decline the emergence of adults in all test concentrations of ZnO−thiamethoxam .Results obtained by Asghar *et al*., (2022) showed that with the increase in NPs concentrations mortality percentage was also increased in all tested instar larvae of *H. armigera*. Moreover in a previous study done by Buhroo *et al*., (2017) they demonstrate that the increased of concentration of Aluminum oxide (Al2O3) nanoparticles reduced the number of adult emergence, it revealed that the reduction percentages of adult emergence were increased with the increase of concentration.

Table 1: Accumulation effects of ZnO NPs synthesized from plant sources against larval stage of mealworm through feeding method after five weeks in ideal incubation conditions:

Plants	Mortality Mean ± SD	F-test	P-Value (Sig.)
Dill	35.76±1.59 (b)	18.514	0.001 (HS)
Eucalyptus	37.60±1.44 (b)	10.550	0.001 (HS)
River oak	61.83±0.94 (a)	17.601	0.001 (HS)
Control	13.30±0.70 (c)	7.370	

Different letters indicate significant differences.

Table 2: Efficiency of ZnO NPs synthesized from plant sources on adult emerge of the mealworm species through feeding method at optimal incubation condition:

Plants	Mean ± SD	F-test	P-Value(Sig.)
Dill	41.68±0.64(c)	86.750	0.001 (HS)
Eucalyptus	42.53±1.74(c)	77.494	0.001 (HS)
River oak	27.50±0.61(b)	29.146	0.001 (HS)
Control	53.30±1.54(a)	55.325	

Different letters indicate significant differences.

The lc50 values of ZnO NPs synthesized from different plant sources against the Mealworm (Tenebrio molitor (Fabricius, 1792)) larval stages through feeding method:

Table (3) shows that the lc50 values of the ZnO NPs derived from the used plants showed a varying effect on the larvae of mealworm with feeding method and that this effect varied according to the plant species in which the River oak was the most effective on the mealworm larvae in which the LC50 value of this plant was 396.27 ppm followed by the Eucalyptus plant with a value of LC50 3630.78 ppm compared with the value of Dill LC50 6280.58 ppm. the results showed that the Dill plant was the least efficient plant among the tested plants, and this was confirmed by the Toxicity Index of these plants as they gradually graded downward in their potency and reached (100, 10.914 , 6.309) for River Oak, Eucalyptus and Dill plants respectively, Although the toxicity curve of all the studied plants showed their effectiveness compared to the control treatment

Table 3: Estimated LC50 of ZnO NPs synthesized from different plant sources against the Mealworm (Tenebrio molitor (Fabricius, 1792)) larval stage with feeding way

Plants	Intervals	Concentrations(ppm)	Corrected mean mortality	LC_{50}	Slope equation	R^2	Toxicity Index LC_{50}
Dill	Week 5	250	23.7	6280.58	y=0.565 x+2.854	0.894	6.309
		1000	27.25				
		2500	42.05				
		5000	50.06				
Eucalyptus	Week 5	250	19.58	3630.78	y=0.788 x+2.194	0.891	10.914
		1000	31.11				
		2500	38.47				
		5000	61.25				
River oak	Week 5	250	39.2	396.27	y=1.056 x+2.256	0.948	100
		1000	53.07				
		2500	76.93				
		5000	88.33				

Data presented in Tables (1,2,3), indicated the presence of variation in the average rates of larval mortality rate depending on the ZnO NPs derived from the used plants (Dill , Eucalyptus, River Oak), through different concentrations by feeding methods. The greatest proportion of death was seen in larvae fed with ZnO NPs derived from River Oak, followed by the Eucalyptus and dill plants, within the period of treatment when applied at all the concentrations after five weeks of therapy, increasing product concentration and exposure period raised mean % mortality. The highest larval mortality of 88.33 % and 76.93% was noticed in the treatment where ZnO NPs derived from River Oak for concentrations 2500ppm and 5000ppm, respectively against the larval stages of mealworm after five weeks of treatments, which was significantly different from untreated control as it's shown in table (1)., all the obtained data of our results revealed that the ZnO NPs derived from River Oak were the most effective against the larval stage of the studied insect and the higher the concentration, the more effective it is .The impact of silver nanoparticles on insect larvae feeding habits causes damage to the cells and tissues of the insect's midgut, resulting in cellular gaps that affect carbohydrate and protein levels in the insect's haemolymph. This was observed by researcher Karthikeyan *et al*., (2014) when exposing fourth instar mosquito larvae to silver nanoparticles. Our study's results are consistent with his findings, suggesting that the feeding of insects during their larval, whether short or long-term, has a significant impact. The use of silver nanoparticles at concentrations of 2500 and 5000 parts per million may reduce the population of these insects, thereby overcoming problems associated with the use of chemical pesticides and their harmful effects.

4.CONCLUSION

We conclude that the Eucalyptus (*Eucalyptus camaldulensis*), river oak (*Casuarina cunninghamiana*) and dill (*Anethum graveolens*) can be used for the synthesis of Zinc Oxide nanoparticlesand, their leaves are thought to contain chemical components that act as a reducing agent and a stabilising agent in the creation of Zinc Oxide nanoparticles. According to the current study, the creation of Zinc Oxide nanoparticles from the studied plants has a high potential effect on the larval stage of mealworm , and the river oak has a high potential insecticidal effect in comparison with the other studied plants ,so it could be a good source for further study in this field .

CONFLICT OF INTEREST STATEMENT

The results of the current study are part of the requirements of the M.Sc. in Insects, Department of Biology, College of Education, University of Salahaddin for the first author. Also, we confirm and declare no the existence of any relationship or conflict of interest with any other party.

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