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Green Synthesis and Evaluation of ZnO NPs and study the effect of Their toxic on Honey Bee (*Apis mellifera*)

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Abstract

The research interest in nanomaterials preparation from natural products as a green method and their application in various fields applications, tremendous attention has been taken to the green composition of nanoparticles. ZnO can be considered one of the most widely used metal oxides for most requirements of daily used products. In this research ZnO NPs prepared by using *Petroselinum crispum* (parsley) extract and to make the first study of toxicological evaluation of ZnO NPs their effect aspects on Honey bees (*Apis mellifera*). ZnO NPs have been charactarized by using SEM, EDX, XRD, UV-Vis and FTIR Spectroscopy. The toxicological evaluation of ZnO NPs has been applied to a honey bee. The lethal ZnO concentration was obtained, and the LC_{50} range calculation values were changed during 288 hours of feeding to ZnO nanoparticles at different concentrations (25, 50, 250, 500 mg per 100 ml) and the obtained LC_{50} values changed from 275, decreasing to 162.55 for the research range times after every 24 hours of exposure feeding calculations. In addition, for the group treated with 500 mg of ZnO per 100 ml, higher mortality was observed compared to other concentrations as it increased more than all other items indicating the above concentrations but not with the control group. The ergonomic design for creating a honey bee shelter was first introduced and no similar investigations were found in the literature.

Keywords: *Apis mellifera*, Green synthesis, Plant extract, Toxic effect, ZnO-NPs.

Introduction

Plant midated synthesis of the nanoparticles like green technique has emerged rapidly during the last decade in a broad range of product domains [1]. Nanotechnology has been used in many of the essential requirements of daily life such as chemistry, biology, agriculture, physics, and medicine [2-6]. Today, due to the reduction of the size of many materials, such as their dimension have between 1 and 100 nm and resulted in unique properties such as their midget dimensions, shapes, flatten features and surface chemistry and their conductivity[7, 8] and create a variety of research field in field of nanotechnology. Therefore, great attention has been given to nanotechnology and has led to the expansion of several technologies[9]. Recently, The ZnO nanoparticle regards one of these materials that has been used in a variety of research fields which is biocompatible and safe that has been used in medical applications easily without overlays[10-13].The increase in the requirements for nanomaterials in numerous

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field produces such as optoelectronic devices , sunscreens, paint pigments, rubber components, cosmetics, and medicines [14] such many of the existence of products in the world use which containing ZnO Nps increases focusing their toxic effect [13] and the fast progress in nanotechnology have caused angst about the environmental effects of nanoparticles and the risk they have constituted on human health.The ZnO nanoparticles can be considered one of the most widely used nanoparticles among nano-sized materials and can be found in many types of products. From ancient times, the leaves of the *Petroselinum crispum* plant were known to be the most obtainable vegetable product that is used in large areas of the world. Parsley was not only used for medical purposes but also, became accepted as a spice or food. Furthermore, The parsley plant extract is known as a plant that includes various types of phytochemicals such as phenylpropanoids, flavonoids, ascorbic acid, carotenoids, tocopherol, apiole, terpenoids, coumarin, phthalides, and furanocoumarins [15]. It is known that these natural compounds have a major effect on the preparation of nanoparticles as they are inhibited and the ability to prevent their aggregation is reduced [16-19]. Currently, we presented an environmentally friendly method for the synthesis of ZnO NPs using *Petroselinum crispum* leaves as a stabilizing agent and reducing agent..

Honey bee (*Apis mellifera*), is regarded as one of the most important producers of honey and wax**,** among pollinators in which threatened by environmental pollution and pest control and it causes huge death in honey bee colonies [20] and This also poses a huge menacing to the agriculture of over the world, by products of nanotechnologies, recently one of the disadvantages of nanotechnology products and their deterministic in wide range may also effect on some insects may also including their effects on honey bees [21]. Consumer products of many of the currently available nanomaterials contain ZnO or TiO2 NPs [22] and due to the finding of zinc oxide nanoparticles in many different products. In ancient times, people over the world practice the breeding of honey bees (*Apis meliferia*). Mortals that are fed with bee products is thought to be affected by nanoparticle in nature. However**,** highly concentrated ZnO nanoparticles have been reported to intoxicate algae and plants, bacteria, aquatic and terrestrial invertebrates, and vertebrates [23]. This research work focuses on the use of plants as a source of green synthesis, ZnO nanoparticles characterization, and their first toxicological effects on *Apis melliffera* honey bee insects.

Materials and Methods

Experimental parts

Materials:

Zinc acetate dehydrate, Zn $(CH_3OO)_2$. $2H_2O$ and Sodium hydroxide (NaOH) were purchased from Sigma-Aldrich, distilled water, deionized water. The plant extract used for synthesis of zinc oxide nanoparticles was prepared using plants *Petroselinum crispum* (pursley) leaves were purchased from Erbil City and identified and clissified by the botanist.

Preparation of plant extract:

The Fig 1 A-E shows the process photographically. It can be seen that; parsley Extract was prepared from 100 gm of thoroughly washed fresh parsley leaves with distilled water Fig 1A. They cutting to small pieces Fig 1B, then 20 grams of cut parsley leaves, made into a paste, using mortar when mixed with 5 ml of distilled water Fig. 1C. After that, 6 gm of the paste is heated and stirred by magnetic stirrer of (500 r.p.m) in 100 ml of distilled water for an hour at 70°C Fig 1D, filtered with Whatmann No 1 filter paper and the resulting extract was cooled down at room temperature Fig 1E and used for synthesis of ZnO nanoparticles.

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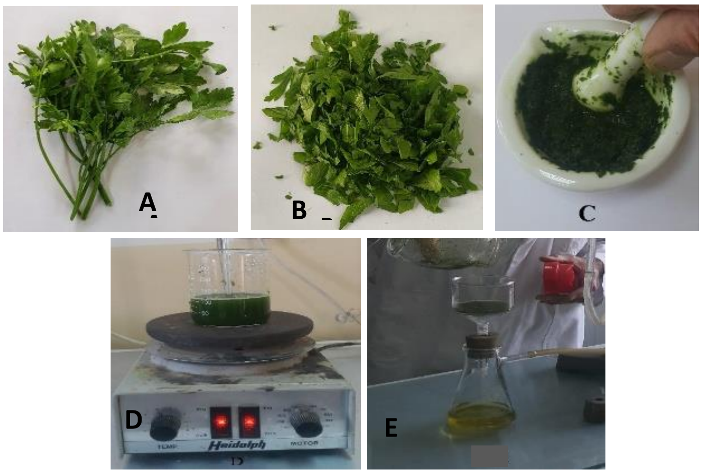

Figure 1. Photographically representation of *Petroselinum crispum* (parsley) leaves extracts preparation procedures: (A) Washing and drying leaves (B) leaves pieces (C) Past formation (D) Heating and stirring (E) Plant extracts separation by filtration.

Synthesis of Zinc oxide nanoparticles:

A plant extract used for the syntheses of Zinc oxide nanoparticles [24]. 30 ml of *Petroselinum crispum* leaf extracts, heated at hotplate magnetic stirrer for about 90 minutes with adding 3 gm of $Zn(CH_3OO)_2 . 2H_2O$ powder to extract. Then the mixture was heated and stirred in a hot plate magnetic stirrer at 90 °C. Solution of 1M sodium hydroxide was added until the colour of the mixture changes to yellow or brawn and the pH reached 8. The mixture, then was heated constantly at the same temperature until it converted to a paste. An air oven was used to dry the paste for 1 hour at temperature 100°C. In order to remove the basics solution, the content was washed thoroughly and repeatedly with distilled water. Finally the result sample was calcinated at 400 °C for 2 hours. And kept in dry area for further characterization.

Test bee insect materials:

First of all, before the start of the experimental process by a month, in order to have the same age and race as the young worker bee, which appeared within the frame of the comb hive, will be obtained by observing and studying the incubation period of the queen bee and during the closed brood period. To obtain more accurate results from the study assessing the acute toxicity effects of the nanoparticles used in the colony, no special chemical was used for pest control against diseases. The same age and racial element as the young worker bees were evenly distributed among the boxes so that there were fifty of them per box unit. The number of wooden boxes is six boxes, one of them for the feeding of the control will be used and five others for the feeding the honey bees with the different ZnO nanoparticles concentrations as shown inTable 1. Bee colonies (*Apis mellifera*) were maintained in the garden at the Science college, University of Salahaddin-Erbil, Iraq according to standard commercial techniques. A special plastic bottle prepared for this purpose was used for the randomly collected adult bees inside the hive during the autumn season and transferred immediately to wooden cages. Before we started our objective, with nanoparticle suspensions, bees were subjected to a 2-hour starvation period. Groups of 50 adult bees were transferred to wooden cages. To study the toxic effects, 250 bees were used and 50 bees were as a control group, so the total number of bees we have been used 300 bees.

The bee's boarding-house box:

To create a very suitable environment for the shelter of bees chosen for study, many types of boxes have been made from different materials and shapes. After several slide experiments, it was found that the most comfortable one for this study purpose was made of the wooden one such as the form shown in Fig 2.

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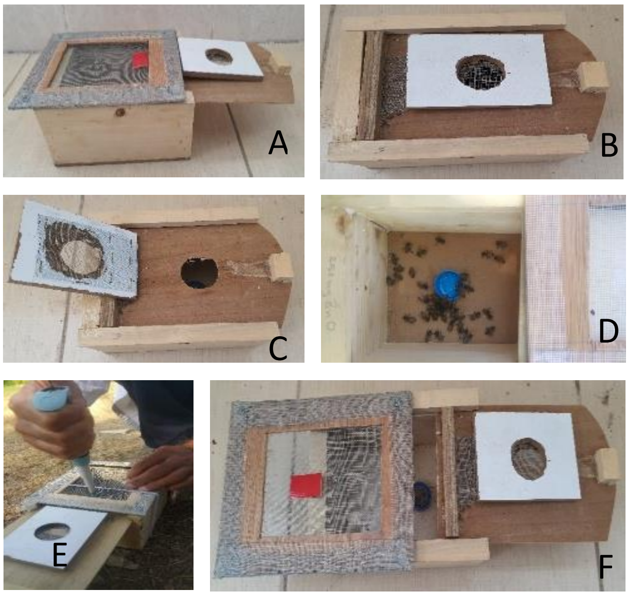

Figure 2. The bee shelter parts, benefits and states of each parts through the test procedure: (A) at day; (B) at night, (C) circular hole for putting the honey bees (D) plastic pan for putting solution feds (E) feeding case (F) all parts together.

In order to shelter the bees during the experiment, 20X4 long 8 cm wide self-covered wooden containers or cages were used. As shown by Figs 2A-E, was made to let easy putting the required number of honey bees inside of the wooden box and let the air in for the bees during the experiment and collect the honey bees that perished during the experiment. Figs 2A-F, shows the parts of the bee shelter, the advantages and conditions of each part through the test procedure. It could be considered the best one of all of the tested box materials of them. In order to shelter the bees for desired time studies hours in wooden boxes as given in the Fig 2A, 20x5x8 cubic cm wooden volume containers were utilized. On the upper side of each of the wooden containers, a movable 15x5 square cm wooden cover with a circular aperture diameter of 5 cm was made to putting the experimental bees' requirements through it. A rectangular mesh cover was made with small holes 2x1cm to allow feeding of ZnONPs and sucrose to honey bees during the experiment, On the middle of the slid latticed cover of these containers, tiny holes were made to let the test solution in and to feed the bees through the use of micro pipette, 1 ml of the solution ZnONPs were added Fig 2E to the small circular plastic pans with diameter 2 cm were fixedly placed on the lower base part of the containers Fig 2D, by means of which the honey bees were made to be fed easily from the plastic pans. The sucrose was put into the small put plastic pan with the help of a micro pipette. In this way, the groups exposed to the test were made to be fed with a test solution every twenty-four hours, on the other hand the control group member were fed with the sucrose solution prepared only with deionized water.

Furthermore, A 15x4 cm movable transparent rectangular piece of window glass was used to cover the test box to calculate the number of honey bees that survived or died after each feeding chain Fig 2E and for the required experiment time. To create a dark and quiet environment for honey bees during our studies, a sliding wooden cover was completely closed at night Fig 2B for all boxes. On the other hand, Fig 5F shows all parts of the honeybee shelter used in our current research. A stainless steel tong was used to collect bees that died during the experiment from the upper sides of the box Fig. 3. This ergonomic design for creating a honey bee shelter was first introduced and no similar investigations were found in the literature. The rectangular piece of window glass was used for the purpose of counting the remaining live bees after each desired experiment test time.

Figure 3. Collecting dead bees and some dead test bees.

Test solutions and suspension preparation:

A suspension of the zinc oxide nanomaterials (ZnO NPs) was prepared by adding ZnO nanopowder to deionized water with ultrasonic (PIO Iskra, Sonnys 2GT; 40 kHz, 2 x 100 W) of the suspension for 24 h. Sucrose ZnO NPs suspension was prepared in 1.5 M sucrose in a final concentration of (25, 50, 100, 250 and 500mg ZnO NPs mL^{-1}, corresponding to 0.25, 0.5, 1.0, 2.5 and 5 mg Zn mL^{-1} [14].

Test honey bee exposure to ZnONPs and mortalities:

In this test procedure, 300 bees were exposed to a suspension solution where the exposure time was 288 hours of exposure tests, where 50 bees as a control group only received sucrose solution via plastic feeders and another 250 bees received suspension solutions of ZnO. The bottom of the cage includes a positioned feeder. The bees were divided into 6 groups and each group of bees was placed into separate wooden cages (9.5 X 4 X 7.5 cm). The control group of 50 bees was fed with 1.5 M only sucrose solution and the other five test groups of altogether 250 bees received a suspension of sucrose ZnO NPs concentrations of (0.25, 0.50, 1, 2.5, and 5 Zn mL-1), for each of the group, respectivel**y**. The wooden cages were placed in a garden of college of science, Salahadden University-Erbil, at a relative humidity of 80% and 28 °C. The daily counted data of survival and dead bees were used for calculating lethal concentration LC_{50} during the 288 hrs exposure period. The gravity feeders were refilled on daily bases from original sucrose solution or ZnO suspension. Before refilling the ZnONPs suspension was vortexed.

Statistical analysis:

After repeating the experiments three times for all the same following used nanoparticle exposure concentrations 20, 50, 100, 250, and 500 mg of ZnO per ml, independently the data on average had been recorded by using the standard deviation. The lethal concentration, LC_{50}, was found through the use of EPA probit statistical analysis**.** The ANOVA were used for performing the other analysis.

Results and Discussion

A novel research work, it includes two main processes such as, characterization studies of Zinc oxide synthesis from parsley (*Petroselinum crispum*) and then determination of the killer concentration (lethal concentration, LC_{50}) for *Apis mellifera* (honey bee) against the green synthesised ZnO exposure nanoparticles. By taking the test results as basis, the lethal concentration LC_{50} values were calculated which were assessed for 288 hours through the probity analysis. The mortality percent and different time lethal concentration LC_{50} values were calculated for ZnO NPs studies in which were assessed for 288 hours.

Nanoparticles characterization:

UV-Vis spectra analysis:

From determining the absorbance obtain by the UV-Vis spectrophotometer, Characterize the nanoparticle's structure properties, confirm previous findings formations and verify that the extracted product from parsley yields synthesis of ZnO nanoparticles in a stable state. The UV-Vis absorption spectrum of Zinc oxide nanoparticles is shown in Fig 4. It shows that the spectrum of the UV-Vis absorption for the green synthesis NPS makes out in the wavelength range 200-400 nm, which corresponds to the ZnO NPs characteristic bands [25, 26]**.** Any other absorption peak disappears in the excitonic absorption spectra between 255 and 335 nm confirming that the synthesized particles are pure ZnO NPs which can be considered as typical characteristic ZnO NPs peaks. This may be attributed to the coulomb attraction between hole and electrons in valence and conduction bands respectively [27]**.** Furthermore, it is reported that the absorption peak position of UV-Visible spectra is related to size of nanoparticles in which as crystal size of the NPs usually depends on the position of the peaks, and due to the columbic interaction as mentioned between holes and electrons in which it has an important role in nano-sized solid materials. The strong absorption was made out at 275 and 310, and the corresponding band gap energies were 4.86 and 3.70 eV, respectively by using the calculation based on the formula: $Eg = 1240/\lambda$, this is due to disagreeing in the NPS crystal size average, in small variance with results values of the previously reported [28]

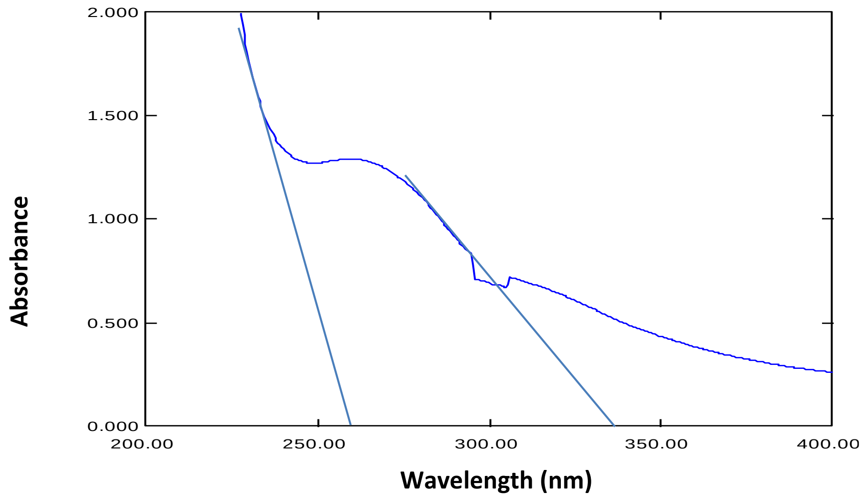

Figure 4. UV-Vis of ZnONPs from *Petroselinum crispum* leaf extract.

FTIR Analysis

In order to determine the functional groups in ZnO nanoparticles synthesized from parsley extract the FTIR spectroscopy technique instruments were used. Fig 5 shows the fingerprint region of green synthesized Zinc Oxide nanoparticles appears in the band width range of 604.37–400 cm^{-1} [29, 30]. The peak around 2924 cm^{-1}, 2852 cm^{-1} corresponds to C-H stretching. The peak at 1440.83 refers to C=C stretching vibrations of the alkene group. Due to the water adsorption of the surface of synthesized Zinc Oxide nanoparticles, the OH stretching bond vibration has appeared as shown as a broad peak around 3435.22 cm^{-1}.

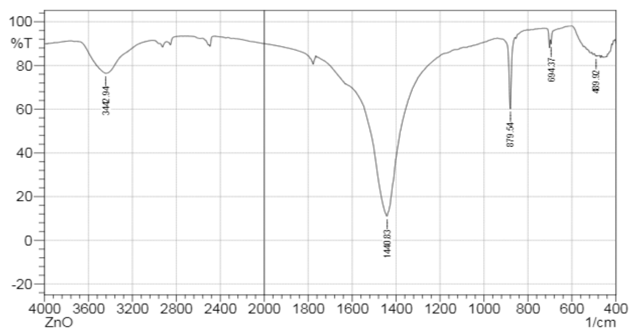

Figure 5. FT-IR spectra of *Petroselinum crispum* (parsley) leaves extract.

XRD Analysis

The XRD result from Fig. 6 showed that the obtained diffraction peaks agreement exactly with the ZnO NPs hexagonal wurtzite phase structures[31-33] with crystalline lattice constants a=b=0.323420 nm and c=0.517720 nm (COD 2300113) [27]. The XRD diffraction peaks, located at a 2ϴ value of the Fig 6, corresponding to the indicated crystal planes at the same figure are consistent well with these previously published work [25-27]. Moreover, the existence of some broad and some other incommodious and strong diffraction peaks gives the meaning that the produced material appears in the nanoscale range [34] with the (15.0774 nm) nm it was calculated from the sharp peaks from the use of the Debye – Scheres famous equation and however also XRD showed that no peaks other than characteristics ZnO peaks, which confirms that the green synthesized NPs were pure and with an optimum crystalline structure due

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to the existence narrow and powerful diffraction peak existence [35, 36].

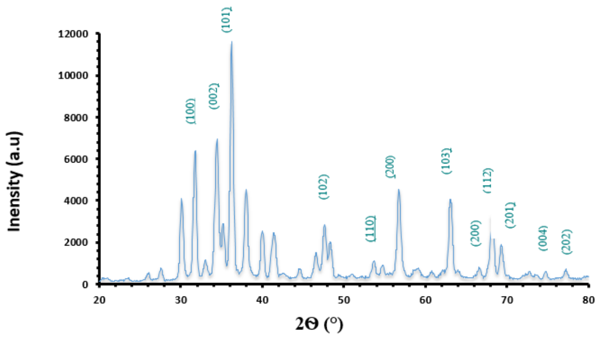
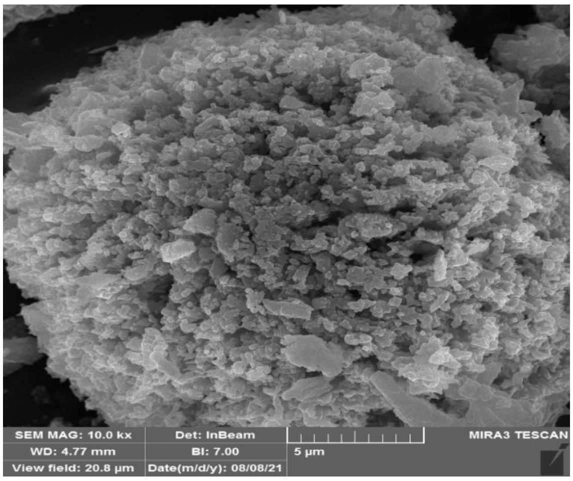
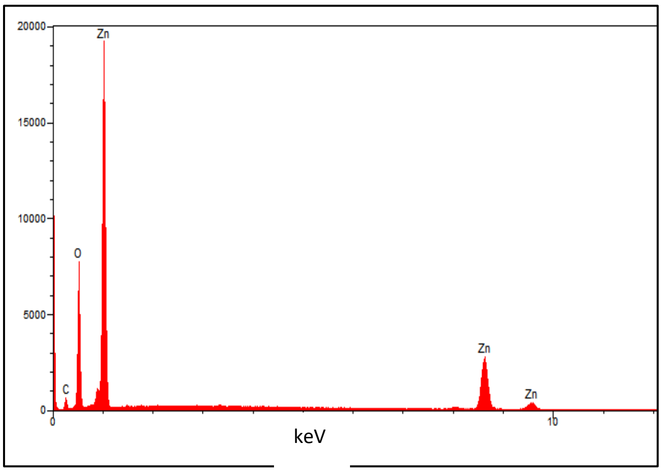


Figure 6. XRD patterns analysis of the synthesized ZnO NPs

SEM analysis

The SEM image of green synthesized ZnO NPs with parsley extract shows the shape and the surface morphology, and the EDX was used to find out the chemical composition Figs 7A, B. According to the results, most of the ZnO NPs had the nanometre scale in various spherical shapes Figs 7A, B, which was very agreement with the results of the studies of some other researchers [37-39]. The image results show in general spherical shapes with careened together, which is typical with green synthesis of NPs and it attributed to the fact that green synthesised NPs possess a higher surface area and nanoparticles strongly stick to each other this is evident as a flower form from our results Fig 7A. [14], therefore the NPs stability and their agglomeration will have been effecting by capping agent factors or this agglomeration is generated due to polarity and ZnO NPs electronic attractions [40] that is due to the capping agent existence which leads to the nanoparticle stabilized [25]. Fig 7B shows the EDX spectrum for the synthesis of ZnO NPs, and it can be seen from the figure that the obtained ZnO powder has high content of Zn and oxygen with high purity compound state [29], which is confirmed with strong main peaks at 0.5, 1 and 2.1, 8.6 and 9.6 kV.





(A) **(B)**

Figure. 7. (A) SEM and (B) EDX of ZnO NPs synthesized from Petroselinum crispum (parsley) leaves extracts.

Acute Toxicity of ZnO Nanoparticles:

For acute toxicity studies of ZnO nanoparticles, green synthesized from *Petrosilum crissum* (parsley), more than 300 bees were accurately selected and repeatedly distributed over the used wooden, so that 50 bees were placed in each container box made of wooden. In order to feed the bees during the experimental test, a volume of 1ml of micro pipette was used. Suspension solutions were prepared with different concentrations of ZnO nanoparticles 25, 50, 100, 250 and 500mg of ZnO NPs per 100ml. By this method, the test solution is fed to the experimental test bee groups with different indicated concentrations for each box every twenty-four hours for 12 days continuously. Whereas the sucrose solution 1.5M, where was prepared with deionized water without Zinc Oxide concentration for the feed control groups. After every 24 hours of feeding, with different concentrations of synthesised zinc oxide nanoparticles, the dead and survival bees were counted and recorded for each group at the 24, 48, 72, 96, 120, 144, 168,192, 216, 240, 264, and 288 hours. The mortality rates are shown in Fig 8. The test was performed at 25-30 °C in the garden of the collage of science, Salahaddin University in Erbil city. The recorded relative humidity throughout the test was between 50-70% in does not exceed normal relative humidity according to the Erbil atmospherically management/central.

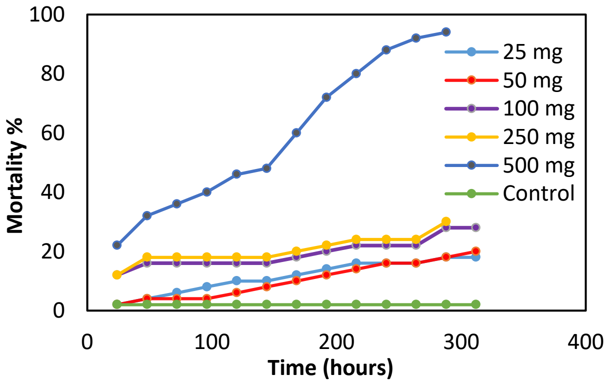


Figure 8. The mortality rates regarding ZnO on *Apis mellifera* to the period of time and concentration.

Our research results during chronic exposure to ZnO nanoparticles and compared to the results of other researchers [14], it is shown that during chronic Fig. 9 exposure to ZnO NPs, overall survival was decreased in all treated groups compared to control in both our results. The maximum toxic effect of ZnO nanoparticles was obtained in honey bees at a concentration (500 mg per 100 ml) more than all other concentrations as shown in Table 1.

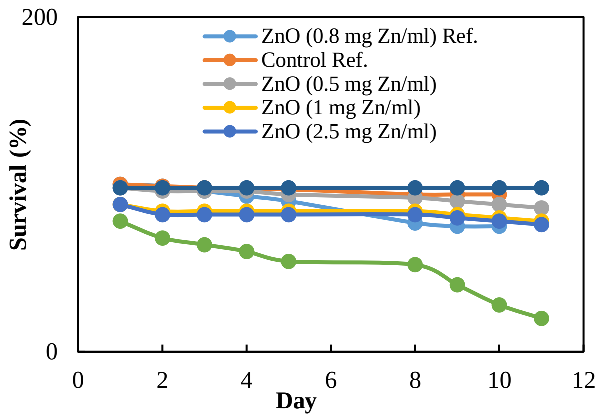


Figure 9. Effects of ZnO NPs on honey bee survival, (0.8 mgZn/l) (our study) Ref. Survival curves of honey bees chronically exposed to ZnO NPs suspension in 1.5 M sucrose as compared to control (1.5 M sucrose) (previus study)

Table 1. Mortality% and different time LC_{50} values calculated for exposure ZnO nanoparticles.

Exposure time hrs	ZnO NPs Exposure concentration mg/100 ml					Control	LC_{50} /100ml
	25	50	100	250	500		
	Mortality %						
24	2	2	12	12	22	2	2754.22
48	4	4	16	18	32	2	1629.296
72	6	4	16	18	36	2	552.38
96	8	4	16	18	40	2	1438.79
120	10	6	16	18	46	2	1285.28
144	10	8	16	18	48	2	1355.189
168	12	10	18	20	60	2	687.068
192	14	14	20	22	72	2	420.726
216	16	16	22	24	80	2	304.789
240	16	16	22	24	88	2	301.3
264	16	16	22	24	92	2	202.768
288	18	18	28	30	94	2	162.554

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To classify specific groups of nanoparticles, there are many ways employed in the literature, based on their chemical composition and properties such as, carbon-based nanoparticles, metal-containing nanoparticles (including metal oxide), quantum dots , dendrimers and zero-valent metals [41-43]. Presently, toxicological find has fundamentally centred on the impacts of carbon-based nanoparticles [44, 45] and metal-oxide nanoparticles (Ag NPs, CuO NPs, TiO_2) [46-48]. In our research work, after synthesizing ZnO NPs from parsley by using an environmentally friendly method and studying their properties, we focused on the toxicological effects of ZnO NPs on honeybees as an application in conducting our research. Specific groups of nanoparticles will be classified according to their chemical composition, properties and using some specific terminologies which refer to them, such as based on their composition, dimension, uniformity, morphology, and agglomeration [8, 26] lowest to the highest ZnO NPs concentrations (25, 50, 100, 250, and 500 mg per 100 ml). The process mentioned and depending on the metal-containing nanoparticles information and some related process occurrences. In our results, honey bee death occurs with exposure ZnONPs concentrations (from lowest to higher). The process steps of cell death occurrs in insects and the effects of the ZnO nanoparticles Table 1. Due to the penetration of the exoskeleton, the organism was damaged [49], and entry into the intracellular space, and then rapid denaturation of enzymes and organelles occurring as a result of binding the nanoparticles to phosphorous from DNA or sulfur from cell death occurs due to the wastage of cellular function [50, 51] due to fall in membrane permeability and disturbance in proton driving force. As shown from the figure the maximum rate of death of the honey bees as a result of the feed of the nanoparticles suspension solutions per a day was after 24 hrs of passing 288 hours for the 500 mg concentration after that it was for the 100 and 250 mg, in which for each of them or 6 groups of honey bees. Metal oxide nanoparticles have received great attention and mass-produced in the past years its extensive use in various fields such as food fields, chemical, biological and physical [47].

Conclusion

This paper research study presents, in order to obtain zinc oxide nanoparticles a fast, economical and non-toxic green synthesis technique using an aqueous extract of Petroselinum crispum parsley with Zinc acetate dihydrate. UV-Vis analysis revealed the absorption peaks at 359 and 300 nm. The synthesized NPs also showed characteristic XRD analysis of the hexagonal wurtzite structure with an average size of 15.0774 nm. The ZnO NPs were characterized by SEM equipped with EDX, which confirmed the presence of NPs with an above indicated average size. Among all the used board house types tested, the size, shape, and wooden cage materials used as honeybee shelters were the best, most comfortable and most successful to keep honey bees tested for monitoring the toxic effects of ZnO nanoparticles on honey bee life and use as a shelter through their experimental tests. As it is known the role of nanoparticles in all different fields of life appears either in the human body or all the technology devices and instruments which are assistance and occupied all the important requirements of daily human life, in this study we have proved that besides all these advantage points of the ZnO nanoparticles, they have disadvantage affects on the environment as toxicological causes the mortality of the honey bee. The maximum toxic effect of ZnO nanoparticles was obtained in honey bees at a concentration (500 mg per 100 ml) more than all other concentrations 20, 50, 100 and 250 mg per 100 ml even at the end of the test mortality rate (94%) vs. (500 mg per 100 ml) while 18% for each concentration 25 and 50 mg per 100 ml and concentrations 100 and 250 mg per 100 ml were (28%) and (30%), respectively. LC_{50} will change from 2754.22 on the first day of the test start to a value of 162.554 at the end of the test (288 hours). In general, the LC_{50} decreases with increasing exposure times of ZnO NPs.

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Data Availability Statement: All available in this paper.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Salahaddin University.

Authors' Contribution Statement

Investigation, A.S.K., F.O.A., N.Z.M; data curation and supervision, A.S.K., F.O.A; writing- original draft preparation, A.S.K., F.O.A.; All authors have read and approved the published version of the manuscript.

References

1. Kalpana V N , Devi Rajeswari V. A review on green synthesis, biomedical applications, and toxicity studies of ZnO NPs. Bioinorg Chem. 2018; 2018: 1-12. https://doi.org/10.1155/2018/3569758
2. Dash D K, Panik R K, Sahu A K, Tripathi V. Role of nanobiotechnology in drug discovery, development and molecular diagnostic. Appl Nanobiotechnology: Intech Open; 2020. https://doi.org/10.5772/intechopen.92796
3. Phull A-R, Abbas Q, Ali A, Raza H, Zia M, Haq I-u. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of Bergenia ciliata. Future J Pharm Sci. 2016; 2(1): 31-6. https://doi.org/10.1016/j.fjps.2016.03.001
4. Ahmed K H, Mohammed A A, Imad M Luaibi. A green synthesis of Iron/Copper nanoparticles as a catalytic of fenton-like reactions for removal of orange G Dye. Baghdad Sci J. 2022; 19(6): 1249-1264 https://dx.doi.org/10.21123/bsj.2022.6508
5. Alwash A. The green synthesize of zinc oxide catalyst using pomegranate peels extract for the photocatalytic degradation of methylene blue dye. Baghdad Sci J. 2020; 17(3): 787–794. https://doi.org/10.21123/bsj.2020.17.3.0787
6. Mahshid M, Shahram S, Fuad O A. Application of magnetic nanomaterials in magnetic-chromatography: A review. Talanta. 2021; 229: 122273. https://doi.org/10.1016/j.talanta.2021.122273
7. Masciangioli T, Zhang W. Environmental technologies at the nanoscal., Environ Sci Technol. 2003; 37(5): 102A-108A
8. Günter O, Eva O and Jan O. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005; 113(7): 823-839. https://doi.org/10.1289/ehp.7339
9. Ajey S, Singh N B, Imtiyaz H, Himani S , Singh S C. Plant-nanoparticle interaction: an approach to improve agricultural practices and plant productivity. Int J Pharm Sci Invent. 2015; 4(8): 25-40. www.ijpsi.org
10. David M C, Ebrahim M, Ada V, Hamed B, Veer S, Jorge L C, et al. Green nanotechnology-based zinc oxide (ZnO) nanomaterials for biomedical applications: A review. J Phys Materials. 2020; 3(3): 034005. https://doi.org/10.1155/2022/3077747
11. Bilal H A, Muzamil S, Syed S H, Munazza N, Sania N, Waqar A. Green bio-assisted synthesis, characterization and biological evaluation of biocompatible ZnO NPs synthesized from different tissues of milk thistle (Silybum marianum). J Nanomater. 2019; 9(8): 1171. https://doi.org/10.3390/nano9081171
12. Yanli G, Dan X, Dan R, Xiyu K, Zeng W. Green synthesis of zinc oxide nanoparticles using Citrus sinensis peel extract and application to strawberry preservation: A comparison study Food. Sci Technol. 2020; 126: 109297. https://doi.org/10.1016/j.lwt.2020.109297
13. Ya-Nan C, Mingyi Z, Lin X, Jun Z, Gengmei X. The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials. 2012; 5(12): 2850-2871. https://doi.org/10.3390/ma5122850
14. Tamara M, Gordana G, Janko B, Kristina S, Tina M , Damjana D. Neurotoxic potential of ingested ZnO nanomaterials on bees. Chemosphere. 2015; 120: 547-554. https://doi.org/10.1016/j.chemosphere.2014.07.054

15. Agyare C, Appiah T, Boakye Y D , Apenteng J. Chapter 25 Petroselinum crispum: a review. Medicinal spices and vegetables from Africa.2017; 527-547. https://doi.org/10.1016/b978-0-12-809286-6.00025-x
16. Devendra K G, Ayush K, Gopal K B, Vikas Kr J , Jagannadha R M. Microbes induced biofabrication of nanoparticles: a review. Inorg Nano-Met Chem. 2020; 50(10): 983-999. https://doi.org/10.1080/24701556.2020.1731539
17. Muhammad W U, Zhijun S, Xudian S, Di Z, Sixiang L, Guang Y. Microbes as structural templates in biofabrication: study of surface chemistry and applications. ACS Sustainable Chem Eng. 2017; 5(12): 11163-11175. https://doi.org/10.1021/cm702152a
18. Sathishkumar M, Sneha K, In S K, Juan M, Tripathy S J , Yun Y-S. Phyto-crystallization of palladium through reduction process using Cinnamom zeylanicum bark extract. J Hazard Mater. 2009; 171(1-3): 400-404. https://doi.org/10.1016/j.jhazmat.2009.06.014
19. Selvaraj M R, Annadurai B, Rajendran K, Venkatesh G K , Arunachalam P. Acaricidal, insecticidal, and larvicidal efficacy of aqueous extract of Annona squamosa L peel as biomaterial for the reduction of palladium salts into nanoparticles. Colloids Surf B . 2012; 92: 209-212. https://doi.org/10.1016/j.colsurfb.2011.11.044
20. Dennis V, Jay D E, Claude S, Chris M, Eric H, Bach K N et al.Colony collapse disorder: a descriptive study. PloS one 2009; 4(8): e6481. https://doi.org/10.1371/journal.pone.0006481
21. Nicole C M, Bernd N. Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol. 2008; 42(12): 4447-4453. https://doi.org/10.1021/es7029637
22. Nowack B, Ranville J F, Diamond S, Gallego-Urrea J A, Metcalfe C, Rose J, et al. Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environ Toxicol Chem. 2012; 31(1): 50-59. https://doi.org/10.1002/etc.726
23. Hongbo M, Phillip L, Stephen A D. Ecotoxicity of manufactured ZnO nanoparticles–a review. Environ. Pollut. 2013; 172: 76-85. https://doi.org/10.1016/j.envpol.2012.08.011
24. Santhoshkumar J, Kumar S V , Rajeshkumar S. Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. Res Efficient Tech. 2017; 3(4): 459-465.https://doi.org/10.1016/j.reffit.2017.05.001
25. Zohra N. K, Farhat S , Iffat B K. Synthesis and characterization of ZnO nanoparticles. Mater Today: Proc. 2015; 2(10): 5619-5621. https://doi.org/10.1016/j.matpr.2015.11.100
26. Meron G D, Fedlu K S, Gemechu D E, Bedasa A G. Synthesis of zinc oxide nanoparticles using leaf extract of lippia adoensis (koseret) and evaluation of its antibacterial activity. J Chem. 2020; 2020: 1. http://dx.doi.org/10.1155/2020/7459042
27. Ali A , Seham A, Ruba A A A, Manal A A , Noura S A, Shouq F A et al. Greener synthesis of zinc oxide nanoparticles: Characterization and multifaceted applications. Molecules. 2020; 25(18): 4198. https://doi.org/10.3390/molecules25184198
28. Thokozani X , Patli P M, and Mahadi L. Decomposition of bis (N-benzyl-salicydenaminato) zinc (II) complex for the synthesis of ZnO nanoparticles to fabricate ZnO-chitosan nanocomposite for the removal of iron (II) ions from wastewater. J Chem. 2019; 20: 19. https://doi.org/10.1155/2019/1907083
29. Sorna P R , Kandasamy S. Synthesis and characterization of zinc oxide and iron oxide nanoparticles using Sesbania grandiflora leaf extract as reducing agent. J Nanotechnol. 2017; 2017: 1-7. https://doi.org/10.1155/2017/8348507
30. Vaezi M and Sadrnezhaad S. Nanopowder synthesis of zinc oxide via solochemical processing. Mater Des. 2007; 28(2): 515-519. https://doi.org/10.1016/j.matdes.2005.08.016
31. Khoshhesab Z M, Sarfaraz M , Asadabad M A. Preparation of ZnO nanostructures by chemical precipitation method. Inorg Nano-Met Chem. 2011; 41(7): 814-819. https://doi.org/10.1080/15533174.2011.591308
32. Sowa H, Ahsbahs H. High-pressure X-ray investigation of zincite ZnO single crystals using diamond anvils with an improved shape. J Appl Crystallogr. 2006; 39(2): 169-175. https://doi.org/10.1107/S0021889805042457/ko5022zno87sup6.hkl
33. Dobrucka R, Długaszewska J. Biosynthesis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract. Saudi J Biol Sci. 2016; 23(4): 517-523. https://doi.org/10.1016/j.sjbs.2015.05.016
34. Satyanarayana T, Srinivasa R K , Nagarjuna G. Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. Int Sch Res Notices, 2012; 2012: 1. https://doi.org/10.5402/2012/372505
35. Elumalai K, Velmurugan S, Ravi S, Kathiravan V, Ashokkumar S. RETRACTED: Green synthesis of zinc oxide nanoparticles using Moringa oleifera leaf extract and evaluation of its antimicrobial activity. Spectrochim Acta A Mol Biomol Spectrosc. 2015; 143: 158–164. https://doi.org/10.1016/j.saa.2015.02.011

36. Yared W, Tesfalem A, Solomon A. Evaluation of antibacterial activity and phytochemical constituents of leaf extract of Lippia adoensis. Asia Pacific J Energy Environ. 2014; 1(1): 45-53. https://doi.org/10.18034/apjee.v1i1.209
37. Ajey S, Singh N B, Imtiyaz H, Himani S, Vijaya Y, Singh S C, Green synthesis of nano zinc oxide and evaluation of its impact on germination and metabolic activity of Solanum lycopersicum. J Biotechnol. 2016;. 233: 84-94. https://doi.org/10.1016/j.jbiotec.2016.07.010
38. Shirin H , Negar M. Green synthesis of zinc oxide nanoparticles using parsley extract. J Nanomed. Res. 2018; 3(1): 44-50. https://doi.org/10.22034/nmrj.2018.01.007
39. Sundrarajan M, Ambika S, Bharathi K. Plant-extract mediated synthesis of ZnO nanoparticles using Pongamia pinnata and their activity against pathogenic bacteria. Adv Powder Technol. 2015; 26(5): 1294-1299. https://doi.org/10.1016/j.apt.2015.07.001
40. Bhatt I , Tripathi B N. Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment. Chemosphere. 2011; 82(3): 308-317. https://doi.org/10.1016/j.chemosphere.2010.10.011
41. Stephen J K, Pedro J J A, Graeme E B, Teresa F F, Richard D H , Delina Y L, et al. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol. 2008; 27(9): 1825-1851. https://doi.org/10.1897/08-090.1
42. Oberdörster E. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. Environ Health Perspect. 2004; 112(10): 1058-1062. https://doi.org/10.1289/ehp.7021
43. Smith C J, Shaw B J, Handy R D. Toxicity of single walled carbon nanotubes to rainbow trout,(Oncorhynchus mykiss): respiratory toxicity, organ pathologies, and other physiological effects. Aquat Toxicol. 2007; 82(2): 94-109. https://doi.org/10.1016/j.aquatox.2007.02.003
44. Buffet P E, Tankoua F, Pan J F, Berhanu D, Herrenknecht C, Poirier L et al. Behavioural and biochemical responses of two marine invertebrates Scrobicularia plana and Hediste diversicolor to copper oxide nanoparticles. Chemosphere. 2011; 84(1): 166-174. https://doi.org/10.1016/j.chemosphere.2011.02.003
45. Margit H, Angela I, Irina B, Henri-C D, Anne K. Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri, crustaceans Daphnia magna and Thamnocephalus platyurus. Chemosphere. 2008; 71(7): 1308-1316. https://doi.org/10.1016/j.chemosphere.2007.11.047
46. Amy H R, Melissa M C, Tonya C B and David L C. The effects of silver nanoparticleson oyster embryos. Mar Environ Res. 2010; 69: S49-S51. https://doi.org/10.1016/j.marenvres.2009.10.011
47. Masala O, and Seshadri R. Synthesis routes for large volumes of nanoparticles. Annu Rev Mater Res. 2004; 34: 41-81. https://doi.org/10.1146/annurev.matsci.34.052803.090949
48. Benay S T. Toxicity of nanoparticles on insects: A review. Environ Sci Pollut Res. 2018; 1(2): 49-61. https://www.researchgate.net/publication/347839379
49. Rai M, Kon K, Ingle A, Duran N, Galdiero S, Galdiero M. Broad-spectrum bioactivities of silvernanoparticles: the emerging trends and future prospects. Appl Microbiol Biotechnol. 2014; 98(5): 1951-1961. https://doi.org/10.1007/s00253-013-5473-x
50. Xiumei J, Teodora M, Liming W, Rasmus F, Duncan S S, Herman Autrup, et al. Fast intracellular dissolution and persistent cellular uptake of silver nanoparticles in CHO-K1 cells: implication for cytotoxicity. Nanotoxicology, 2015; 9(2): 181-189. https://doi.org/10.3109/17435390.2014.907457
51. Benelli G. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res. 2016; 115(1): 23-34 https://doi.org/10.1007/s00436-015-4800-9

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التصنيع الامن وتوصيف وتقييم التأثيرات السامة لـ ZnO NPs على نحل العسل Apis (mellifera)

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الخلاصة

إن الاهتمام البحثي لتحضير المواد النانوية من المنتجات الطبيعية كطريقة التصنيع الخضراء واستخدمها في مختلف التطبيقات الميدانية ، نال اهتمام كبير من خلال التركيب الأخضر للجسيمات النانوية. يمكن اعتبار جسيمات **ZnO** أكسيد الزينك أحد أكاسيد المعادن الأكثر استخدامًا لمعظم متطلبات المنتجات المستخدمة يوميًا. في هذا البحث تم تحضير جزيئات اكسيد الزينك النانويه باستخدام مستخلص البقدونس (Petroselinum crispum) ولإجراء الدراسة الأولى للتقييم السام لمركب جزيئات اكسيد الزينك النانويه وجوانب تأثيرها على نحل العسل (Apis mellifera) . تم تشخيص جزيئات اكسيد الزنك النانويه باستخدام SEM و EDX و XRD و UV-Vis و FTIR الطيفي. تم تطبيق التقييم السمي لـجزيئات اكسيد الزينك النانويه في نحل العسل. تم الحصول على تركيز اكسيد الزنك القاتلة ، وتم تغيير قيم حساب نطاق LC_{50} خلال 288 ساعة من التغذية لجسيمات اكسيد الزنك النانوية بتركيزات مختلفة (25 ، 50 ، 250 ، 500 مجم لكل 100 مل) وتغيرت قيم التركيز المميت النصفي التي تم الحصول عليها من 275 ، تتناقص إلى 162.55 لمدى البحث بعد كل 24 ساعة من اجراء حسابات التغذية التعرضية . بالإضافة إلى ذلك ، بالنسبة للمجموعة التي عولجت بـ 500 مجم من اكسيد الزنك لكل 100 مل ، لوحظ معدل وفيات أكبر بلمقارنة مع التركيزات الأخرى حيث زادت أكثر من جميع العناصر الأخرى التي تشير إلى التركيزات المذكورة أعلاه ولكن ليس مع المجموعة الضابطة. تم تقديم التصميم الاساس لإنشاء خلية النحل العسل لأول مرة ولم يتم العثور على دراسات سابقة مماثلة في الأدبيات.

الكلمات المفتاحية: Apis mellifera ، التصنيع الامن، مستخلص نباتي، تأثير سام، جسيمات اوكسيد الزنك النانوية .