Biosynthesis of Zinc Oxide Nanoparticles and effect on seed germination of wheat (*Triticum aestivum*) in vitro

Mohammed Jasim Ali , Ahmed Saeed Mohmed and Rafid Ahmed Abbas Al-khaldy
College of Agriculture/ Al-Qasim Green University/ Republic of Iraq.
Corresponding author Email: ahmedsa@agre.uoqasim.edu.iq

DOI: https://doi.org/10.36077/kjas/2024/v16i2.11312

Received date: 18/2/2023

Accepted date: 30/3/2023

Abstract

Zinc Oxide nanoparticles (ZnO NPs) are considered an effective factor in plant growth due to their high absorption because of their small size and effect on the plant's physiological system. This study included the biosynthesis of zinc nanoparticles by plant extract from the leaves of the *Myrtus communis* L. and used the following analyses ultraviolet- X-ray diffraction, Fourier transform infrared (FITR), and visible (UV-vis) spectroscopy (XRD). Using field emission scanning electron microscopy (FESEM), it was possible to analyze the ZnO NPs and determine how their concentration affected the germination rate and several other aspects of the growth of wheat *Triticum aestivum* seeds in vitro. The study results showed that a distinctive absorption peak at 366 nm caused by surface plasma resonance visible in the UV-Vis spectroscopy. A FESEM revealed that ZnO NPs were semi-spherical and had an average diameter of 30 nm.. The results also revealed the effectiveness of the concentration of 100 ppm in the germination percentage of wheat seeds which reached the mean of 59.17% compared with 24.17 % in the control treatment, While the length of plumule and radicle recorded 5.767 cm and 3.400 cm respectively at the same concentration. The study suggests found the effectiveness of biosynthetic zinc nanoparticles in the germination characteristics of wheat seeds.

Keywords: Biosynthesis ZnO NPs, *Myrtus communis*, Seed germination

Introduction

Recently, many challenges have emerged in the cultivation of wheat such as climate change and exposure to pests, which has become necessary for researchers to search for ways to increase the wheat production. Nanoparticles are the primary building block of nanotechnology which has been applied in many medical and agricultural fields (8). The greatest promise for application as nano fertilizers is offered by metal oxide nanoparticles especially zinc oxide nanoparticles as indicated by many previous studies , and nanoparticles can affect growth, crop production, as well as the growth of plant organs and seeds. They can also be absorbed through leaves and roots (14). According to these, zinc oxide nanoparticles have drawn a lot of interest because of their distinctive and useful qualities in agriculture. This nanoparticle can be used as a catalyst, chemical adsorbent, additive to polymers, and antibacterial agent due to its features, including Long life, adequate photoluminescence, a sizable specific surface area, and minimal toxicity (7). Moreover, because zinc oxide nanoparticles are very soluble in water, therefore plants can take them up and store them in their biomass. In fact, it has been shown that using nanoparticles can help provide zinc nanofertilizers with highly effective solubility and distribution properties, which major a turning point in the agricultural sector (4). Furthermore, the process of biosynthesis of zinc oxide nanoparticles is considered a process that is environmentally friendly, cheap, with high efficiency, and has a large yield of nanoparticles where many plants were used as effective and good reducers in the production of zinc nanoparticles (1). Our study aimed to investigate the effectiveness of extract leaves of *M. communis* in synthesising of zinc nanoparticles and the effect of these particles on the germination rate and some growth characteristics of wheat seeds.

Material and Methods

Plant collection and preparation

The fresh leaves of *M. communis* were obtained from the University of Babylon Gardens in the Babylon Governorate of Iraq in November 2022. The leaves were cleaned, air dried for five days in the shade, crushed, and stored until needed.

Extract Preparation

In an orbital shaker for 24 hours, a 10g of dried plant powder from *M. communis* leaves was extracted with 100 ml of ethanol 98%. Whatman No. 1 filter paper and a sterile sieve were used to filter the outcome. The resultant extract was airtight-preserved at 4 °C for further use after being evaporated-dried.

Synthesis of zinc oxide nanoparticles

ZnO NPs were synthesized by adding 1 mM of zinc acetate dihydrate, to 100 ml of double-distilled water was added. Then completely blended at 40°C for 20 minutes using a heater stirrer. followed by adding 10 ml of *M. communis* extract plant leaves to the initial solution for 45 minutes at 40 °C with a magnetic stirrer. The pH of the mixture was maintained at 12. The method created a white precipitate, which was then overnight dried at 60 C. Before drying, the precipitate was centrifuged twice with sterile de-ionized water for 20 minutes at 4,000 rpm. full transformation Finally, nanoparticles were air-dried and kept at 4 C for further investigation(6).

Characterization of ZnO NPs

In the Baghdad-Iraq-Nanotechnology Research Center, the produced ZnO NPs by *M. communis* leaves extract were analyzed by ultraviolet-visible (UV-vis), Fourier transform infrared (FITR), X-ray diffraction (XRD), and field emission scanning electron microscopy (FESEM).

Test of ZnO NPs in wheat seeds

Ten wheat seeds of uniform size were placed in perti-dishes (100 mm x 15 mm) lined with two layers of sterilized filter paper to promote seed germination. Applying 3 ml of each nanoparticle concentration (25, 50, and 100 ppm) using a hand sprayer. In the control treatment, just sterile distilled water was used to mist Petri dishes. Three replicates of each concentration were used in the experiment. They were kept at 26° C in an incubator for 12 days. After 3 and 6 days of incubation, the % germination was calculated. The plumule length, radicle length, fresh weight, and dry weight were measured after 9 days.

Statistical Analysis

The collected data on different parameters were analyzed statistically by one and two-factor. A completely Randomized Design (CRD) was followed to carry out the experiment. .and the least significant difference L.S.D test at the 5 % level was implied in statistical analysis. by using the software GenStat package 11

Results and Discussion

Characterization of zinc oxide nanoparticles

UV-Vis spectral analysis

UV–Visible absorption spectrum of synthesized nanoparticles by using UV-Spectrophotometer in the range of 200–1000 nm. (Figure 1). Various peaks were observed under the UV region but The clear and typical peak centred around 350 nm is specific for ZnO NPs because of the effect of which conclude that the phytochemicals present in *M. communis* extract plant as reducing agent of zinc ions and stabilizing agents in addition to the electron transitions from the valence band to the conduction band which can be assigned to the intrinsic band-gap absorption of ZnO. The absorbance peak is reported between 310 nm and 360nm of wavelength (15).

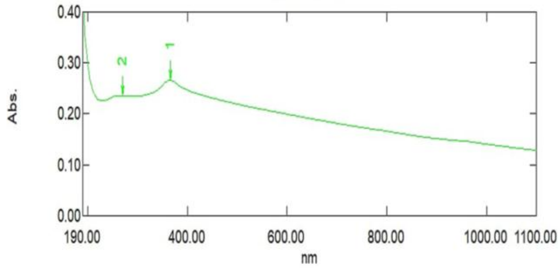

Fig. 1 UV-vis spectrum pattern of ZnO NPs

In the process of ZnO NPs, the reduction of zinc acetate dihydrate into ZnO NPs was made by finding several functional groups such as steroids, polyphenols, flavonoids, tannins, terpenes, and amines in the extract plant of *M. communis* that consider stabilizing, capping, and reducing agents. The 4000-500 cm $^{-1}$ range was used to record the spectra for FT-IR analysis. The peak 416.62 cm^{-1} within the absorption region 400 cm^{-1} to 600 cm $^{-1}$ by demonstrating the production of ZnO nanoparticles through stretching vibrations of the zinc and oxygen bonds. These findings are in agreement with previous studies reported by (16). The peak 3375.43 cm^{-1} coincides with OH and NAH stretching out from hydroxyl groups (O-H) extending from phenolic compounds identified in the plant extract could be hydrogen-bonded groups (5). The 3000–2850 cm^{-1} absorption bands in CH3 groups are used to identify stretching vibrational modes. And the computed wavenumber is 2924.09 cm^{-1}, which expresses CH3 symmetric stretching The results came out similar to (9).

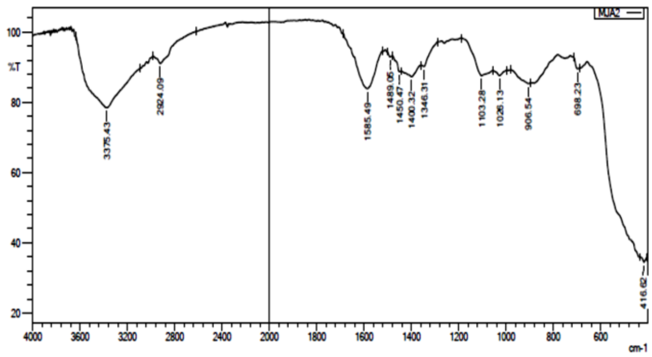

Figure 2. FTIR spectrum pattern of ZnO NPs

Fig. 3 showed the XRD patterns of ZnO nanoparticles synthesised by *M. communis* leaf extract to ascertain the average particle size and crystallinity of synthetic nanoparticles. By applying Bragg's Law or the Debye-Scherrer equation to analyze the XRD spectra, peaks at 2 values that agree with the hexagonal phase of ZnO were found. These 2 values correspond to 31°, 34°, 36°, 47°, 56°, 62°, 66°, 68°, 69°, 72°, and 76°. (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202) respectively planes .where The broad peaks show a decrease in crystallinity, which points inward to the creation of smaller-sized particles.(12).

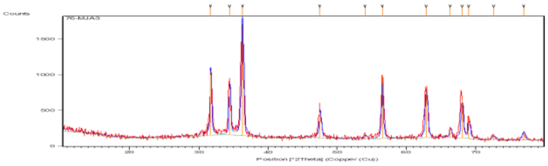

Fig. 3 : X-ray diffraction (XRD) results of zinc oxide nanoparticle

The field emission scanning electron microscope (FESEM) used to distinguish the diameter and shape of the synthesized zinc oxide nanoparticles by M. communis leaves extract, the particle shape was spherical and the diameter of the ZnONPs was found to be within 31-49 nm in (Figure 4). our results were consistent with what was reached (3).

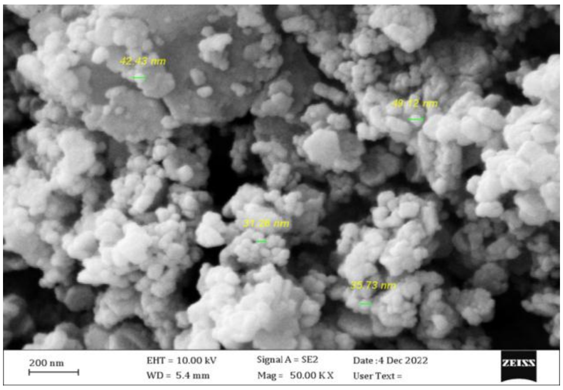

Fig. 4 : FESEM images of ZnO NPs

The significant Table 1 displays the impact of ZnO NPs on wheat seed germination where the high concentration of 100 ppm recorded the maximum germination percentage of 51.67and 66.67% after 3 and 6 days of treatment while the minimum germination percentage was observed with the control treatment to recorded21.67 and 26.67% at the same period. previous study showed that treatment seed on *T. aestivum* at 50 ppm ZnO-NPs had a positive effect on germination. (4). These results would suggest that a high concentration of ZnO NPs content has a crucial physiological function in seeds during seed germination (11).

Table 1 shows the impact of various ZnO NP concentrations on seed germination (%) of wheat

Concentration Ppm	Time period / days 3	6	Mean

0	21.67	26.67	24.17
25	33.33	43.33	38.33
50	46.67	58.67	52.67
100	51.67	66.67	59.17
Mean	38.33	48.83	
L.S.D≤ 5 %	Con.3.533 Time p. 2.498 Conc .× Time p. 4.997		

The results showed in table 2 a significant difference among the treatments that the length of plumule and radicle increased with the increase in concentrations of ZnO NPs recorded at 100ppm 5.767 and 1.941cm compared with 4.207 and 1.333 cm at 25ppm. The results of our study agreed with(13) that low ZnO-NP concentrations positively impact Rootlet and plumule growth. Indole acetic acid (IAA), a phytohormone (auxin) that strongly controls plant growth, is secreted by an enzyme of which zinc is a component. Where Zinc promotes seed germination by raising IAA levels(10).

Table (1) Effect of different Concentration of ZnO NPs on length of plumule and radicle of seed wheat

Concentration Ppm	length of plumule cm	length of radicle cm
0	3.580	0.730
25	4.207	1.333
50	4.873	2.300
100	5.767	3.400
Mean	4.607	1.941
L.S.D ≤ 5 %	0.6457	0.4550

Conclusion

In the present study, we have confirmed the biosynthesis of zinc nanoparticles by plant extract from the leaves of the *M. communis* and evaluated the wheat germination seed in addition to its effect on the length of plumule and radicle of seed wheat. We obtained ZnO NPs within diameter 31-49 nm by using FESEM In addition to the tests such as UV–Visible, FTIR, and XRD which confirmed the biosynthesis of zinc oxide nanoparticles. The results indicated a positive effect of the Concentrations ZnO NPs on germination, plumule length, and seed wheat radicle. The obtained results open up prospects for using ZnO NPs as a crop growth stimulant.

Conflict of interest

The authors have no conflict of interest.

References

1- Abel ,S.; J. L. Tesfaye; N. Nagaprasad; R. Shanmugam; L. P. Dwarampudi and Krishnaraj, R.2021. “Synthesis and characterization of zinc oxide nanoparticles using moringa leaf extract,” Journal of Nanomaterials, vol. Article ID 4525770, 6 pages.

2- Alamdari, S.; M. Sasani Ghamsari; C. Lee; W. Han; H. H. Park; M. J. Tafreshi; H. Afarideh snd Ara, M. H. M. 2020. Preparation and Characterization of Zinc Oxide

Nanoparticles Using Leaf Extract of Sambucus ebulus. Appl. Sci., 10, 3620.

3-Alrubaie, E. A. and R. F. Kadhim, .2019. Synthesis of Zno nanoparticles from Olive plant extract. Plant Archives, 19 (Supplement 2): 339-344.

4- Awasthi, A.; S. Bansal; L. K. Jangir; G. K. Awasthi; K. Awasthi and Awasthi, K. 2017. Effect of ZnO nanoparticles on germination of *Triticum aestivum* seeds," Macromolecular Symposia, 376(1):1–5.

5- Awwad, A. M.; N. M. Salem and Abdeen, A.O.2013. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. Int. J. Ind. Chem. 4:29. http://dx.doi.org/10.1186/2228-5547-4-29.

6- Gnanasangeetha, D. and D. S. Thambavani.2013. Biogenic production of zinc oxide nanoparticle using *Acalypha indica*. J. Chem. Biol. Phys. Sci., 4(1): 238–246.

7- He, X.; H. Deng and Hwang, H. M. 2019. The current application of nanotechnology in food and agriculture. Journal of Food and Drug Analysis, 27(1):1.

8- Hilal, S. M.; A. S. Mohmed; N. M. Barry and Ibrahim, M. H.2021. Entomotoxicity of TiO2 and ZnO Nanoparticles against Adults *Tribolium Castaneum* (Herbest) (Coleoptera: Tenebrionidae). IOP Conf. Series: Earth and Environmental Science 910, doi:10.1088/1755-1315/910/1/012088.

9- Kalpana V.N.; P. Chakraborthi and Devi, R. V.2017. Lagenaria siceraria aided green synthesis of ZnO NPs: Anti-dandruff, Anti-microbial and Anti-arthritic activity. Res. J. Chem. Environ., 21(11): 14-19.

10- Pandey A.C.; S. S. Sanjay and Yadav R.S 2010. Application of ZnO nanoparticles in influencing the growth rate of *Cicer arietinum*. J. Exp. Nanosci., 5:488–497.
doi: 10.1080/17458081003649648.

11- Prasad T.N.V.K.V. ; P. Sudhakar; Y. Sreenivasulu; P. Latha; V. Munaswamy; K. Raja Reddy; T. S. Sreeprasad; P. R. Sajanlal and Pradeep T. 2012. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J. Plant Nutr., 35:905–927. doi: 10.1080/01904167.2012.663443.

12- Pung S.; L. Wen-Pie and Azizan, A .2012. Kinetic study of organic dye degradation using ZnO particles with different morphologies as a photocatalyst. International Journal of Inorganic Chemistry. Article:1–9.

13-Sarkhosh, S.; D. Kahrizi,; E. Darvishi; M. Tourang; S. Haghighi-Mood; P. Vahedi and Ercisli, S.2022. Effect of zinc oxide nanoparticles (ZnO-np's) on seed germination characteristics in two Brassicaceae family species: Camelina sativa and Brassica Napus L Journal of Nanomaterials, Article.1-15. https://doi.org/10.1155/2022/1892759.

14- Solanki, P. and J. S. Laura.2018. Effect of ZnO nanoparticles on seed germination and seedling growth in wheat (*Triticum aestivum*). Journal of Pharmacognosy and Phytochemistry; 7(5): 2048-2052.

15- Verma, P. R.; F. Khan and Banerjee, S.2020. *Salvadora persica* root extract-mediated fabrication of ZnO nanoparticles and characterization. Inorganic and nano-metal chemistry. doi.org/10.1080/24701556.2020.1793355.

16- Yuvakkumar R.; J. Suresh; B. Saravanakumar B; A. Joseph Nathanael; S. L. Hong and Rajendran.2015. Rambutan peels promoted biomimetic synthesis of bioinspired zinc oxide nanochains for biomedical applications. Spectrochim Acta A Mol Biomol Spectrosc; 137:250–258. doi: 10.1016/j.saa.2014.08.022.