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Determination of the Effects of Biogenic Metal Nanoparticles Concentrations on Soil Properties, Vicia faba Growth and the Toxicity on Black Aphid

Ayoub Sabir Karim
Department of Physics, College of Education, Salahaddin University, Erbil, Iraq

Halala Rahman Qadr
Environmental Science and Health Department, College of Science, Salahaddin University, Erbil, Iraq

Sakar Abdulqadr Saeed
Environmental Science and Health Department, College of Science, Salahaddin University, Erbil, Iraq

Nashmeel Saeed Khudhur
Environmental Science and Health Department, College of Science, Salahaddin University, Erbil, Iraq, nashmeel.khudhur@su.edu.krd

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Abstract

The development of nanomaterials could support more extensive agricultural uses, including nano-pesticides. Current study was conducted to observe the effects of different concentrations (25, 50, 75, 100, 150 and 200 ppm) of biosynthesized zinc oxide nanoparticles (ZnO NPs) and aluminum oxide nanoparticles (Al_2O_3 NPs) on soil, faba bean and toxicity on black aphid (*Aphis fabae*). The synthesized ZnO NPs from *Musa paradisiaca* L. characterized by ultraviolet (UV)-visible spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray diffraction and Transmission electron microscopy. Al_2O_3 NPs was prepared by calcination of Iraqi kaolin via evaporation process. Highest pH (8.21) and soil organic matter (2.62%) were observed in the sprayed pots with 75 ppm of ZnO NPs, while the highest values in the sprayed pots with Al_2O_3 NPs were 8.24 in 100 ppm and 1.73% in 75 ppm. Taller plants (53 cm) and more leaf numbers (54 leaf per plant) were observed in 75 ppm of ZnO NPs sprayed pots. Greater branch number was detected in 100 ppm ZnO NPs sprayed pots. All sprayed pots with ZnO NPs showed less chlorophyll contents when compared with control. Spraying pots with Al_2O_3 NPs showed different results. Taller plants (56 cm) were observed in 75 ppm. More leaf numbers (40 leaf per plant) were observed in 25 ppm. Whereas negative effects were observed on number of branches and leaf chlorophyll contents. Faba bean spraying with ZnO NPs and Al_2O_3 NPs induced significant effects on black aphid mortality indicating their great insecticidal activities at different doses, and this considered as the first performance in Kurdistan Region regarding to insecticidal habit of nanoparticles.

Keywords

Biosynthesis; Nanoparticles; Toxicity; Nano-pesticides; Vicia faba L.; Soil Pollution

Cover Page Footnote

The authors are appreciative to Salahaddin University-Erbil, Iraq

RESEARCH PAPER

Determination of the Effects of Biogenic Metal Nanoparticles Concentrations on Soil Properties, *Vicia faba* Growth and the Toxicity on Black Aphid

Ayoub S. Karim [a], Halala R. Qadr [b], Sakar A. Saeed [b], Nashmeel S. Khudhur [b,*]

[a] Department of Physics, College of Education, Salahaddin University, Erbil, Iraq
[b] Environmental Science and Health Department, College of Science, Salahaddin University, Erbil, Iraq

Abstract

The development of nanomaterials could support more extensive agricultural uses, including nano-pesticides. Current study was conducted to observe the effects of different concentrations (25, 50, 75, 100, 150 and 200 ppm) of biosynthesized zinc oxide nanoparticles (ZnO NPs) and aluminum oxide nanoparticles (Al_2O_3 NPs) on soil, faba bean and toxicity on black aphid (*Aphis fabae*). The synthesized ZnO NPs from *Musa paradisiaca* L. characterized by ultraviolet (UV)-visible spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray diffraction and Transmission electron microscopy. Al_2O_3 NPs was prepared by calcination of Iraqi kaolin via evaporation process. Highest pH (8.21) and soil organic matter (2.62 %) were observed in the sprayed pots with 75 ppm of ZnO NPs, while the highest values in the sprayed pots with Al_2O_3 NPs were 8.24 in 100 ppm and 1.73 % in 75 ppm. Taller plants (53 cm) and more leaf numbers (54 leaf per plant) were observed in 75 ppm of ZnO NPs sprayed pots. Greater branch number was detected in 100 ppm ZnO NPs sprayed pots. All sprayed pots with ZnO NPs showed less chlorophyll contents when compared with control. Spraying pots with Al_2O_3 NPs showed different results. Taller plants (56 cm) were observed in 75 ppm. More leaf numbers (40 leaf per plant) were observed in 25 ppm. Whereas negative effects were observed on number of branches and leaf chlorophyll contents. Faba bean spraying with ZnO NPs and Al_2O_3 NPs induced significant effects on black aphid mortality indicating their great insecticidal activities at different doses, and this considered as the first performance in Kurdistan Region regarding to insecticidal habit of nanoparticles.

Keywords: Biosynthesis, Nanoparticles, Toxicity, Nano-pesticides, *Vicia faba* L., Soil pollution

1. Introduction

Nanotechnology is the newest and fastest-growing sector that has an impact on the environment, society, and economy [1]. It has a wide application approach ranges as in fields of agriculture, targeted-drug delivery, cancer and cosmetic industry [2]. The two most commonly utilized nanomaterials are zinc oxide (ZnO NPs) and aluminium oxide nanoparticles (Al_2O_3 NPs) which are produced from any applications which are vary from the use of a sunscreens and antimicrobials to blue optic-electronic devices [3]. These nanoparticles have a wide range of biological applications, including waste water treatment, biofiltration, drug delivery, and the creation of therapeutic cancer vaccines [4]. Hence, their environmental and health effects are of great importance due to their wide application fields [5]. They have potential entities for the remediation of soil as well as they may change the soil quality and plant growth after entering the soil [6]. Moreover, giving out of NPs into the ecosystem, particularly the soil, has their probable toxic effects [2]. Recently, nanomaterials application has expanded in modern agriculture including nano-fertilizers, nano-pesticides, nano-biosensors. Such applications may increase the concentration of these nanomaterials in

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* Corresponding author.
E-mail addresses: ayoub.karim@su.edu.krd (A.S. Karim), halala.qader@su.edu.krd (H.R. Qadr), sakar.saheed@su.edu.krd (S.A. Saeed), nashmeel.khudhur@su.edu.krd (N.S. Khudhur).

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the environment and the exposed plants to these nanosized particles manifest either positive or negative effects [7,8]. Numerous studies have demonstrated that nanomaterials have impacts on plant development, cell structure, function, seed germination, and yield; these effects vary according on the plant species, concentration, type, size, treatment method, and exposure period. Moreover, such nanoparticles produce different effects on other soil organisms [6]. *Vicia faba* L. known commonly as broad bean, considered as the most important and valuable nutrition for human by conferring the best nitrogen source and improving soil fertility. In the recent years, there are observations on cultivation decline and production of broad bean due to insect infections, destructive diseases, low soil fertility, yield instability and lack of effective and compatible strain of *Rhizobium* for nitrogen fixing in the soil [9,10]. The most yield losses (35–40 %) refer to black aphids through sucking the plant sap. Thus they are the most economically important insect belonging to Hemiptera order infecting crops [11]. Unlike previous studies that primarily focused on general impact of ZnO and Al_2O_3 NPs on plants and soil, this study introduces several novel aspects. Biosynthesis of ZnO and Al_2O_3 NPs from leaf plant extracts and their characterization was the first aim of the study. With the objectives of ensuring the safe use of such nanomaterials in modern agriculture, detailed studies on their impacts on growth, productivity and seed germination of faba bean was the another aim of the study. Insect pests become resistant and are ineffectively managed as a result of life cycle plasticity and abuse of insecticides over time; thus, alternative and efficient pest management methods are required. Another novel aspect of the study is the lethal toxicity of varying dosages of ZnO NPs and Al_2O_3 NPs to aphids.

2. Materials and methods

2.1. Materials

Leaf extract of previously identified *Musa paradisiaca* L. was used for synthesis of ZnO NPs which obtained from Erbil City. Deionized and distilled-water, sodium hydroxide (NaOH) as well as zinc acetate dihydrate [Zn $(CH_3CO_2)_2 \cdot 2H_2O$] were purchased from sigma-Aldrich. However, powdered Al_2O_3 was synthesized from Iraqi Kaolin (an abundant material in Iraq) after calcination process through precipitation by evaporation method. H_2SO_4 1 M was used as a solvent for powdered material extraction [12]. The produced nanoparticles were then characterized by X-ray powder diffraction analysis with the use of Mac Science M18XHF diffractometer [13].

2.1.1. Plant extract preparation

Preparation of plant extract is photographically presented inFig. 1(A–E). *Musa paradisiaca* L. extract was prepared, 100 g of leaves were washed thoroughly with distilled-water (Fig. 1A), cut to small parts (Fig. 1B), later a paste was prepared from 20 g of chopped leaves with 5 mL of distilled-water (Fig. 1C). From the paste, 6 g was heated, stirred an hour in 100 mL of distilled-water at 70 °C (Fig. 1D), filtered (Whatman No.1), air-dried in the room (Fig. 1E) and used for ZnO NPs synthesis.

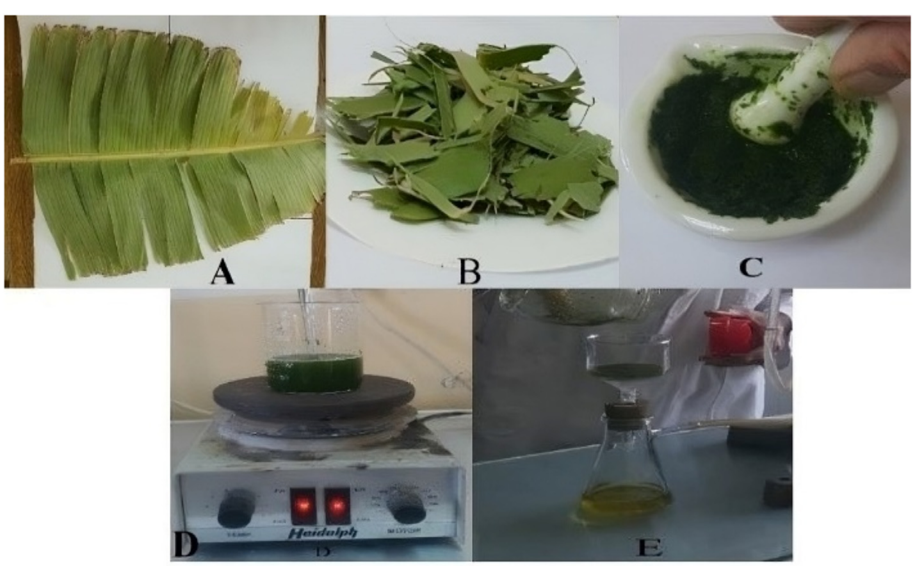

Fig. 1. Preparation procedures for M. paradisiaca *L. leaf extract: (A) Rinsing then drying, (B) Partitioning, (C) Past composition, (D) Heating and inspiring, and (E) Extract filtration.*

2.1.2. Bio-synthesizing of ZnO NPs

The ZnO NPs were biologically created following the illustrated steps in Fig. 2(A–F). From the aqueous extract, 30 mL was mixed with 3 g dehydrated zinc acetate, boiled at 90 °C and stirred at 400 rpm, then few drops of 1 M NaOH solution was added to extract till the color observed to be changed from a dark yellow to yellowish-white color. Then, 50 mL deionized-water was poured into the paste, allowed to settle for 24 h to precipitate the NPs, oven-dried at 90 °C to hung out the NPs, calcinated inside crucibles at 400 °C for 2 h to get pure ZnO NPs and the basic solution was discarded, then thorough and repeated wash with distilled-water was performed and the resulting content was stored dry for subsequent determinations [14,15].

2.1.3. Preparation of aluminum oxide NPs (Al_2O_3 NPs)

Iraqi kaolin was calcinated by evaporation process to prepare X % of Al_2O_3 (X = 17.85, 28.05, 87.98 and 95.63). In the frequency-range of 1 KHz − 1 MHz, the annealed powder temperature-dependence of various Al_2O_3 dielectric constant was examined. The dielectric constant frequency-dependent values of the powder contents were varied between 9.21 − 12.29 and 6.99–12.063, respectively for 87.98 % Al_2O_3 and 95.63 % Al_2O_3 and changed between 22.195 − 151 and 13.015–21.09, respectively for 17.85 % Al_2O_3 and 28.05 % Al_2O_3 powder-content samples. The frequency examination of 10 kHz were gave for the annealing temperature impact on the dielectric-constant. The structure was transformed by the annealing temperature and consequently affected the dielectric-constant. It has observed that the dielectric-constant for all the examined samples was decreased with increasing temperature from 17.85 % Al_2O_3 to 95.63 % Al_2O_3 [12,16].

2.2. Methods

2.2.1. Soil sample preparation

On the superior layer (0–30 cm depth), a sandy-loam soil was taken randomly [17]. The soil was taken from Aski-Kalak area with the coordinates North 36°15' 22.9″ and East 43°38' 20.6″ in Erbil province during October 2022. The properties of the studied soil was [pH (7.79); organic matter (12.5 g/kg); electrical conductivity (695 μS/cm); particle size distribution: clay (52.1 g/kg), sand (251.1 g/kg) and sand (696.8 g/kg)] [18].

2.2.2. Experiment layout

Two completely randomized design each with three replications and seven treatments were performed including the following doses for each of ZnO NPs and Al_2O_3 NPs 25, 50, 75, 100, 150 and 200 ppm and control (treatment without nanoparticle). For this purpose, 42 similar and pre-labeled plastic pots (27 cm internal diameter and 25 cm height) were organized, filled to 5 kg soil, and each was contributed with a saucer to collect the drained irrigation water. During autumn 2022, all the prepared pots were planted with *Vicia faba* L. seeds (Spanish variety-Lashbona and obtained from Alwa of Erbil for agricultural materials). The critical effect of choosing *Vicia faba* is that it is plant species broadly used in plant stress research [19]. The

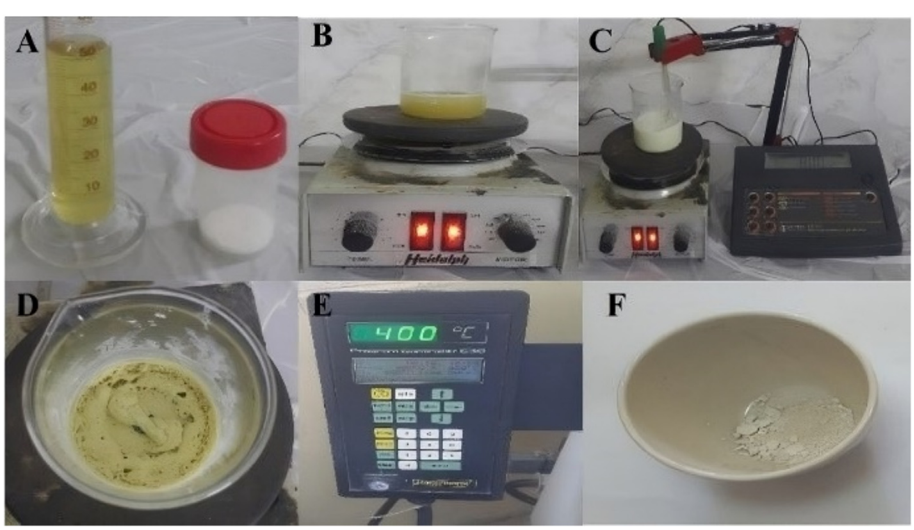

Fig. 2. Pictographic presentation of ZnO NPs production via **M. paradisiaca** *L. leaf extract: (A) Zinc acetate with plant extract, (B) Heating and stirring, (C) pH assessment to 8 by NaOH, (D) Past production, (E) Nanoparticles assessment by calcination at 400 °C, and (F) Produced nanoparticles.*

experiment was performed in a glasshouse of Science College/Salahaddin University at 25/14 °C and natural light [20–22]. Four seeds were sown 3–6 cm aside from each other and in 2–5 cm depth, then they were diminished to one plant. Later, each sample was separately sprinkled with described concentrations of ZnO NPs and Al_2O_3 NPs and left. Daily irrigation to 60 % field capacity was applied and the study lasted four months. Vegetative growth characteristics then measured and soil samples were collected from each pot separately inside pre-labeled glass containers.

2.2.3. Laboratory analysis

The soil samples were assessed for chemical properties in the laboratory at the Departments of Environmental Science and Health and Biology.

a. The Characteristics of Vegetative growth

Growth features of faba bean comprised: fresh and dry weights, plant height (cm), leaf numbers, branches per plant and leaf chlorophyll.

b. Seed germination %

Ten days after seed planting in the laboratory, seed germination % was measured. Separate petri dishes in-contained with the studied doses of ZnO NPs and Al_2O_3 NPs imbedded filter papers were sown with 10 seeds and control was used by absorbing filter papers with distilled-water. The filter papers retained moist and the germinated seed numbers were reported. Below equation was used for calculation of seed germination given by Ref. [23].

$$\%\ \text{Seed germination} = \frac{\text{seeds germinated in nanoparticle sprayed plants}}{\text{seeds germinated in control}} \times 100 \quad (1)$$

c. Difference from control (% DFC)

The percentage of Difference from control (% DFC) for the emerged seeds was estimated from the Mhatre and Chaphekar's (1982) formula given by Ref. [24]:

$$\%\ \text{DFC} = \frac{\%\ \text{germination in control} - \%\ \text{germination in nanoparticle sprayed plants}}{\%\ \text{germination in control}} \times 100 \quad (2)$$

d. Chemical analysis

Soil pH and EC were determined according to Ref. [25] in 1:1 soil solution. Walkley-Black method was applied for soil organic matter (SOM) described by Ref. [25].

e. Toxicity testing via the use of black aphid (*Aphis fabae*)

In toxicity, mortality is the use of any analytical technique to determine death of insects in response to multiple causes, thus, it has potentially far-reaching implications for population ecology [26]. The planted pots were sprayed with different doses of ZnO NPs and Al_2O_3 NPs after the swarming the aphid in 96 h. Aphid feeding effects were determined on the same-sized plants inside the glasshouse at initial infestation levels as 10 aphids per plant.

i Death rate calculation

The below equation was used to calculate death rate given in Ref. [27].

$$\text{Death rate} = \frac{\textit{death number}}{\textit{total number}} \times 100 \quad (3)$$

ii Corrected-mortality rate

Mortality rate counting was corrected referring to mortality of control referring to Abbott's formula [27].

$$\text{Mc} = \frac{Mo - Me}{100 - Me} \times 100 \quad (4)$$

Mo = Mortality rate of treated adults (%)
Me = Mortality rate of control (%)
Mc = Corrected Mortality rate (%).

f. Statistical analysis

Microsoft Office Excel 2019 was used for statistical expressions and the data represented as mean values. For each experimental values control was used for comparison. Results were resolved by one-way ANOVA accompanied by Duncan test at significant level 0.05 [28] applied on Statistical Package for Social Sciences (SPSS) Version 26. Pearson correlation was applied to determine the effects of pH and SOM on the vegetative growth parameters (***P* ≤ *0.005***). Unpaired t test (***P* ≤ *0.001***) was used to compare the effects of both ZnO NPs and Al_2O_3 NPs on the studied parameters during this study.

3. Results and discussion

3.1. ZnO NPs characterization

3.1.1. UV−visible spectra analysis

Within the spectrum of 200−800 nm, UV−vis spectrophotometer was used for confirmation of ZnO NPs formation. At 274 nm, strongest absorption was recorded (Fig. 3) parallel to band-gap energy 4.52ev, when calculated on the base of formula (E.g. = 1240/λ) and this was barely different with the outcomes of [29] and previously reported values of [14,30] where this variance might be attributable to the NPs' average crystal-size disagree.

3.1.2. FTIR analysis

Fourier-transform infrared (FTIR) spectroscopy was used to identify the functional groups present in the biosynthesized ZnO NPs. As illustrated in Fig. 4, the FTIR spectrum reveals distinct absorption bands, confirming the interaction of biomolecules with the synthesized ZnO NPs. The characteristic ZnO fingerprint region appears in the bandwidth range of 604.37−400 cm^{-1}, which corresponds to the stretching vibrations of Zn−O bonds, verifying the successful formation of ZnO NPs [14,15,31]. Additionally, peaks at 2924 cm^{-1} and 2852 cm^{-1} indicate C−H stretching vibrations, suggesting the presence

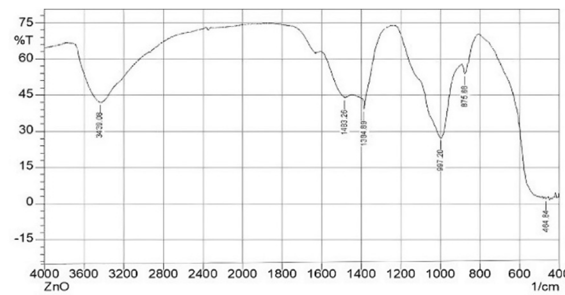

Fig. 4. FT-IR spectra from aqueous extract of M. paradisiaca *L. leaf.*

of organic residues from plant metabolites involved in the synthesis process. The peak at 1440.83 cm^{-1} corresponds to C=C stretching, indicating the presence of alkene groups, which may have originated from phytochemicals in *M. paradisiaca* L. acting as capping or stabilizing agents. Furthermore, a broad absorption peak at 3435.22 cm^{-1} is attributed to O−H stretching vibrations, which is a result of water adsorption on the ZnO NP surface. This peak suggests the presence of hydroxyl groups, which may contribute to the stability and dispersion of the NPs in aqueous solutions. The FTIR results confirm that bioactive molecules from *M. paradisiaca* L. played a significant role in the biosynthesis of ZnO NPs, likely acting as reducing and stabilizing agents. These organic functional groups not only facilitate nanoparticle formation but may also enhance their biocompatibility and potential applications in biomedical, catalytic, and environmental fields.

3.1.3. XRD analysis

The X-ray diffraction (XRD) paradigm of synthesized ZnO NPs is explained in Fig. 5.

The biosynthesized ZnO NPs displayed dominant crystalline peak 2θ values, were at 31.9, 34.55 and 36.4 °C referring to three main planes 100,002 and

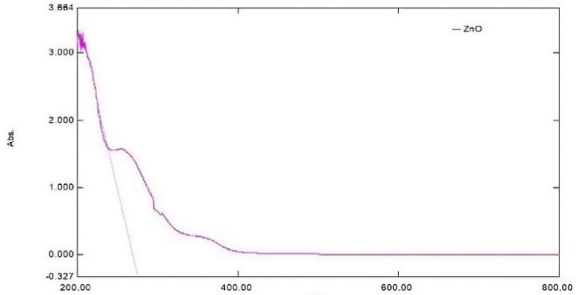

Fig. 3. UV−visible spectrum of synthesized ZnO NPs from M. paradisiaca *L. leaf extract.*

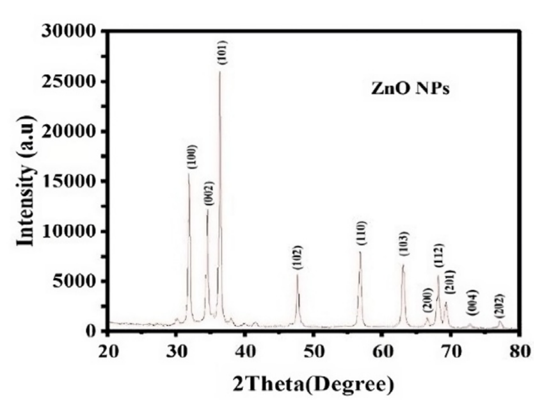

Fig. 5. XRD-pattern of biosynthesized ZnO NPs from M. paradisiaca *L.*

101 respectively, and eight low peaks at 47.8, 56.85, 63.15, 66.5, 68.1, 69.2, 72.7 and 77.15 °C linked respectively to the 102, 110, 103, 200, 112, 201, 004 and 202 planes. The presence of pure hexagonal wurtzite phase production was verified by the crystalline-peaks. In order to detect the dominant diffraction peak on plane 101 and crystal-dimension, the Famous Shearers equation was used as below:

$$D = \frac{0.94\lambda}{\beta cos\theta} \quad (5)$$

When: the dimension of the crystalline is D, the X-ray wave length is λ and equal to 1.5406 A°, peak full-width is β. The ZnO NPs average crystal-size calculated was 49 nm which was compatible with both [32,33] for different plant-mediated ZnO NPs production.

3.1.4. TEM analysis

The Transmission electron microscopy (TEM) was used to analyze the morphology and size of the biosynthesized ZnO NPs. As shown in Fig. 6, the ZnO NPs appear predominantly spherical in shape, with a measured average size of approximately 47 nm. The image, captured at a 50 nm scale, provides clear insight into the structural characteristics of the nanoparticles, confirming their uniformity and nanoscale dimensions. The observed spherical morphology is consistent with previous reports on biologically synthesized ZnO NPs [14,15,31], suggesting that the synthesis conditions influenced the particle formation and growth. The relatively small size of the NPs enhances their surface area, which is a critical factor in their applications in catalysis, biomedicine, and environmental remediation. Moreover, the uniformity of the ZnO NPs suggests efficient biosynthesis using *M. paradisiaca* L., which may have played a role in controlling particle nucleation and growth due to the presence of bioactive compounds acting as stabilizing and capping agents.

3.2. Chemical analysis

In majority of soils, the impact of NPs-induced pH changes brought on by plant root transudation is generally unknown [34]. Through their interactions with plant roots, NPs influence the pH of the soil when plants are present. In this regard [29], showed that CeO_2NPs decreased rhizospheric pH, possibly due to the increased excretion of root exudate. In the current investigation, in 25 and 75 ppm sprayed pots with ZnO NPs, maximum pH of 8.21 was detected and with a 100-ppm treatment of Al_2O_3 NPs, the highest pH of 8.24 was recorded (Table 1). It has been explained by Ref. [35] that with the increase of soil pH, the dissolution of ZnO NPs has also been shown to decrease, hence in this study we observed highest pH values accompanied by lowest dose of ZnO NPs in comparing with the higher doses used and this led to the production of highest branches in faba bean plant (Fig. 7) which shows a significant positive correlation between pH and different doses of ZnO NPs.

Table 1. Effects of different doses of both ZnO NPs and Al_2O_3 NPs on pH and organic matter two weeks after pot spraying.

Treatments	*ZnO NPs treatments*		*Al_2O_3 NPs treatments*	
	pH	O.M. %	pH	O.M. %
25 ppm	**8.21**^{a}	0.62^{e}	8.23^{ab}	0.54^{e}
50 ppm	8.20^{b}	0.85^{c}	8.21^{c}	0.49^{f}
75 ppm	**8.21**^{a}	**2.62**^{a}	8.22^{bc}	**1.73**^{a}
100 ppm	8.20^{b}	0.44^{g}	**8.24**^{a}	0.74^{d}
150 ppm	8.20^{b}	0.74^{d}	8.22^{bc}	1.24^{b}
200 ppm	8.20^{b}	0.87^{b}	8.24^{a}	0.78^{c}
Control (without treatment)	8.20^{b}	0.47^{f}	8.21^{c}	0.47^{g}

Different letters express significant differences among the treatments ($P < 0.005$).

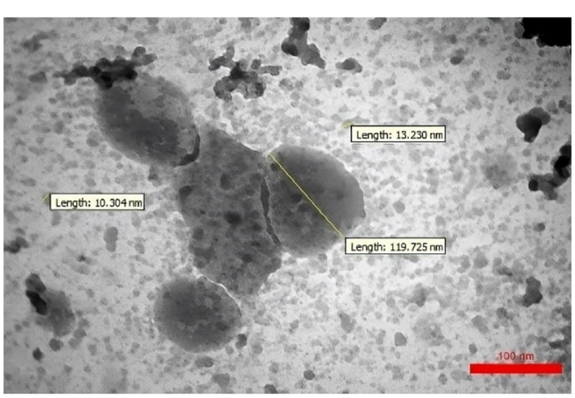

Fig. 6. TEM-image of biosynthesized ZnO NPs from M. paradisiaca *L.*

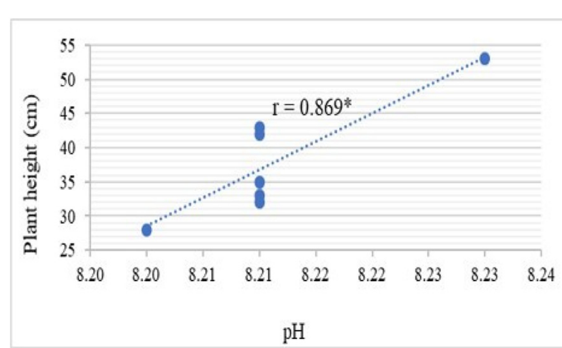

Fig. 7. Significant correlation between pH and plant height ($P \leq 0.005$) after ZnO NPs spraying.

However, yet the influences of nanoparticles on the soil organic matter characteristics are rare [36,37].

According to the current study's findings, 75 ppm sprayed pots containing ZnO NPs had the highest organic matter, 2.62 % (Table 1). Furthermore, a significant positive correlation was observed between organic matter and different doses of ZnO NPs (Fig. 8). Whereas, the highest organic matter was 1.73 % in the sprayed pots with 75 ppm of Al_2O_3 NPs (Table 1).

Moreover, unpaired t test showed significant differences between ZnO NPs and Al_2O_3 NPs effects on pH by t values of 0.025** (***P<0.001***) (Fig. 9).

3.3. Vegetative growth characteristics

Depending on their characteristics, nanoparticles (NPs) can enter plant systems through the roots and leaves and interact with them at the cellular and subcellular levels to accelerate morphological and physiological changes that are either suppressive or simulative [38]. Lower NP concentrations may have advantages for the plants by improving their vegetative growth characteristics, physio-biochemical processes such as those of chlorophyll *a* and *b*, soluble sugars, carotenoid levels, protein and amino acid, proline, and defence enzyme activity [39].

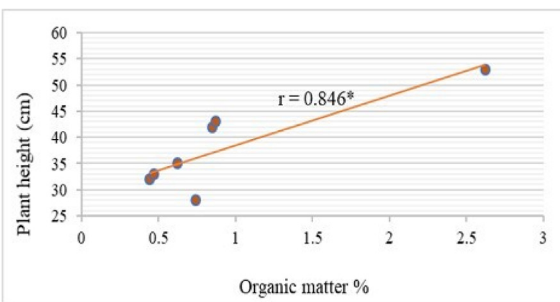

Fig. 8. Significant correlation between organic matter and plant height (P ≤ 0.005) after ZnO NPs spraying.

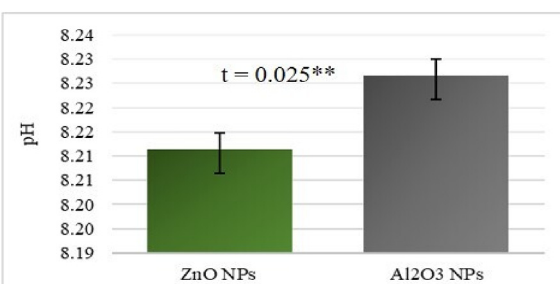

Fig. 9. Significant differences between ZnO NPs and Al_2O_3 NPs effects on pH.

Here we uncovered that, taller plants of 53 cm and more leaf numbers of 54 leaf per plant were detected in pots sprayed with 75 ppm of ZnO NPs. Greater number of branches were determined in pots sprayed with 100 ppm of ZnO NPs (Table 2) in which these results were consistent with the observations of [40] who found an increase in the number of branches when broad bean were treated with 100 and 200 ppm ZnO NPs. Furthermore [41], showed that foliar spraying with ZnO NPs at 50 and 100 ppm either in the absence or presence of salinity stress enhanced the growth of faba bean. However, it has been documented by Ref. [42] that under salinity conditions, foliar sprinkling with ZnO NPs induced growth characteristics, photosynthetic efficacy and biochemical reactions in faba bean.

Greater leaf chlorophyll content 48.5 was detected in control when compared to all of the treated pots with ZnO NPs which showed lesser chlorophyll content (Table 2) this might indicate either no or adverse effects of ZnO NPs in contrast to Ref. [41], where they showed that the highest amounts of chlorophyll *a*, *b*, carotenoids, and total pigments were recorded in those plants which received 50 ppm ZnO NPs, whereas the studies of [2,43] declared that higher concentrations (100−200 ppm) of ZnO NPs produced genotoxic and phytotoxic effects. Moreover [44], demonstrated the highly potential toxic consequences of ZnO NPs on chlorophyll synthesis.

Whereas, Al_2O_3 NPs showed different effects. Plants that were sprayed with 75 ppm grew to a height of 56 cm which was recorded as the taller plants. When Al_2O_3 NPs were sprayed at a concentration of 25 ppm, more leaves (40 per plant)

Table 2. Growth parameter averages of V. faba *after two weeks of sprinkling with different doses of ZnO NPs.*

Treatments	Plant height (cm)	No. leaves	No. branches	Chlorophyll at leaf	Seed germination %
25 ppm	35^{bc}	30^{cd}	16^{cd}	46.46^{a}	60^{b}
50 ppm	42^{b}	41^{b}	23^{bc}	38.64^{c}	70^{a}
75 ppm	**53^{a}**	**54^{a}**	26^{b}	45.33^{a}	70^{a}
100 ppm	32^{bc}	50^{bc}	**44^{a}**	47.36^{a}	**90^{a}**
150 ppm	28^{c}	28^{d}	14^{d}	41.1^{bc}	80^{a}
200 ppm	43^{b}	52^{b}	27^{b}	39.7^{b}	80^{a}
Control (without treatment)	33^{b}	16^{e}	24^{bc}	**48.5^{a}**	**90^{a}**

Different letters express significant differences among the treatments (P < 0.005).

Table 3. Mean growth parameters of V. faba *14 days after sprinkling by different doses of Al_2O_3 NPs.*

Treatments	Plant height (cm)	No. leaves	No. branches	Chlorophyll at leaf	Seed germination %
25 ppm	48^{b}	$\mathbf{40^{a}}$	22^{ab}	41.26^{c}	70^{c}
50 ppm	45^{bc}	38^{b}	20^{c}	40.9^{c}	60^{d}
75 ppm	37^{de}	36^{c}	21^{bc}	43.56^{b}	$\mathbf{90^{a}}$
100 ppm	$\mathbf{56^{a}}$	38^{b}	16^{e}	38^{d}	70^{c}
150 ppm	35^{e}	30^{d}	18^{d}	34.93^{e}	80^{b}
200 ppm	38^{de}	20^{e}	9^{f}	41.73^{bc}	60^{d}
Control (without treatment)	33^{cd}	16^{f}	$\mathbf{24^{a}}$	$\mathbf{48.5^{a}}$	$\mathbf{90^{a}}$

Different letters express significant differences among the treatments ($P < 0.005$).

were seen (Table 3). Similar findings were detected by Ref. [39], where they recorded the 0.01 % Al_2O_3 NPs had an augmented effect on growth properties, like dry weight, fresh weight, root length, shoot length and leaf-area. Whereas, greater number of branches and leaf chlorophyll contents were detected in control (Table 3) could imply the negative effects of Al_2O_3 NPs. Results from Ref. [40] may support current findings in this regard. It was demonstrated that treating beans (*Phaseolus vulgaris* L.) with 100, 10, and 1 ppm of Al_2O_3 NPs reduced pollen viability, increased the thickness of the intone layer, and caused irregularities in the structure and arrangement of the tetrads of the pollen grains.

3.4. Germination of seeds and difference from control (DFC%)

Both control pots and those the 100 ppm ZnO NPs-sprayed pots had the highest seed germination rate (90 %) which means no difference from the control (Table 2), whereas the 25 ppm pots had the biggest deviation from control (33.3 %) (Fig. 10). A relative study on ZnO NPs [45] showed their prospective role in promoting growth and development of faba bean seedlings, acting at a post-germinative phase, probably by developing the stem cell mitosis. In addition [43] recorded that 10−25 ppm ZnO NPs induced higher seed germination in faba bean plant. In contrast [46] showed that ZnO NPs decreased seed germination and germination parameters of faba bean.

While both the 50 and 200 ppm exhibited the largest difference from control with a percentage value of 33.3 %, the 75 ppm Al_2O_3 NPs sprayed pots and the control pots (Table 3) demonstrated the highest seed germination of 90 % with no difference from control (Fig. 11). There is no competent literature regarding to the impacts of Al_2O_3 NPs on seed germination, as it was seen by Ref. [47] that no significant variation in the faba bean seeds germination was recorded upon treatment with (50, 100, 200, 400, and 8000 ppm) Al_2O_3 NPs.

3.5. Black aphid (Aphis fabae) mortality analysis

This study represents the first accomplishment in the Kurdistan Region concerning the insecticidal activities of nanoparticles at varying doses. It was demonstrated that foliar faba bean sprinkling with ZnO NPs and Al_2O_3 NPs caused a tremendous effect on mortality of *A. fabae*, indicating the vast insecticidal activities of nanoparticles at different doses. The production of reactive oxygen species, oxidative stress, membrane disruption, and protein unfolding are all considered to be part of the toxicity pathway [48]. 96 h after sprinkling faba bean plants with varying doses of ZnO NPs, the dose of 150 ppm of ZnO NPs demonstrated a 100 % mortality rate of *A. fabae*, followed by both doses of 100 and 50 ppm of

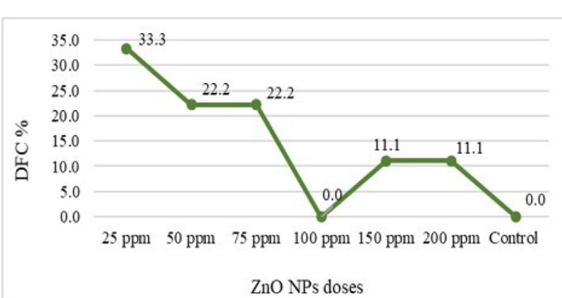

Fig. 10. Difference from control (DFC %) of V. faba *seeds treated with different doses of ZnO NPs.*

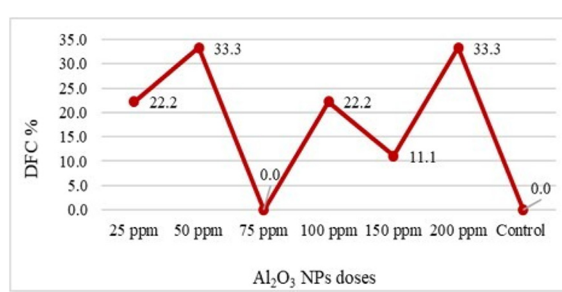

Fig. 11. Difference from control (DFC %) of V. faba *seeds treated with different doses of Al_2O_3 NPs.*

ZnO NPs, which demonstrated 87.5 and 75% death rates, respectively (Fig. 12).

The greatest corrected mortality rate (Mc %) was observed after spraying of faba bean planted pots with 150 ppm of ZnO NPs followed by 100 ppm with values of 7.22 and 6.19 % respectively (Fig. 13). The statement of [49] may confirm this observation who stated that zinc, copper, aluminum and silver NPs have important pest control capacities giving them acceptable biopesticide potentiality. Lowest mortality was detected in 75 ppm ZnO NPs treatment and the statements of [50] who declared that climatic conditions and soil characteristics have great influence on aphid population Since sufficient soil moisture is vital for plant health, it may also indirectly attract the aphid colonies by identifying the vulnerable host plant and encouraging the growth of fungi that could destruct aphids.

After spraying faba bean plants with varying doses of Al_2O_3 NPs, the dose 150 ppm exhibited a 75 % mortality rate of *A. fabae* 96 h following the spraying. This was followed by the doses of both 100 and 50 ppm of Al_2O_3 NPs which showed 73.3 and 66.7 % death rate respectively (Fig. 14). The greatest corrected mortality rate (Mc %) was observed after spraying of faba bean planted pots with 100 ppm of Al_2O_3 NPs and was 5.1 5% (Fig. 15). There was insufficient literature about the effects of Al_2O_3 NPs on *Aphis fabae.* The study of [51] was in line with the present findings who observed that Al_2O_3 NPs was highly effective against turnip aphid but it has deleterious effects on plant growth.

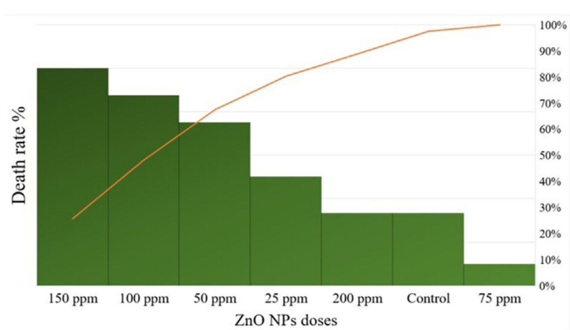

Fig. 12. A. fabae *death rate % 96 h after infestation followed spraying of faba bean by different doses of ZnO NPs.*

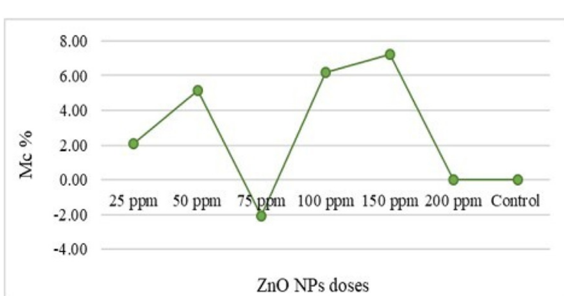

Fig. 13. Mc % of A. fabae *96 h after infestation followed sprinkling of faba bean with different doses of ZnO NPs.*

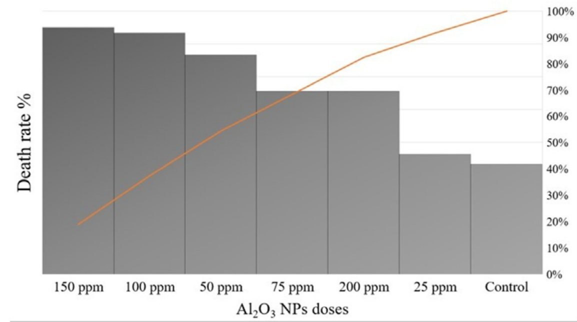

Fig. 14. Death rate % of A. fabae *after 96 h of infestation of sprinkled faba bean by different doses of Al_2O_3 NPs.*

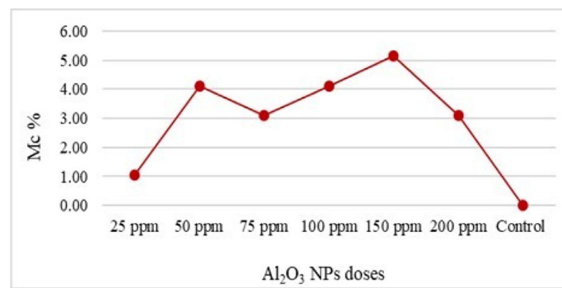

Fig. 15. Mc % of A. fabae *96 h after infestation of sprinkled faba bean with different doses of Al_2O_3 NPs.*

4. Conclusion

From the present study we concluded that, nanoparticle biosynthesis was successfully employed. Reducing the use of synthetic and chemical pesticides and replacing them with naturally occurring and biosynthesized nanomaterials is the goal of sustainable pesticide use management and regulation. According to our findings, ZnO and Al_2O_3 nanoparticles may be used to eradicate pests and can be used as effective instruments against *Aphis fabae* in pest management programs. Given the low chance of insect resistance, is one of the likely evident advantages of long-term use of nanoinsecticides. However, more research are needed to determine how employing ZnO and Al_2O_3 nanoparticles as insecticides affects the ecosystem.

Authors' declaration

We hereby confirm that all the Figures and Tables in the manuscript are ours.

Ethical clearance

The project was approved by the local ethical committee at University of Salahaddin University/ College of Science.

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Conflicts of interest

The authors declare that they have no competing interests.

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