Biosynthesis of Zinc Oxide Nanoparticles Using *Myrtus communis* L. Leaf Extract and Their Effects on *Lolium temulentum* L. Seeds Under Laboratory Conditions.

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Abstract:

The growing reliance on synthetic herbicides has intensified interest in eco-friendly nanotechnology-based alternatives. Here, nanoparticles (ZnO NPs) were green-synthesized using an aqueous *Myrtus communis* L. leaf extract, and were then evaluated for phytotoxic activity against darnel (*Lolium temulentum* L.) seeds. Ultraviolet-visible (UV-Vis) spectroscopy confirmed the synthesis of nanoparticles by identifying a distinctive absorption peak at 366 nm, while Fourier-transform infrared (FTIR) spectroscopy corroborated the presence of the zinc–oxygen bond at 416.62 cm^{-1}. Field emission scanning electron microscopy (FESEM) analysis revealed that the nanoparticles were nearly spherical and homogeneous, with an average size of approximately 30 nm. In an experimental setup employing a completely randomized design with three replicates, seeds were subjected to treatments of 0, 50, 100, and 200 ppm of zinc oxide nanoparticles (ZnO NPs). Observations taken eight days following sowing indicated a substantial decline in germination rates from 96.7% in the control group to 23.0% at the highest concentration of 200 ppm. Additionally, there was a notable reduction in radicle and plumule lengths by 54.6% (from 8.853 to 4.023 cm) and 54.0% (from 8.573 to 3.943 cm), respectively, with significant concentration-dependent effects observed at intermediate ZnO NP levels. These findings illustrate that biosynthesized ZnO NPs hold potential as an environmentally sustainable option for bioherbicide applications in integrated weed management systems. Further empirical investigations are required to determine optimal application rates under diverse agricultural conditions.

Keywords: *Myrtus communis*, zinc oxide nanoparticles, *Lolium temulentum* seed

Introduction:

Weeds are one of the major challenges facing agricultural production globally. They compete with staple crops for essential resources like nutrients, water, and light, resulting in significant yield losses and diminished product quality. A particularly dangerous weed is *Lolium temulentum* L., prevalent in wheat fields, especially in temperate and warm regions. This weed presents a dual threat: it competes fiercely with crops and hosts a symbiotic fungus that produces toxic alkaloids, which can cause acute poisoning in humans and animals if its contaminated seeds are consumed [12].

The extensive and repeated use of conventional chemical herbicides over the years has led to the development of herbicide-resistant weed strains and environmental pollution affecting soil and

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groundwater. This pollution, in turn, raises health concerns for non-target organisms, highlighting the urgent need for sustainable and environmentally friendly solutions [14].

In this context, nanotechnology has rapidly emerged as a transformative scientific field, influencing various sectors including agriculture. Nanoparticles, owing to their minute size and high specific surface area, offer promising tools for enhancing plant productivity and combating pests and diseases, including harmful weeds. This can be achieved through mechanisms such as inducing oxidative stress or disrupting the fundamental physiological processes of seeds and plants [1, 13].

Traditionally, methods to synthesize nanoparticles are categorized into physical, chemical, and biological (green synthesis) techniques. However, physical and chemical methods often depend on hazardous chemical reagents, involve harsh conditions of high temperature and pressure, consume significant energy, and generate toxic byproducts that pose risks to the environment and public health [15]. To address these issues, researchers have increasingly adopted biosynthesis (green synthesis), which utilizes plant extracts as natural reducers and stabilizers. These extracts contain bioactive compounds like phenols, flavonoids, and terpenes that help reduce metal ions and stabilize the resulting nanoparticles without relying on toxic chemicals. Myrtus communis L., known for its abundant phenolic compounds, volatile oils, and flavonoids, is particularly promising for the biosynthesis of zinc oxide nanoparticles. Studies have shown that these nanoparticles possess suitable crystalline properties and nanometer-scale size [1, 5, 8].

The objective of this research is to:

1. Biosynthesize zinc oxide nanoparticles (ZnO NPs) utilizing an aqueous extract from myrtle leaves and characterize them through various physical and chemical techniques.
2. Assess the impact of these nanoparticles at concentrations of 50, 100, and 200 ppm on certain seed germination traits of Lolium temulentum L. in laboratory conditions. This includes evaluating germination rate, radicle length, and plumule length to explore their potential as a sustainable, environmentally friendly solution in integrated weed management strategies.

Materials and methods:

2.1. Plant Material Collection and Extract Preparation

This research was carried out at the College of Agriculture, Al-Qasim Green University during the 2025–2026 academic year. Leaves of healthy myrtle (M. communis L.) were sourced in November from the Tahmaziya region in the Babil Governorate. These leaves underwent a cleaning process using distilled water and were subsequently dried in a shaded environment at a stable room temperature of 25 ± 2°C for a duration of seven days. Once dried, the leaves were ground into a fine powder and preserved in airtight containers to maintain their integrity. For the preparation of the aqueous extract, 25 grams of leaf powder were combined with 250 milliliters of distilled water, then subjected to heating at 100°C for one hour with continuous stirring to ensure thorough mixing. The resulting extract was allowed to cool naturally, then filtered using Whatman No. 1 filter paper, and finally stored at a temperature of 4°C until required for further use.

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2.2. Green Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide nanoparticles (ZnO NPs) were synthesized through a biosynthesis process utilizing myrtle leaf extract, which served both as a reducing agent and a stabilizer. This method was adapted from a previously established protocol with minor adjustments, as documented in source [13]. Briefly, 50 mL of the aqueous extract was added to 5 g of zinc acetate dihydrate [$Zn(CH_3\ COO)_2 \cdot 2H_2\ O$] under continuous magnetic stirring until complete dissolution and subsequent evaporation, yielding a white precipitate. The precipitate was dried at 80°C for 2 h, ground into a fine powder, and calcined in a muffle furnace at 400°C for 3 h [13]. After cooling to room temperature in a desiccator, the resulting ZnO NPs were stored in sealed containers until further characterization.

3. Characterization of ZnO Nanoparticles

The biosynthesized ZnO NPs were characterized using UV-Vis spectroscopy, FTIR, and FESEM.

3.1. UV-Vis Spectroscopy

The formation of ZnO NPs was confirmed by recording the absorption spectrum of the nanoparticle dispersion in distilled water using a UV-Vis spectrophotometer in the 200–800 nm range, based on the appearance of the characteristic absorption peak [1].

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded in the 400–4000 cm^{-1} range to identify the functional groups involved in the reduction and stabilization of the nanoparticles [15].

3.3. Field Emission Scanning Electron Microscopy (FESEM)

The surface morphology, size, and size distribution of the nanoparticles were examined by FESEM. Samples were prepared by drying a nanoparticle suspension on a copper stub and gold-coating prior to imaging [1].

4. Preparation of ZnO Nanoparticle Suspensions

Stock suspensions of ZnO NPs were prepared by dispersing appropriate amounts of the nanoparticle powder in sterile distilled water, followed by ultra-sonication for 15–20 min to ensure homogeneous dispersion and prevent particle aggregation. Serial dilutions were then prepared to obtain the following concentrations: 0, 50, 100, 200, 400, and 800 ppm (mg/L), with sterile distilled water (0 ppm) serving as the control treatment.

2.3. Collection and Preparation of Darnel (*Lolium temulentum* L.) Seeds

Darnel seeds were collected from infested wheat fields in the Al-Kifl district during the 2024 growing season. Seeds were manually sorted to remove impurities and damaged grains, then stored in paper bags at room temperature until use. Prior to experimentation, seeds were surface-sterilized in 1% sodium hypochlorite (NaOCl) solution for 5 min, followed by three rinses with sterile distilled water to remove residual sodium hypochlorite.

2.4. In Vitro Germination Assay

Germination bioassays were carried out in sterile 9 cm glass Petri dishes lined with double-layered Whatman No. 1 filter paper. Twenty sterilized seeds were evenly placed in each dish, and 10 mL of the respective ZnO NP suspension was added according to treatment, with distilled water used for

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the control. Dishes were sealed with Parafilm to minimize moisture loss and contamination, then incubated in a germination chamber at 25 ± 2°C under 12 h light/12 h cycle. The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment [2].

2.5. Germination and Growth Parameters

Germination and seedling growth were assessed over an 8-day period based on the following parameters:

- **Final Germination Percentage (FGP):** calculated as the number of germinated seeds relative to the total number sown, expressed as a percentage [2].
- **Radicle Length (cm):** measured from the seed coat to the root tip using a graduated ruler [2].
- **Plumule Length (cm):** measured as the length of the aerial shoot [2].

2.6. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using GenStat statistical software. Treatment means were compared using the least significant difference (LSD) test at $p \leq 0.05$, and results are expressed as mean values

Results and discussion:

3. Characterization of zinc nanoparticles.

3.1. UV-Vis Spectroscopic Analysis

Figure 1 presents the UV-Vis absorption spectrum of the biosynthesized ZnO NPs, showing a distinct absorption peak at 366 nm, which confirms the successful formation of ZnO nanoparticles. This was accompanied by a visible color change of the reaction mixture from pale yellow to yellow, attributed to surface Plasmon resonance (SPR) resulting from the collective oscillation of free electrons on the nanoparticle surface when excited by light of a matching wavelength [10]. This result is in close agreement with the findings of [6]. Who reported a maximum absorption peak at 370 nm for ZnO NPs.

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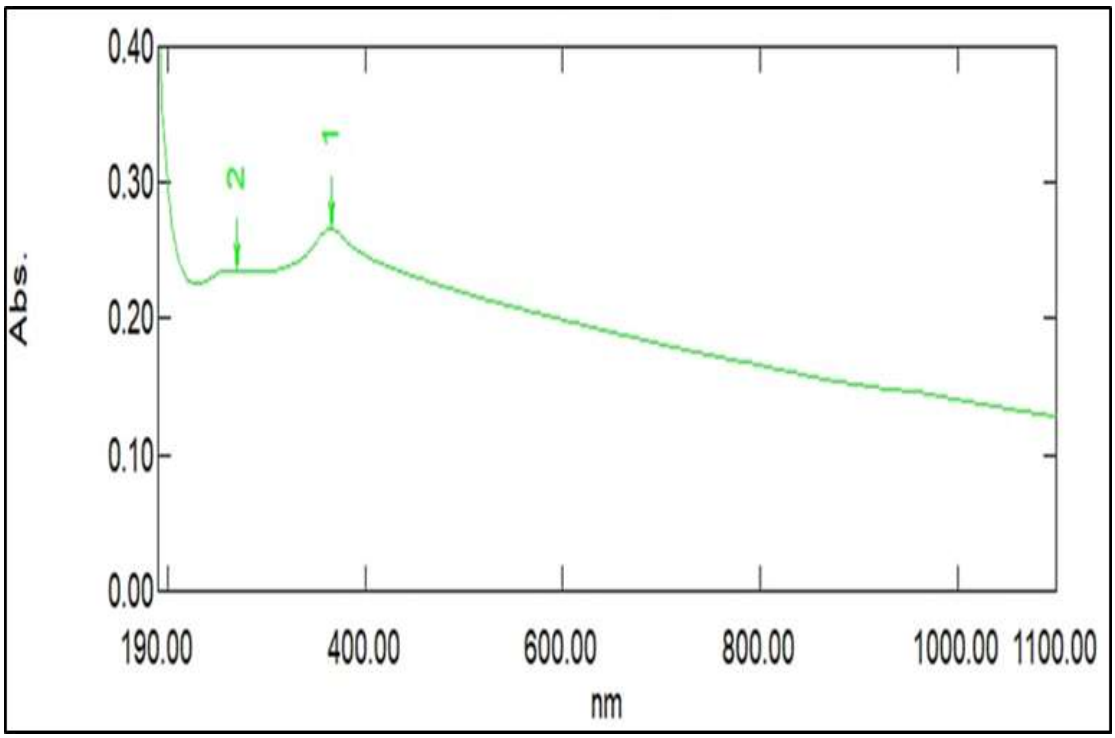


Figure 1. UV-Vis spectrum of ZnO NPs synthesized using myrtle leaf extract.

3.2. FTIR Analysis

The FTIR spectrum of the biosynthesized ZnO NPs, recorded in the range of 400–4000 cm^{-1}, was used to identify the functional groups of the myrtle leaf extract involved in the reduction of zinc acetate dihydrate and the subsequent stabilization of the nanoparticles (Figure 2). The process of reduction is associated with the bioactive phytoconstituents in the extract, including flavonoids, terpenoids, and amine compounds, which serve as natural reducing and capping agents. The absorption band identified at 416.62 cm^{-1}, located within the typical range of 400–600 cm^{-1}, aligns with Zn–O stretching vibrations. This finding corroborates the formation of ZnO nanoparticles, consistent with the outcomes reported in reference [16].

The broad band observed at 3375.43 cm^{-1} is attributed to the O-H stretching vibrations of hydrogen-bonded hydroxyl groups linked to phenolic compounds found in the plant extract [4]. Furthermore, the peak at 2924.09 cm^{-1}, situated within the 2850–3000 cm^{-1} range, aligns with the symmetric stretching vibration of C-H bonds in CH_3 groups, which corroborates the observations made in [7].

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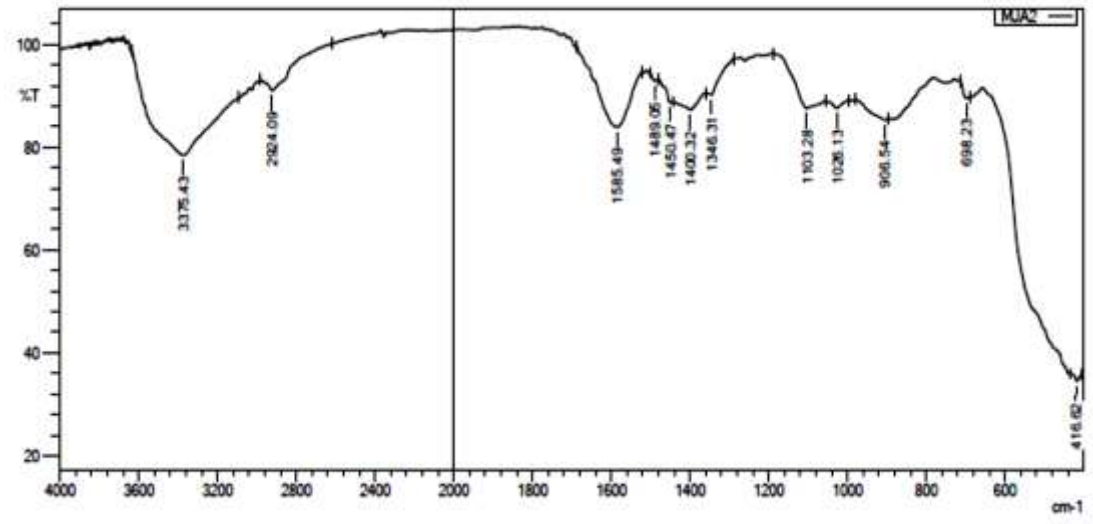


Figure 2. FTIR spectrum analysis of biosynthesized ZnO nanoparticles.

3.3. FESEM Analysis

Field emission scanning electron microscopy (FESEM) was employed to examine the morphology, size, and distribution of biosynthesized ZnO nanoparticles. According to Figure 3, the ZnO nanoparticles synthesized using Myrtus communis leaf extract primarily displayed a spherical shape with an average size of about 39 nm. They tended to aggregate into irregular clusters, a phenomenon often linked to the capping and stabilizing effects of phytochemical components found in the plant extract

. This finding is consistent with the results reported by [3], who obtained ZnO NPs with diameters ranging between 20 and 50 nm using olive leaf extract.

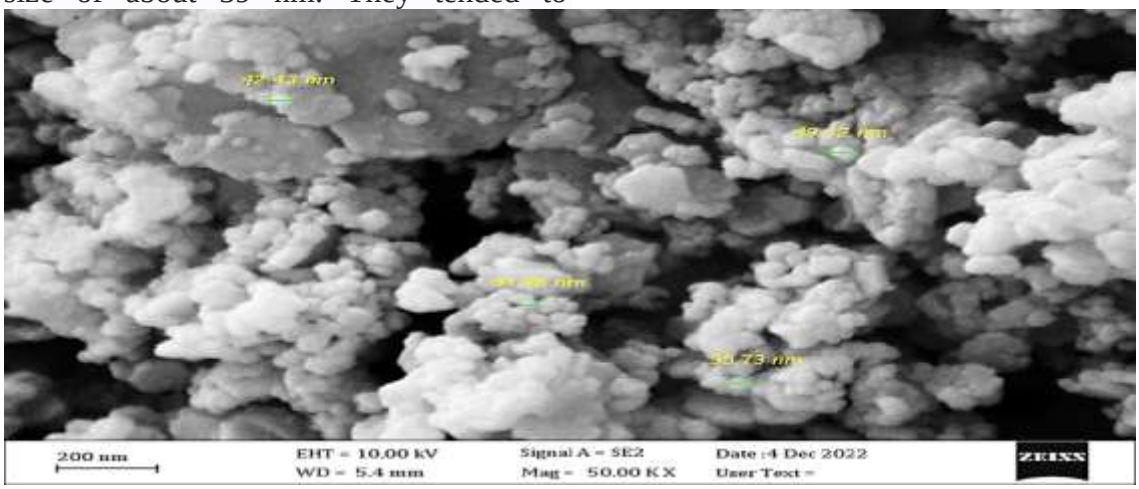


Figure 3. FESEM image of biosynthesized ZnO nanoparticles.

4 - The effect of different concentrations of ZnO NPs on the seeds of the Darnel

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4-1 Effect of different concentrations of ZnO NPs on the germination rate of Darnel seeds

Germination percentages differed significantly among the four treatments ($LSD_{0.05}$ = 8.24; $P < 0.05$), declining progressively from 96.7% in the control to 71.0%, 52.7%, and 23.0% at 50, 100, and 200 ppm ZnO NPs, respectively — a 76.2% inhibition at the highest concentration, confirming a clear dose-dependent phytotoxic effect. This inhibition is likely attributable to ROS-mediated oxidative damage to cellular membranes and macromolecules [13], Zn^{2+} -induced disruption of nutrient uptake and cytotoxicity [2], physical impairment of water imbibition and gas exchange via seed coat adhesion, and interference with germination-regulating hormones such as gibberellins and cytokinins [9]. These findings corroborate previous reports of ZnO NP phytotoxicity in wheat [2] and darnel [9], while the low CV (7.2%) supports the reliability of the results.

Table 1 Effect of different concentrations of biosynthesized zinc oxide nanoparticles (ZnO NPs) using myrtle leaf extract on the germination rate of Darnel (*Lolium temulentum* L.) seeds after 8 days of sowing.

Treatment	Concentrations	The germination rate Darnel
Nano-extract	control	96.7
	50 ppm	71
	100 ppm	52.7
	200 ppm	23
Mean		60.85
L.S.D 0.05		8.24

4-2 Effect of different concentrations of zinc oxide nanoparticles (ZnO NPs) on plumule and radicle length.

The average plumule and radicle lengths of the four treatments are presented in Table 2. Both growth parameters differed significantly among treatments ($LSD_{0.05}$ = 0.4991 and 0.5009 for plumule and radicle length, respectively; $P < 0.05$), declining progressively with increasing ZnO NP concentration. Plumule length decreased from 8.573 cm in the control to 7.003, 5.297, and 3.943 cm at 50, 100, and 200 ppm, respectively, corresponding to a 54.0% inhibition at the highest concentration, while radicle length declined from 8.853 cm in the control to 7.190, 5.287, and 4.023 cm at the same concentrations, confirming a similar dose-dependent phytotoxic pattern. This inhibitory effect can be attributed to several interrelated mechanisms: translocation of NPs/Zn^{2+} via the xylem to apical Meristematic tissues and root growth zones, disrupting cell division and elongation [13]. ROS-mediated oxidative damage to cell membranes, walls, and macromolecules, impairing metabolic and growth processes [2] reduced chlorophyll content and disrupted photosynthetic electron transport, limiting energy available for vegetative growth; hormonal imbalance affecting auxins and gibberellins responsible for cell elongation [9]; altered membrane permeability affecting water and nutrient uptake; and physiological drought stress arising from impaired root water

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absorption. These findings are consistent with previous reports of ZnO NP-induced growth inhibition in wheat [2] and dose-dependent suppression in perennial ryegrass at concentrations exceeding 20 mg/L [9], while the low coefficients of variation (CV = 4.3% and 4.2% for plumule and radicle length, respectively) confirm the reliability and reproducibility of the experimental data.

Table 2 Effect of different concentrations of biosynthesized zinc oxide nanoparticles (ZnO NPs) using myrtle leaf extract on the average plumule and radicle length of Darnel seeds (*Lolium temulentum* L.) after 8 days of sowing.

Treatment	Concentrations	Plumule length	Radical length
Nano-extract	control	8.573	8.853
	50 ppm	7.003	7.19
	100 ppm	5.297	5.287
	200 ppm	3.943	4.023
Mean		6.204	6.338
L.S.D 0.05		0.4991	0.5009

Conclusion

This study successfully achieved the green biosynthesis of zinc oxide nanoparticles (ZnO NPs) using myrtle leaf extract and demonstrated their dose-dependent phytotoxic effect on darnel (*Lolium temulentum* L.), significantly inhibiting germination rate, plumule length, and radicle length with increasing concentration. These findings align with global research on ZnO NP phytotoxicity and highlight the potential of biosynthesized ZnO NPs as a promising, eco-friendly nano-bio herbicide for weed management. Further field trials, molecular studies, and environmental risk assessments are recommended to validate these findings and support practical application.

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