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EFFECT OF SYNTHESIZED ZINC OXIDE NANOPARTICLES ON THE GERMINATION OF OKRA *(Abelmoschus esculentus)* AND MUNG BEAN (*Vigna radiata)*

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ABSTRACT

The present study aims to synthesize zinc oxide (ZnO) nanoparticles and study its effect on the germination of okra *(Abelmoschus esculentus*) and mung bean *(Vigna radiata*). The chemical co-precipitation method was used for the synthesis of zinc oxide nanoparticles. Zinc oxide nanoparticles were characterized using Zeta sizer, Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy, and Fourier- Transform Infrared Spectroscopy. The effect of synthesized ZnO NPs on seed germination, root-shoot growth, fresh and dry biomass, and its translocation was studied in okra and mung bean. A significant improvement was seen in root-shoot length, germination rate, overall zinc content, and biomass in ZnO nanoparticles treated seeds.

Keywords: Zinc Oxide Nanoparticles, Nanoparticles Synthesis, Germination Rate, Translocation Factor, Seed Germination.

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INTRODUCTION

Nanotechnology is an evolving field with its scope in nutritional security, improving food quality, and advancing processing techniques.[1] Nanoparticles (NPs) are structures with a size of less than 100 nm and an extensive surface area. Various methods are applied for the synthesis of nanoparticles. Preparation of nanoparticles using chemical methods mainly involves sol-gel, co-precipitation, and chemical reduction methods. Reactivity, small size, shape, and structure are responsible for the mechanical, optical, and magnetic properties of nanoparticles.[2] Chemical fertilizers have negative environmental impacts, such as eutrophication, environmental harm, and the buildup of surplus fertilizers in groundwater and soil. [3] By delivering the nutrients to the plants, the nano-carriers can overcome the challenge of nutrient availability to different plant parts as they carry complex payloads across biological barriers and to specific tissue targets with prolonged release. [4] Long-term continued release of nanoparticles provides sustained support for plant growth activities which can bring agricultural sustainability ensuring global food security.[5]

Different nutrients are required for the desirable growth of plants. Zinc is one of the essential micronutrients for the optimal growth of plants which is readily solubilized by root secretion .[6] Zinc oxide nanoparticles promote growth and productivity of several plants.[7] Zinc plays an indispensable function in the development of all crop plants. Zn contributes to the maintenance and functioning of several enzymes, including RNA polymerases, DNA polymerases, dehydrogenases, transphorylases, and proteinases. It also promotes plant growth, cell division, chlorophyll generation, and membrane structure.[8]

Zinc requirement in plants is quite significant being a micronutrient otherwise it can limit desired plant growth. The present study involves the synthesis and characterization of ZnO nanoparticles and analyzes the impact of ZnO NPs on the growth and development of *okra* (*Abelmoschus esculentus) and* Mung bean (*Vigna radiate*). Phytotoxic effects of zinc oxide NPs at varying concentrations in-vitro-grown seedlings have been depicted. Measure to counter the phytotoxicity without compromising the seed yield should be focused in the upcoming research studies.

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EXPERIMENTAL

Seeds of okra (Arka Anamika) were purchased from the local market and Mung bean (MH-421) from Krishi Vigyan Kendra, Rohtak (Haryana). Chemicals such as sodium hydroxide (NaOH), Zinc Chloride ($ZnCl_2$), 2-propanol, sodium hypochlorite (NaClO), and distilled water were used in the study. About 10 g of NaOH (1.0 M) and 17 g of $ZnCl_2$ (0.5 M) were taken separately and each dissolved in 100 ml of deionized water. NaOH solution was stirred so that the salt was properly dissolved. This solution was added gradually into $ZnCl_2$ (drop by drop for 20-60 minutes). The precipitate was separated from the supernatant dispersion and washed five times with distilled water to ensure the complete removal of NaCl. The particles were sonicated using 2-propanol for 15 minutes at room temperature. Then, the particles were centrifugated at 6000 rpm for 15 min. Precipitated nanoparticles were rinsed several times with distilled water to get rid of impurities. The nanoparticles were kept for drying overnight at 70°C. [9] Synthesized zinc oxide nanoparticles were characterized using various techniques. The absorbance peak was obtained using an 1800 UV Spectrophotometer, Shimadzu at 330 nm. Zeta potential was assessed utilizing the Malvern Zetasizer Nano ZS, revealing both particle dimensions and charge stability. The structural characteristics of the synthesized nanoparticles were examined through Scanning Electron Microscopy (SEM) (JSM-6390LV, JEOL). Elemental composition was determined via Energy Dispersive X-ray spectroscopy (EDX) using X-act (Oxford Instruments) in conjunction with SEM. Fourier-transform infrared spectroscopy (FTIR) analysis, conducted with an FTIR Spectrometer (Shimadzu), targeted specific functional groups for testing. To conduct the germination test, seeds were surface sterilized for 10 minutes using a 0.5% NaClO (sodium hypochlorite) solution, followed by five times rinsing with distilled water and subsequent drying with filter paper. Subsequently, filter paper was positioned within each petri dish, and 5 ml of suspension was added to each dish, each having a diameter of 90 mm. Seeds were then evenly dispersed within each petri dish. About 50 mg of synthesized zinc nanoparticles were added to 1000 ml of distilled water in a beaker and stirred well. Simultaneously, solutions of 100 mg/L, 150 mg/L, 200 mg/L and 250 mg/L concentration solutions were prepared. The seeds were grown in prepared solutions under a controlled environment using an incubator, kept in darkness at a temperature of 25ºC with a relative humidity of 70%. Radicles were deemed to have germinated once they exceeded half the length of the seed. The number of germinated seeds was counted every 24 hours. [10] Shoot and root elongation of all germinated seeds were measured after 7 days of culture. The germination index was calculated by using the formula given below:

$$\text{Germination index} = \frac{\mathrm{Gs} \times \mathrm{Ls}}{Gc \times Lc}$$

In this study, Gc represents the seed germination rate of the control group (%), while Lc denotes the root length of the control group (in centimeters). Similarly, Gs indicates the seed germination rate, and Ls signifies the root length following ZnO NPs treatment.[11] Measurements of root and shoot length were taken using a scale. The fresh weight of both the root and shoot of seedlings was determined individually using a weighing balance. Subsequently, the seedlings were desiccated in a hot air oven at 80°C for 72 hours after which the dry matter weight was recorded.[12] After 7 days of ZnO NPs treatment, the shoots and roots of okra and mung bean plants were separated, washed with deionized water, and then oven-dried for 24 hours at 70°C. The dried plant samples underwent digestion with 10 ml of digestion reagent (nitric acid: perchloric acid in a 3:1 ratio) on a hot plate at 70-100°C until yellow nitric chloride fumes and white perchloric acid fumes were produced. The digestion process was repeated until a clear solution was obtained after acid volatilization. Once the residue in the flask became transparent, the digestion was stopped. The digested samples were dissolved in distilled water and filtered to remove impurities. The samples were then analyzed using an Atomic Absorption Spectrophotometer to determine the levels of zinc in different plant samples.[13] The translocation of zinc was calculated using the zinc concentration in the shoot and root according to the formula, Translocation factor (TF) = Zn concentration in plants shoot / Zn concentration in plants root.

RESULTS AND DISCUSSION

Synthesized zinc oxide nanoparticles (of range 50-100 nm) were analyzed using different characterization techniques such as Zeta sizer, Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy, and Fourier- Transform Infrared Spectroscopy. The particle size distribution by intensity shows a nanoparticle formation peak at 92nm (Fig.-1).

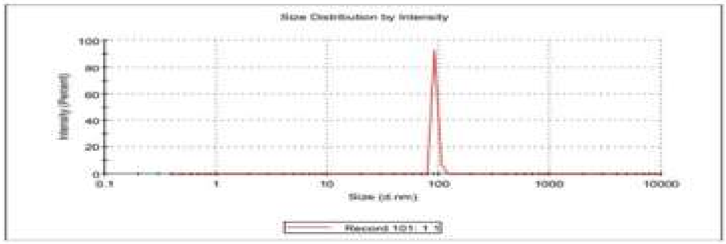

Fig.-1: Particles Size Distribution of Zinc Oxide Nanoparticles

The Zeta potential of ZnO nanoparticles was found 19.6 mV.[14] Definitive image was created using SEM which involves electrons as a substitute of light. Various dimensions and diffusion of synthesized nanoparticles are distinguished by using SEM. The SEM examines the exterior of the sample with high-energy electron beams.[15] Fabricated zinc oxide nanoparticles were analyzed using SEM. It depicted the morphology of the particles at 1 µm and 5 µm resolution showing the nanoparticles with leaf-like appearance (Fig.-2).

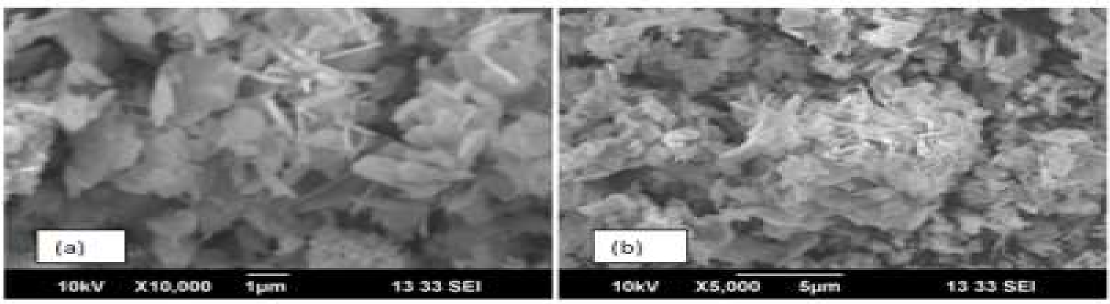

Fig.-2: SEM Images of Zinc Oxide Nanoparticles at (a) 1µm at (b) 5µm

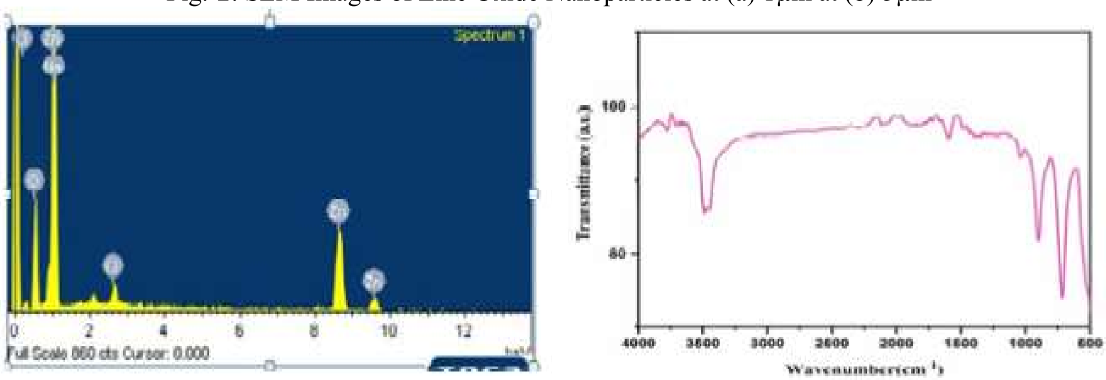

Fig.-3: EDX and FTIR Spectrum of Zinc Oxide Nanoparticles

EDX depicted elevated signals for Zinc, Chloride, and Oxygen confirming the appearance of ZnO. The weight of different elements such as Oxygen, Sodium, Chlorine, and Zinc are 25.89, 10.83, 2.15, and 61.13 respectively. Atomic weight (%) of different elements such as Oxygen, Sodium, Chlorine, and Zinc were 52.46, 15.27, 1.97, and 30.31 respectively. The composition of different elements in the analyte was obtained with EDX with peaks. FTIR spectrum of the sample was obtained in the range of 4000 to 500 cm^{-1}. Distinct peaks represent the particular functional group existing in synthesized nanoparticles. High absorption peaks were detected at 3500 cm^{-1}, 800 cm^{-1}, 700, and 500 for stretching vibration between C-H, O-H, O-H, and Zn-O bonds respectively, depicting the peak of various elements present in the synthesized nanoparticles (Fig.-3). The seed germination rate was calculated by dividing the number of germinated seeds by the total number of seeds used.

$$\text{Seed germination rate (\%)} = \frac{\text{number of seed germinated}}{\text{total number of seed taken}} \times 100$$

The germination index of okra and mung bean were maximum at 200 mg/L and 150 mg/L concentrations respectively. Nanoparticles have been observed to release essential nutrients in a controlled manner, responding to various environmental changes such as temperature and moisture. Germination rate was found to be maximum at 50 and 100 mg/L i.e. 90 % for okra and 85 % at 50 and 100 mg/L for mung bean. The controlled release of nanoparticles can lead to more efficient plant growth compared to traditional fertilizers.[16] Okra root length as well as shoot length showed consistency with maximum growth at 200 mg/L. In mung been, inhibitory effect of ZnO NPs was observed at high concentrations and optimum effect was observed at 100 mg/L. ZnO NPs affected the activity of crucial enzymes like carbonic anhydrase, which facilitates the transport of CO_2 to carboxylation sites in plants by catalyzing the hydration of CO_2.[17] Zinc oxide nanoparticles produce varying effects on okra and mung bean. The highest fresh weight of okra was observed at concentrations of 200 mg/L and thereafter showed consistency at 250 mg/L, while for mung bean, it was maximum at 100 mg/L. Conversely, the lowest fresh weight of okra seeds was recorded at 50 mg/L, and for mung bean, it was at 250 mg/L showing phytotoxicity. Maximum dry weight was observed in okra seeds at a concentration of 200 mg/L, while for mung bean, it was at 100 mg/L. Minimum dry weight was absorbed in okra at 50 mg/L and minimum of mung bean at 250 mg/L showing phytotoxicity by hampering the growth.

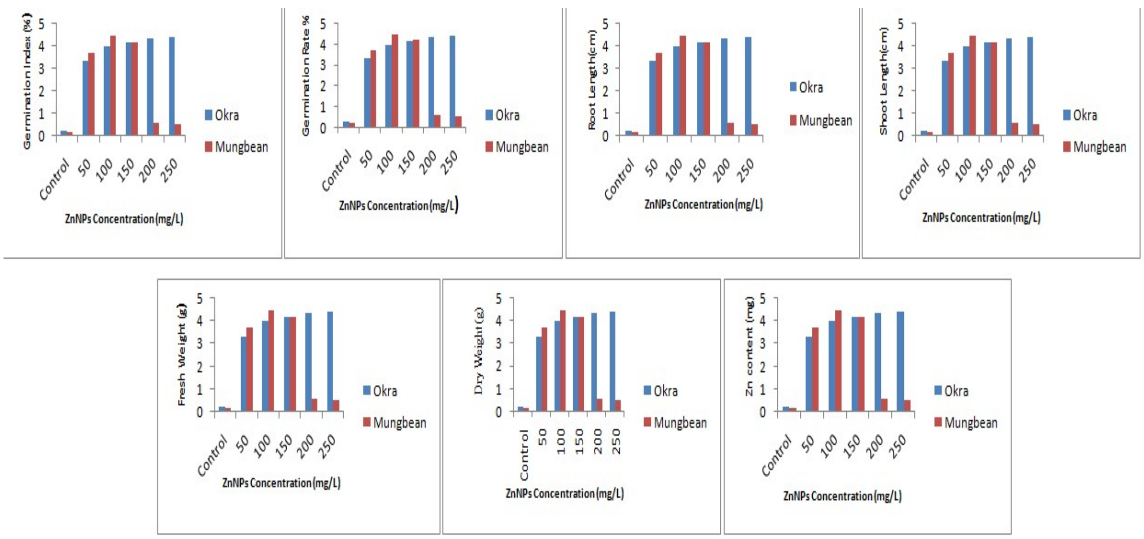

Fig.-4: Impact of Zinc Oxide Nanoparticles on Plant Growth Parameters

The translocation factor was calculated to find out the translocation of metal from the root to the aerial parts. Intake, transport, and accumulation of NPs into the food chain are all likely to occur through plants, which constitute an important part of the environment.[18] Maximum translocation factor of okra was found at 200 mg/L, and the maximum translocation factor of mung bean at 100 mg/L. ZnO NPs at high concentrations can be phytotoxic and reduce plant production. The effect of zinc oxide nanoparticles on various plant growth parameters has been depicted in Fig.-4. Like other micronutrients, it's essential to administer minimal doses of zinc oxide nanoparticles (ZnO NPs) to plants to achieve optimal biomass development.[19] The application of nanofabricated chemical fertilizers is still in its early stages, so additional study is required to evaluate their long-term impacts on plant growth, soil health, and environmental safety, despite the promised benefits.[20] To support the safe and responsible implementation of smart nanofertilisers in agricultural systems, standardized procedures for nanomaterial manufacturing, risk evaluation, and product control are essential.[21]

CONCLUSION

Zinc oxide nanoparticles were synthesized via the co-precipitation method to assess their impact on the germination of Mung bean and Okra seeds. Synthesized ZnO NPs were observed by using various characterization techniques. Significant growth was shown in the yield of okra and mung bean in terms of root-shoot development, total biomass, and optimum germination rate at suitable nanoparticle concentrations. The highest accumulation was observed at 200mg/L in okra and 100mg/L in mung bean.

Nanoparticles represent a promising solution as an efficient nutrient source for crops, especially amidst the escalating demand for food and the prevailing scarcity of staple crops.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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