IMPACT OF MAGNESIUM OXIDE NANOPARTICLES ON THE GROWTH AND DEVELOPMENT OF WHEAT (*Triticum aestivum* L.) SEEDS

UDIN, S.[1] – KHAN, N. U.[2] – SHAKEEL, M.[1] – LAW, D.[3] – ELSADEK, M. F.[4] – AL-NUMAIR, K. S.[5] – TAN, D. K. Y.[6] – YASIN, M.[1*]

[1]*Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, KP, Pakistan*

[2]*Department of Plant Breeding and Genetics, Gomal University, Dera Ismail Khan, KP, Pakistan*

[3]*Faculty of Health and Life Sciences, INTI International University Nilai, Negeri Sembilan, Malaysia*

[4]*Department of Biochemistry, College of Sciences, King Saud University, Riyadh, Saudi Arabia*

[5]*Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia*

[6]*Plant Breeding Institute, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, Australia*

[*]*Corresponding author*
e-mail: drmywazir-biotech@gu.edu.pk

(Received 27th Dec 2024; accepted 26th May 2025)

Abstract. Wheat (*Triticum aestivum* L.) is staple cereal across the world. However, its yield is constantly challenged by various biotic and abiotic factors. Nanotechnology has the capability to defend plants, monitor plant development, detect plant diseases and enhance crop production. Magnesium (Mg) is essential for the plant metabolism and acts as structural components or enzyme co-factor, thus helps in activating the metabolic pathways such as photosynthesis which leads to better growth and higher yields of plants. However, very little information is available about the dosage dependant delivery of Mg in the form of MgO nanoparticles and its effect on the growth and development of vital cereal crop wheat. This study positively reported the impact of MgO NPs on wheat growth and development. Different concentrations (50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}) of MgO NPs were applied on wheat seeds in-order to check both morphological parameters and biochemical parameters. Results showed that only 50 mg L^{-1} and 100 mg L^{-1} concentrations enhanced both physiological as well as biochemical parameters as compared to the control. While the 200 mg L^{-1} concentration decreased the growth-related parameters**.** This work infers that MgO NPs 50 mg L^{-1} and 100 mg L^{-1} concentrations can easily penetrate the plant cell. However, the 200 mg L^{-1} concentration may aggregate in the seed pores which only permits slow water absorption and other essential nutrients hence slow down the growth and development of wheat seeds. The current study is significant for future nano-based improvement of the growth, development and yield related aspects of staple wheat crop.
Keywords: *agricultural innovation, wheat (Triticum aestivum L.), nanoparticles, MgO NPs, food production, seed germination, seed vigour*

Introduction

Agriculture plays an important role as the backbone of most developing countries which directly or indirectly provides food for human beings. By 2025, the world's

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

population is estimated to grow to eight billion and will reach to nine billion at the end of 2050. Hence, there is a dire need to increase agricultural production globally to meet the demand for food of the fast-developing world population. One of the key drivers of economy is the agri-food production which is of vital importance. It is predicted by UN (Food and Agriculture Organization) that annually 200 million tons meat production will be required by the end of 2050 in-order to fulfil the needs of foods of increasing world's population (Daszkiewicz, 2022).

Bread wheat (*Triticum aestivum* L.) is one of the top three most cultivated crops, along with rice and corn and is grown globally for the production of highly beneficial and nutritious grains. Wheat is used commercially in many products like bread, feeds, confectionary and biscuits as well as in other utilizations (Siddiqui et al., 2022). Globally, it is the most extensively grown cereal crop that annually covers nearly 237 million hectares, totally accounts for four hundred and twenty million tonnes (Kirilenko and Dronin, 2022; Teixeira et al., 2023) in which one fifth is for the consumption of a man's calorie. However, the growth, development and final yield of wheat is challenged by various biotic (diseases) and abiotic stresses.

Agricultural land which is used for the food crops is also facing an increasing competition for other various purposes like biofuels and pharmaceuticals productions. Hence, the capacity of food production, especially wheat production, is facing with many challenges, which includes a decreasing ratio of the arable land to the population (Liu et al., 2020). Due to increase in the world population, it is necessary to use the latest technologies like nanotechnology as well as nano-biotechnology in food sciences and agriculture. Currently nano-agriculture focuses on target farming to improve crops and livestock productivity that involves the use of nano-sized particles with unique properties (Garg and Payasi, 2020). Application of nanotechnology to the food and agriculture sectors is relatively new avenue associated with its use in pharmaceuticals and drug delivery (Kheiriabad et al., 2024). Nanotechnology has the capability to defend plants, monitor plant development, detect animal and plant diseases, enhance world production of foods, improve quality of food and decrease wastes for "ecological growth" (Pramanik et al., 2020; Munir et al., 2023; Nizamani et al., 2024). Nanoparticles offers wide spread applications in agriculture by boosting both growth and productivity of crops.

The thriving scientific and technological sector of nanotechnology is in charge of creating new materials at the nanoscale (1–100 nm) (Ying et al., 2022). At this scale, the novel materials have distinct qualities that set them apart from bulk compounds (Hamza et al., 2023). Through the manipulation and creation of individual atoms and molecules, nanotechnology allows researchers to create novel materials, systems, and technologies with intriguing features and functions (Liu et al., 2022). Along with various nanoparticles, MgO NPs acts as an excellent bactericide against some bacteria especially *Ralstonia solanacearum* as being nontoxic, safe as well as easy to obtain hence offers a substantial promise to inhibit stem and root diseases (Rabea et al., 2023).

Magnesium (Mg) is of vital importance in agriculture, it acts as the most limiting macro-nutrient element at global level. Besides, it is one of the most essential nutrients involved in the biochemical processes, reactive oxygen species (ROS) synthesis, photooxidation, utilization and partitioning of photo-assimilates, photophosphorylation and protein synthesis of plants (Cai et al., 2018). Mg performs an active function in operating essential organic poly-phosphate compound such as RNA, DNA as well as ATP and therefore needed to all living cells. It is also significant for the plant

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

metabolism as structural components or enzyme co-factor. However, latest studies have surprisingly revealed that over time Mg elements in old seeds of cereal crops have obviously decayed, hence in developed countries 2/3rd populations receive less than their minimum daily magnesium requirements and suffered from severe disease known as hypomagnesemia (Ahmed and Mohammad, 2019). Hence, there are two urgent practical problems in plants, ways to increase Mg contents and the mechanisms of response to magnesium deficiency (Chaudhry et al., 2021). Therefore, developments of such unique metals and its use like nano-nutrients have a large potential to enhance food quality, nutrient efficiency and to reduce adverse environmental effects. Finally, the nanotechnology-created method is reliable for the benefits of plants due to nutrition regulation in plants and soil. In almost 50% of world's soil nutrients insufficiency have certainly lowers food quality as well as food amounts thus badly influence the human health (Chaudhry et al., 2021).

There are many benefits to providing Mg to plants in the form of magnesium oxide NPs (MgO-NPs). The first advantage of MgO-NPs is that they assist to nano-sized delivery method for magnesium ions, increasing the efficiency of plants to absorb and utilize the mineral. The MgO-NPs' larger surface area enhances their interaction with plant roots and accelerates magnesium absorption (Cai et al., 2018). Additionally, MgO-NPs can be used to improve the solubility and bioavailability of magnesium in soil. If traditional magnesium fertilizers do not breakdown rapidly in the soil, magnesium may be leached and released gradually. Because MgO-NPs have a larger surface area, they dissolve more quickly and provide plants with easy access to magnesium ions. MgO-NPs can also be designed to release magnesium ions gradually, which will provide a steady supply of magnesium over time. This regulated magnesium release can help maintain the proper levels of magnesium in plants, which is beneficial for their growth and development (Gautam et al., 2023). Consequently, MgO-NPs can boost plant growth and productivity while reducing waste when used as a magnesium ion source.

Mg nanoparticles have been improved and extensively used in activating the metabolic pathway like photosynthesis which leads to better growth and higher yields of plants (Abbas et al., 2024). However, very little information is available about the dosage dependant delivery of Mg in the form of MgO nanoparticles and its effect on the growth and development of vital cereal crop wheat. Therefore, this study was designed to identify both positive and negative impact of MgO nano-particles on the growth and development of bread wheat crop.

Materials and methods

The research study was divided into two main sections. In the first section synthesis of MgO nanoparticles were carried out and in the second section the nanoparticles were applied to wheat seeds. Local elite wheat cultivar AZRC Dera was used in this study. The whole experiment was conducted in Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Pakistan.

Synthesis of Magnesium oxide nanoparticles

MgO nanoparticles were carefully synthesized via co-precipitation method by means of $NaHCO_3$, NaOH and $MgNO_3$ (Wang et al., 2018). Polyvinyl acetate was used as surfactant under room temperature. Under constant stirring condition, 1 molar solution of $NaHCO_3$ of 50 ml was gradually poured to it while using addition funnel. Under

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

stirring also one molar NaOH of 50 ml was gradually added to the above consequential solution. The entire process was carried out under continuous strong stirring condition (130 rpm). The constituent mixture was permitted to mix for 3 hrs. Consequently, a very fine powder magnesium oxide white precipitate was deposited at the round bottom flask bottom. The fine powder was carefully separated while using Buckner funnel. The whole precipitation was thoroughly washed with the ddH_2O. The resulting substrate Mg $(OH)_2$ precursor was kept in air oven for about 1 hour at 80 °C. Finally, MgO NPs were obtained through Controlled Calcination Method, using muffle furnace for a period of 5hrs at a temperature of 350 ºC.

Preparation of stock solutions

After synthesis of Magnesium oxide nanoparticles by co-precipitation method it is suspended in double distilled water and stock solutions i.e. 50 mg L^{-1}, 100mg L^{-1} and 200mg L^{-1} of Magnesium oxide nanoparticles were prepared and the solution was not renewed. Finally, for the dispersion of MgO nanoparticles, solutions were kept in a sonicator for about 35-45 minutes at 25˚C.

Seed treatment

Wheat was preferred as the ideal plant due to its significance for human diet. Therefore, seeds of wheat (*Triticum aestivum*) were selected for the present study to test the impact of MgO nanoparticles on seeds germination, vigor index, root/shoot length, protein and sugar contents, effect on chlorophyll and effect on proline. The seeds were washed with distilled water and then sterilized with 5% sodium hypochlorite followed by washing with distilled water. About five seeds were transferred to every petri plate containing filter paper. The prepared 50 mg L^{-1}, 100 mg L^{-1} and 200 mg L^{-1} concentration of Magnesium oxide solutions were poured into petri plate containing wheat seeds followed by placing in growth chamber at temperature 25°C (Model: BJPX-L500) for germination and growth for 24 hrs. Distilled water was used in the control treatment instead of MgO nanoparticles concentrations. The complete randomized design (CRD) with four replications was used for all the experiments.

Study of different parameters

After few days of seed treatment, different parameters, such as seeds germination, vigor index, root and shoot length, protein contents, chlorophyll contents, sugar and proline accumulation were observed using different equipment (*Tables 1 and 2*).

Table 1. *NP type, characterization, concentrations level and parameters studied*

Nanoparticle	Characterizations	Concentrations level	Parameters analysis
Magnesium Oxide (MgO)	Scanning Electron Microscopy (SEM)	50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}	Germination percentage, Root/Shoot length, Chlorophyll, protein, proline, sugar contents

Table 2. *Final germination percentage of Tritium aestivum seeds at various concentrations*

Final Germination Percentage	Control	50 mg	100 mg	200 mg
	84%	80%	100%	60%

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

Seeds germination and vigor index

Four petri dishes were used for seeds germination, one as control and the rest three as 50 mg L^{-1}, 100 mg L^{-1} and 200 mg L^{-1} concentrations. Five seeds were placed in each petri dish so that there is enough space for leaves and roots of wheat plants. Seed germination and vigor index were observed and noted according to the following formula.

$$Germination\ \% = \text{No of seeds germinated/total no of seeds*100} \quad \text{(Eq.1)}$$

$$Vigor\ Index = (\text{root length} + \text{shoot length}) \times \text{Germination percentage} \quad \text{(Eq.2)}$$

Determination of root/shoot length

Wheat plants were exposed to MgO nanoparticles for about fifteen days in plant growth chamber. After fifteen days of growth and development, the wheat plants were taken out from petri plates and measured by simple metric scale method.

Chlorophyll estimation

The chlorophyll contents were extracted with 80% acetone solution. Three treated (Magnesium oxide treated plants) and untreated wheat plant leaves were selected and isolated from plants. Fresh leaves of both treated and untreated wheat plants were weighted by electric balance. About 250 mg fresh leaves were homogenized in 80% acetone with help of homogenizer. After homogenization, the contents were centrifuged at 1000 rpm for a period of 15 minutes at 4°C. After centrifugation, two layers were made i.e. upper and lower layers. The upper layer supernatants consist of chlorophyll and the lower layer containing pellet was discarded. The supernatants were transferred to fresh Eppendorf tube. The green supernatants were further analysed by spectroscopic analysis. The spectroscopic analysis was carried out at wave length 700 nm under UV/VIS spectrophotometer (PG T80+ UV/VIS Spectrometer) against 80% acetone blank (Chung et al., 2019).

Total protein determination

Total protein contents were determined by Bradford assay method (Bradford, 1976). About 250 mg fresh tissues from each treated and untreated plants and emersed in phosphate buffer saline followed by grinding using chilled pestle and mortar. After the homogenization, the contents were transferred to Eppendorf tube and centrifuged at 10,000 rpm for 15 minutes at 4°C. After centrifugation, the supernatant was discarded and 1ml of 10% trichloro acidic acid (TCA w/v) was added into the pellet. The product was again centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant was discarded and the pellets were washed with 80% acetone. The obtained protein was dissolved in 1 ml of 0.1M NaOH and read at 595 nm in spectrophotometer (PG T80+ UV/VIS Spectrometer), the Bovine Serum Albumin (BSA) keep as a standard (Bradford, 1976).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

Proline estimation

Proline contents were determined by the method of Bates et al. (1973) from the roots and shoots of treated (50 mg L^{-1}, 100 mg L^{-1} and 200 mg L^{-1}) and control wheat plants. Wheat roots/ shoots samples were frozen in liquid nitrogen and homogenized with 3% aqueous sulphosalicylic acid (0.02 *g*/1 ml). The samples were then centrifuged at 12000 rpm for about 10minutes. Supernatants were transferred to fresh test tube after centrifugation. 1 ml acid ninhydrin with 1 ml of glacial acidic acid was added to test tube containing supernatants at a temperature of 100°C in a water bath. As a result, acid-ninhydrin was made in glacial acidic acid with 6M phosphoric acid of 2:1. Reactions were completed in ice bath after 1 hr. About 4 ml toluene was added, continuously mixed it at room temperature for about 35 minutes. Finally, optical density was measured with UV/VIS spectrophotometer model PG T80+ UV/VIS Spectrometer at 520 nm for upper pinkish layer of proline. Hence toluene was used as blank whereas D-proline was used as a standard (Bates et al., 1973).

Total soluble sugar content in leaf

The soluble sugar was measured according to Dey (1990) method. Fresh leaves of 0.5 g were kept in 10 ml alcohol at 600°C in incubator for 1 hr. The materials were transferred into volumetric flask of 25 ml and re-extracted the remaining. By the addition of alcohol, the final quantity was made up to 25 ml. To the test tube 1 ml aliquot was added and further 5% phenol of 1.0 ml was poured to it then carefully mixed it. 5 ml analytical grade H_2SO_4 was added to it, thoroughly mixed with the help of a rod glass. Here test tube was cooled in fresh environment for exothermic reaction. Finally, absorbance was checked with the help of spectrophotometer at 485 nm.

Statistical analysis

Data for all the recorded parameters were subjected to analysis of variance, by the Statistix 8.1 ($P \leq 0.05$) for statistical analysis.

Results

Our results revealed that at 50 mg L^{-1} and 100 mg L^{-1} concentration of MgO NPs positively affected the growth of wheat seeds. Hence enhanced both physiological and biochemical parameters while the 200 mg L^{-1} concentration decreased the seedling process as compared to control.

Characterization of MgO nanoparticles

To observe the morphology of MgO NPs Scanning Electron Microscopy (SEM) was used in the University of Peshawar at the department of physics. Images obtained from SEM confirmed the presence of sponge, porous and spherical structure of MgO NPs. *Figure 1* shows the MgO NPs taken by SEM (JEOL JSM-6460 SEM) at 50.0 kx magnification.

Effect of MgO NPs on Triticum aestivum seeds germination

After two days, wheat seeds started germination and after four days a short germination has been observed as shown (*Figure 2*). Two seeds germinated in control as

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

well as in 200 mg L^{-1} concentration petri dishes while four seeds germinated in 100 mg L^{-1} concentration and finally three seeds germinated in 50 mg L^{-1} concentration at day four.

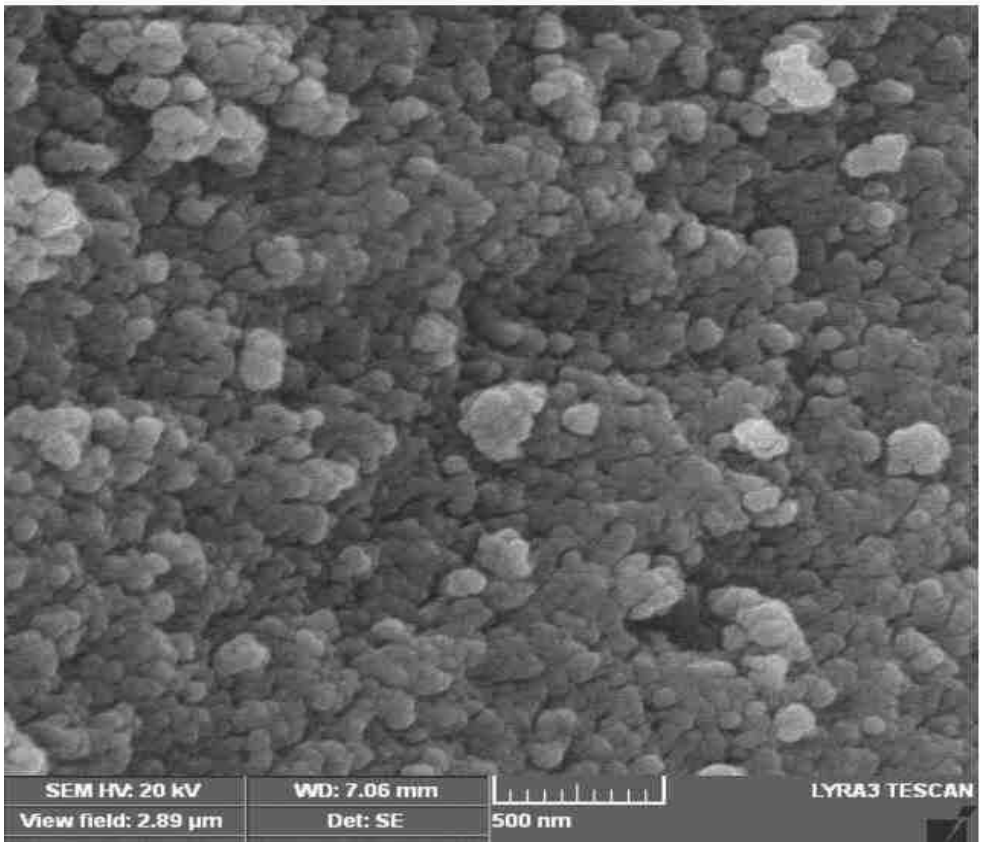

Figure 1. *Scanning Electron Microscopy image of MgO NPs at X 50.0 kx*

Figure 2. *Observations of Triticum aestivum seeds germination at day 4. The first plate on the left most corner shows control*

As shown in *Figure 3*, at day six a very clear germination has been observed with well-developed leaves and roots. Clear germination of three seeds in control and in lower concentration (50 mg L^{-1}) and also the fourth seed started germination at day fifth while the fifth seed did germinate yet in control as well as in lower MgO concentration. Better result of 100 mg L^{-1} has been observed so far in which four seeds germinated with more developed leaves and roots as compared to the 50 mg L^{-1} and 100 mg L^{-1}

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

concentration and control one. The fifth seed started germination at day fifth. Finally, in 200 mg L^{-1} MgO concentration two seeds germinated with green leaves and white roots. The third seed started germination at day fifth but the fourth and fifth seeds did not germinate.

Figure 3. *Observation of Triticum aestivum seeds germination at day 6. The third plate from the left side shows control*

As shown in *Figure 4*, third time the germination has been observed after eight days. Seeds of untreated plant (control) developed more long leaves and roots. The fourth seed was developed short to about one cm and the fifth one was germinated at day seven. Similarly, the roots and leaves of 50 mg L^{-1} concentration got enough length of the three seeds. The fourth seed growth to about half cm with little roots but the fifth seed did not start germination at day 7. At 100 mg L^{-1} concentration four seeds germinated equally with fine roots, shoots and leaves and also the fifth seed was growing well at day 8^{th}. Finally, slow germination had occurred at 200 mg/l. The third seed started a little developing and the fourth and fifth seeds did not germinate yet.

Figure 4. *Observation of Triticum aestivum seeds germination at day 8. The first plate on the left most corner shows control*

Figure 5 shows the fourth time observation of *Triticum aestivum* seeds germination at day 10. All the seeds of control and lower concentration were growing well except the fifth seed was growing slowly in control but did not germinate in lower

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

concentration yet at day 10. All the seeds of 100 mg L^{-1} concentration were growing well with enough roots, shoots and leaves but at 200 mg L^{-1} concentration three seeds developed and the fourth/fifth seeds were unable to start germination at day10.

Figure 5. *Final observation of Triticum aestivum seeds germination at day 10. The first plate on the left most corner shows control*

The final observation was recorded at day 12 (*Figure 6*). At day twelve, four seeds in control and 50 mg L^{-1} MgO concentration developed more as compared to day ten. But the fifth seed was developed less in control and did not germinate in 50 mg L^{-1} concentration. Finally, at 100 mg L^{-1} concentration all the seeds were developed to a maximum length but at 200 mg L^{-1} concentration just three seeds were developed more than day 10 and the two seeds were unable to start germination still at final observation at day 12.

Figure 6. *Final observation of Triticum aestivum seeds germination at day 12. The first plate on the left most corner shows control*

Germination percentage

After twelve days of growth and development of wheat seeds, their germination% was taken. The present study revealed that the 100 mg L^{-1} concentration of MgO NPs increased the seed germination percentage in wheat seeds (*Figure 7*). At 100 mg L^{-1} concentration, seed germination had occurred 100%, hence increased the seed germination by 16% in comparison to the control. At 50 mg L^{-1} and 200 mg L^{-1} concentrations 80% and 60% seed germination had occurred which reduced the seeds germination by 4% and 24% respectively as compared to the control.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

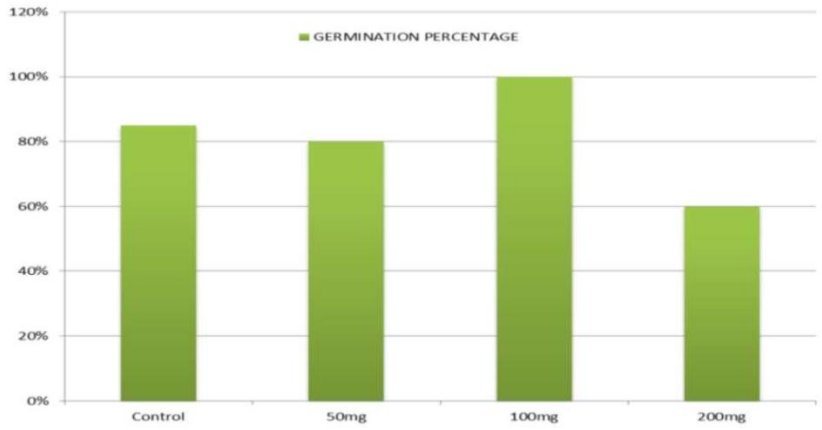

Figure 7. *Germination percentage of Triticum aestivum seeds*

Vigor index

The vigor index exhibited sharp increase by the 100 mg L^{-1} and 50 mg L^{-1} concentration of MgO NPs while decrease at 200 mg L^{-1} concentration as compared to the control (*Figure 8*).

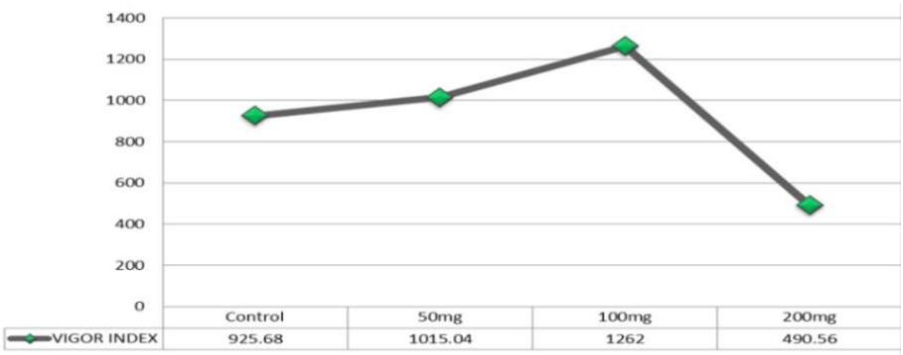

Figure 8. *Vigor index of Triticum aestivum seeds at various concentrations*

Roots/shoots responses to MgO nanoparticles

The current research determined that the 50 mg L^{-1} and 100 mg L^{-1} concentration of MgO NPs positively affected the growth of roots and shoots hence increased the length of roots and shoots of wheat plants. But the 200 mg L^{-1} concentration of Magnesium Oxide has negatively affected on the roots and shoots of wheat in comparison to control one (*Figure 9*). As shown in *Figure 10*, at 50 mg concentration wheat roots increased by 13% while shoots increased by 2% as compared to control. Similarly, wheat treated with 100 mg increased the roots length by 12% and shoots length by 9% in comparison to the untreated wheat plants. Highest concentration of MgO NPs (200 mg) has negatively affected on treated wheat plants roots and shoots lengths and decreased was reported. At 200 mg concentration *Triticum aestivum* roots and shoots were declined and decreased the length by 2% and 3% respectively in comparison to the control one.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

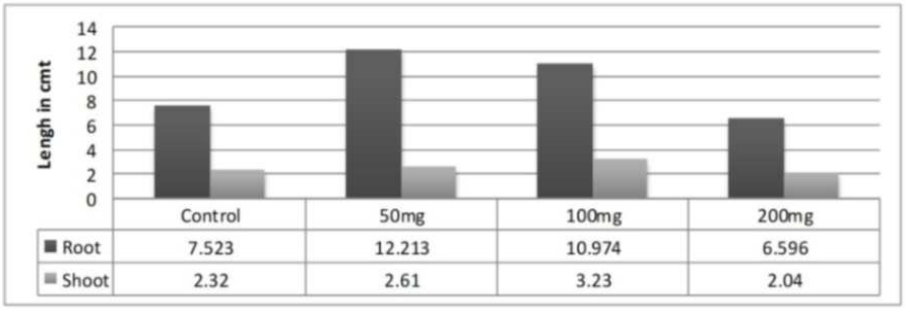

Figure 9. *Root/shoot length of Triticum aestivum seeds at various concentrations*

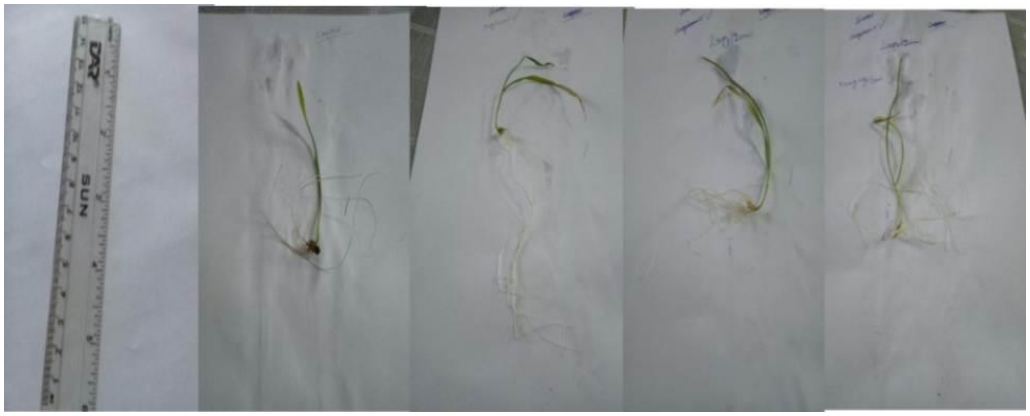

Figure 10. *The effect of different concentrations of MgO NPs on wheat plants (Triticum aestivum) from left to right indicate control, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}*

Chlorophyll responses to MgO nanoparticles

The 50 mg L^{-1} and 100 mg L^{-1} concentration of MgO NPs increased the chlorophyll contents of *Triticum aestivum* plants (*Figure 11*). At 50 mg L^{-1} and 100 mg L^{-1} concentration MgO NPs treated wheat plants increased the chlorophyll contents by 20% and 13% respectively as compared to the untreated wheat plants. While at 200 mg L^{-1} concentration (200 mg) the chlorophyll contents were just increased to 3% only which did not show much difference with the control one.

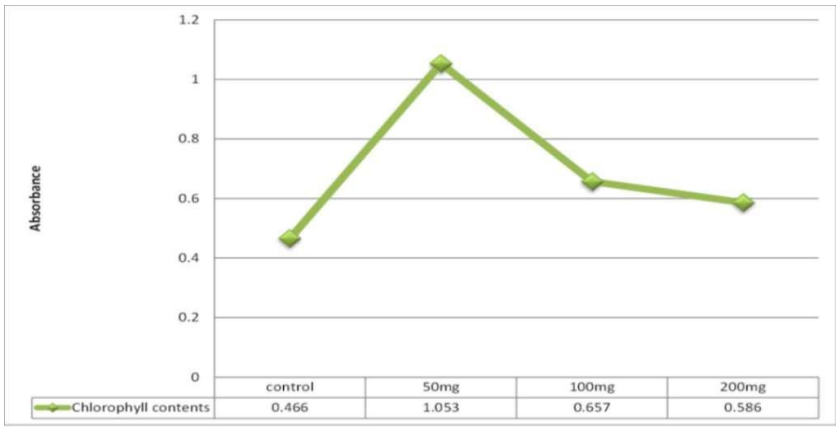

Figure 11. *Chlorophyll contents of Triticum aestivum seeds at various concentrations*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

Protein responses to MgO nanoparticles

The effect of magnesium oxide nanoparticles on protein contents of wheat plants were checked and found that 100 mg L^{-1} concentration of MgO NPs promoted the total protein contents in *Triticum aestivum* plants followed by the 50 mg L^{-1} concentration. While the highest concentration of MgO NPs decreased protein contents in *Triticum aestivum* plants (*Figure 12*) illustrates that a significant increase in protein contents were found when wheat plants were exposed to 100 mg L^{-1} and 50 mg L^{-1} concentrations of MgO nanoparticles. About 17% at 100 mg L^{-1} and 13% at 50 mg L^{-1} protein contents increased were reported in *Triticum aestivum* plants. While at 200 mg L^{-1} concentration the results were opposite and the protein contents were decreased. At 200 mg L^{-1} MgO NPs treated wheat plants showed 6% decreased in protein contents as compared to untreated wheat plants.

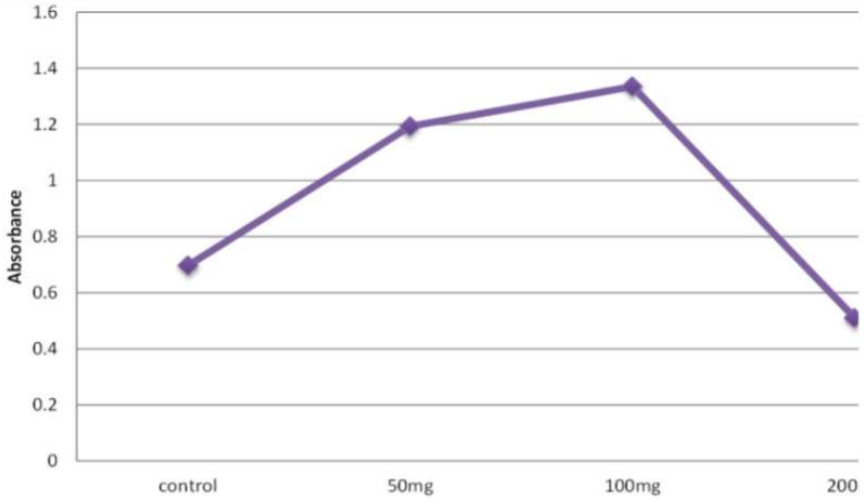

Figure 12. *The total protein contents of Triticum aestivum seeds*

Proline responses to MgO nanoparticles

Proline is a key amino acid that plays a vital role in all plants once it is exposed to different stress environmental conditions. An effective correlation exists between plant stress conditions and proline accumulation. During stressful environments, plants excrete over proline which turn the propagate stress tolerance due to maintaining of cell turgor, cell osmotic balance, and the membrane stabilization. Thus avoiding electrolyte leakage and within optimum range import the concentration of reactive oxygen species (ROS) in-order to avoid the oxidative burst in plant species (Li et al., 2018).

Biochemical analysis of proline resulted that very small amount of proline were accumulated in roots and leaves of MgO NPs treated *Triticum aestivum* plants as compared to untreated plant. At 50 mg L^{-1} and 100 mg L^{-1} concentrations of MgO NPs, more proline accumulation were reported while at 200 mg L^{-1} concentrations small amount of proline accumulations were reported in *Triticum aestivum* roots and leaves. It was also reported that the level of proline accumulation was less in *Triticum aestivum* leaves than proline accumulations in *Triticum aestivum* roots. At 50 mg L^{-1} MgO nanoparticles treated plants proline level increased by 2.6 folds in leaves while 3.2 folds in roots. At 100 mg L^{-1} proline level increased by 2.0 and 2.5 folds in wheat leaves and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

roots respectively than untreated wheat plants. Similarly, at 200 mg/l proline level increased by 1.5 folds in leaves and 2.1 folds in roots of *Triticum aestivum* plants as compared to untreated plants as shown in *Figure 13*.

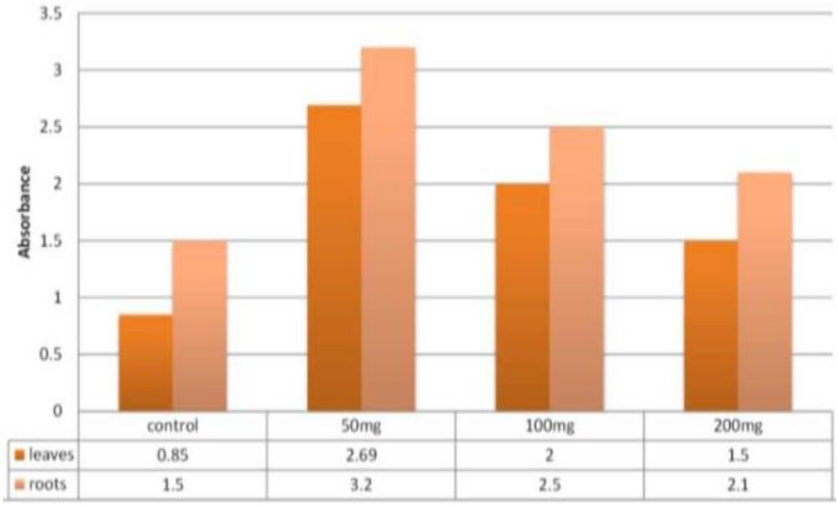

Figure 13. *The proline accumulation in leaves and roots of Triticum aestivum plants*

Total sugar contents responses to MgO nanoparticles

Effect of magnesium oxide nanoparticles (MgO NPs) on total sugar contents of *Triticum aestivum* plants was also checked and a significant improvement was found at 50 mg L^{-1} concentration followed by 100 mg L^{-1} concentration. While at 200 mg L^{-1} concentration, MgO NPs reduced the total sugar contents in *Triticum aestivum* plants (*Figure 14*). A significant increase in sugar contents was observed when wheat plants were exposed to 50 mg L^{-1} and 100 mg L^{-1} concentrations of MgO nanoparticles. Total sugar contents were enhanced by 11% at 50 mg L^{-1} and 10% at 100 mg L^{-1} as compared to control. While the 200 mg L^{-1} concentration resulted a 5% decreased in total sugar contents as compared to untreated wheat plants.

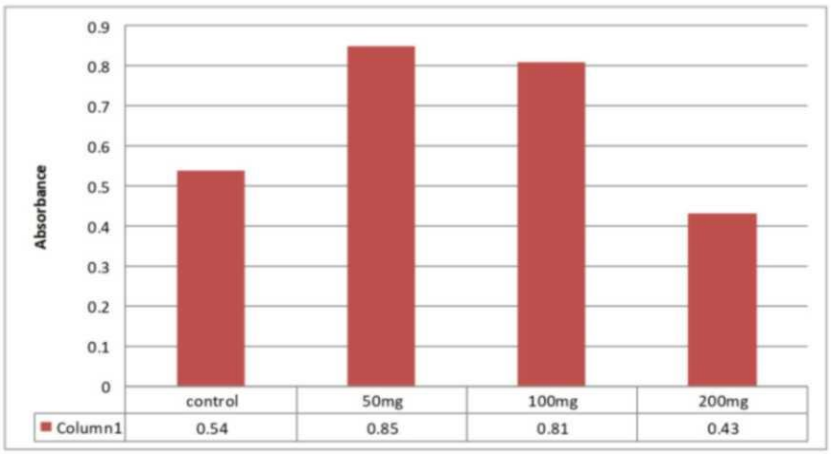

Figure 14. *The total sugar contents in Triticum aestivum plant leaves at various concentration*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

Discussion

Our result revealed that MgO NPs stimulated the seed germination of wheat plants. The highest GP (100%) was obtained at 100 mg L^{-1} and the lowest GP (60%) at 200 mg L^{-1}. Teixeira et al. (2023) also showed similar result in maize plant and stated that the highest and the lowest GP (95% and 80%) were obtained at 100 mg concentration of nano-sized MgO and control, respectively. Rodriguez-Morales et al. (2020) reported that it is possibly expected that magnesium hydroxide NPs bind with waxy surface layer that is present on *Z. mays* seeds while nano-size through seed coat make easy their dispersion inside the maize seeds, employs a positive impact on seed germination development. Seeds water absorption capacity is generally increased through nanoparticles. H_2O immersed by the seeds therefore solubilizes gibberellic acid (GA) that exists in its embryo then moves to the aleuronic cytoplasm through seed tissues in-order to produce enzyme amylase (Abbas et al., 2024). Hence amylase hydrolyses seed starch into sugars (maltose) thus helps to supply energy to seed cells needed for the germination process.

Rashid et al. (2022) examined mung bean seeds with three various concentrations i.e. (50,100 and 150 mg) of zinc oxide nanoparticles and also examined without ZnO NPs. Both treated as well as untreated seeds were germinated at day 10 but germination percentage in treated zinc oxide nanoparticles suspension solution was significantly higher than control one. Recommended zinc oxide nanoparticles suspension solution was greatly effective on seed germination of mung bean at 50 and 100 mg concentrations when compared to higher concentration (150 mg) and control (Rashid et al., 2022). Similarly, Mazhar et al. (2023) also observed a significant positive effect of ZnO NPs on shoot and root elongation of mung bean seeds in 50 and 100 mg concentrations as compared to unexposed control germination. Using 50 and 100 mg concentration of ZnO NPs treated seeds, germination time, germination percentage and vegetative biomass shown results as compared to untreated seeds. Navya et al. (2021) resulted similarly that mungbean plant was grown in 50 and 100 mg developed well long stems as compared to control (Navya et al., 2021). Seed germination percentage, biomass and root/shoot length of mungbean was significantly recoded at 50 and 100 mg concentration of ZnO NPs. This significance improvement may be due to the penetration of seed coat penetration by zinc oxide NPs. Indicated that seed germination as well as seedling growth was promoted by ZnO NPs at 50 and 100mg concentration, but a significant decrease was observed in seed germination of moungbean plants at 150 mg concentration of zinc oxide NPs. These results are in accordance with our MgO 100 mg L^{-1} concentration NPs positive affect and the growth and development of wheat seed, roots and leaves. Our current research resulted that MgO NPs at 50 mg and 100 mg concentration positively affected roots & shoots growth of wheat hence increased their length. But at the highest concentration (200 mg) MgO NPs has negatively affected the shoots and roots of wheat plants in comparison to control one.

Wang et al. (2024) reported that the effect of SNPs looks like concentration dependent. Nanoparticles could stimulate plant growth at short dosage but high doses retard its growth. Alghofaili et al. (2024) also reported similar result. He observed that increasing the silver nanoparticles (SNPs) concentrations from 20 ppm to 60 ppm led to an enhance in plant growth parameters of common bean and corn such as root/shoot lengths, the surface area of a leaf, some biochemical parameters like protein, chlorophyll and also carbohydrate contents. However, further enhancement of silver nano-particles resulted in retardation of plant growth (Alghofaili et al., 2024).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

Sheteiwy et al. (2021); Tymoszuk and Wojnarowicz (2020) worked on different plants with zinc oxide nanoparticles such as wheat, soybean, peanut and onion. They stated that lower concentrations of zinc oxide NPs positively impacted on the seed germination, however, their higher concentrations reduced germination (Tymoszuk and Wojnarowicz, 2020; Sheteiwy et al., 2021). The effectiveness of nanoparticles in seed germination depends upon the NPs amount and different plant species that vary from each other. Gupta et al. (2022) applied various concentrations of ZnO NPs on cucumber, tomato and alfalfa, found only cucumber with enhanced seedling processes.

Our experiment clearly observed two concentrations of MgO NPs i.e. 50 mg and 100 mg concentrations. So, 100 mg showed increase in seed germination percentage over control while both 50 mg and 100 mg of MgO NPs significantly enhanced the physiological parameters (germination percentage, vigor index, root/shoot length) as well as biochemical parameters (chlorophyll, total protein, proline, total sugar contents) of wheat plants than control. But 200 mg L^{-1} concentration is the main reason in our experiment that inhibited both physiological and biochemical parameters of *Triticum aestivum* plants in comparison to untreated plants. The inhibition reason at 200 mg L^{-1} concentration may be due to the aggregation of MgO NPs in the seed pores that permit only slow water absorption as well as other essential nutrients inside the seed hence slow down their growth.

Conclusions

In this study, the positive role of eco-friendly synthesized magnesium oxide nanoparticles (MgO NPs) was investigated. The study revealed that MgO nanoparticles have a positive effect on plant growth and showed that significantly affected the seed germination and plant growth regulation in *Triticum aestivum* L. plants. Our research results pointed out no seed germination inhibition at 50 mg L^{-1} and 100 mg L^{-1} concentrations but the 200 mg L^{-1} concentration of MgO NPs reduced the germination and plant development process due to aggregation and their accumulation in seed pores that only allow slow entrance of water and other essential molecules. The present study validated that Mg accumulation in roots and stems as well as in leaves tissues at various applied concentrations, especially 50 mg L^{-1} and 100 mg L^{-1} concentrations, excluding 200 mg L^{-1} MgO concentration was the basic and actual factor that enhanced chlorophyll contents, roots/shoots lengths, other biochemical parameters and plant growth. Notably, it was confirmed by the MgO NPs to serve as excellent magnesium supplement for the plant growth.

Acknowledgements. The authors would like to extend their sincere appreciation to the Ongoing Research Funding program (ORF-2025–349), King Saud University, Riyadh, Saudi Arabia. The authors are grateful to the Gomal Centre of Biochemistry and Biotechnology (GCBB), Gomal University, Dera Ismail Khan, Pakistan for facilitating this research by providing resources.

REFERENCES

[1] Abbas, Z., Hassan, M. A., Huang, W., Yu, H., Xu, M., Chang, X., Fang, X., Liu, L. (2024): Influence of Magnesium Oxide (MgO) Nanoparticles on Maize (*Zea mays* L.). – Agronomy 14(3): 617. https://doi.org/10.3390/agronomy14030617.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

[2] Ahmed, F., Mohammed, A. (2019): Magnesium: the forgotten electrolyte—a review on hypomagnesemia. – Med. Scie. 7(4): 56. doi: 10.3390/medsci7040056.
[3] Alghofaili, F., Tombuloglu, H., Almessiere, M. A., Akhtar, S., Tombuloglu, G., Turumtay, E. A., Turumtay, H., Baykal, A. (2024): Fine-tuning the element dose in nanoparticle synthesis is the critical factor determining nanoparticle's impact on plant growth. – Plant Physiol. Biochem. 216: 109110. https://doi.org/10.1016/j.plaphy.2024.109110.
[4] Bates, L. S., Waldren, R. P., Teare, I. D. (1973): Rapid determination of free proline for water-stress studies. – Plant Soil 39: 205-207. https://doi.org/10.1007/BF00018060.
[5] Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3.
[6] Cai, L., Chen, J., Liu, Z., Wang, H., Yang, H., Ding, W. (2018): Magnesium oxide nanoparticles: effective agricultural antibacterial agent against *Ralstonia solanacearum*. – Front. Microbiol. 9: 790. https://doi.org/10.3389/fmicb.2018.00790.
[7] Cai, L., Liu, M., Liu, Z., Yang, H., Sun, X., Chen, J., Xiang, S., Ding, W. (2018): MgO NPs Can boost plant growth: evidence from increased seedling growth, morphophysiological activities, and Mg Uptake in tobacco (*Nicotiana tabacum* L.). – Molecules (Basel, Switz) 23(12): 3375. DOI: 10.3390/molecules23123375.
[8] Chaudhry, A. H., Nayab, S., Hussain, S. B., Ali, M., Pan, Z. (2021): Current understandings on magnesium deficiency and future outlooks for sustainable agriculture. – Int. J. Mol. Sci. 22(4): 1819. https://doi.org/10.3390/ijms22041819.
[9] Chung, S., Chung, J., Lee, Y. W. (2019): Use of TiO 2 media by sputtering and peroxy gel method for wastewater reuse in the electronics industry. – Sci. Adv. Mater. 11(11): 1547-1555. DOI:10.1166/sam.2019.3613.
[10] Daszkiewicz, T. (2022): Food production in the context of global developmental challenges. – Agriculture 12(6): 832. https://doi.org/10.3390/agriculture12060832.
[11] Garg, D., Payasi, D. K. (2020): Nanomaterials in agricultural research: an overview. – Environ. Nanotechnol. 3: 243-275. https://doi.org/10.1007/978-3-030-26672-1_8.
[12] Gautam, A., Sharma, P., Ashokhan, S., Yaacob, J. S., Kumar, V., Guleria, P. (2023): Magnesium oxide nanoparticles improved vegetative growth and enhanced productivity, biochemical potency and storage stability of harvested mustard seeds. – Environ. Res. 229: 116023. https://doi.org/10.1016/j.envres.2023.116023.
[13] Gupta, N., Jain, S. K., Tomar, B. S., Anand, A., Singh, J., Sagar, V., Kumar, R., Singh, V., Chaubey, T., Abd-Elsalam, K. A., Singh, A. K. (2022): Impact of foliar application of ZnO and Fe_3O_4 nanoparticles on seed yield and physio-biochemical parameters of cucumber (*Cucumis sativus* L.) seed under open field and protected environment vis a vis during seed germination. – Plants 11(23): 3211. https://doi.org/10.3390/plants11233211.
[14] Hamza, M. F., Abd El-Hamid, A. A. M., Guibal, E., Abdel-Rahman, A. A. H., El Araby, R. (2023): Synthesis of a new pyrimidine-based sorbent for indium (III) removal from aqueous solutions – Application to ore leachate. – Sep Purif Technol. 314: 123514. https://doi.org/10.1016/j.seppur.2023.123514.
[15] Kheiriabad, S., Jafari, A., Namvar Aghdash, S., Ezzati Nazhad Dolatabadi, J., Andishmand, H., Jafari, S. M. (2024): Applications of advanced nanomaterials in biomedicine, pharmaceuticals, agriculture, and food industry. – Bionanoscience 14: 4298-4321. https://doi.org/10.1007/s12668-024-01506-w.
[16] Kirilenko, A., Dronin, N. (2022): Recent grain production boom in Russia in historical context. – Clim. Change. 171: 22. https://doi.org/10.1007/s10584-022-03332-z.
[17] Li, J., Wang, X., Zhao, G., Chen, C., Chai, Z., Alsaedi, A., Hayat, T., Wang, X. (2018): Metal-organic framework-based materials: superior adsorbents for the capture of toxic and radioactive metal ions. – Chem. Soc. Rev. 47: 2322-2356. DOI:https://doi.org/10.1039/C7CS00543A.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

[18] Liu, X., Shi, L., Qian, H., Sun, S., Wu, P., Zhao, X., Engel, B. A., Wang, Y. (2020): New problems of food security in Northwest China: A sustainability perspective. – Land Degrad. Dev. 31(8): 975-989. https://doi.org/10.1002/ldr.3498.

[19] Liu, Z., Fu, R., Yuying, Y. (2022): Preparation and evaluation of stable nanofluids for heat transfer application. – In: Ali, H. M. (ed.) Advances in Nanofluid Heat Transfer. Amsterdam: Elsevier, pp. 25-57. https://doi.org/10.1016/B978-0-323-88656-7.00013-1.

[20] Mazhar, M. W., Ishtiaq, M., Maqbool, M., Akram, R. (2023): Seed priming with zinc oxide nanoparticles improves growth, osmolyte accumulation, antioxidant defence and yield quality of water-stressed mung bean plants. – Arid Land Res. Manag. 37: 222-246. https://doi.org/10.1080/15324982.2022.2132547.

[21] Munir, N., Gulzar, W., Abideen, Z., Hancock, J. T., El-Keblawy, A., Radicetti, E. (2023): Nanotechnology improves disease resistance in plants for food security: Applications and challenges. – Biocatal Agric Biotechnol. 51: 102781. https://doi.org/10.1016/j.bcab.2023.102781.

[22] Navya, P. P., Akhila, M., Dawson, J. (2021): Effect of plant growth regulators on growth and yield of Zaid Mung bean (*Vigna radiata* L.). – J Pharmacogn. Phytochem. 10(2): 1228-1230.

[23] Nizamani, M. M., Hughes, A. C., Zhang, H. L., Wang, Y. (2024): Revolutionizing agriculture with nanotechnology: Innovative approaches in fungal disease management and plant health monitoring. – Sci. Total Environ. 13: 172473.

[24] Pramanik, P., Krishnan, P., Maity, A., Mridha, N., Mukherjee, A., Rai, V. (2020): Application of nanotechnology in agriculture. – Environ. Nanotechnol. 4: 317-348. https://doi.org/10.1007/978-3-030-26668-4_9.

[25] Rabea, A., Naeem, E., Balabel, N. M., Daigham, G. E. (2023): Management of potato brown rot disease using chemically synthesized CuO-NPs and MgO-NPs. – Bot. Stud. 64(1): 20. doi: 10.1186/s40529-023-00393-w.

[26] Rashid, M. H., Rahman, M. M., Halim, M. A., Naidu, R. (2022): Growth, metal partitioning and antioxidant enzyme activities of mung beans as influenced by zinc oxide nanoparticles under cadmium stress. – Crop and Pasture Sci. 73(7-8): 862-876. https://doi.org/10.1071/CP21598.

[27] Rodriguez-Morales, A. J., Bonilla-Aldana, D. K., Balbin-Ramon, G. J., Rabaan, A. A., Sah, R., Paniz-Mondolfi, A., Pagliano, P., Esposito, S. (2020): History is repeating itself: probable zoonotic spillover as the cause of the 2019 novel coronavirus epidemic. – Infez. Med. 28(1): 3-5.

[28] Sheteiwy, M. S., Shaghaleh, H., Hamoud, Y. A., Holford, P., Shao, H., Qi, W., Hashmi, M. Z., Wu, T. (2021): Zinc oxide nanoparticles: potential effects on soil properties, crop production, food processing, and food quality. – Environ. Sci. Pollut. Res. 28: 36942-36966. https://doi.org/10.1007/s11356-021-14542-w.

[29] Siddiqui, S. A., Mahmud, M. C., Abdi, G., Wanich, U., Farooqi, M. Q., Settapramote, N., Khan, S., Wani, S. A. (2022): New alternatives from sustainable sources to wheat in bakery foods: Science, technology, and challenges. – J. Food Biochem. 46: e14185. https://doi.org/10.1111/jfbc.14185.

[30] Teixeira, D. S., Koblianska, I., Kucher, A. (2023): Agricultural production in Ukraine: An insight into the impact of the Russo-Ukrainian war on local, regional and global food security. – J. Agric. Sci. 68(2): 121-140. https://doi.org/10.2298/JAS2302121T.

[31] Tymoszuk, A., Wojnarowicz, J. (2020): Zinc oxide and zinc oxide nanoparticles impact on in vitro germination and seedling growth in *Allium cepa* L. – Materials 13(12): 2784. DOI:10.3390/ma13122784.

[32] Ying, S., Guan, Z., Ofoegbu, P. C., Clubb, P., Rico, C., He, F., Hong, J. (2022): Green synthesis of nanoparticles: Current developments and limitations. – Environ Technol Innov. 26: 102336. https://doi.org/10.1016/j.eti.2022.102336.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288

[33] Wang, L., Yang, Y. X., Shi, X., Mignani, S., Caminade, A. M., Majoral, J. P. (2018): Cyclotriphosphazene core-based dendrimers for biomedical applications: an update on recent advances. – J. Mater. Chem. 6: 884-895. https://doi.org/10.1039/C7TB03081A.
[34] Wang, J., Wu, H., Wang, Y., Ye, W., Kong, X., Yin, Z. (2024): Small particles, big effects: How nanoparticles can enhance plant growth in favorable and harsh conditions. – J. Integr. Plant Biol. 66(7): 1274-1294. https://doi.org/10.1111/jipb.13652.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2401_271288