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DETERMINATION OF PHYSIOLOGICAL PARAMETERS AND DMDH ENZYME ACTIVITY IN MAIZE (*ZEA MAYS L.*) SPROUTS GROWN UNDER COMBINED STRESS CONDITIONS

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SUMMARY

Maize (*Zea mays* L.) sprouts growth and activity dynamics of DMDH (decarboxylating malate-dehydrogenase, Malic enzyme EC:1.1.1.40) enzyme have undergone studies under combined stress conditions developed by gamma rays, salt solution, and nanoparticles. Three different factors comprising various doses of gamma radiation (250, 500, and 750 Gy), NaCl solution (100 mM), and iron oxide nanoparticles (maghemite, γ- Fe_2O_3) have been in use by the study to develop artificial stress conditions. These combined stress conditions have significantly affected and delayed the plant growth compared with the control variant. The higher doses of radiation (500 and 750 Gy) have slowed down the plant growth and caused destruction. The effect of gamma radiation (250 Gy) delayed the growth of plants. However, during the combined influence of nanoparticles (Fe_2O_3) with a dose of 250 Gy, plant development was better than with an individual dose of 250 Gy. This difference was not evident in the medium with high doses (500 and 750 Gy) of gamma radiation combined with nanoparticles (Fe_2O_3) and with salt stress (NaCl 100 mM). During the first 10 days of the experiment, DMDH enzyme activity, induced in the root and leaf cells in all variants, included the control, and inhibition occurred on the 15th day. This reaction product can be due to enzyme inhibition. However, the enzyme activity was higher during the combined stress conditions.

Keywords: Maize (*Zea mays* L.) seedlings, gamma radiation, salt stress, iron oxide nanoparticles, decarboxylating-malate dehydrogenase, combined stress conditions, growth traits

Key findings: Adding maghemite nanoparticles to the medium slightly reduced the stress effects, and thus, the stimulating effect of nanoparticles has manifested. In variants with NaCl solution (100 mM), no stimulating effect appeared; however, on the contrary, a retarding effect was noticeable. The

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DMDH enzyme (ME, EC:1.1.1.40) activity regarding corn plant growth was prominent at the beginning of the experiment (5^{th} and 10^{th} days) in the root and stem tissues under the combined stress conditions and both in control. Gamma irradiation has reduced the plant's organic matter in applied variants compared with the control variant. However, this reduction was superior for the variants with NaCl solution.

INTRODUCTION

Abiotic stress factors, including salt stress, radiation, drought, pollution with heavy metals, temperature fluctuation, and other stress factors, adversely affect the growth and productivity of crop plants (Mei *et al*., 2018; Mirza *et al*., 2019; Iqbal *et al*., 2020). Although the defense mechanism against the various stress effects has numerous separate studies, information about combined stress conditions (caused by many factors) effects on plant growth and the mechanisms of the plant's defense system is insufficient (Sara *et al*., 2021).

Like other crop plants, maize also suffers from constantly changing ecosystems, which requires the study of plant growth under different stress factors individually and in combination. The biological effects of gamma rays rely on the interaction with atoms and molecules in the cell, especially with water, for the formation of free radicals that can damage various important compounds of plant cells (Kovacs and Keresztes, 2002; Delia *et al*., 2013). Salt stress has a stimulating effect in the initial phases of plant growth at low concentrations; however, plant growth retardation happens under increased salt concentrations (Shanhu *et al*., 2021). The exact value of the salt concentration depends upon diverse factors, including salt level and plant types (Hongqiao *et al*., 2021).

In most cases, salt stress enhance the concentration of Na^+ and Cl^- ions in cells, decreasing K^+ ions, and later, it weakens photosynthesis by causing stomatal closure, which results in the retardation of most physiological processes in crop plants (Saiema *et al*., 2022). Numerous studies demonstrated that several nanoparticles, including the maghemite (γ- Fe_2O_3), can accelerate various physiological processes in plants. Thus, nanoparticles protect the cell membrane and the photosynthetic apparatus, increase the photosynthetic efficiency, and improve antioxidant activity, enhancing plant resistance to several stress conditions (Fabian *et al*., 2020; Adnan *et al*., 2022; Jan *et al*., 2022). However, studies also determined that their high concentration could produce toxic effects on crop plants. These nanoparticles can also enter the animal and human body through the food chain, and produce highly poisonous effects (Anshu *et al*., 2017; Heba *et al*., 2022; Neda *et al*., 2022).

Therefore, in this regard, nanoparticles' form, size, and concentration are specific for each living being. Probing the effects of various stress factors, including nanoparticles, on the corn plant requires proper investigation of these nanoparticles (for concentration and size). In some studies, the influences of many nanoparticles (magnetite - Fe_3O_4, maghemite - γ-Fe_2O_3, silver nanoparticles, gold nanoparticles) have reached numerous investigations on maize plants, with their positive and negative effects on plant growth with specific concentrations also determined (Junli *et al*., 2016; Vladimir *et al*., 2022).

It is also a fact that various stress factors in nature (salinity, drought, pollution with heavy metals, and radiation) can influence living systems simultaneously. Unlike plants' exposure to only one stress, exposure to two or more stresses results in specific physiological, molecular, and metabolic stress reactions (Hera *et al*., 2023). Such combined stress effects can cause antagonistic and synergistic effects (Haina and Sonnewald, 2017; Lidor *et al*., 2017). According to past studies, in most cases, the delay in plant growth occurs more intensively under many stress-causing factors (Prachi *et al*., 2015).

The occurrence of stress conditions activates the plants' defense mechanisms. One of the vital factors regulating the defense mechanism is the NADPH dehydrogenases, required during the glutathione reductase cycle in the cells, especially participating in cell growth, detoxification, and other vital physiological processes (Francisco and Juan, 2014). One of these enzymes is the enzyme decarboxylating-malate dehydrogenase (DMDH, Malic enzyme, NADP-ME EC:1.1.1.40), which catalyzes the oxidative decarboxylation of malic acid and participates in numerous metabolic processes. Studies on multiple isoforms of the DMDH enzyme have progressed in mitochondria, cytoplasmic stroma, and chloroplasts. The features of this enzyme—increasing the efficiency of water use and accelerating photosynthesis—enhance the resistance to stress conditions (Xi *et al*., 2019). The presented research sought to determine the effects of iron oxide nanoparticles on the maize plants individually and in combination with gamma radiation and salt stress.

MATERIALS AND METHODS

Three maize (*Z. mays* L.) genotypes (locally improved Zagatala, Zagatala-420, and Zagatala-68) widely distributed in Azerbaijan were samples in the presented research. Given that the genotype Zagatala-420 was more resistant to gamma rays thus, the preference for the said genotype. The stress conditions in the following variants to study the maize sprouts' control sample and the samples affected by individual stress factor and combined stress conditions comprised three different factors, i.e., gamma radiation (250, 500, and 750 Gy), NaCl solution (100 mM), and iron oxide nanoparticles (Fe_2O_3).

Irradiation with ionizing gamma rays utilized the RUHUND-20000 device with Co^{60} on the variants, exposing it to ionizing gamma radiation at doses of 250, 500, and 750 Gy. The irradiated maize seeds received treatments of γ-Fe_2O_3 (Iron Oxide Nanoparticles gamma-Fe_2O_3, 99%, 20-40 nm, SkySpring Nanomaterials, Inc. 2935 Westhollow Dr., Houston, TX 77082) nanoparticles using a vortex blender. Seed cultivation in soil continued for 15 days in the Plant Growth Chamber (GVS 940) at room temperature (23 °C), 70% relative humidity, 300 μmol m^{-2} s^{-1} light intensity, and with 16/8 hours (light/dark) regime. Calculations for recorded biometric indicators of sprouts and identification of enzyme activity progressed every five days. Enzyme activity determination spectrophotometrically had a wavelength of 340 nm, based on the rate of reduction of NADP in an MRC (Israel) spectrophotometer. The μM NADPH/min/g wet weight was the basis for the enzyme unit. The reaction ran at 23 °C, with measurements repeated 3-5 times. The tissue extraction solution was at a ratio of 1 g: 5 ml.

The 100 mM Tris-HCl (pH 7.5) buffer solution containing 5 mM $MgCl_2$, 0,5 mM EDTA, 10 % glycerol, 10 mM mercaptoethanol, and 1 mM phenylmethylsulfonyl comprised the preparation of the DMDH enzyme, and 50 mM Tris-HCl (pH 7.0) solution containing 10 mM $MgCl_2$, 0.5 mM NADP, and 4 mM malate determined the activity of the said enzyme.

After drying, formulation of the organic and inorganic content and wet and dry weight of plant roots and stems proceeded separately. Determining heavy metals ensued using the ICP-MS and atomic absorption spectrometer (Sequential X-ray Fluorescent Spectrometer (S8 TIGER)). Drying maize plant samples in an electric oven at 65 °C continued with grinding in an electric ball mill to obtain the mass (Herzog). Taking 2 g of plant powder gained the addition of 2 ml of solid nitric acid, with the resulting solution kept in a closed oven for a day. Afterward, the specimen drying at 65 °C temperature had them placed in a microwave oven (Sineo MBES 86), and kept at 150 °C – 165 °C temperature for one hour. Then, transferring the solutions into graphite cups, they continued drying at 65 °C temperature until a moist residue resulted. After adding the liquid 2% HNO_3 to each of the samples, the samples' filtration on filter paper continued to transfer them to volumetric flasks. After storing the prepared samples for two hours, their measurements ran in the ICP-MS apparatus.

Statistical analysis

The experiment employed three biological replicates, with each replicate reproduced thrice independently. A statistical compilation of the data ensued using the licensed IBM SPSS Statistics software package. Assessing the reliability of arithmetic means variations was dependent on the Student's coefficient. Differences among the various groups were significant at the two-tailed significance level ($p \leq 0.05$). The diagram construction employed the Graph Pad Prism-8 software.

RESULTS AND DISCUSSION

The effects of combined stress conditions comprising different doses of gamma radiation (250, 500, and 750 Gy), NaCl solution (100 mM), and nanoparticles maghemite (γ- Fe_2O_3) on the maize (*Z. mays* L.) genotypes attained scrutiny. The morpho-physiological characteristics of the maize plants and the activity dynamics of the decarboxylating-malate dehydrogenase enzyme (DMDH, ME, EC: 1.1.1.40) gained studying in abiotic stress variants and the control treatment.

Following the experiments, plant growth weakened by a high percentage compared with the control variant under the effects of individual and combined stress factors. The effect of gamma radiation (250 Gy) delayed plant growth, while an increased radiation dose (500 and 750 Gy) further deepened these effects and became lethal. Both the irradiation and salt solution variants undermined the maize plant growth. However, adding the maghemite nanoparticles (γ- Fe_2O_3) to the medium produced a relatively stimulating effect in the plants. However, the nanoparticles could not prevent the delay in the variants with high doses of gamma rays and salt solution. The work (Martin *et al.*, 2017) shows that treating plants with maghemite accelerates plant and leaf development and increases chlorophyll content.

Adding maghemite also decreases hydrogen peroxide and MDA in the leaves after drought. Hematite, a less enzymatic form of iron oxide, helped increase catalase activity, which is effective under stress conditions, as stressful conditions cause toxic accumulation of H_2O_2. Other functions, such as hastening leaf growth rate, are crucial properties in agriculture. These results are consistent with this study's outcomes.

The gamma rays' effects, compared with the control variant, weakened the growth of the root and stem system of the maize plants by 36% and 32% with 250 Gy, 83% and 81% with 500 Gy, and 84% and 82% with 750 Gy, accordingly (Figure 1A, B). The combined effects of gamma rays and salt stress formed a more severe inhibitory effect on the maize plant development. Thus, the combined effects of gamma radiation at the doses of 250, 500, and 750 Gy and salt solution caused a considerable delay in the maize roots and stem system progress by 74%, 84%, 86% and 78%, 87%, and 88%, respectively. The growth of the root and stem system of the maize sprouts exposed to gamma radiation with the 250, 500, and 750 Gy doses and treated with magnetic nanoparticles has slowed down by 34%, 79%, 82%, and 31%, 80%, and 81%, compared with the control variant.

The combined effects of all three abiotic stress factors on the root and stem system of the maize plants made an inhibitory effect of 77%, 84%, 85% and 83%, 88%, and 89% with different gamma-ray doses (250, 500, and 750 Gy). Since 500 and 750 Gy doses of gamma rays destroyed the plant, the evaluation of enzyme activity became unfounded, with the experiments continued with the study comprising only the effects of gamma rays at the dose of 250 Gy. The gamma-ray effect on corn seeds at 250 Gy resulted in a 33%–34% delay compared with the control variant in the maize plant growth. Adding maghemite nanoparticles to the medium slightly reduced the stress effects, and even provided a stimulating effect. However, in the variants with the salt solution (NaCl 100 mM), a stimulating result did not appear.

On the contrary, a retarding effect was noticeable. In the combined stress conditions developed by the influences of gamma rays (250 Gy) and salt stress (NaCl 100 mM), the weakening of maize plant growth has been extremely sharp, whereas adding iron-oxide magnetic nanoparticles (γ-Fe_2O_3) to the medium could not prevent the delay in progress of maize plants. The DMDH enzyme

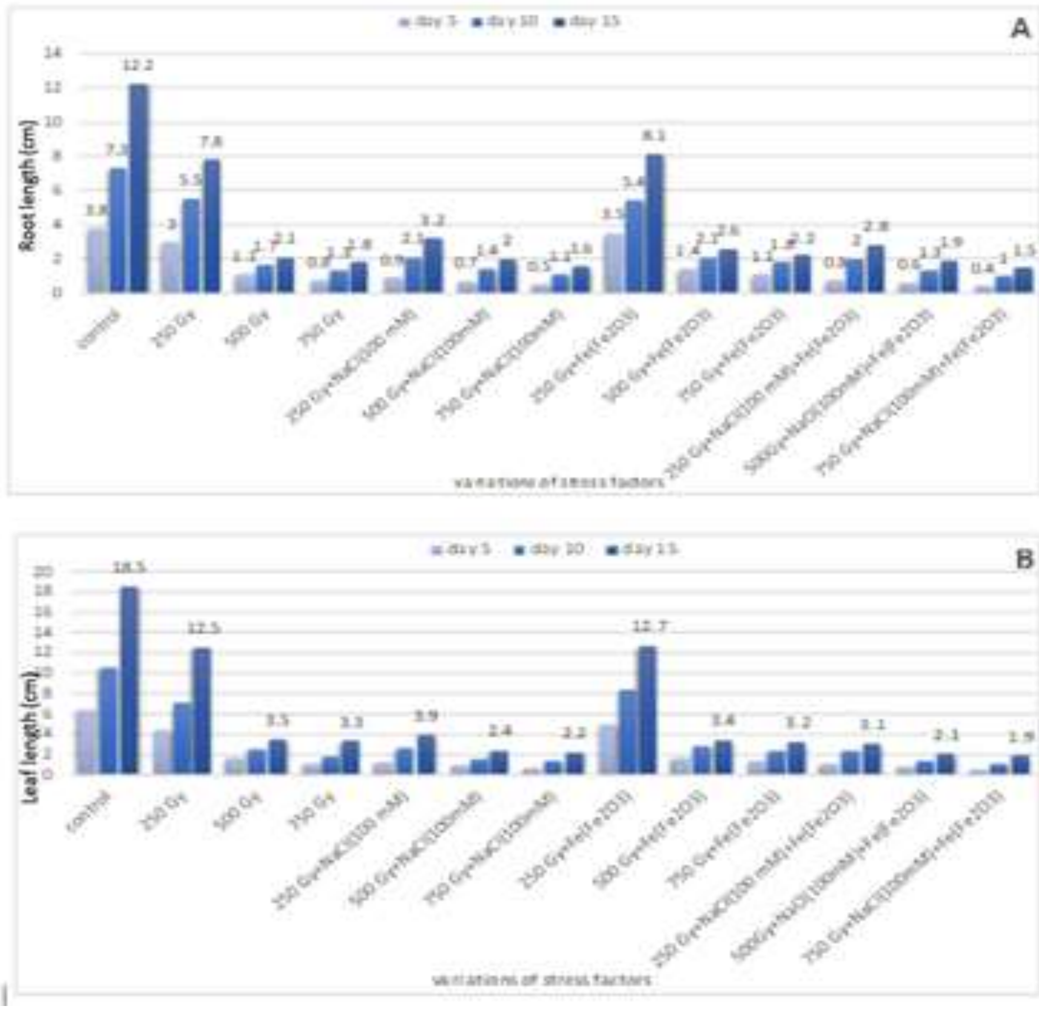

Figure 1. Effect of stress conditions developed individually and with combination of different abiotic stress factors (γ-irradiation (250, 500, and 750 Gy), NaCl solution (100 mM), and iron oxide (Fe_2O_3) nanoparticles on the growth of roots (A) and leaves (B) of maize plants.

(ME, EC:1.1.1.40) activity regarding maize plant growth has shown an increasing pattern at the beginning of the experiment (fifth and 10th day) in the root and stem tissues under combined stress conditions (gamma rays, salt solution, and nanoparticles) and control (Figure 2). With stress-persistence conditions, inhibition of enzyme activity has appeared in all the studied variants since day 15 of the experiment. The stress exacerbation has strengthened the intensity between induction and inhibition of the DMDH enzyme. The abiotic stress conditions developed by the combined effects of gamma rays, salt solution (NaCl 100 mM), and iron oxide nanoparticles (γ-Fe_2O_3) have altered the metabolic characters of the maize plants and also affected the values of organic and inorganic content in roots and leaves. These values are available in the graphs (Figures 3A, B and 4A, B).

A work (Junli *et al*., 2016) examined the absorption and distribution of γ-Fe_2O_3 NPs in corn (*Zea mays* L.) and determined its effect on seed germination, antioxidant enzyme activity, malondialdehyde (MDA) content, and chlorophyll content. The 20 mg/L γ-Fe_2O_3 NPs significantly promoted root elongation by 11.5% and increased the germination index and viability index by 27.2% and 39.6%,

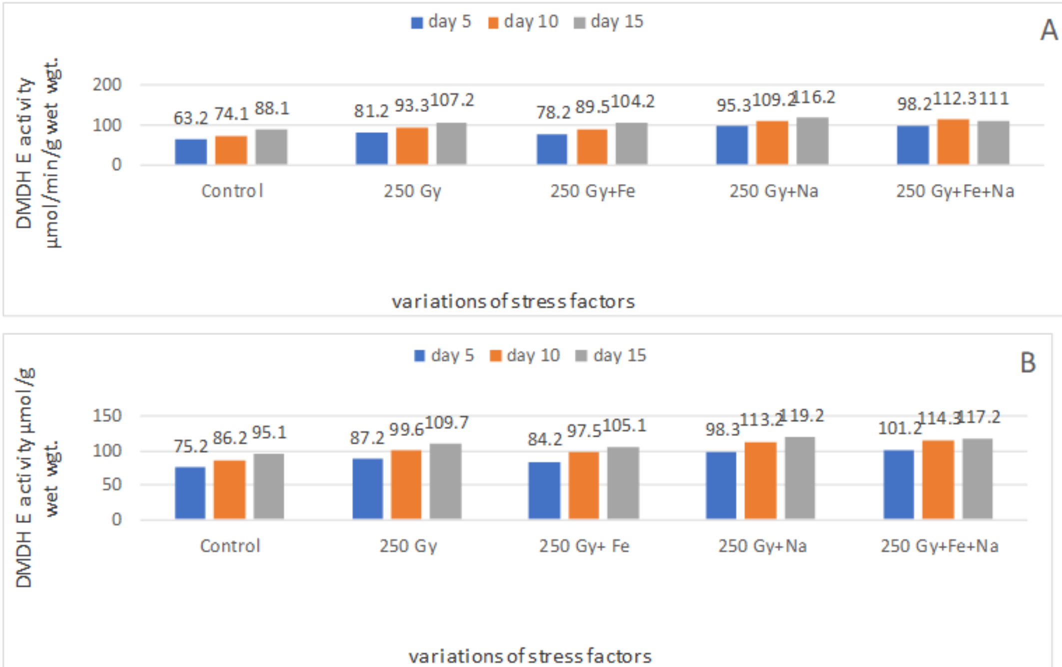

Figure 2. Activity dynamics of DMDH enzyme in root (A) and leaf (B) cells of maize plants under the individual and combine effects of gamma rays (250 Gy), NaCl solution (100 mM), and iron oxide (γ-Fe_2O_3) nanoparticles.

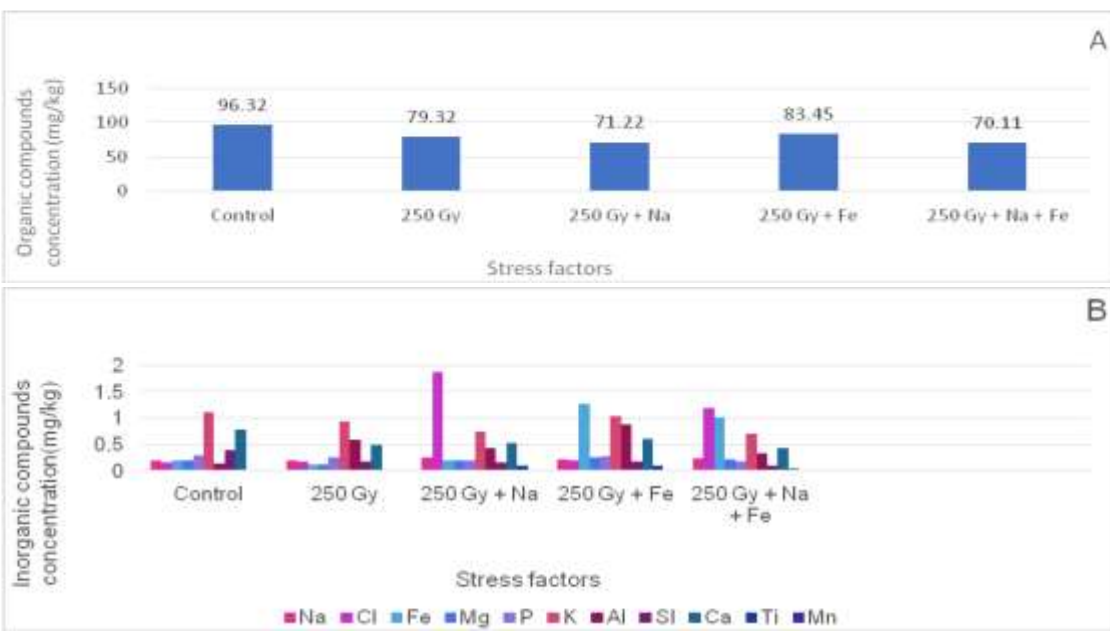

Figure 3. Analysis (organic [A] and inorganic [B]) (mg/kg in ppm) of maize plant's samples (dried at 105 °C) under stress conditions developed by the individual and combined effects of gamma rays (250 Gy), salt solution (NaCl 100 mM), and iron oxide (γ-Fe_2O_3) nanoparticles in root cells of maize genotype Zagatala-420.

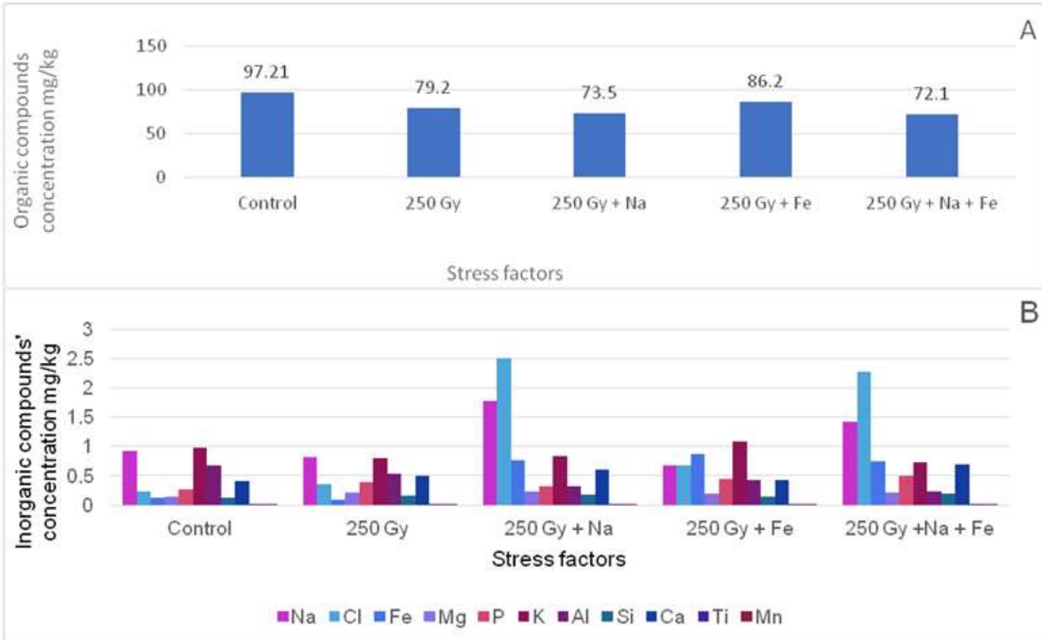

Figure 4. Analysis (organic [A] and inorganic [B]) compounds (mg/kg in ppm) of maize plant's samples (dried at 105 °C) under stress conditions developed by the individual and combined effects of gamma rays (250 Gy), salt solution (NaCl 100 mM), and iron oxide (γ-Fe_2O_3) nanoparticles in leaf cells of maize genotype Zagatala-420.

respectively. However, γ-Fe_2O_3 NPs at concentrations of 50 and 100 mg/L significantly reduced root length by 13.5% and 12.5%, respectively. In addition, evidence that γ-Fe_2O_3 NPs cause oxidative stress emerged exclusively in the root. Exposure to different concentrations of NPs caused markedly high levels of MDA in maize roots, and MDA levels in maize roots treated with γ-Fe_2O_3 NPs (20-100 mg/L) were 5–7 times higher than in the control. At the same time, the chlorophyll content during treatment with NPs decreased by 11.6%, 39.9%, and 19.6%, respectively, compared with the control group. Fluorescence and transmission electron microscopy (TEM) images showed that γ-Fe_2O_3 NPs could penetrate plant roots and migrate apoplectically from the epidermis to the endodermis and accumulate in the vacuole. In addition, the study found that NPs mainly exist around the root epidermis, and no translocation of NPs from roots to shoots manifested. These results are valuable for understanding the fate and physiological effects of γ-Fe_2O_3 NPs in plants.

In comparison to the control variant, the effects of various doses of gamma rays have reduced the organic matter of maize plants (Figures 3 and 4). An observation also has shown that this difference was greater in the variants added with NaCl solution. Even though applying magnetic nanoparticles to variants exposed to gamma rays has reduced these effects, observations were in variants exposed only to gamma rays and NaCl solution. The comparison of the inorganic content in the control and stressed variants showed that individual elements have different indicators in the maize roots and leaf tissues. The said phenomenon can be due to the effects of stress conditions on various biochemical mechanisms of the maize plants, including photosynthesis.

A study (Vladimir *et al.*, 2022) revealed the effect of treating wheat seeds with various concentrations of iron nanoparticles Fe_3O_4 and Fe_2O_3 on biomass accumulation, the rate of photosynthesis and respiration, photochemical activity, and antioxidant balance. The seeds had the following procedure: treated for 3 h, germinated for two days in Petri dishes, transplanted into the sand, and grown under light for 18 days without mineral nutrition until the third leaf appeared. At a Fe_3O_4 concentration of 200 mg L^{-1}, a significant increase in the dry biomass of the second leaf by 45% and the rate of photosynthesis by 16% was evident. At a concentration of nanoparticles in the form of Fe_2O_3 at 200 and 500 mg L^{-1}, an increase in the rate of photosynthesis in the second leaf also occurred but not in the biomass of the leaves. The activity of photosystem 2, estimated from the Fv/Fm value, also increased in experiments with nano iron. However, the activity of antioxidant enzymes, guaiacol-dependent peroxidase and superoxide dismutase, decreased. An assumption is that the acceleration of growth at an early stage of wheat development correlates with increasing photosynthetic processes (Abdul-Mohsin and Farhood, 2023; Mahdi *et al*., 2024).

CONCLUSIONS

With combined stress conditions, the inhibition of the DMDH enzyme activity was notable in all variants from the 15th day of the maize experiment. Compared with the control variant, all stress factors negatively affected the organic compounds of the plant, especially during the combinative effect. Although, the application of magnetic nanoparticles (γ-Fe_2O_3) only to the variants exposed to low gamma rays (250 Gy) reduced this effect; however, such effects were not apparent in the variants with combined exposure to low gamma rays (250 Gy) and salt solution. Outcomes of maize seeds exposure to gamma rays at the rate of 250 Gy revealed a delay in the plant growth by 33%–34% compared with the control treatment. Adding iron oxide nanoparticles to the medium slightly reduced the influence of stress conditions, with the stimulating effect of nanoparticles emerging. Under the combined stress conditions developed by maize plants' exposure to the gamma ray (250 Gy) and NaCl stress (100 mM), the weakening of plant growth was extremely sharp, and even applying nanoparticles could not prevent the retardation of maize plants growth.

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