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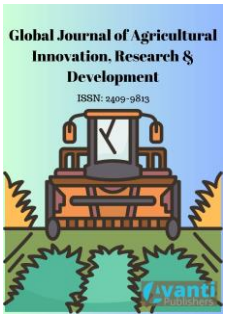

Investigating the Effect of Static Magnetic Field and Magnetic Iron Oxide Nanoparticle on Enzymatic Antioxidant Defense in *Dracocephalum polychaetum* Cell Suspension Culture

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ABSTRACT

This study was conducted to investigate the effect of magnetic iron oxide nanoparticles (MNP) and static magnetic field (SMF) on the activity of antioxidant enzymes in the cell suspension culture of *Dracocephalum polychaetum* (Lamiaceae family). The treatment procedure was done by cultivating the cells either with 100 ppm MNP, SMFs, or simultaneous exposure to both MNP and SMFs. The SMF at 30 mT was uniformly applied to the cells either for 3 or 4 days with 3 hours per day or 5 hours per day intervals, respectively. The highest activity of polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase (CAT), malondialdehyde (MDA) content, and electrical conductivity (EC) were observed under the elicitation of the cells with simultaneous exposure to both MNP and SMFs, but the highest amount of FRAP value was observed under the elicitation of the sample with the MNP treatment. Also, the results of this study showed that the greatest activity of peroxidase (POX) was observed under SMF and MNP treatments. In general, SMF and MNP treatments caused various changes in cell structure and metabolism by inducing oxidative stress and having a direct effect on the membrane. The cell activated its enzymatic antioxidant defense system in response to these treatments, which caused changes in its activity and amount compared to the control cell.

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1. Introduction

Plants are usually exposed to a wide range of environmental stresses during their life cycle, among which magnetic fields and nanoparticles can be mentioned. Magnetic fields are undoubtedly an integral part of modern life today. The static magnetic field exists naturally (Earth's magnetic field) and artificially (resulting from electric and magnetic devices) in the environment around plants and other living organisms [1, 2]. In our living environment, magnetic fields with very low frequency exist in the range of 0.01 to 1 mT. The intensity of these fields reaches 10 and 30 mT near power lines. Since the use of magnetic and electric devices in medicine, industry, and daily life is increasing rapidly, the effect of magnetic fields on living systems, especially in recent years, has attracted attention. There are contradictory reports on the effects of magnetic fields on living systems. Almost in most studies, the magnetic field has caused a decrease in germination percentage [2]. However, the exact mechanism of the effect of magnetic fields on living cells has not been determined yet. The inhibitory or stimulatory effect of the magnetic field on plant growth and development depends on the plant species, type of magnetic field, intensity and duration of the magnetic field, and its duration. There is a potential correlation between the magnetic field and its effects on living organisms, so the magnetic field directly affects the cell membrane and indirectly causes a kind of oxidative stress that increases the activity, concentration, and lifespan of free radicals [1]. Free radicals as a consequence of oxidative stress have a dual role: on one hand, they cause cell damage and on the other hand act as a signal molecule to initiate cell defense mechanisms [3]. Also, the magnetic field can cause changes or increase the activity of enzymes such as catalase, peroxidase, polyphenol oxidase, and superoxide dismutase. Alterations of the enzymatic activities, such as superoxide dismutase, catalase, and peroxidase, by an external magnetic field suggest their potential function as magnetoreceptors. Thus, another proposed mechanism for how the magnetic field works is by affecting materials with magnetic properties, the most important of which are ferromagnetic materials such as iron and diamagnetic materials such as starch. Based on previous studies, the effect of a 30 mT magnetic field on biological systems has been studied in the past on plant and animal cells [4]. The intensity of 30 mT magnetic fields has been introduced as the lowest threshold intensity for displacing magnetic materials in living organisms [5].

One of the other non-biological stimuli is nanoparticles. The effects (positive or negative) of nanoparticles on higher plants depend on the physical and chemical properties of nanoparticles and the species studied. Previous studies have shown that nanoparticles can cause free radicals production and ultimately oxidative stress in cells. Also, the attachment of magnetic nanoparticles to the membrane causes changes in membrane activity and structure, channels and membrane transporters, and ultimately changes in permeability and integrity of the membrane and ion exchange [6]. Magnetic iron oxide nanoparticle, like iron metal, has a supermagnetic property that becomes highly magnetic under a magnetic field [4]. Magnetic iron oxide contains reduced iron and oxidized iron, which is often described as iron II, and III oxide. Iron as an essential element has a dual role in plants: on the one hand, it participates in oxidation-reduction reactions and the structure of many intracellular enzymes such as catalase, peroxidase, superoxide dismutase, and on the other hand, it produces oxygen species through the Haber-Weiss reaction. Therefore, regulating cellular iron content is very important in this situation. However, increased iron in cells can be toxic to cells, so plants adopt different mechanisms to remove excess iron from cells in various ways [7]. Based on previous studies, iron as a ferromagnetic material can be affected by a magnetic field. The magnetic field may affect the iron homeostasis in certain cells, resulting in increased free iron in the cytoplasm and nucleus. *D. polychaetum* is a medicinal plant from the mint family, endemic to Iran and the Kerman province, which is used in the traditional medicine of the Kerman region due to its pleasant smell and as an anti-stomachache (3). Despite the few studies on the effects of magnetic fields and magnetic nanoparticles, their specific effects on plant defense systems are still unclear. This study was conducted to investigate the effects of physical treatment (static magnetic field), chemical treatment (a magnetic iron oxide nanoparticle), and co-treatment on enzymatic antioxidant defense system responses upon these treatments in *D. polychaetum* cells. It may help us increase human knowledge in the field of plant physiology’s response to magnetic fields, magnetic nanoparticles, and co-treatment.

2. Materials and Methods

2.1. Optimization of Cell Suspension Culture of *D. polychaetum*

The seeds of *D. polychaetum* collected from the Hezar mountains, in the southeast of Kerman (altitude of about 4000 meters), were transferred to the laboratory. Sterilized seeds were transferred into the Murashige and Skoog medium (MS) [8] containing 30 g L^{-1} of sucrose and 8 g L^{-1} of agar with a pH of 5.8, along with 25 mg L^{-1} of gibberellin. Then seeds were placed in a germinator under 16 hours light / 8 hours dark, 60% humidity at 23 ± 1 °C conditions. After six weeks in laboratory conditions, leaf samples were cultured on a MS medium containing 30 g L^{-1} of sucrose and 8 g L^{-1} agar with a pH of 5.8, with 1 NAA mg L^{-1} + 4.5 BAP mg $L^{-1}$and placed in a growth chamber under dark condition, at 23 ± 1 °C and 60% humidity. For cell suspension culture, 2.5 g of suitable callus were poured into 100 mL Erlenmeyer flasks containing 25 mL of liquid culture medium. Cells were stored in suspension culture medium at 25 ± 1 °C in darkness on an orbital shaker at 120 rpm and sub-cultured every 10 days at their logarithmic growth phase After several times sub-culturing them, a suitable suspension culture was provided for studying the growth curve and environmental treatments [9].

2.2. Cell Treatment with Magnetic Field

For elicitation purposes, the SMF intensity commonly ranged from 10 to 50 mT, and the field intensity of 30 mT was frequently applied to plant cell models [10, 11]. The elicitation period was chosen based on some preliminary experimental works and a literature review [12]. Exposure to SMF was performed by a locally designed SMF generator. The elicitation groups were located in SMF producing system (30 mT), and control cells were kept far from the system, and they were exposed only to the very low SMF of the earth (about 60 μT, according to the Geophysics Institute of Tehran University). The present study was undertaken to test the effects of static magnetic fields on *D. polychaetum* cells when the cells are in their exponential growth phase (from day 7 to 10 after sub-cultures). The cells were exposed to 30 mT SMF in their exponential growth phase for 3 days at 3 h per day interval (so-called SMF1) and for 4 days at a 5 h per day interval (so-called SMF2). Control cells were kept in similar conditions but away from the magnetic field. Every day after treatment, the samples were transferred to the shaker.

2.3. Cell Treatment with Magnetic Iron Oxide Nanoparticles (Fe_3O_4 MNPs)

The Fe_3O_4 MNP was supplied from the US Research Nanomaterial Inc., Houston, TX, USA; according to the manufacturer, the diameter of Fe_3O_4 MNP was 10-20 nm and purity 99.99%. For uniform distribution and dispersion of magnetic iron oxide nanoparticle, 0.05% dimethyl sulfoxide (DMSO) solvent and an ultrasonic bath was used. The DMSO was selected because it is a significant polar aprotic solvent dissolving both polar and noMNPolar compounds. Moreover, it is miscible in many organic solvents as well as in water [13]. Magnetic iron oxide nanoparticle was poured into DMSO solvent and deionized water and placed in an ultrasonic bath for thirty minutes. The resulting solution was added under sterile conditions to the Erlenmeyer flasks containing seven-day-old cells and the samples were transferred to the shaker room. to omit the effects of DMSO on the parameters, DMSO was only added to one group of cells and considered as the second control group.

2.4. Cell Treatments with Fe_3O_4 MNPs and SMF

The cells in their exponential growth phase (7 days after cell subculture) were treated with 100 ppm Fe_3O_4 MNPs. These samples were then divided into two groups: One group containing seven-day-old cells was placed in the SMF generating apparatus under 30 mT SMF for 3 days at 3 h per day intervals (MNPs+ SMF1) and the other was exposed to the same SMF for 4 days at 5 h per day intervals (MNPs+ SMF2). The control cells consisted of two groups, the first group was grown in the culture medium without Fe_3O_4 MNPs, and the second group was grown in the culture medium with 0.05% DMSO, then they kept far from the SMF-producing system.

At the end of all elicitations, the cells were separated from the suspension cultures by filtration via a Buchner funnel with a nylon mesh under vacuum, and after weighing, they were fixed in liquid nitrogen and stored at -80 °C for subsequent analyses.

2.5. Measurement of Electrical Conductivity

The electrical conductivity of the cell-free culture medium was measured at room temperature using an EC meter.

2.6. Malondialdehyde (MDA) Measurement

The extent of damage to the membranes was determined by measuring the amount of MDA as the final product of lipid peroxidation of cell membranes. 200 mg of frozen cells were crushed with 3 mL of 10% trichloroacetic acid (TCA). The samples were then centrifuged for 15 minutes at 12000 rpm. 1 mL of 25% thiobarbituric acid (TBA) was added to 1 mL of the filtered samples and placed for 30 min at 100 °C and then immediately cooled on ice. The amount of MDA was calculated by measuring the absorption at wavelengths of 532 nm and 600 nm using a constant coefficient ε = 155 $mM^{-1}cm^{-1}$ [10].

2.7. Measurement of Iron-Reducing Power (FRAP)

200 milligrams of frozen cells were homogenized in methanol and centrifuged for 10 minutes at 12000 rpm. Phosphate buffer M 0.2 (pH: 6.6) and 1% potassium ferricyanide solution were added to the resulting extract and placed in a warm water bath at 50 °C for 20 minutes. Then 10% trichloric acid was added and centrifuged for 20 minutes at 12000 rpm. Finally, supernatant, deionized water, and 0.1% ferric chloride were mixed and the absorption of aliquot was read at 700 nanometers. The reducing power was calculated as a percentage compared to the blank [11].

2.8. Enzyme Extraction and Activity Measurement

200 mg of frozen cells were extracted in 50 mM tris buffer with pH 6 and centrifuged at 12000 rpm for 20 minutes at 4°C [14]. The supernatant was used to measure enzyme activities.

2.8.1. Peroxidase (POX)

The reaction mixture contained 60 mM sodium phosphate buffer (pH 6.1), 28 mM guaiacol, 5 mM H_2O_2, and 250 µl enzyme extract. Enzyme activity was calculated as the increase in absorbance at 470 nm per minute per milligram of protein in the sample [15].

2.8.2. Catalase (CAT)

The reaction mixture contained 25 mM sodium phosphate buffer (pH 6.8), 10 mM H_2O_2 and 200 µl enzyme extract. Enzyme activity was measured by the decomposition of H_2O_2 and the decrease in absorbance at 240 nm per milligram of protein in the enzyme extract [16].

2.8.3. Superoxide Dismutase (SOD)

200 mg of frozen cells were extracted in 50 mM KOH-HEPES buffer pH 8.7 containing 0.1 mM EDTA and centrifuged at 12000 rpm for 20 minutes at 4 °C. The supernatant was used to measure SOD activity. The reaction mixture contained 50 mM KOH-HEPES buffer pH: 8.7 containing 0.1 mM EDTA, 50 mM Na_2CO_3, 12 mM L-methionine, 75 µM NBT (Nitro Blue Tetrazolium chloride), 1 µM riboflavin and 250 µl of enzyme extract. The tubes were exposed to light for 10 minutes. Their absorption before and after exposure to light was measured at 560 nm. Measuring the activity of this enzyme also requires a light control sample. The enzyme activity was measured in comparison with the light control sample. Due to the lack of enzyme in this tube, NBT reduction in the presence of light was completely performed and all nitroblue tetrazolium present in the reaction mixture was converted to formazone therefore, the color of this tube was darker than the rest. The OD of this sample at 560 nm shows a 100% light reduction of NBT. The difference in absorption of samples and light control sample shows the inhibition of NBT light reduction in the presence of superoxide dismutase enzyme in samples. Based on this method, one unit of superoxide dismutase enzyme is the amount of enzyme that causes 50% inhibition of light reduction of NBT. SOD enzyme activity was expressed as an enzyme unit per milligram protein extract [17].

2.9. Polyphenol Oxidase (PPO) Activity

For extraction of polyphenol oxidase solution, 200 mg frozen cell was homogenized in 20 mM sodium phosphate buffer (pH: 8.6) on ice, and then the samples were centrifuged at 12000 rpm for 15 minutes. The supernatant was used to measure PPO activity. The reaction mixture consisted of 20 mM sodium phosphate buffer (pH: 6.5), 20 mM 4-methyl catechol and enzyme extract. The absorption of the samples was read over one minute at 410 nm and the enzyme activity was expressed in milligrams of protein [18].

2.10. Phenylalanine Ammonia-lyase (PAL)

PAL activity was determined by homogenizing a 200 mg frozen cell with *β*-mercaptoethanol (15 mM) in Tris-HCl (pH 8.2, 100 mM). The homogenate was centrifuged at 10000 rpm for 20 min, and the supernatant was collected for enzyme assay. To 1 mL of the extraction buffer, 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of double distilled water, and 0.1 mL of enzyme extract were added and incubated at 37 °C for 1 h. The reaction was stopped by adding 0.1 mL of 6 M HCl, and the product *i.e.,* cinnamic acid was extracted three times with ethyl acetate, followed by evaporation to remove the extracting solvent. The solid residue was suspended in 3 mL of sodium hydroxide (0.05 M) and the cinnamic acid concentration was quantified spectrophotometrically at 290 nm. One unit of PAL activity was considered equal to 1 µmol of cinnamic acid produced per min per mg of protein [19]. Total protein was determined according to the Bradford assay using bovine serum albumin as the standard [14].

2.11. Statistical Analysis

The experiments followed a completely randomized design, and the values were presented as means ± SE (standard error). All the experiments and observations were replicated three times with at least three samples. The significant differences between treatments were evaluated using the Duncan's test at $p \leq 0.05$.

3. Results

3.1. Electrical Conductivity

Based on the results, treatments applied in this research project caused a significant increase in the electrical conductivity of the culture medium compared to the control medium. EC was much higher in the NP+MF1 treatment (176.57) than in other treatments. The lowest electrical conductivity was related to the control (143) and iron oxide nanoparticle treatment (Fig. **1a**).

3.2. MDA Content

Based on Fig. (**1b**), iron oxide nanoparticle and magnetic field treatment caused an increase in lipid peroxidation of the membrane compared to the control cell, so that the highest amount of MDA was related to the cells under MNP+SMF2 treatment (2.39 µmol. g^{-1} FW). The amount of MDA in the magnetic field and iron oxide nanoparticle treatments did not show a significant difference, but with the addition of magnetic iron oxide nanoparticles, the amount of MDA was increased.

3.3. CAT Activity

Catalase is one of the important enzymes involved in removing H_2O_2 radicals. Changes in CAT enzyme activity after treatment with iron oxide nanoparticles and magnetic field alone and together are shown in Fig. (**1c**). The highest increase in enzyme activity was related to the combined treatments of magnetic field and magnetic iron oxide nanoparticles compared to the control. The highest CAT activity including 2.37 and 2.24 (Abs 240/mg protein), were observed in MNP+SMF2 and MNP+ SMF1 treatments respectively. Also, the enzyme activity in SMF2 and MNP treatments decreased, while no change was observed in SMF1 treatment compared to the control.

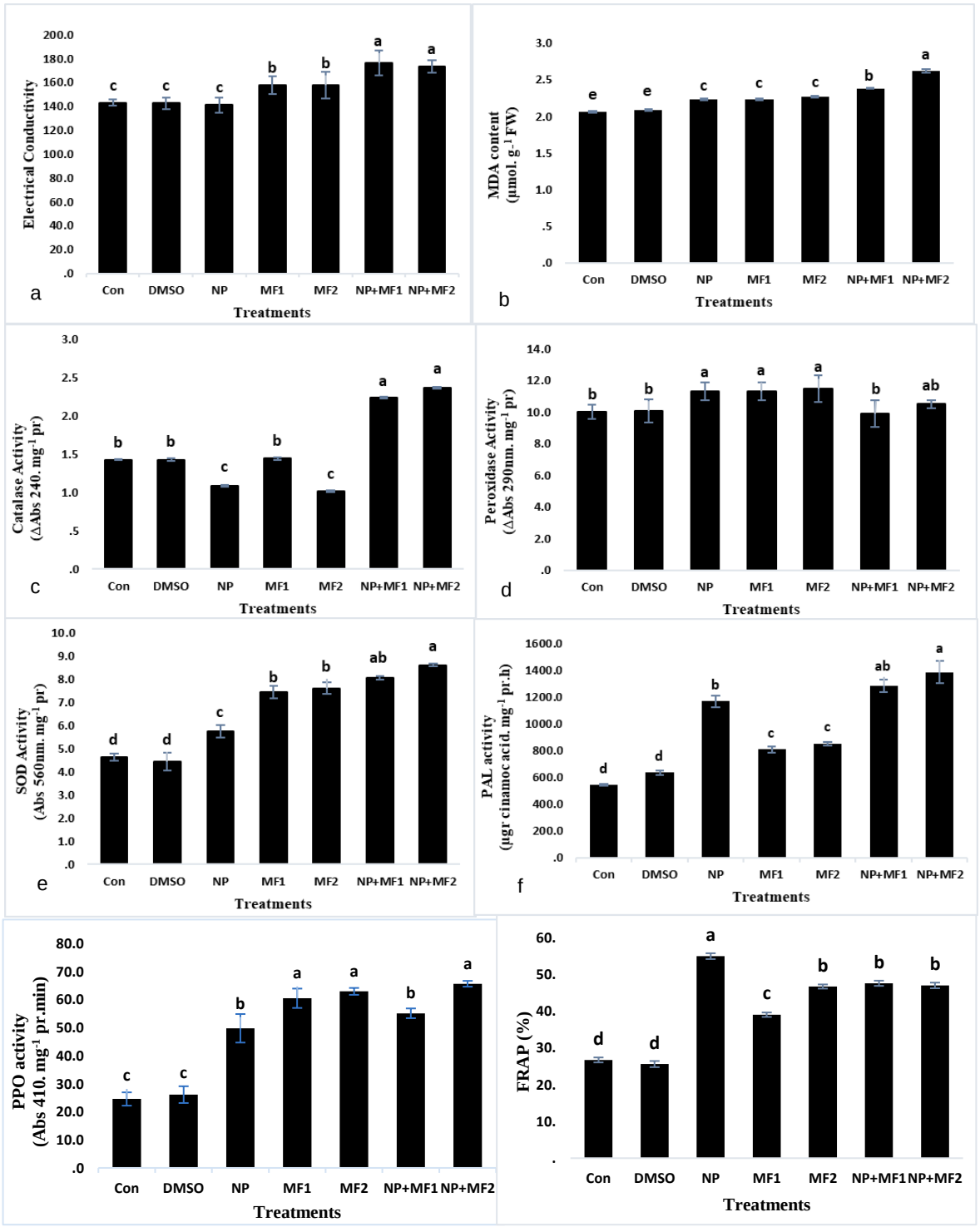

Figure 1: Effect of magnetic field and iron oxide nanoparticle on electrical conductivity of culture medium (a), MDA amount (b), catalase activity (c), peroxidase activity (d), superoxide dismutase activity SOD (e), phenylalanine ammonia lyase activity PAL (f), polyphenol oxidase activity PPO (g) and FRAP analysis (h), in *D polychaetum* cell culture. Control (Con). Control with DMSO (DMSO) 0.05%. Iron oxide nanoparticle 100 ppm (MNP), magnetic field 30 mT, three days, three hours a day (SMF1), magnetic field 30 mT, four days, five hours a day (SMF2), magnetic field 30 mT, three days, three hours a day with 100 ppm iron oxide nanoparticle (MNP+SMF1), magnetic field 30 mT, four days, five hours a day with 100 ppm iron oxide nanoparticle (MNP+SMF2).

The values are mean of at least 3 replicates and ±SE. Different letters indicate significant differences at P>0.05.

3.4. POX Activity

The values related to POX enzyme activity are shown in Fig. (**1d**). Based on the results, the activity of this enzyme did not change significantly in the combined treatments of MF+NP compared to the control, while it increased in the MF and NP treatments. According to the result, the highest enzyme activities including 11.49, 11.32, and 11.3 (Abs 290 nm/mg protein) were observed in SMF2, SMF1, and MNP treatments, respectively.

3.5. SOD Activity

The pattern of changes in SOD enzyme activity in control and iron and magnetic field treated cells separately and together is shown in Fig. (**1e**). Based on the results, the activity of SOD increased by all treatments, so that the enzyme activity reaches its maximum level in cells treated with MNP+SMF2 as recorded as 8.62 (Abs 560 nm/mg protein). The lowest amount of enzyme activity was related to control cells of 4.64 (Abs 560 nm/mg protein). The lowest amount of enzyme activity was related to control cells (4.64 Abs 560 nm/mg protein). The activity of this enzyme in magnetic field treatments showed a higher increase than in iron oxide nanoparticle treatment.

3.6. PAL Activity

The results showed that PAL activity increased after treatment with magnetic iron oxide nanoparticles and magnetic field so that the increase was especially higher in MNP+SMF2 (1088.1 µg CA. mg^{-1} proteins. h^{-1}) and MNP+SMF1 (1021.4 µg CA. mg^{-1} proteins. h^{-1}). These treatments resulted in 1.79 and 1.7-fold increase in PAL activity compared with control cells, respectively. Based on the results (Fig. **1f**) the enzyme activity in magnetic iron oxide nanoparticle treatment showed more increase than magnetic field treatment.

3.7. PPO Activity

Fig. (**1g**), shows the PPO activity which it increased in cells under all applied treatments compared to the control. The highest increase in PPO activity was observed in SMF1 (59.79 Abs 410 mg^{-1} proteins. min^{-1}), SMF2 (62.23 Abs 410. mg^{-1} proteins. min^{-1}) and MNP+SMF2 (65.67 Abs 410. mg^{-1} proteins. min^{-1}) treatments. The PPO enzyme activity did not show a significant change in MNP+SMF1 and MNP treatments.

3.8. FRAP Analysis

Based on the results given in Fig. (**1h**), the FRAP value increased significantly under all magnetic treatments compared to the control. The highest amount of FRAP was related to cells treated with MNP (56.43%). This treatment resulted in 2.04- a fold increase in the FRAP value compared with control cells. No significant difference was observed in FRAP content in cells under SMF2 and combined treatments.

4. Discussion

In the present study, the magnetic field (30 mT) caused an increase in membrane permeability, changes in the activity of catalase, superoxide dismutase, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes, changes in electrical conductivity and MDA content of cells. The mechanism of the effect of magnetic fields on living cells has not been determined precisely yet, but the effect of magnetic field on cells depends on the type and intensity of the magnetic field, and duration of exposure to the field [1]. Changes in the electromagnetic properties of biological molecules of cells and the electric potential of the membrane ultimately lead to changes in membrane integrity and permeability [20, 21]. Also, a magnetic field has been shown to increase the activity and lifespan of free radicals which causes oxidative stress. Free radicals have a dual role. On the one hand, they cause damage in cells and on the other hand, they act as signal molecules and cause activation of defense mechanisms in cell [22]. Based on previous results, detoxification of H_2O_2 happens by antioxidant enzyme system such as catalase and peroxidase and non-enzymatic system. At the enzyme level, it has been shown that a magnetic field leads to an increase in the activity of CAT, PPO, POX, and SOD. [1, 13, 23, 24]. Another proposed mechanism explaining how a magnetic field works is through its effect on materials with magnetic properties, most importantly ferromagnetic materials such as iron and diamagnetic materials such as starch

which perceive field by iron-containing compounds and chemical receptors. Therefore, according to previous results, in this study, the magnetic field may induced cellular response through a direct effect on membrane and cellular molecules, as well as creating oxidative stress in cells, which ultimately has changed cellular structure and function such as enzyme activity, membrane structure in response to magnetic field effects. Magnetic fields probably affect unpaired electrons of metals in the structure of these enzymes and affect their biological activity by changing their spatial structure. SOD, POX, and CAT are among the enzymes that can be affected by magnetic field due to having iron in their structure [25-27]. Previous studies have shown that catalase probably plays a more effective and prominent role in sweeping and removing H_2O_2 and regulates peroxidase enzyme activity [16, 21]. Regoli and colleagues [28] also attributed changes in catalase enzyme activity in the presence of magnetic field to changes in the spatial structure of this enzyme in the presence of a field [29]. Thus even magnetic field in some experiments has caused an increase in the probability of substrate binding to enzymes [23, 24]. Due to having a high ratio of surface-to-volume, nanoparticles showed high energy and very high reactivity compared to conventional particles [30]. Previous studies have shown that nanoparticles cause free radicals production and ultimately oxidative stress in cells. The MNP's attachment to the membrane molecules can cause changes in membrane activity and structure, channels, and membrane transporters' membrane integrity [6]. Iron, as an essential micronutrient, plays a crucial role in physiological processes in plant cells [31]. Iron has a dual role on the one hand it participates in oxidation-reduction reactions and the structure of many intracellular enzymes such as catalase, peroxidase, and superoxide dismutase, and on the other hand it produces oxygen species through the Fenton reaction [32, 33]. Recent studies have shown that Iron magnetic nanoparticles have an intrinsic CAT-like and POX-like activity, they are capable of catalyzing H_2O_2 to oxidize various peroxidase substrate. Mechanism studies revealed that Iron magnetic nanoparticles first catalyze H_2O_2 to produce hydroxyl radicals, which are then responsible for substrate oxidation. Thus, one of the potential mechanisms involved in the cytotoxicity of MNPs may be their peroxidase-like activities, and hydroxyl radicals would attack numerous intracellular molecules, and ultimately oxidative stress in cells [34].

Numerous reports have shown that increasing iron content in culture medium usually leads to an increase in plant cells [31]. According to the results obtained in this study, iron oxide nanoparticle treatment caused changes in antioxidant enzyme activity and changes in MDA content. It seems that iron nanoparticle affects cell structure and function directly and through stimulation of oxidative stress and induction of defensive responses in cells.

Iron in the cell can absorb magnetic energy, therefore the magnetic field causes the torque generation of magnetic iron oxide nanoparticles and their migration into the cells. Accordingly, the MNPs may get new shape and feature under SMF and may affect the cell function and induce more oxidative stress [35, 36]. Comparing results from MNP treatment, with SMF and combined treatment of MNP with SMF showed a higher effect of MNP under the influence of SMF on plant cells and increased oxidative stress in cells under combined treatment. Higher antioxidant enzyme activities support this claim, as electrical conductivity and MDA content of cells under combined treatment were higher compared to other treatments. Production of peroxidized lipids such as MDA is known as an indicator of oxidative stress in a biological system. The increase in MDA level in this study indicates the increase in lipid peroxidation that happened through SMF MNP and combined treatment, which has been either a direct effect of these treatments on membrane structure and function or an indirect effect through oxidative stress. The increase in electrical conductivity of the culture medium under these treatments compared to control confirms lipid peroxidation of membrane and leakage of electrolytes from cell to culture medium. In confirmation of the results obtained in this study, in the *Cucumis sativus* plant, 150-200 mT magnetic field treatment caused an increase in H_2O_2 and MDA production. Also, treatment of *Allium ascalonicum* with the magnetic field of 7 mT and 20 kV/m electromagnetic field caused an increase in H_2O_2 and MDA [1]. Hazelnut cell treatment with silver nanoparticle caused an increase in H_2O_2 and MDA content in this plant [28]. Also, wheat, soybean, and rapeseed plants treated with iron oxide and zinc oxide nanoparticles caused an increase in H_2O_2 and MDA content [37]. Antioxidant enzymes are defensive enzymes that usually control free radical production and prevent oxidative. As a result of increased free radical production, the balance between oxidants and antioxidants may be disturbed and cause a decrease in antioxidant activity [38]. Also, iron increase can inhibit catalase enzyme activity by inhibiting –SH groups in its structure [38-42]. It is well-accepted that the expression and activity of CAT and POX enzymes are regulated in response to oxidative stress. Based on the results obtained in this study, the amount of CAT enzyme activity decreased in magnetic field and magnetic iron oxide nanoparticle treatment.

However, in combined treatments, the amount of catalase enzyme activity increased, which is responsible for detoxifying H_2O_2. Since CAT is an iron-containing protein, probably in SMF, MNP treatments, spatial structure, and function or even gene expression of this enzyme have changed, which ultimately has resulted in decreased enzyme activity observed. In combined treatment, the amount of catalase enzyme activity increased, probably during these treatments gene expression of this enzyme increased or this treatment had a synergistic effect on enzyme activity and substrate binding with enzyme increased. Another reason for increasing catalase enzyme activity could be increasing stress and H_2O_2 concentration [43]. Both catalase and peroxidase enzymes collect H_2O_2, but catalase has a faster turn-over, while peroxidase has more tendency to H_2O_2 [44].

Based on the results obtained, the peroxidase activity increased in MNP, SMF, and SMF2+MNP treatments, and MNP and SMF treatments showed a higher increase compared to control and combined treatment. These results could probably be due to a decrease in catalase activity. Peroxidase is a metalloprotein, and probably it changed in some way under magnetic treatments. In cells under combined treatments with higher catalase activity, the peroxidase activity decreased. Probably due to decreased activity or gene expression of catalase, peroxidase enzyme intervened and caused sweeping of H_2O_2. Catalase and peroxidase have complementary statuses, meaning that wherever catalase enzyme activity increases, due to sweeping and removing a large amount of hydrogen peroxide, there is no need for peroxidase activity and vice versa. SOD is one of the antioxidant enzymes belonging to metalloenzymes, and catalyzing and dismutating superoxide anion to oxygen and hydrogen peroxide plays an important role in protection against free radicals [45]. The antioxidant activity of these enzymes affects the content of free oxygen radicals and H_2O_2 content. SOD has Cu^{+2} and Zn^{+2} and iron in its structure, each of which can be affected by the magnetic field, which can affect the function and activity of the enzyme [45]. It is assumed that the first response of the plant defense system to increased free radical content is the intervention of SOD and small molecules, especially ascorbate [46]. In addition, it seems that a pathway other than SOD such as pathways present in peroxisome can interfere with the production of H_2O_2 in these treatments [47]. Previous studies have shown that tobacco cell treatment with magnetic field 30 and 10 mT caused an increase in SOD activity and MDA content, and decreased catalase activity so that increased SOD activity and decreased catalase activity in 30 mT was more intense than 10 mT [16]. It has been reported that the effect of magnetic field on soybean has led to increased SOD activity [12]. Jamshidi and colleagues showed that hazelnut cell treatment with silver nitrate nanoparticle (10ppm) caused a decrease in cell survival percentage, increased peroxidase activity, decreased catalase activity, increased SOD activity, and increased H_2O_2 content compared to control [28]. Wheat seedlings treated by the magnetic field and electromagnetic field caused an increase in CAT activity and decreased POX activity compared to the control [48]. Studies have shown that in response to iron increase, SOD activity increases in *Bacopa monnieri* roots and decreases in its aerial organ compared to control plants [49]. *Coffea arabica* treatment with a magnetic field of 2 mT caused a decrease in catalase, peroxidase, and SOD enzyme activities [1]. Watermelon treatment with magnetic iron oxide nanoparticle caused an increase in catalase, peroxidase activities and a decrease in SOD activity [43]. *Lolium perenne* and *Cucurbita mixta* treatment with magnetic iron oxide nanoparticle caused an increase in CAT, POX activities and a decrease in SOD activity [50]. In this study, SOD activity increased in cells under all treatments in response to increased free radicals, so that in combined treatment with increased intensity of treatment and free radicals more increase was observed in the activity of this enzyme. Since SOD has metal in its structure, its structure and function can be affected by the magnetic field and magnetic nanoparticle, and as a result its activity increases, because one of the effects of the magnetic field is the effect on metals inside the cell. In addition, like CAT and POX, it may be possible that increased activity is due to increased gene expression of this enzyme under the influence of magnetic field treatment, magnetic iron oxide nanoparticles, and combined treatment.

In this study, total antioxidant activity was measured using the FRAP method. Our results showed that total antioxidant activity increased in all treatments, which was higher in the combined treatment. In fact, in all these treatments, cells transferred extra electrons to reducing compounds such as phenolic compounds to reduce cell damage. It has been reported that soybean seedling treatment with a 20 mT magnetic field caused an increase in FRAP content compared to control [12]. Probably, the increase in FRAP is due to the increase in phenolic compounds and secondary metabolites during treatment [9]. The more phenolic content of a cell, the higher the antioxidant power of the cell.

In general, increasing antioxidant enzyme activity and MDA and FRAP content in cells of *D. polychaetum* plant under magnetic field, magnetic iron oxide nanoparticle, and combined treatments were due to changes in electric potential of membrane and effect on magnetic and charged molecules and ultimately changes in membrane and cellular structure and function. Also, magnetic field treatment, magnetic iron oxide nanoparticle, and combined treatment caused a disturbance in redox level and ROS formation that caused oxidative damage and physiological damage. Also, combined treatments led to more increase in the antioxidant enzyme activity of cells, which can be a sign of cell resistance mechanisms against magnetic treatments.

5. Conclusion

Taken together, the results presented here showed that magnetic field, magnetic iron oxide nanoparticle, and combined treatments can increase the FRAP, and MDA content and deteriorate the antioxidant defense system of *D. polychaetum* cells. Furthermore, combined treatments led to more increase in the antioxidant enzyme activity of cells, which can be a sign of cell resistance mechanisms against magnetic treatments.

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Conflict of Interests

All authors declared that they have no conflict of interest.

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