

Research Article

Electromagnetic Field Improved Nanoparticle Impact on Antioxidant Activity and Secondary Metabolite Production in *Anthemis gilanica* Seedlings

Halimeh Hassanpour ¹, Ali Eydi,² and Malak Hekmati²

¹Aerospace Research Institute, Ministry of Science Research and Technology, Tehran 14665-834, Iran

²Department of Organic Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medicinal Sciences, Islamic Azad University, Tehran, Iran

Correspondence should be addressed to Halimeh Hassanpour; hassanpour@ari.ac.ir

Received 27 May 2021; Revised 26 June 2021; Accepted 7 July 2021; Published 15 July 2021

Academic Editor: Maria Serrano

Copyright © 2021 Halimeh Hassanpour et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Electromagnetic field (EMF) causes fundamental alternations in biological systems. In this study, we studied the effects of EMF on physiological responses and secondary metabolites production in SiO₂ NP-treated *Anthemis gilanica* plants. The results indicated that EMF improved plant growth by inducing chlorophyll and carotenoid content, which led to enhanced biomass in SiO₂ NP-treated plants. EMF enhanced adventitious roots in SiO₂ NP-treated plants. EMF treatment improved the activity of antioxidative enzymes such as superoxide dismutase, catalase, and peroxidase in both control and SiO₂ NP-treated plants. EMF and SiO₂ NP treatments significantly declined hydrogen peroxide content in *A. gilanica* plants. Although protein content was reduced by SiO₂ NP treatment, combined application of EMF with SiO₂ NP caused a significant induction in protein content. Our results presented that EMF induced secondary metabolites accumulation such as flavonoid and phenol in SiO₂ NP-treated *A. gilanica* plants. This work can open prospects for the production of the pharmaceutically high-value secondary metabolites.

1. Introduction

The *Anthemis* genus belongs to the Asteraceae family and is distributed across Asia, Europe, Saudi Arabia, and Africa [1]. *Anthemis gilanica* grows spontaneously in the Eshkevar-e Sofla mountains of Rudсар, Gilan province of Iran [2]. It is a rich source of natural antioxidants and is used in folk medicine and various industries of food, cosmetics, aromatherapy, perfumery, and pharmaceuticals [3, 4] and has an economical application in endemic areas. It is traditionally used for the treatment of spasms, stomachaches, earaches, gastrointestinal disorders, and deafness [3]. Anti-inflammatory, antispasmodic, and antibacterial properties of Anthemis extract have been reported due to the existence of main chemical constituents, including phenolic, flavonoid, and terpenoid compounds [3–6]. Therefore, access to the specific condition of *A. gilanica* culture with more growth,

antimicrobial activity, and secondary metabolites is so valuable.

Magnetic field (MF) is an unavoidable environmental agent affecting the growth and development processes of living organisms due to the usage of many manmade devices and the industrial revolution. MF may induce paramagnetic characteristics of some molecules in the cells [7] and affect electrical characteristics and permeability of cell membrane, the activity of free radicals, and ionic transports of the membrane [8, 9]. It has been reported that MF at suitable intensities can have an inductive effect on secondary metabolite production and antioxidant capacity [4, 7, 10], which may be related to MF impact on plant metabolism.

Silicon (Si) is a beneficial element that affects plant growth and development. It has been reported that Si can enhance the antioxidative defense system and improves resistance to environmental stress [4, 11]. Nanoparticles

(NPs) are very small particles with at least two dimensions in the size range of 1–100 nm. The small scale of these particles and a greater proportion of surface atoms produce specific behaviors in their physical properties compared to their bulk materials. Most of these changes are associated with the appearance of quantum effects when size is reduced and converted to the origin of an event like superparamagnetism [12]. The negative and positive impacts of NPs in agriculture associate significantly with physiochemical properties, concentration, exposure method of NPs, and/or plant species. For example, SiO₂ NPs application caused a significant increase in photosynthetic pigments in *Zea mays* [13]. Ag NPs are reported to have an inductive effect on the secondary metabolite content of *Bacopa monnieri* [14]. Moreover, SiO₂ NPs significantly changed antioxidant capacity and vascular tissues in *A. gilanica* [4]. Therefore, the goal of this article was to study the impact of SiO₂ NPs as a chemical elicitor, MF as a physical elicitor, and their combinations on the growth and bioactive compounds production metabolite in *A. gilanica* seedlings. Few studies have been conducted about the impact of SiO₂ NPs and EMF on antioxidative defense mechanisms in medicinal plants, and this is the first study in *A. gilanica*. Data from this research help us to gain biological knowledge about the response mechanisms to NPs, MF, and interaction between them, which may also help in getting more induction of valuable phenolic and flavonoid compounds that can be used in the pharmaceutical and food industries.

2. Materials and Methods

2.1. Plant Material and Culture Conditions. Seeds of *A. gilanica* Bornm. and Gauba with voucher number of 603_AUPF were gathered from Rahimabad of Rudsar mountains, Gilan providence of Iran. Seeds were sowed in distilled water and were put on a wet towel paper in dark condition. After one week, the germinated seeds were exposed to the different MF intensities (0 and 4 mT) for 1 hour during three days (Figure 1). A homogenous MF was applied in the middle of a polyethylene tube covered with the copper wire based on the method described by Hassanpour and Niknam [15]. Then, the seedlings were transferred in plastic pots with a 17 cm diameter filled with perlite and fed with ½ Hoagland nutrient solution (pH 7) once a week. SiO₂ NP (10–20 nm, CAS No. 7631-86-9) was commercially bought from Merck. The SiO₂ NP (6 gL⁻¹) suspension was prepared in deionized water and then was homogenized with a homogenizer (IKA, T 18 digital ULTRA-TURRAX) for 2 min, 21000 rpm to avoid aggregation. The SiO₂ NP solution (100 ml for each pot) was added to the roots once a week at the vegetative stage. The pots were put in a growth chamber with a temperature of 25/18°C (day/night), 56% humidity, and 16/8 (light/dark) photoperiod. The selection of EMF intensity and SiO₂ NP level was conducted according to the published works [4, 15]. After four weeks, the seedlings were harvested for physiological and anatomical analyses. Six plants per treatment were used for analyses in all the experiments.

2.2. Growth Parameters. For determination of fresh (FW) and dry weight (DW), five plants per treatment were dried in an oven at 45°C for three days. Relative water content (RWC) was determined based on the method of Weatherley [16] using the equation of $RWC (\%) = ((FW - DW) / (SW - DW)) \times 100$. Water-saturated weight (SW) was quantified by putting the leaves in deionized water for 24 h at 4°C.

2.3. Photosynthetic Pigments. The photosynthetic pigments were measured according to Lichtenthaler [17] using leaf extraction in 80% acetone. The absorptions were recorded at 480, 649, and 665 nm with a UV-Vis Double Beam Spectrophotometer (model SQ-4802, UNICO, USA).

2.4. Proline and Total Soluble Sugars. Proline content was evaluated through the procedure described by Bates et al. [18]. Total soluble sugars were estimated by the procedure of anthrone-sulphuric acid using the protocol of Fales [19], and the standard curve was drawn by different glucose concentrations.

2.5. H₂O₂ Content. The hydrogen peroxide (H₂O₂) content was estimated spectrophotometrically at 390 nm via the method described by Velikova et al. [20]. The standard curve was drawn with different H₂O₂ concentrations.

2.6. Protein and Enzyme Assays. The fresh leaves (0.5 g) were homogenized in 3 ml of cold extraction buffer containing Tris-HCl 1 M (pH 6.8), and then the homogenates were centrifuged at 13000 × g for 20 minutes at 4°C. The supernatants were separated and used for protein and enzymes analyses. Total protein was determined according to Bradford [21].

The superoxide dismutase (SOD) activity was quantified using an assay solution composed of sodium phosphate buffer (0.1 M, pH 7.8), EDTA (0.1 mM), methionine (13 mM), riboflavin (1.3 μM), NBT (63 μM), and plant extract (0.1 ml) [22]. The assay solution was put in front of the light for 20 minutes, and the enzyme activity was recorded at 560 nm.

The peroxidase (POX) activity was assayed based on Abeles and Biles [23] method with an assay solution of benzidine (0.1 ml), 0.2 ml H₂O₂ (3%), 2 ml acetate buffer (0.2 M, pH 4.8), and enzyme extract (60 μl). The changes in absorbance were read at 530 nm.

The catalase (CAT) activity was determined by the described method of Aebi [24]. The assay mixture was 0.075 ml H₂O₂ (3%), 0.625 ml potassium phosphate buffer (50 mM, pH 7.0), and enzyme extract (5 μl). The changes in absorbance were recorded at 240 nm.

2.7. DPPH Activity, Phenol, and Flavonoid Contents. Reducing the activity of the 2, 2-diphenyl-2-picryl hydrazyl (DPPH) was considered as the scavenging activity of enzyme extract and was assayed according to the method of Patro

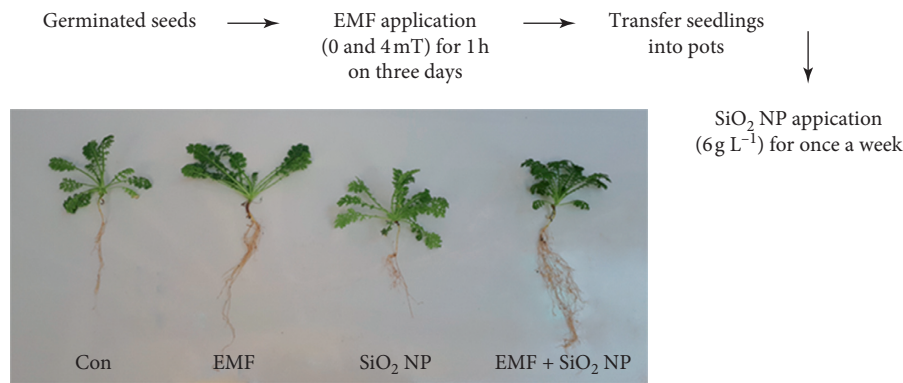


FIGURE 1: Depiction of EMF and SiO₂ NPs on *A. gilanica* plants.

et al. [25]. The 500 mg of leaf dry powder was homogenized in 10 ml of methanol (80%) and centrifuged at 4000 rpm for 5 min. The 150 μ L extract was added to 2.5 mL of DPPH (0.5 mM in methanol). The absorbance was recorded using a UV-visible spectrophotometer at 517 nm at room temperature after 30 min darkness.

Total phenolic content was determined spectrophotometrically at 725 nm using the Folin-Ciocalteu procedure [26]. The assay solution was 500 μ L Folin-Ciocalteu reagent, 400 μ L sodium carbonate 0.7 M, and 100 μ L extract, which was kept at room temperature for 30 min in the dark. The total phenolic was monitored as mg of gallic acid in 1 g DW.

Total flavonoid content was quantified using the aluminum chloride method [27]. The assay solution was 500 μ L leaf extract, 50 μ L sodium nitrate solution (5%), 50 μ L aluminum chloride solution (10%), and 250 μ L sodium hydroxide solution (4%), which was kept at room temperature for 30 min. The absorbance was immediately recorded at 415 nm and expressed as mg of rutin equivalents in 1 g DW.

2.8. Statistical Analysis. The experiment was designed in a randomized complete block design, and the analysis of variance (ANOVA) and mean comparison (Duncan's multiple range test at $P \leq 0.05$) were done with SPSS software (version 18). All data were represented as mean \pm standard errors (SE) of three or five independent replicates of each treatment.

3. Results

The results shown in Table 1 indicated that EMF significantly enhanced growth traits in terms of FW, DW, root length, and the number of the adventitious roots as compared to nonmagnetized plants. EMF treatment increased FW and DW by 66.6% and 46.2%, respectively, compared with the control. The increment of root length and the number of adventitious roots were significant under EMF treatments and an increase of 30.21% and 51.11% compared to control was observed at both parameters, respectively. In contrast to EMF, SiO₂ NP application did not change significantly fresh and dry weights, root length, and adventitious root compared to control. However, shoot length showed a 23.11%

enhancement under SiO₂ NP length in comparison to control plants. The interaction treatment of EMF/SiO₂ NP induced root length and number of the adventitious roots markedly compared to untreated plants.

Application of EMF, SiO₂ NP, and EMF/SiO₂ NP enhanced RWC compared to control condition, and the highest content (65.7%) was observed in plants treated to EMF (Table 1). EMF application resulted in a 31.85% increase in protein content as compared with the control plants. Treatment with SiO₂ NP showed no effect on protein content in plants grown under normal conditions. In plants treated to SiO₂ NP, EMF application induced protein content significantly compared with the control. EMF significantly enhanced photosynthetic pigment contents in *A. gilanica* plants, especially Chl *a* compared to Chl *b* and carotenoid. The contents of Chl *a*, Chl *b*, and carotenoid were reduced following SiO₂ NP exposure in control plants. EMF treatment caused a significant enhancement in Chl *a* and carotenoid in plants treated with SiO₂ NP, but this treatment had no significant effect on Chl *b* content (Figure 2).

Proline content increased significantly under SiO₂ NP and EMF compared to control; however, the highest level was observed in EMF and EMF/SiO₂ NP-treated plants. Proline content in EMF/SiO₂ NP treatment was 86.5% higher than control (Figure 3(a)). Total sugar content increased significantly under EMF and decreased under SiO₂ NP treatment. EMF treatment induced a 47.49% increase in total sugars compared to control. EMF also stimulated the soluble sugar accumulations in EMF/SiO₂ NP-treated plants (Figure 3(b)).

H₂O₂ content was approximately 44.44% lower in plants exposed to EMF than in control plants. H₂O₂ content was increased by exogenous SiO₂ NP application in control plants. The level of H₂O₂ in EMF-treated plants following SiO₂ NP application was reduced compared to control plants (Figure 4(a)). EMF treatment improved SOD activity in *A. gilanica* plants when compared with control. A significant increase was observed in SOD activity in both EMF- and SiO₂ NP-treated plants. There was no significant difference in SOD activity between EMF-treated plants and cotreated EMF/SiO₂ NP plants (Figure 4(b)). EMF and SiO₂ NP treatment significantly induced POX activity compared to control, and the effect of EMF was more pronounced in *A. gilanica* plants. In plants exposed to SiO₂ NP, POX

TABLE 1: Effect of EMF and SiO₂ NPs on some growth parameters and protein content in *A. gilanica* seedlings.

Parameters	Treatments			
	CON	EMF	SiO ₂ NP	EMF + SiO ₂ NP
FW (g plant ⁻¹)	0.72 ± 0.038 ^{cd}	1.20 ± 0.065 ^a	0.78 ± 0.033 ^c	0.83 ± 0.046 ^b
DW (g plant ⁻¹)	0.067 ± 0.0041 ^c	0.098 ± 0.0039 ^a	0.071 ± 0.0044 ^c	0.069 ± 0.0032 ^b
Shoot length (cm)	2.25 ± 0.091 ^b	2.15 ± 0.083 ^b	2.77 ± 0.064 ^a	2.11 ± 0.085 ^b
Root length (cm)	7.76 ± 1.091 ^{bc}	11.12 ± 0.835 ^a	8.85 ± 0.723 ^b	11.50 ± 0.698 ^a
No. of adventitious roots	5.5 ± 0.431 ^b	11.25 ± 0.335 ^a	5.85 ± 0.463 ^b	12.5 ± 0.227 ^a
RWC (%)	52.8 ± 1.956 ^c	73.8 ± 3.982 ^a	65.7 ± 2.173 ^b	65.7 ± 2.361 ^b
Protein (mg g ⁻¹ FW)	25.1 ± 1.723 ^c	42.7 ± 2.791 ^a	23.5 ± 1.380 ^{cd}	36.8 ± 2.297 ^b

Bars indicate means ± SE ($n = 5$) in each group. Different letters indicate significant differences at $P < 0.05$.

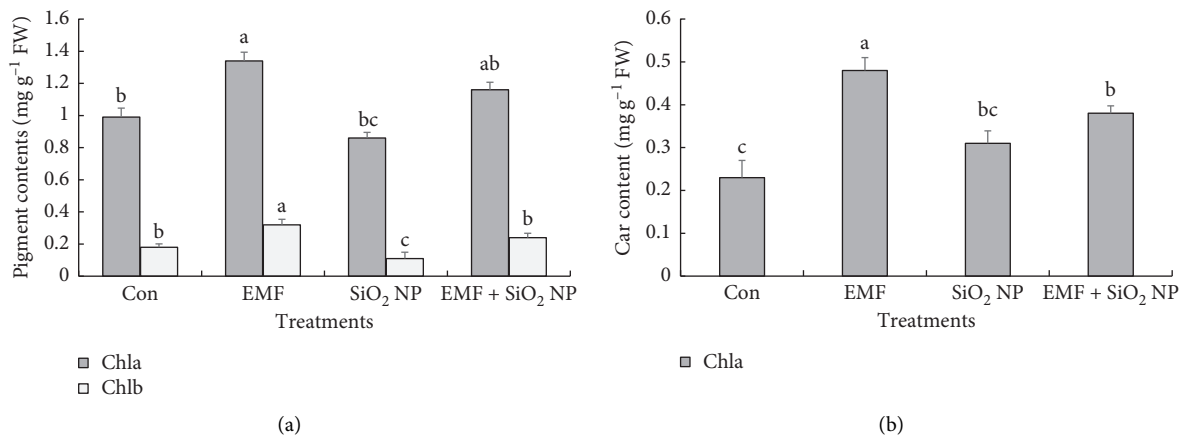


FIGURE 2: Effect of EMF and SiO₂ NP treatments on chlorophyll a and b (Chl) (a) and carotenoid (Car); (b) contents of *A. gilanica* plants. Bars indicate means ± SE ($n = 5$) in each group. Different letters indicate significant differences at $P \leq 0.05$.

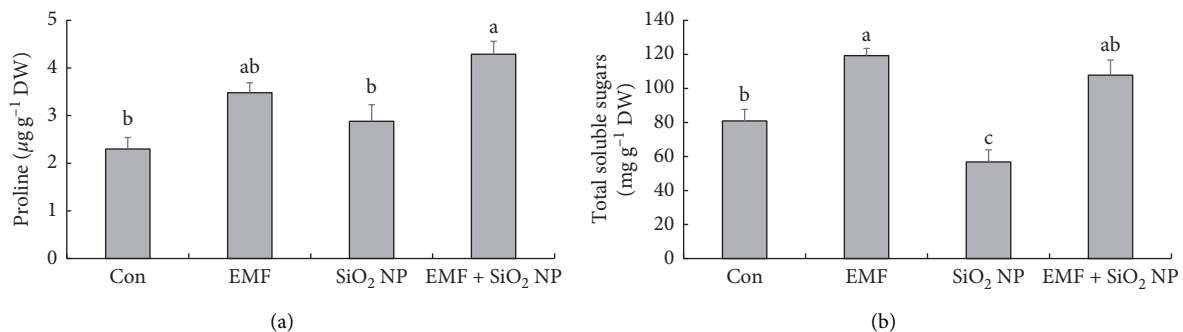


FIGURE 3: Effect of EMF and SiO₂ NP treatments on the content of proline (a) and total soluble sugars (b) of *A. gilanica* plants. Bars indicate means ± SE ($n = 3$) in each group. Different letters indicate significant differences at $P \leq 0.05$.

activity was induced following EMF treatment (Figure 4(c)). EMF led to a significant rise in CAT activity compared to control. SiO₂ NP exposure elevated the CAT activity in plants grown in both control and EMF conditions. The highest CAT activity (37.5%) was observed in plants treated to EMF/SiO₂ NP (Figure 4(d)).

Total phenolic and flavonoid contents were more eminent in leaves than those in roots. EMF caused a considerable increase (43.51%) in total phenol content in the leaf compared to control plants. Treatment with SiO₂ NP and

EMF/SiO₂ NP resulted in the induction of total phenol content in the leaf of *A. gilanica* plants (Figure 5(b)). In roots, all treatments enhanced the total phenol content compared to control, and the maximum increase was detected in plants cotreated with EMF and SiO₂ NP. Flavonoid content of leaf was induced in the plants exposed to EMF compared to control. Enhancement of flavonoid content in leaf was detected in both EMF- and EMF/SiO₂ NP-treated plants (Figure 5(a)). No significant change in flavonoid content was identified following EMF and SiO₂ NP

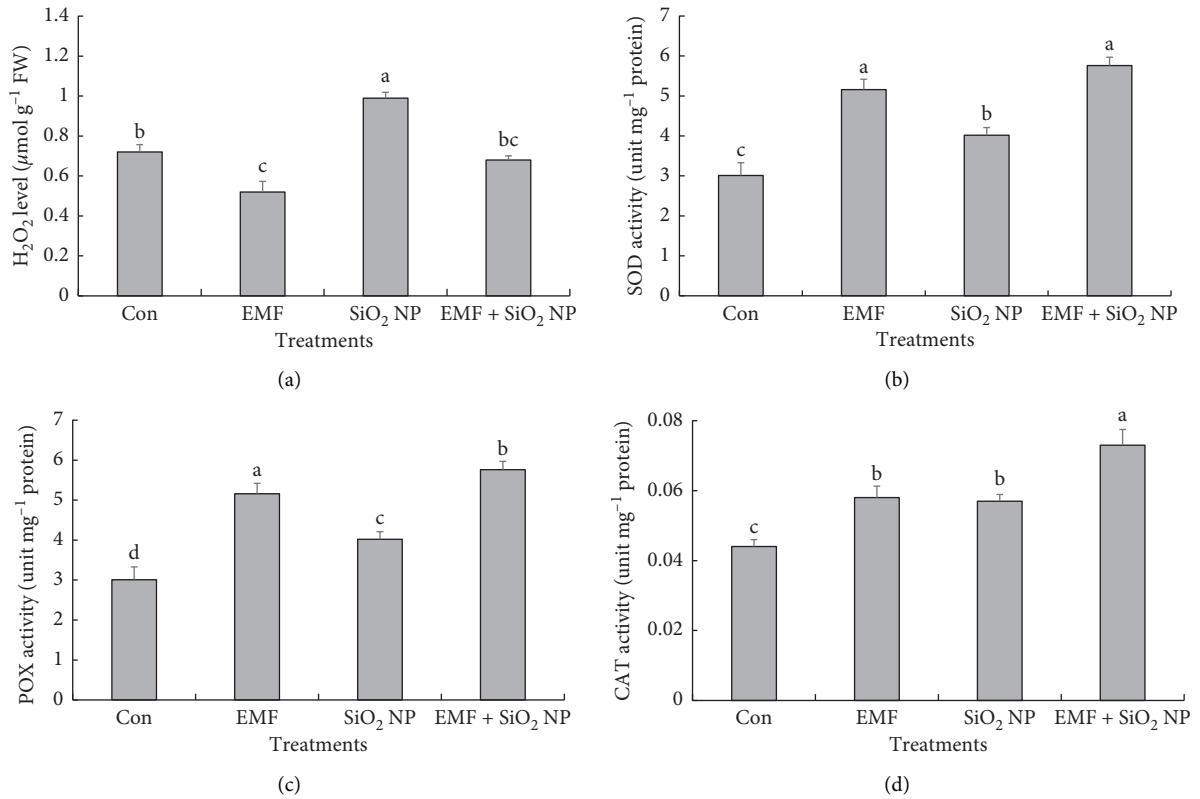


FIGURE 4: Impact of EMF and SiO₂ NP treatments on H₂O₂ content (a), SOD activity (b), POX activity (c), and CAT activity (d) in *A. gilanica* plants. Bars indicate means ± SE (*n* = 3) in each group. Different letters indicate significant differences at *P* ≤ 0.05.

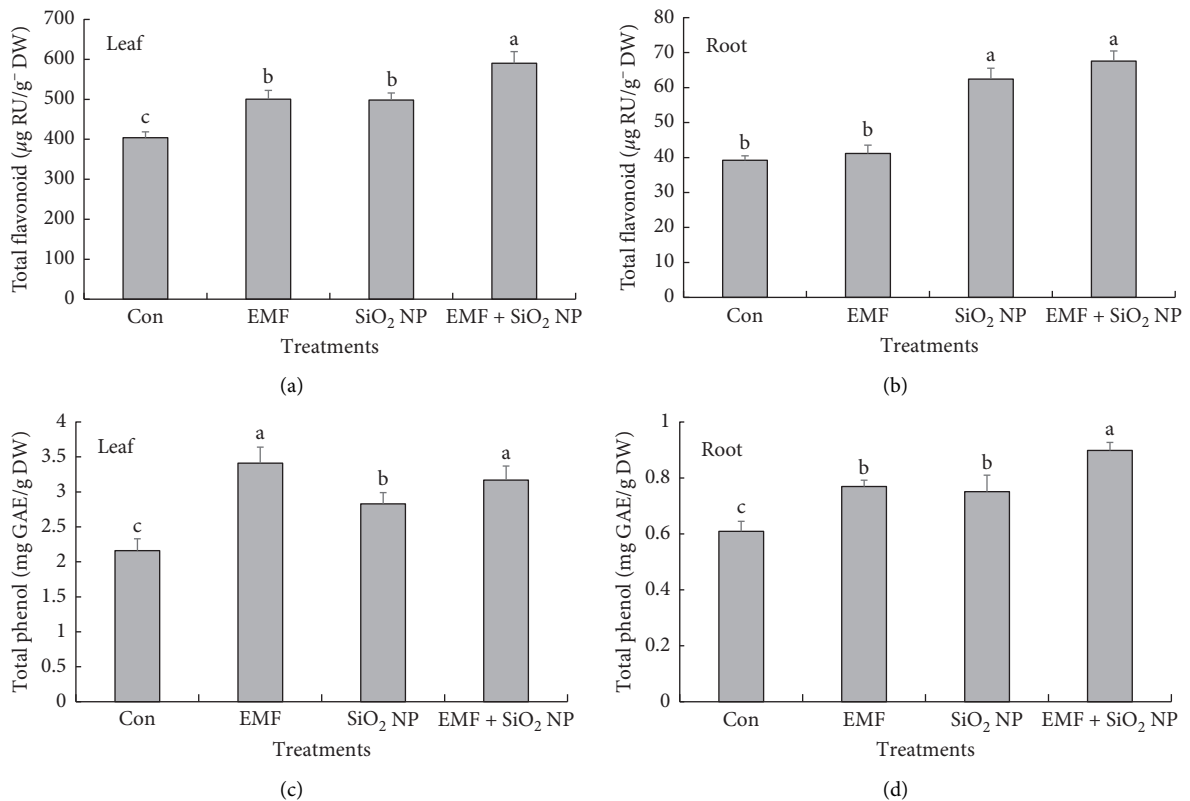


FIGURE 5: Changes in flavonoid (a, b) and phenol (c, d) contents in *A. gilanica* plants under EMF and SiO₂ NP treatments. Bars indicate means ± SE (*n* = 3) in each group. Different letters indicate significant differences at *P* ≤ 0.05.

treatments in leaves, but SiO₂ NP showed a considerable effect in both conditions on flavonoid content in the root of *A. gilanica* (Figure 5(b)). EMF caused a sharp induction (1.78-fold) in DPPH scavenging activity as compared to control plants. EMF treatment also induced DPPH scavenging activity in SiO₂ NP-treated plants compared to control (Figure 6).

4. Discussion

This study was conducted to investigate the impact of the EMF, SiO₂ NPs, and their interactions on stimulating the growth and production of phenolic and flavonoid compounds in *A. gilanica* seedlings. Our results showed that fresh and dry weights, root growth, and number did not significantly change in SiO₂ NP-treated plants in roots as compared to control, but EMF application markedly promoted effect of SiO₂ NP on the growth (Table 1 and Figure 1). The small NPs size and high surface area make them very active [12] and move into the roots through the apoplastic and symplastic pathways. At special concentrations, NPs may reduce the absorption of macro- and microelements and affect vital parts of plant cells such as photosynthetic apparatus, resulting in the decline of growth and yield [28, 29]. On the other hand, the primary response of plant cells to MF is the alteration of electrical properties and permeability of membranes, which can be due to changes in the spine of paramagnetic molecules and the related-physiochemical reactions. Following MF, ionic flows in cell membranes are changed and can result in increasing ion and organic molecule transports and finally growth induction [8]. Moreover, MF may play an important role in cation uptake capacity and has a positive effect on immobile plant nutrient uptake [30].

RWC is a physiological parameter to evaluate the water status of plants. In our study, an enhanced level of RWC was perceived in EMF and SiO₂ NP-treated plants. The rise of RWC in plants could be associated with the impact of EMF and SiO₂ NP on osmolyte accumulation and induction of membrane permeability [4, 31]. The compatible solutions, proline, and total soluble sugars are effective compounds in cell protection under stress situations. These compounds can decrease the osmotic potential of cells by sustaining high turgor level and storage reserves to improve cell metabolism and maintaining plant growth. Increased proline content may be associated with protein destruction and generation of amino acids like proline [32], activation of the proline biosynthesis enzyme, and/or decrease of proline oxidation under stress conditions [33]. On the other hand, soluble sugars and proline may also act as protective osmolytes for ROS scavenging and prevention of lipid peroxidation and protein denaturation [6, 29]. In this research, SiO₂ NP application against EMF did not change proline content significantly and decreased total soluble sugars in *A. gilanica*. These results confirmed the data obtained from ZnO NPs impact on okra (*Abelmoschus esculentus*) [34]. Lower accumulation of osmotic adjustments in *A. gilanica* plants exposed to SiO₂ NP may cause less water sustaining and

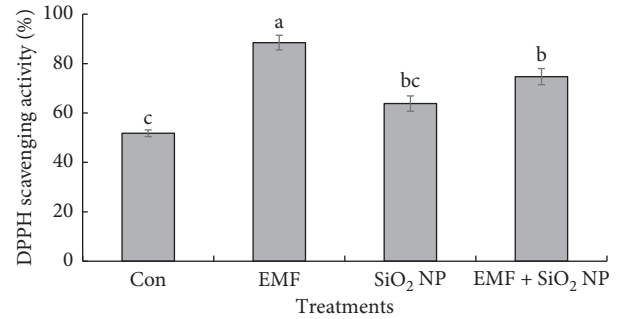


FIGURE 6: Impact of EMF and SiO₂ NP treatments on DPPH scavenging activity in *A. gilanica* plants. Bars indicate means \pm SE ($n = 3$) in each group. Different letters indicate significant differences at $P \leq 0.05$.

protection against oxidative damage, which affect growth parameters.

Photosynthetic pigments are highly sensitive to stress conditions and can be an indicator to investigate the impact of stress on growth parameters. Our results stated that SiO₂ NP adversely influenced photosynthetic pigments and protein content. Zarafshar et al. [35] showed that SiO₂ NP reduced chlorophyll and carotenoid contents in pear seedlings. The decline in chlorophyll pigments and protein may be due to oxidative injuries through the ROS accumulation on stroma-lamellar proteins and other proteins under stress conditions [36, 37]. EMF and its interaction with SiO₂ NP showed a positive effect on protein and photosynthetic pigment in *A. gilanica* plants. Similarly, seeds exposed to MF displayed a significant rise in chlorophyll content and photosynthesis in Indian maize [38]. MF also increased significantly Chl *a* and Chl *b* and carotenoid in *Phoenix dactylifera L.* [39] and protein content maize [40]. Increased photosynthetic pigment may be associated with the production of oxidative injury by improving antioxidant capacity, which is in agreement with the stimulation of enzymatic antioxidant activities in *A. gilanica*.

Antioxidative enzymes such as SOD, CAT, and POX deactivate or scavenge free radicals before they attack cellular components [41, 42]. SOD scavenges the superoxide anions and generates hydrogen peroxide, and CAT and POX convert hydrogen peroxide to water and O₂ [43]. Results obtained from our experiments showed that antioxidant enzyme and DPPH scavenging activities were induced with the SiO₂ NP application compared to control, but the increased levels improved markedly after EMF treatment (Figure 4). H₂O₂ content was also induced in SiO₂ NP-treated plants compared to control and EMF-treated plants indicating the toxicity impact of SiO₂ NP on the *A. gilanica* growth (Figure 4(a)). Disruption in ROS homeostasis by NPs may lead to impaired organelle function, membrane damage, and eventually phytotoxicity [44]. It can be proposed that the rise of antioxidant enzymes in SiO₂ NP-treated plants could not regulate ROS levels, resulting in the reduction of pigment and growth parameters. On the other hand, ROS suppression is triggered after plant exposure to EMF. Enzymatic activities such as CAT, POX, and SOD had

a twofold to a fourfold rise in MF-treated seedlings [45, 46]. Induction of antioxidant enzyme activities under EMF suggests that these enzymes may act as a magnetoreceptor. Magnetic characteristics of molecules define their capability of absorbing and then altering the energy into other forms and transferring them to other molecules in cells, thus making them more active [7].

Antioxidant compounds are molecules that quench free radical reactions and inhibit cellular injuries. These compounds neutralize the ROS in a process called radical scavenging and carry them away [47]. Flavonoids are known for their scavenging ability of H_2O_2 and are considered to play a vital function in the phenolic/ascorbate-peroxidase cycle [48]. Our results showed that EMF had a positive effect on nonenzymatic antioxidants. These findings illuminate the EMF-improved capability of scavenging free radicals, leading to growth enhancement in *A. gilanica* plants. Induction in the content of nonenzymatic antioxidants such as phenol and flavonoid has been described in other plants [49, 50]. The results of our study showed that nonenzymatic antioxidants are enhanced with the addition of SiO_2 NP (Figure 5). Suriyaprapha et al. [51] reported that SiO_2 NP increased nonenzymatic antioxidants. EMF treatment induced more accumulation of nonenzymatic antioxidants in SiO_2 NP-treated plants. It was shown that EMF enhanced the growth of *A. gilanica* plants by inducing organic combinations such as phenol and flavonoid.

5. Conclusions

This study demonstrated some beneficial impacts of germinated seeds exposed to EMF on growth parameters, antioxidative enzymes, and bioactive compounds (phenols and flavonoids) in control and SiO_2 NP-treated plants. EMF also declined the toxicity of SiO_2 NP on photosynthetic pigments, ROS accumulation, and growth in *A. gilanica*. It can be proposed that EMF with the stimulation of antioxidative capacity may be used as a tool for enhancing stress resistance under environmental stress. The mechanism of antioxidant enzyme activities as a magnetoreceptor is not clear under EMF and needs to be more investigated in the future.

Data Availability

All data are means \pm SE with three or five replications of treatment parameters: CON EMF SiO_2 NP EMF + SiO_2 NP FW (g plant⁻¹): 0.72 \pm 0.038 cd, 1.20 \pm 0.065 a, 0.78 \pm 0.033 c, 0.83 \pm 0.046 b; DW (g plant⁻¹): 0.067 \pm 0.0041 c, 0.098 \pm 0.0039 a, 0.071 \pm 0.0044 c, 0.069 \pm 0.0032 b; shoot length (cm): 2.25 \pm 0.091 b, 2.15 \pm 0.083 b, 2.77 \pm 0.064 a, 2.11 \pm 0.085 b; root length (cm): 7.76 \pm 1.091 bc, 11.12 \pm 0.835 a, 8.85 \pm 0.723 b, 11.50 \pm 0.698 a; no. of adventitious roots: 5.5 \pm 0.431 b, 11.25 \pm 0.335 a, 5.85 \pm 0.463 b, 12.5 \pm 0.227 a; RWC (%): 52.8 \pm 1.956 c, 73.8 \pm 3.982 a, 65.7 \pm 2.173 b, 65.7 \pm 2.361 b; protein (mg g⁻¹ FW): 29.1 \pm 1.723 c, 42.7 \pm 2.791 a, 23.5 \pm 1.380 cd, 33.8 \pm 2.297 b; Chl a: 0.99 \pm 0.056 b, 1.34 \pm 0.054 a, 0.86 \pm 0.035 bc, 1.16 \pm 0.047 ab; Chl b: 0.18 \pm 0.021 b, 0.32 \pm 0.034 a,

0.11 \pm 0.039 c, 0.24 \pm 0.027 d; Car: 0.23 \pm 0.041 c, 0.48 \pm 0.032 a, 0.31 \pm 0.029 bc, 0.38 \pm 0.017 b; H_2O_2 : 0.93 \pm 0.037 a, 0.52 \pm 0.053 c, 0.78 \pm 0.029 bc, 0.68 \pm 0.021 b; SOD: 3.01 \pm 0.32 c, 5.16 \pm 0.26 a, 4.02 \pm 0.19 b, 5.76 \pm 0.21 a; POX: 2.82 \pm 0.22 d, 4.35 \pm 0.24 a, 3.31 \pm 0.27 c, 3.98 \pm 0.19 b; CAT: 0.044 \pm 0.0026 c, 0.058 \pm 0.0035 b, 0.057 \pm 0.0014 b, 0.073 \pm 0.0044 a; phenol (leaf): 2.16 \pm 0.17 c, 3.41 \pm 0.23 a, 2.83 \pm 0.16 b, 3.17 \pm 0.20 a; phenol (root): 0.609 \pm 0.036 c, 0.769 \pm 0.023 b, 0.751 \pm 0.059 b, 0.898 \pm 0.029 a; flavonoid (leaf): 404.01 \pm 14.7 c, 500.5 \pm 21.9 a, 498.26 \pm 17.7 b, 590.18 \pm 29.3 a; flavonoid (root): 39.19 \pm 1.3 b, 41.14 \pm 2.4 b, 62.44 \pm 3.1 a, 67.57 \pm 2.9 a; DPPH: 51.8 \pm 1.36 d, 88.5 \pm 2.97 a, 63.8 \pm 3.17 c, 74.81 \pm 3.27 b.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

A. E. took part in the bench experiments. H. H. and M. H. have designed and analyzed the data. All authors read and approved the final version of the article.

Acknowledgments

The authors acknowledge Paya Kesh Company for the laboratory support and EMF application.

References

- [1] K. Bremer, *Asteraceae, Cladistics and Classification*, Timber Press, Portland, OR, USA, 1994.
- [2] S. Houshmand, H. Abasalipour, A. Tadayyon, and H. Zinali, "Evaluation of four chamomile species under late season drought stress," *International Journal of Plant Production*, vol. 5, pp. 9–24, 2012.
- [3] F. Eser, A. Sahin Yaglioglu, M. Dolarslan, E. Aktas, and A. Onal, "Dyeing, fastness, and cytotoxic properties, and phenolic constituents of *Anthemis tinctoria* var. *tinctoria* (Asteraceae)," *The Journal of the Textile Institute*, vol. 108, no. 9, pp. 1489–1495, 2017.
- [4] N. Ahmadi, H. Hassanpour, M. Hekmati, and M. Ghanbarzadeh, "Effect of SiO_2 nanoparticles on phytochemical and anatomical alterations in *Anthemis gilanica*," *Iranian Journal of Plant Physiology*, vol. 10, pp. 3223–3231, 2020.
- [5] N. Jafari, S. Nazari, Z. Rabiei, S. T. Enferadi, and R. Behroozi, "Growth inhibitory effect of *Anthemis haussknechtii* root extract, as a source of Parthenolide, on breast cancer cell line," *Journal of Medicinal Plants and By-Product*, vol. 5, pp. 205–210, 2013.
- [6] H. Hassanpour, V. Niknam, and S. Salami, "Acceleration breaks the cells defense mechanisms against vibration in *Anthemis gilanica* calli," *International Journal of Agronomy*, vol. 2021, pp. 1–12, 2021.
- [7] A. Aladjadjiyan, "Influence of stationary magnetic field on lentil seeds," *International Agrophysics*, vol. 24, pp. 321–324, 2010.
- [8] O. Çelik, N. Büyüksulu, C. Atak, and A. Rzakoulieva, "Effects of magnetic field on activity of superoxide dismutase and

- catalase in *Glycine max* (L.). Merr. Roots," *Polish Journal of Environmental Studies*, vol. 18, pp. 175–182, 2009.
- [9] P. Galland and A. Pazur, "Magnetoreception in plants," *Journal of Plant Research*, vol. 118, no. 6, pp. 371–389, 2005.
- [10] H. Hassanpour and S. Hassanpour, "Promoting impact of electromagnetic field on antioxidant system and performance of vascular tissues in *Physalis alkekengi*," *Russian Journal of Plant Physiology*, vol. 68, no. 3, pp. 545–551, 2021.
- [11] A. A. Berahmand, A. Ghafariyan Panahi, H. Sahabi et al., "Effects silver nanoparticles and magnetic field on growth of fodder maize (*Zea mays* L.)," *Biological Trace Element Research*, vol. 149, no. 3, pp. 419–424, 2012.
- [12] M. Auffan, J. Rose, M. R. Wiesner, and J.-Y. Bottero, "Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro," *Environmental Pollution*, vol. 157, no. 4, pp. 1127–1133, 2009.
- [13] J. Karimi and S. Mohsenzadeh, "Effects of silicon oxide nanoparticles on growth and physiology of wheat seedlings," *Russian Journal of Plant Physiology*, vol. 63, no. 1, pp. 119–123, 2016.
- [14] C. Krishnaraj, E. G. Jagan, R. Ramachandran, S. M. Abirami, N. Mohan, and P. T. Kalaichelvan, "Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism," *Process Biochemistry*, vol. 47, no. 4, pp. 651–658, 2012.
- [15] H. Hassanpour and V. Niknam, "Establishment and assessment of cell suspension cultures of *Matricaria chamomilla* as a possible source of apigenin under static magnetic field," *Plant Cell, Tissue and Organ Culture (PCTOC)*, vol. 142, no. 3, pp. 583–593, 2020.
- [16] P. E. Weatherley, "Studies in the water relations of cotton plants. The field measurement of water deficit in leaves," *New Phytologist*, vol. 49, pp. 81–97, 1973.
- [17] H. K. Lichtenthaler, "Chlorophylls and carotenoids: pigments of photosynthetic biomembranes," *Methods in Enzymology*, vol. 148, pp. 350–382, 1987.
- [18] L. S. Bates, R. P. Waldren, and I. D. Teare, "Rapid determination of free proline for water-stress studies," *Plant and Soil*, vol. 39, no. 1, pp. 205–207, 1973.
- [19] F. Fales, "The assimilation and degradation of carbohydrates by yeast cells," *Journal of Biological Chemistry*, vol. 193, no. 1, pp. 113–124, 1951.
- [20] V. Velikova, I. Yordanov, and A. Edreva, "Oxidative stress and some antioxidant systems in acid rain-treated bean plants," *Plant Science*, vol. 151, no. 1, pp. 59–66, 2000.
- [21] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1–2, pp. 248–254, 1976.
- [22] C. N. Giannopolitis and S. K. Ries, "Superoxide dismutases," *Plant Physiology*, vol. 59, no. 2, pp. 309–314, 1977.
- [23] F. B. Abeles and C. L. Biles, "Characterization of peroxidases in lignifying peach fruit endocarp," *Plant Physiology*, vol. 95, no. 1, pp. 269–273, 1991.
- [24] H. Aebi, "[13] Catalase in vitro," *Methods in Enzymology*, vol. 105, pp. 121–126, 1984.
- [25] B. S. Patro, A. K. Bauri, S. Mishra, and S. Chattopadhyay, "Antioxidant activity of *myristica malabarica* extracts and their constituents," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 17, pp. 6912–6918, 2005.
- [26] V. L. Singleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents," *American Journal of Enology and Viticulture*, vol. 16, no. 3, pp. 144–158, 1965.
- [27] C. C. Chang, M. H. Yang, H. M. Wen, and J. C. Chern, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *Journal of Food and Drug Analysis*, vol. 10, no. 3, pp. 178–182, 2002.
- [28] A. Rastogi, M. Zivcak, D. K. Tripathi, S. Yadav, and H. M. Kalaji, "Phytotoxic effect of silver nanoparticles in *Triticum aestivum*: improper regulation of photosystem I activity as the reason for oxidative damage in the chloroplast," *Photosynthetica*, vol. 57, no. 1, pp. 209–216, 2019.
- [29] D. Torabzadeh, H. Hassanpour, J. Asgarpanah, and M. Rezayian, "Nanoparticles induced antioxidative compounds in *Matricaria chamomilla*," *Iranian Journal of Plant Physiology*, vol. 9, no. 4, pp. 2955–2961, 2019.
- [30] A. Esitken and M. Turan, "Alternating magnetic field effects on yield and plant nutrient element composition of strawberry (*Fragaria ananassa* cv. Camarosa)," *Soil and Plant Science*, vol. 54, pp. 135–139, 2004.
- [31] M. Mansourkhaki, H. Hassanpour, and M. Hekmati, "Effect of static magnetic field on growth factors, antioxidant activity and anatomical responses of *Silybum marianum* seedlings," *Journal of Plant Process and Function*, vol. 7, pp. 10–17, 2019.
- [32] M. Ashraf and M. R. Foolad, "Roles of glycine betaine and proline in improving plant abiotic stress resistance," *Environmental and Experimental Botany*, vol. 59, no. 2, pp. 206–216, 2007.
- [33] P. D. Hare, W. A. Cress, and J. Van Staden, "Dissecting the roles of osmolyte accumulation during stress," *Plant, Cell and Environment*, vol. 21, no. 6, pp. 535–553, 1998.
- [34] N. M. Alabdallah and H. S. Alzahrani, "The potential mitigation effect of ZnO nanoparticles on [*Abelmoschus esculentus* L. Moench] metabolism under salt stress conditions," *Saudi Journal of Biological Sciences*, vol. 27, no. 11, pp. 3132–3137, 2020.
- [35] M. Zarafshar, M. Akbarinia, H. Askari, S. M. Hosseini, M. Rahaie, and D. Struve, "Toxicity assessment of SiO₂ nanoparticles to pear seedlings," *International Journal of Nanoscience and Nanotechnology*, vol. 11, pp. 13–22, 2015.
- [36] T. Dobroviczka, B. Pirsellova, P. Meszaros et al., "Effects of cadmium and arsenic ions on content of photosynthetic pigments in the leaves of *Glycine max* (L.) Merrill," *Pakistan Journal of Botany*, vol. 45, no. 1, pp. 105–110, 2013.
- [37] H. Hassanpour, V. Niknam, and B. S. Haddadi, "High-frequency vibration improve callus growth via antioxidant enzymes induction in *Hyoscyamus kurdicus*," *Plant Cell, Tissue and Organ Culture (PCTOC)*, vol. 128, no. 1, pp. 231–241, 2016.
- [38] A. Anand, S. Nagarajan, A. P. Verma, D. K. Joshi, P. C. Pathak, and J. Bhardwaj, "Pre-treatment of seeds with static magnetic field ameliorates soil water stress in seedlings of maize (*Zea mays* L.)," *Indian Journal of Biochemistry & Biophysics*, vol. 49, pp. 63–70, 2012.
- [39] F. Dhawi and J. M. Al-Khayri, "Magnetic fields induce changes in photosynthetic pigments content in date palm (*Phoenix dactylifera* L.) seedlings," *The Open Agriculture Journal*, vol. 3, no. 1, pp. 1–5, 2009.
- [40] A. Shabrangi, H. Hassanpour, A. Majd, and M. Sheidai, "Induction of genetic variation by electromagnetic fields in *Zea mays* L. and *Brassica napus* L.," *Caryologia*, vol. 68, no. 4, pp. 272–279, 2015.
- [41] M. J. Merati, V. Niknam, H. Hassanpour, and M. Mirmasoumi, "Comparative effects of salt stress on growth and antioxidative responses in different organs of pennyroyal (*Mentha pulegium* L.)," *Journal of Plant Research*, vol. 28, no. 5, pp. 1097–1107, 2015.

- [42] H. Hassanpour and V. Niknam, "Effect of water deficit stress on growth and antioxidant enzyme activity of *Mentha pulegium* L. at flowering stage," *Journal of Plant Process and Function*, vol. 3, no. 8, pp. 25–36, 2014.
- [43] K. Das and A. Roychoudhury, "Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants," *Frontier in Environmental Science*, vol. 2, no. 53, 2014.
- [44] P. Sharma, A. B. Jha, R. S. Dubey, and M. Pessarakli, "Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions," *Journal of Botany*, vol. 2012, Article ID 217037, 26 pages, 2012.
- [45] H. P. Singh, V. P. Sharma, D. R. Batish, and R. K. Kohli, "Cell phone electromagnetic field radiations affect rhizogenesis through impairment of biochemical processes," *Environmental Monitoring and Assessment*, vol. 184, no. 4, pp. 1813–1821, 2012.
- [46] H. Zare, S. Mohsenzadeh, and A. Moradshahi, "Electromagnetic waves from GSM a mobile phone simulator and abiotic stress in *Zea mays* L.," *Journal of Nutrition & Food Sciences*, vol. 11, p. 3, 2015.
- [47] S. B. Nimse and D. Pal, "Free radicals, natural antioxidants, and their reaction mechanisms," *RSC Advances*, vol. 5, no. 35, pp. 27986–28006, 2015.
- [48] A. Ashouri-Sheikhi, H. Hassanpour, P. Jonoubi, M. G. Nohooji, and M. Nadimifar, "The effect of gamma irradiation on in vitro total phenolic content and antioxidant activity of *Ferula gummosa* Bioss.," *Journal of Medicinal Plant*, vol. 15, no. 59, pp. 122–131, 2016.
- [49] S. M. Y. Azizi, S. Hosseini Sarghein, A. Majd, and M. Peyvandi, "The effects of the electromagnetic fields on the biochemical components, enzymatic and non-enzymatic antioxidant systems of tea *Camellia sinensis* L.," *Physiology and Molecular Biology of Plants*, vol. 25, no. 6, pp. 1445–1456, 2019.
- [50] T. Cebulak, J. Oszmiański, I. Kapusta, and S. Lachowicz, "Effect of UV-C radiation, ultra-sonication electromagnetic field and microwaves on changes in polyphenolic compounds in chokeberry (*Aronia melanocarpa*)," *Molecules*, vol. 22, no. 7, p. 1161, 2017.
- [51] R. Suriyaprapha, G. Karunakaran, R. Yuvakkumar, P. Prabu, V. Rajendran, and N. Kannan, "Growth and physiological responses of maize (*Zea mays* L.) to porous silica nanoparticles in soil," *Journal of Nanoparticle Research*, vol. 14, pp. 2–14, 2012.