Synthesized Fe_3O_4 nanoparticles induce antioxidant activity and total phenolic and flavonoid contents in *Matricaria chamomilla* seedlings

Zeinab Rastegaran[1], Halimeh Hassanpour[2]*, Hakimeh Ziyadi[1]

1. Department of Organic Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

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

Abstract

This study was conducted in order to determine the effects of different concentrations of synthesized Fe_3O_4 nanoparticles (NPs) on the growth, the content of secondary metabolites, and antioxidant capacity in *Matricaria chamomilla*. To this end, four levels of Fe_3O_4 NPs concentrations were applied as follows: basic Murashige and Skoog solution (control), 25, 50, and 100 mg $L^{-1}Fe_3O_4$ NPs, and sterilized seeds were cultured in these media. Results indicated that the biomass was higher in the plants treated with 25 mg $L^{-1}Fe_3O_4$ NPs than in the control plants. On the other hand, biomass declined in 50 and 100 mg L^{-1} Fe_3O_4-exposed plants compared with the unexposed plants. Relative water content gradually decreased with the enhancement of Fe_3O_4 concentration. Fe_3O_4 NPs in 50 and 100 mg L^{-1} caused a significant induction in the number of roots. Fe_3O_4NPs treatment enhanced the production of secondary metabolites such as total phenol and total flavonoid in roots and leaves of *M. chamomilla*. Moreover, Fe_3O_4 NPs increased the antioxidant capacity of the roots and leaves by inducing DPPH scavenging activity in 25 mg L^{-1} Fe_3O_4 NPs. The results may suggest that the application of Fe_3O_4 NPs can be a useful way for increasing the higher content of secondary metabolites in the *M. chamomilla* plants.

Keywords: flavonoid, growth, *Matricaria chamomilla*, nanoparticles, phenol

Rastegaran, Z. H. Hassanpour, H. Ziyadi. 2022. 'Synthesized Fe_3O_4 nanoparticles induce antioxidant activity and total phenolic and flavonoid contents in *Matricaria chamomilla* seedlings'. *Iranian Journal of Plant Physiology* ,12 (1),4003-4012.

Introduction

Plant tissue culture of medicinal plants has been shown to be a valuable method in the study of secondary metabolite production and plant regeneration. Elicitation of plant cells under in vitro conditions has been identified as a valuable biotechnological method to improve the quality and quantity of the beneficial metabolites (Tahsili et al., 2014).

*Matricaria chamomila (*chamomile*)* is a traditional medicinal plant from the Asteraceae family. It is native to Iran and is cultured in Europe, America, and Asia. In traditional medicine, chamomile is commonly used for stomachaches, headaches, colds, etc. Apigenin as a flavonoid with antioxidant activity has been detected in chamomile seedlings, which has various biological activities, including anti-cancer, anti-

* *Corresponding Author*
E-mail Address: hassanpour@ari.ac.ir
Received: December, 2020
Accepted: July, 2021

inflammatory, neuro-protective, anti-microbial, and anti-allergic effects (Sayadi et al., 2014; Zemestani et al., 2016; Hassanpour and Ghanbarzadeh, 2021). Pharmacological compounds of chamomile include secondary metabolites such as sesquiterpenoids, coumarin, and flavonoids (Hassanpour and Niknam, 2020; Greger, 1977).

Nanoparticles (NPs) are engineered structures with at least two dimensions, a diameter of less than 100 nm, high surface energy, and very large surface area. They can be manufactured via inorganic synthesis or by exploiting living organisms (Ma et al., 2010). These properties have shown different physiological and biochemical responses in living organisms compared with their bulk counterparts (Ma et al., 2010). Specific attention has been given to the usage of NPs on plants for agricultural and horticultural targets (Dimkpa et al., 2012). The negative and positive impact of NPs on higher plants is significantly related to the physico-chemical characteristics of the metal NPs and plant species. Beyer et al. (2011) showed NPs could increase light absorption by chlorophyll molecule due to plasmon resonance effect of metal nanoparticles and decreased quantum yield by photosystem due to nanoparticles energy transfer. Krishnaraj et al. (2012) reported on the effect of biologically synthesized Ag NPs on hydroponically grown *Bacopa monnieri* growth metabolism and found that biosynthesized Ag NPs significantly increased seed germination, protein, and carbohydrate syntheses. Also, SiO_2 NPs could increase the total phenol and flavonoid contents of *Anthemis gilanica* seedlings (Ahmadi et al., 2020). Silver NPs enhanced artemisinin content in the hairy roots of *Artemisia annua* (Zhang et al., 2013). Moreover, Fe_2O_3 magnetic nanoparticles increased considerably the content of rosmarinic acid, naringin, and apigenin in *Dracocephlum polychaetum* cell suspension culture (Taghizadeh et al., 2018).

There is a lack of information about the impact of Fe_3O_4 NPs on the secondary metabolites of chamomile seedlings. So, the objective of this study was to study the impacts of Fe_3O_4magnetic NPs as a chemical elicitor on the secondary metabolites of in vitro chamomile seedlings. Data from this investigation can aid us in promoting our knowledge about the mechanism(s) of the in vitro seedling reactions to Fe_3O_4NPs. It also may help us to enhance the biosynthesis of the valuable phenolic and flavonoid metabolites under in vitro conditions.

Material and Methods

Plant material and culture conditions

Seeds of *M. chamomilla* were surface disinfected in 10% (v/v) sodium hypochlorite solution for 15 min, 70% (v/v) ethanol for 1 min, followed by three washes with sterile distilled water. The sterilized seeds were cultured in Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) containing different Fe_3O_4 NPs concentrations (0, 25, 50, and 100 mgL^{-1}) and transferred to culture room with 25 ± 2 ℃ temperature, 55% relative humidity, and a 16-h photoperiod with a light intensity of 46 $\mu mol^{-1}m^{-2}$ s^{-1}. Each treatment was carried out in triplicate, and seedlings were collected after 5 weeks, kept at −70 ℃, and used for all the experiments.

Synthesis of Fe_3O_4 nanoparticles

Magnetic Fe_3O_4 nanoparticles were prepared according to the method described by Massart et al. (1981). Briefly, 5.83 g of FeCl3 was dissolved completely in 100 ml deionized hot water at 85 ℃ (solution A). Then, 2.74 g $FeCl_2$ was added to the solution for 15 min with stirring. In solution B, 24 g NaoH was dissolved in 200 ml deionized water. Solution B was added to the solution a drop wise under inert gas at 70 ℃ in a flask equipped with mechanical stirring. After increasing pH to 11 and observation of black sediment, the solution was cooled at room temperature and the magnetic Fe_3O_4 nanoparticles were collected by the magnet. The nanoparticles were washed three times with deionized water and then placed in the furnace at 300 ℃ for 2 h.

Characterization of Fe_3O_4nanoparticles

The synthesized nanoparticles were characterized by energy-dispersive X-ray **(**EDS), fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), vibrating sample magnetometer (VSM), and Brunauer-Emmett-Teller (BET)

analyses. FT-IR spectra were measured in the region of 400 cm^{-1} to 3900 cm^{-1} with Shimadzu 8400 s, Japan and spectroscopic grade KBr. XRD spectrum was documented by a diffractometer (Philips X'Pert XL 30) using CuK*a* radiation in Bragg-Brentano geometry. VSM analyses was studied by vibrating sample magnetometer/ alternating gradient force magnetometer (VSM/AGFM, MDK Co., Iran), and the BET content was measured by a Micromeritics TriStar II Plus model device.

Growth parameters

Fresh and dry weights of five seedlings per treatments were measured as the growth parameters. Relative water content (RWC) of leaves was estimated according to Wheatherley (1973) and based on the following equation:

RWC (%) = [(FW- DW)/(SW- DW)] × 100

Saturated weight (SW) of the plants was determined by keeping them in de-ionized water at 4 ℃ in the dark for 24 h, and DW was obtained after oven drying at 45 ℃ for 72 h.

Total phenol and flavonoid content

In order to prepare methanolic extract, 1 g dry tissue of the plants was homogenized in 5 ml methanol 80% and then was centrifuged at 5000 rpm for 20 minutes. The supernatant was used for measuring total phenolic, flavonoid, and antioxidant activity.

For determination of total phenol content, 0.1 ml methanolic extract was mixed with 2.5 ml Folin–Ciocalteu reagent 10%. The mixtures were neutralized by sodium bicarbonate 7% and then absorbance was read at 765 nm (Vermerris and Nicholson, 2008).

Flavonoid content was measured using aluminum chloride colorimetric method. The reaction mixture was pure methanol (1.5 ml), 10 % aluminum chloride (0.1 ml), 1 M potassium acetate (0.1 ml), distilled water (2.8 ml), and methanol extract (0.5 ml). The mixture was placed at room temperature for 30 min. The absorbance of the reaction mixture was recorded at 415 nm and expressed in µg g^{-1} fresh weight (Chang et al., 2002).

Measurement of DPPH-radical scavenging activity

For determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity method of Patro et al. (2005) was used. Accordingly, 100 µl of metabolic extract was mixed with 800 µl of DPPH (0.5 mM in ethanol). The absorbance of the resulting solution was measured at 517 nm after 30 min in darkness. The ability to scavenge the DPPH radical was calculated by the following equation:

Inhibition of DPPH radical (%) = [(Absorbance of control - Absorbance of sample) / (Absorbance of control)] × 100

Statistical Analyses

Each data point was an average of three or five replicates. The obtained data were submitted to the analysis of variance (ANOVA) using SPSS (Version 21). The significance of differences was determined according to Duncan's multiple range tests at 0.05 level of probability.

Results

Characterization of synthesized nanoparticles are shown in Fig. I. EDS spectra showed the Fe (4.4%), O_2(50.3%), and sodium (45.2%) peaks (Fig. I. a). FT-IR analysis identified the broad peak at about 1591 and 3381 cm^{-1}, which can correspond to O–H bonds. Also, the peak at about 580 and 610 cm^{-1}can be attributed to Fe-O radiation (Fig. I. b). The nature of nanoparticles was quantified using XRD analysis. The peaks at 30, 36, 38, 44, 58, and 63 are attributed to the (98), (011), and (1285) Bragg reflections, respectively and the formation of Fe_3O_4 NPs (Fig. I. c). Magnetic properties of NPs were quantified using VSM analysis with the field sweeping from -15000 Oe to +15000 Oe. The NPs showed high permeability and magnetization with the saturation magnetization value of 18 emu/g (Fig. I. d). Moreover, the BET analysis showed the BET surface area of 0.87 m^2/g and a pore diameter of 15.353 nm for the Fe_3O_4 NPs. All characterized analyses confirmed the formation of magnetic

Fe_3O_4 NPs before the synthesized NPs were used for in vitro culture studies.

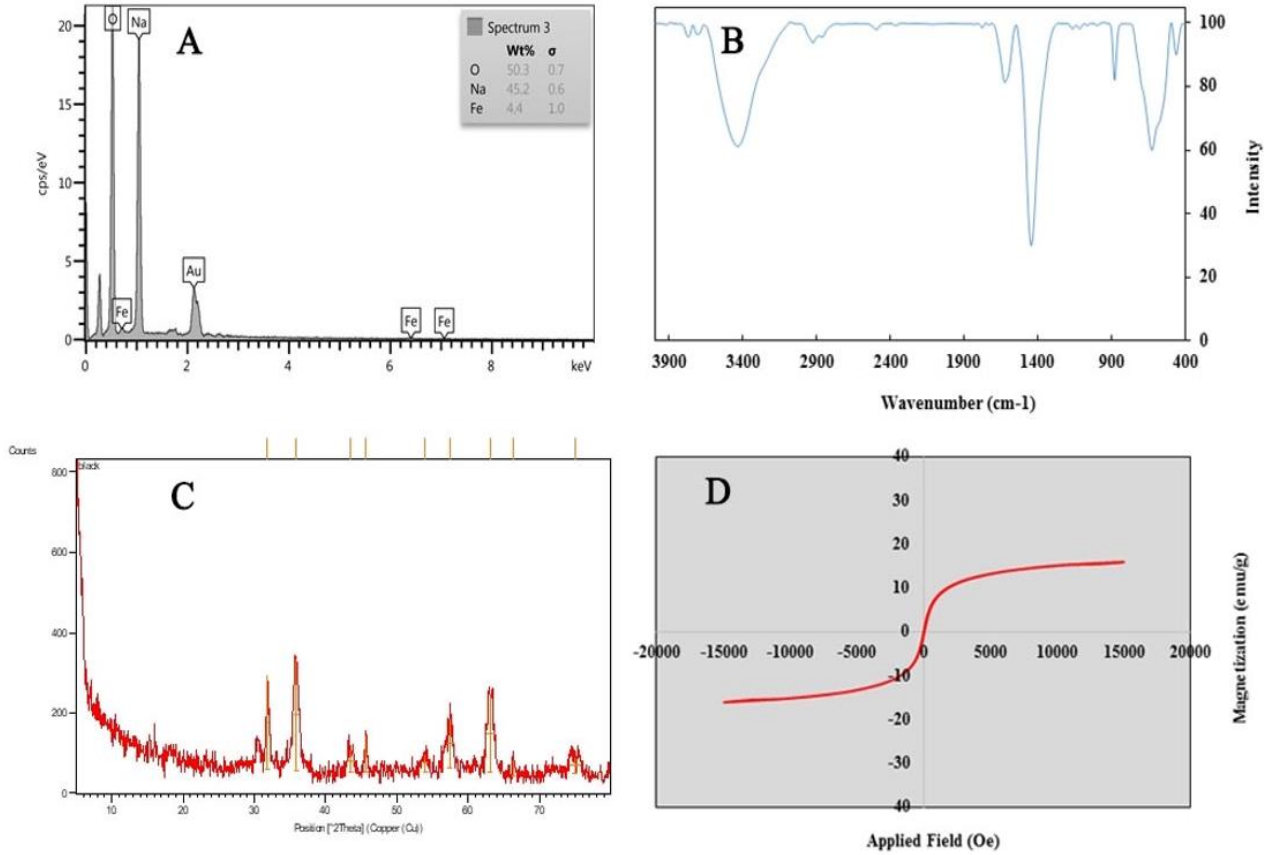

Fig. I. Characterization of Fe_3O_4 NPs using different analyses of EDS (A), FT-IR (B), XRD (C), and VSM (D).

Different concentrations of Fe_3O_4 NPs affected differently the *M. chamomilla* growth. Our results indicated that the Fe_3O_4 NPs had a positive effect on the fresh weight of *M. chamomilla* plants at 25 mg L^{-1}, but this parameter was reduced at higher concentrations. Fresh weight in the plants treated with the 25 mg L^{-1} concentration increased by 23.52%, compared to the control plants (Fig. II. b). No change in dry weight was detected following 25 mg L^{-1} exposure. Dry weight of plants subjected to 50 and 100 mg L^{-1} treatments reduced in comparison with the corresponding control plants. The dry weight declined by 43.75% and 62.5%, respectively, in comparison with the non-Fe_3O_4 NPs-exposed plants (Fig. II. C). Shoot length of 25 mg L^{-1}-exposed plants was higher than that of control plants, but 100 mg L^{-1} treatment caused a significant reduction in this parameter (Table 1). Treatment with 50 mg L^{-1} induced root length by 65.78 % compared to control (Table 1). The number of root in plants treated with 25 and 50 mg L^{-1} improved by 47.05% and 56.7%, respectively as compared with the Fe_3O_4-unexposed plants (Table 1). RWC enhanced by

Table 1
Effect of different Fe_3O_4 NPs concentrations on shoot length, root length, root number and relative water content (RWC) of chamomile seedlings.

Parameters	Fe_3O_4 NPs (mg L^{-1})			
	0	25	50	100
Shoot length (cm)	2.3 ± 0.11 b	3.1 ± 0.15 a	2.5 ± 0.14 b	1.8 ± 0.13 c
Root length (cm)	3.8 ± 0.14 b	4.3 ± 0.23 b	6.3 ± 0.35 a	2.6 ± 0.18 c
Number of root	4.3 ± 0.12 b	6.2 ± 0.17 a	6.66 ± 0.19 a	3.75 ± 0.25 bc
RWC	57.33 ± 1.24 ab	68.49 ± 3.32 a	50.12 ± 2.45 b	20.32 ± 1.83 c

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

19.46% with 25 mg L^{-1} of Fe_3O_4 NPs. However, treating with 50 and 100 mg L^{-1} concentrations decreased RWC. The maximum decrement in RWC was recorded in plants treated with 100 mg L^{-1} (Table 1).

The content of total phenol in leaves of plants treated with different concentrations of Fe_3O_4 NPs is compared with the control plants in Fig. II. a). Accordingly, the total phenol content of leaves significantly enhanced following the application of different Fe_3O_4 NPs concentrations. However, the highest leaf phenol content was observed in the plants treated with 50 mg L^{-1} concentrations. The total phenol content of leaves was elevated as 2.22-fold in the 50 mg L^{-1}-treated plants compared with the control condition. As shown in Fig. III.a, the content of total phenol in the roots of *M. chamomilla* plants exposed to Fe_3O_4 NPs was higher than that in the unexposed plants. Content of total phenol in the root tissues exposed to 25, 50, and 100 mg L^{-1} induced by 90.38%, 45.19%, and 11.19%, respectively, as compared with the control.

The total flavonoid content of leaves in plants subjected to 25 and 50 mg L^{-1} of Fe_3O_4 NPs was higher than that of the control plants. Application of 100 mg L^{-1} of Fe_3O_4 NPs reduced total flavonoid content in leaves by 39.68%. According to the obtained results, total flavonoid content in the *M. chamomilla* roots treated with 25 and 50 mg L^{-1} of Fe_3O_4 NPs was enhanced as compared to the normal condition. The 100 mg L^{-1} treatment caused a sharp decline (1.66-fold) in total flavonoid content in roots (Table 1).

DPPH scavenging activity increased significantly by about 47.6% and 10. 46% at 25 and 50 mg $L^{-1}Fe_3O_4$ NPs in leaves, respectively. However, it sharply reduced in leaves as a result of 100 mg L^{-1} of Fe_3O_4 NPs application (Fig. IV). The treatment with 25 mg L^{-1} Fe_3O_4 NPs also showed a positive effect on DPPH scavenging activity in the roots of *M. chamomilla* plants (Fig. IV).

Discussion

In this experiment, the effects of Fe_3O_4 NPs were evaluated on plant growth (Fig. I). According to the findings, the effects of Fe_3O_4 NPs on *M.*

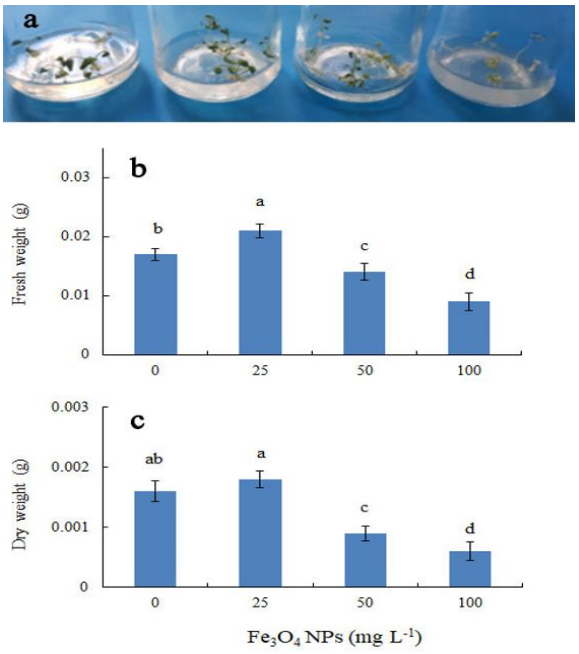

Fig. II. Depiction of in vitro chamomile seedlings under different Fe_3O_4 NPs concentrations(A); fresh (B) and dry (c) weights after 5 weeks; bars indicate ± SE (n = 5) in each group. Different letters indicate significant differences at $P \leq 0.05$ (LSD).

chamomilla plants were either positive or negative, depending on the applied concentrations. Low concentrations of Fe_3O_4 NPs had positive effects on *M. chamomilla* plant growth, whereas high concentrations of Fe_3O_4 NPs appeared to be adversely affecting the plants in this study. The application of Fe_3O_4 NPs at 25 mgL^{-1} concentration had positive effects on *M. chamomilla* growth. Seemingly, the stimulated growth potential in response to the Fe_3O_4 NPs application is attributed to increasing the root number, RWC, and antioxidant capacity of the plants. Hassanpouraghdam et al. (2020) reported nano-Fe foliar application had a positive influence on the *Rosmarinus officinalis* plant growth. Shankramma et al. (2015) stated that Fe_2O_3 magnetic nanoparticles enhanced the growth of *Solanum lycopersicum* plant. Usually, the performance of nanoparticles is dependent on some characteristics including chemical structure, size of particles, surface covering, and rate and concentration of application (Khodakovskaya et al., 2012). Fe is a necessary element for plant growth and production (Fageria, 2016). This microelement is also essential in stimulation of

some enzymes present in photosynthetic tissues and also the need for chlorophyll construction (Ramani and Kannan, 1985). The results of this study indicated that the growth of *M. chamomilla* plants decreased 50 and 100 mg L^{-1} of Fe_3O_4 NPs. This suggests adverse and toxic effects of Fe_3O_4 NPs on *M. chamomilla*. The observed decline in shoot and root lengths as well as dry and fresh weights of the plants subjected to Fe_3O_4 NPs might be due to a metabolic disorder. Different studies have shown the impacts of Fe_3O_4 NPs on the growth parameters of plants. Lee et al. (2010) stated the inhibition of root elongation in Arabidopsis under 400, 2000, and 4000 mg L^{-1} concentrations of Fe_3O_4 NPs. Toxic impacts of nanoparticles on plants are mostly expressed as decreasing root length, shoot length, and biomass, and also cellular damages such as protein damage, chlorophyll content, transpiration, and photosynthesis (Ghosh et al., 2015).

RWC is a widely used index that shows the water status of a plant (Shabrangi et al., 2015; Merati et al., 2015). RWC enhanced in plants treated with 25 mg L^{-1} concentration of Fe_3O_4 NPs while the lowest content was recorded in 100 mg L^{-1} treated plants (Table 1). We thus assume that the RWC in the leaves of Fe_3O_4 NPs-treated plants changed depending on the amount of applied Fe_3O_4 NPs. Application of iron nanoparticles in in vitro culture of strawberries increased RWC (Mozafari et al., 2018). Martínez-Fernández and Komárek (2016) showed that Fe_2O_3 treatment decreased RWC in *Solanum lycopersicum* L.

In this study, the content of secondary metabolites such as total flavonoid and total phenol in leaf and root of the Fe_3O_4 NPs-exposed plants were higher than those in the unexposed plants. It seems that Fe_3O_4 NPs had negative impacts on *M. chamomilla* plants; hence, the *M. chamomilla* plants tried to reduce these negative effects by enhancing the accumulation of these metabolites. The heightened accumulation of flavonoid and phenol metabolites in the *M. chamomilla* of Fe_3O_4 NPs-exposed plants is displayed as a strategy to avoid injury to the plant cells. Phenolic compounds are biosynthesized via the shikimate/phenylpropanoid pathway and play an important function as chemopreventive agents. These combinations act as effective free

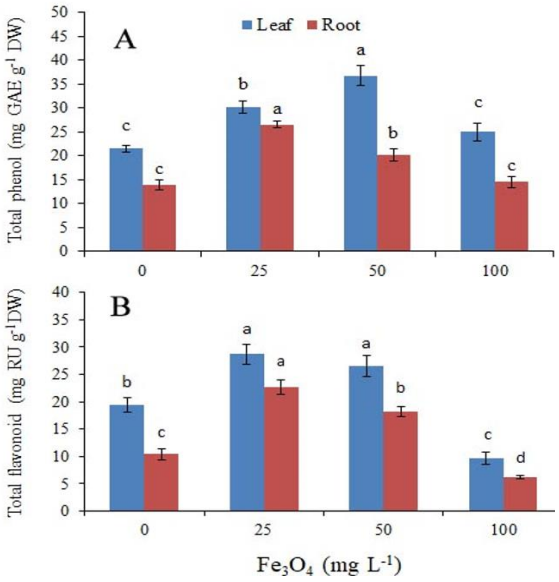

Fig. III. Effect of different concentrations of Fe_3O_4 NPs on total phenol (A) and flavonoid (B) contents of chamomile seedlings; bars indicate ± SE (n = 3) in each group. Different letters indicate significant differences at $P \leq 0.05$ (LSD).

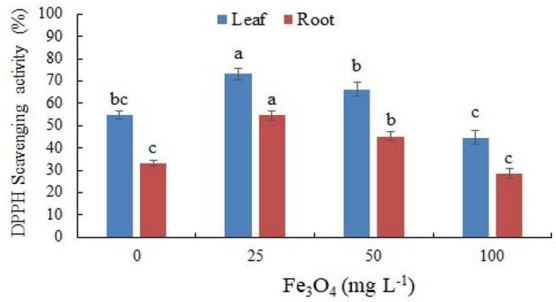

Fig. IV. Effect of different Fe_3O_4NPs concentrations on the DPPH scavenging activity of chamomile seedlings. Bars indicate ± SE (n = 3) in each group. Different letters indicate significant differences at $P \leq 0.05$ (LSD).

radical scavengers in cells. The antioxidant ability of phenolics relies on the reduction capabilities of the free radicals (Hassanpour et al., 2016). These composites have been found to have many therapeutic characteristics comprising anticancer, antimicrobial, and anti-inflammatory properties as well as enhancing the immune system. These properties make them a suitable candidate for therapeutic and medicinal applications (Sulaiman and Balachandran, 2012). Enhanced phenolic compounds might be a basic mechanism in Fe_3O_4 NPs-treated *M. chamomilla* to detoxify ROS in cells. Plant phenolic combinations such as flavonoids are powerful antioxidants and can act as metal chelators, reducing causes, and radical scavengers, so they may contribute in the

suppression of ROS produced by oxidative conditions (Asif, 2012). Consistent with these data, in the studies conducted by Mahmoud et al. (2019) and Hassanpouraghdam et al. (2020) it was observed that Fe_3O_4 NPs boosted the levels of phenol and flavonoid in plants.

The antioxidant ability was measured indirectly only by DPPH. Root and leaf extracts of 25 mg L^{-1} Fe_3O_4 NPs-treated plants showed the greatest DPPH scavenging activity in comparison with the other treatments. Increasing Fe_3O_4 NPs at 100 mg L^{-1} significantly declined the DPPH scavenging activity of the root and leaf extracts compared to the control. According to our data, antioxidant capacity of *M. chamomilla* increased under 25 and 50mg L^{-1} Fe_3O_4 NPs treatment and its growth improved. However, at higher concentrations of Fe_3O_4 NPs, the plant's antioxidant capacity decreased, and it was not able to overcome the stress conditions caused by Fe_3O_4NPs, and its growth has reduced. Environmental factors, the nutrient concentration of medium, developmental stage of the plant, and genotype could influence its antioxidant capacity (Zhang et al., 2013; Hassanpour and Niknam, 2014). In fact, stimulation of DPPH radical-scavenging activity has been previously reported under stress conditions (Rezayian et al., 2018; Ashouri Sheikhi et al., 2016).

Conclusion

The study of plant response to various concentrations of nanoparticles is important and can lead to a better understanding of the reaction of plants to elements. Fe_3O_4 NPs influenced growth and metabolic processes of *M. chamomilla* plants in a concentration-dependent manner. Based on the obtained data, 25 mg L^{-1} Fe_3O_4 NPs had a positive impact on the plant growth. Also, the findings showed that the elevated Fe_3O_4 NPs has injurious effects on the growth of *M. chamomilla*. Generally, Fe_3O_4 NPs had a positive effect on the content of secondary metabolites such as total phenol and total flavonoid. Considering the high pharmacological value of secondary metabolites, the findings can be promising for many medical purposes. However, further comprehensive analyses are desired to complete our understanding of how elevated Fe_3O_4 NPs concentrations regulate the metabolism of secondary metabolites in *M. chamomilla* and other plants.

Acknowledgment

The authors thank the Aerospace Research Institute, Ministry of Science, Research and Technology for preparing instruments for this study.

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