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The Effects of some Inorganic-, Synthetic- and Organic-Fertilizers on the Vegetative Growth and Iron Content in Pistachio cv. Ghazvini under Alkaline Conditions

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A R T I C L E I N F O

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A B S T R A C T

In the current research, the effects of some organic fertilizers as fulvic and humic acids on the vegetative growth of pistachio seedlings in a semi-hydroponic culture containing Fe-EDDHA (as a synthetic fertilizer) and $FeSO_4$ (as an inorganic fertilizer) were investigated. The alkalinity is recognized as a main problem in pistachio orchards, therefore the pH of nutrient solution was adjusted to 7.5 and 8.5 (as two alkaline pHs) versus 6.5 (as the optimum pH of hydroponics) in this study. Plants were treated for 9 months under greenhouse conditions. The results demonstrated that the organic fertilizers (especially humic acid) reduced plant growth in acidic condition (pH 6.5). It can be related to precipitation of humic acid under acidity and thereby removing some essential elements for instance Fe form plant growth medium. In contrast, humic substances increased plant growth up to 2.3 times under alkalinity. The analysis indicated that these organic-stimulants can improve plant growth under alkaline conditions by increasing Fe content to about 2 times in pistachio seedlings, especially in shoots. It was noted that this result was somewhat similar in plants grown in media containing Fe-EDDHA and $FeSO_4$. In addition, environmental and economic importance of the application of humic and fulvic acids makes them proper candidates to substitute synthetic fertilizers for agricultural improvement under alkalinity.

Introduction

Pistachio (*Pistacia vera L.*) belongs to the nut crops and is recognized as a strategic agricultural production in arid and semi-arid regions of Iran. Currently, the area of pistachio cultivation in Iran is over 360000 hectares, with Kerman province producing more than 270000 hectares (Rezaei *et al*., 2014; Amiri and Rezvani, 2016; Jalali *et al*., 2018). The most of pistachio orchards in Iran suffer from alkalinity which is considered critical because of its effects on pH of soil solution and also on plant growth and quality (Roosta and Mohammadi, 2013). The appropriate pH for most of crop plants is between 5.5 and 7.5. But the soil pH in Kerman and Rafsanjan fields is in the range of 7.2-8.5 with an average of 7.9 (Talaie and Panahi, 2002; Jalali *et al*., 2018). Soil alkalinity may be mostly originated from low rainfall which leads to accumulate exchangeable bases such as carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-) ions in the soil (Valdez-Aguilar and Reed, 2010; Roosta, 2011). The increase of pH may detrimentally affect plant growth and development by reducing the solubility of plant essential elements especially irons (Fe) (Roosta,

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2011). Fe is an important micronutrient which plays different critical roles in the growth, development and reproduction of plants (Kobayashi and Nishizawa, 2012; Vigani *et al*., 2013). Iron is involved in some metabolic processes as DNA synthesis, photosynthesis and respiration (Rout and Sahoo, 2015). Furthermore, many metabolic pathways affected by Fe because it is an important co-factor of many enzymes such as those involved in the pathway of chlorophyll biosynthesis (Hu *et al*., 2017). So, leaf chlorosis under alkaline conditions is attributed to Fe deficiency due to reduced Fe avalibility and uptake (Roosta, 2011).

Angiosperm species uptake Fe by the acidification of the rhizosphere via H^+-ATPase activity, the reduction of Fe(III) to Fe(II) by ferric chelate reductase, and the uptake of Fe(II) by iron transporters (Kobayashi and Nishizawa, 2012). Using some Fe-fertilzers and also suitable elemental sources may reduce some detrimental effects of alkalinity on plant growth and production (Colla *et al*., 2010; Roosta *et al*., 2015; Sida-Arreola *et al*., 2015). There are three main classes of iron fertilizers: inorganic and soluble Fe compounds such as iron (II) sulfate ($FeSO_4$), synthetic Fe chelates for example ethylenediamine-di(O-hydroxy phenyl acetic acid) (EDDHA), and finally natural Fe complexes like humic substances (Abadía *et al*., 2011; Roosta *et al*., 2015; Sida-Arreola *et al*., 2015).

It has been proven that under alkaline conditions, the solubility and availability of iron in inorganic fertilizer of $FeSO_4$ would be remarkably reduced. Chelates such as EDDHA are organic molecules that envelope certain micronutrients and protect them from being rendered unavailable by interaction with other elements under alkalinity (Ferrarezi *et al*., 2007; Mordoğan *et al*., 2013). Roosta *et al*. (2015) demonstrated that leaf Fe content and overall growth of lettuce (*Lactuca sativa* L.) was increased significantly by Fe-EDDHA application in alkaline solutions. They found the lowest content of Fe, chlorophyll, carotenoids and soluble sugars were determined in $FeSO_4$ treatment.

Humic substances originated from microbial degradation of plant and animal residues provide the main source of carbon in the soil (Mikkelsen, 2005; Canellas *et al*., 2015). It has been demonstrated that they can stimulate plant nutrient uptake and also improve root, shoot and leaf growth as well as crop germination via interacting with physiological and metabolic processes (Piccolo *et al*., 1993; Nardi *et al*., 2002; Jia *et al*., 2019; Jomhataikool *et al*., 2019).

Moreover, the effect of humic acid on some growth parameters and ion concentrations of Mexican Lime (Citrus aurantifolia Swingle) have been reported under stressed condition (Abootalebi Jahromi, and Hassanzadeh Khankahdani, 2016). Not only humic acid positively affect growth traits of fruit tress but also increases vegetables and ornamentals commercial yield (Barzegar, 2016; Abdipour et al., 2019; Zaferanch et al., 2019). So, they are agriculturally recognized as organic fertilizers.

Humic substances are characterized as diverse and low molecular weight compounds providing dynamic associations by hydrogen bounds and hydrophobic interactions. The ratios of hydrophilic/hydrophobic properties control their environmental reactivity (Piccolo, 2012). These substances can be divided into three sections based on their solubility in different media: humin, humic and fulvic acids (Peña-Méndez *et al*., 2005). Although humin presented as the insoluble residue, humic acid dissolves under alkalinity but precipitates in acidic pH. On the other hand, fulvic acid remains soluble in both alkaline and acidic solutions (Hayes, 2006).

The aim of the current study was to investigate the effects of humic substances (fulvic and humic acids) on plant growth and also Fe content in *P. vera* seedlings under different pHs (6.5, 7.5 and 8.5) in a semi-hydroponic culture. We also compared the effects of two forms of iron such as Fe-EDDHA and $FeSO_4$ in the nutritional solution.

Materials and Methods

The seeds of *P. vera* L. cv. 'Ghazvini' were prepared in the spring of 2018 from Pistachio Research Center in Rafsanjan, Kerman, Iran. To break dormancy, dehisced seeds of pistachio were pre-chilled for 10 days at 4°C and then soaked in water for 48 h. They were cultivated into black plastic pots (12 cm diameter) containing water washed perlite (Pakdaman *et al*., 2013).

One week after the germination, seedlings were nourished with modified Hoagland’s solutions. The basic nutrient medium contained 1.5 mM $Ca(NO_3)_2$, 1mM KNO_3, 0.9 mM NaOH, 0.5 mM $MgSO_4$, 0.5 mM NH_4NO_3, 0.02 mM KH_2PO_4, 0.1 mM NaCl, 0.05 mM ferrous ethylendiamine-N,N'-bis(2-hydroxyphenylacetic acid) (FeEDDHA), 0.001 mM H_3BO_3, 0.0007 mM $MnSO_4$, 0.0005 mM $ZnSO_4$, 0.0001 mM $(NH_4)_6Mo_7O_{24}$, 0.0001 mM $CuSO_4$ (Parker and Norvell, 1999). In the current study, the effects of three factors such as organic-fertilizers (control, fulvic and humic acids), chelators (sulfate as inorganic- and EDDHA as synthetic-fertilizers) as well as pH (at 3 levels of 6.5, 7.5 and 8.5) were evaluated on the dry weight and Fe content of pistachio seedlings (totally 3×2×3 treatments). For this purpose, basic nutrient solution (as the control) was modified with 25 mg/l potassium humate and fulvic acid. In the other part of the research, sulfate (SO_4^{-2}) replaced the chelator of EDDHA to bind Fe^{+2}. Meanwhile, 0.05 mM $FeSO_4$ used in the basic and modified nutrient solutions (as 2 treatments of Fe-EDDHA and $FeSO_4$). The pH of all nutrient media adjusted to 6.5, 7.5 and 8.5 by using weak acids and alkalines such as HCl and NaOH.

It is noted that nutrient solutions were being replaced completely every 2 weeks. Nine months after treatment application, plants were harvested and divided into roots and shoots, oven-dried at 70 °C for 72 h and their dry weights were separately measured. Fe analyses were performed on the grounded dried materials which passed through 40-mesh screen. Then, 1 g of this material was turned into ash at 550°C and extracted with 6N HCl (Jones Jr *et al*., 1991; Nadi *et al*., 2011). Fe concentration in plant materials was determined using inductively-coupled plasma (ICP) spectrometry (Optima, model 7000 DV).

Three pots, each containing 3 seedlings (totally 9 replicates), were considered for every treatments. They were arranged in a randomized complete block design in a greenhouse. Statistical analyses were performed using SPSS software (version 21), then the results were analyzed through one-way analyses of variance (ANOVA) and finally the means were compared using Duncan's test (P<0.05).

Results

The results of variance analysis (Table 1) indicated the effects of different levels of organic-fertilizers (control, fulvic and humic acids), chelators (sulfate and EDDHA), pH (6.5, 7.5 and 8.5) and their interactions on some factors as dry weights and Fe concentration in pistachio roots and shoots.

The effects of fulvic and humic acids on dry weight of pistachio roots and shoots grown in nutrient solution containing different Fe binding agents of EDDHA and SO_4^{-2} at pH 6.5 is shown in Fig. 1. As the results related to Fe-EDDHA show, root dry weights of plants grown in the basic medium (control) and also the treatment containing fulvic acid were significantly higher than the medium containing humic acid(Fig. 1a). There was no significant difference between root dry weight of plants in control and fulvic acid treatment (Fig. 1a). Fulvic and humic acids had no significant effect on root dry weights of plants grown in $FeSO_4$ containing solution (Fig. 1a).

As the results of Fe-EDDHA in Fig. 1b show, there was no significant difference between shoot dry weights of plants in control and fulvic containing media at pH 6.5. But the shoot dry weight of plants grown in humic acid containing solution was significantly lower than two others (control and fulvic containing medium). When iron was used in acidic nutrient solution as $FeSO_4$, humic and fulvic acid did not have any significant effect on shoot dry weights (Fig. 1b).

Fig. 2 shows the effects of fulvic and humic acids on root (Fig. 2a) and shoot (Fig. 2b) dry weights of plants in Fe-EDDHA and $FeSO_4$ containing media at pH 7.5. Humic acid in the presence of Fe-EDDHA increased root dry weight in comparison with the control (Fig. 2a). There was no significant difference between root dry weights of plants grown in control and fulvic acid containing solutions. In the presence of $FeSO_4$, there was not any significant difference between root dry weights of control plants with those treated with fulvic and humic acids (Fig. 2a).

By using Fe chelating agent (EDDHA) at pH 7.5, the highest shoot biomass was seen in plants treated with humic acid compared to plants grown in control and fulvic acid containing media (Fig. 2b). Shoot dry weights did not change by treating with humic and fulvic acids in the presence of $FeSO_4$ (Fig. 2b).

The effects of fulvic and humic acids and also different Fe binding agents (EDDHA and SO_4^{-2}) on root and shoot dry weights of *P. vera* grown in modified Hoagland's solution adjusted to the alkaline pH of 8.5 are shown in Fig. 3. In the presence of Fe-EDDHA, humic and fulvic acids increased root dry weights significantly, in comparison with control plants (Fig. 3a). There was no significant difference between root dry weights of control plants under alkalinity (pH 8.5) with those treated with fulvic and humic acids in the presence of $FeSO_4$ (Fig. 3a).

Table 1. The variance analysis effects of organic-fertilizers (control, fulvic and humic acids), chelators (sulfate and EDDHA), pH (6.5, 7.5 and 8.5) and their interactions on some factors studied.

Source of variation	Root dry weight		Shoot dry weight		Root Fe concentration		Shoot Fe concentration	
	df	Mean Square	df	Mean Square	df	Mean Square	df	Mean Square
Chelators	1	0.068	1	1.058*	1	47118.072*	1	1478.940*
pH	2	0.209*	2	0.265	2	11974.736*	2	1413.312*
Organic-fertilizers	2	0.494*	2	3.637*	2	33596.977*	2	5416.731*
Chelators*pH	2	0.001	2	0.385	2	15945.622*	2	730.949*
Chelators*organic-fertilizers	2	0.130*	2	0.742*	2	3581.266*	2	191.941*
pH*organic-fertilizers	4	0.328*	4	3.998*	4	17743.496*	4	4536.089*
Chelators*pH*organic-fertilizers	4	0.408*	4	5.073*	4	7332.904*	4	264.321*
Error	36	0.027	36	0.139	36	136.515	36	30.468
Total	54		54		54		54	

*Significant at $P<0.05$.

At the highest pH (8.5), plants treated by fulvic and humic acids had higher shoot dry weight than those grown in nutrient solution containing Fe-EDDHA (Fig. 3b). There was no significant difference between humic and fulvic acid treated plants under this condition (Fig. 3b). In the presence of $FeSO_4$, plants grown in humic acid containing medium had the highest shoot dry weight compared with control and fulvic acid containing solutions at pH 8.5 (Fig. 3b).

The effect of organic-fertilizers based on fulvic and humic acids on root and shoot Fe concentration in plants grown in the modified Hogland's solution containing Fe-EDDHA or $FeSO_4$ is shown in Fig. 4. As the results at pH 6.5 show, these fertilizers had no significant effect on Fe concentrations in *P. vera* roots under Fe-EDDHA treatment (Fig. 4a). By treating with $FeSO_4$, the highest root concentration of Fe was seen in plants grown in nutrient solution containing humic acid (Fig. 4a). There was no significant difference among Fe concentration in roots under control and fulvic acid treatments (Fig. 4a).

At acidic pH of 6.5, fulvic and humic acids significantly reduced Fe concentration in shoots under

Fe-EDDHA treatment (Fig.4b). By using $FeSO_4$, iron concentration in shoots significantly decreased in plants grown in fulvic and humic media (Fig. 4b).

The effect of Fe-EDDHA and $FeSO_4$ and also fulvic and humic acid fertilizers on the concentration of Fe in plants grown in modified Hoagland's solution at pH 7.5 is shown in Fig. 5. As it can be seen, plants treated with humic acid had the highest root Fe concentration in the presence of Fe-EDDHA or $FeSO_4$ (Fig. 5a). There was no significant difference between control and fulvic acid treatments in this condition (Fig. 5a). Just like roots, the highest Fe concentration of shoots can also be seen in plants treated with humic acid in Fe-EDDHA or $FeSO_4$ containing solutions at pH 7.5 (Fig. 5b).

The effect of fulvic and humic acids and also different Fe binding agents (EDDHA and SO_4^{-2}) on iron concentration in *P. vera* roots and shoots grown in modified Hoagland's solution adjusted to the alkaline pH 8.5 is shown in Fig. 6. As the results indicate, humic acid increased root Fe content in plants grown in Fe-EDDHA containing solution and there was no significant difference between control and fulvic treated ones (Fig. 6a). By using $FeSO_4$ in nutrient medium, organic-fertilizers significantly increased the concentration of Fe in *P. vera* roots at pH 8.5 (Fig. 6a).

It was also mentioned that the highest Fe concentration in plant shoots grown in medium containing both Fe binding agents (EDDHA and SO_4^{-2}) was seen under treatment of humic acid (Fig. 6b).

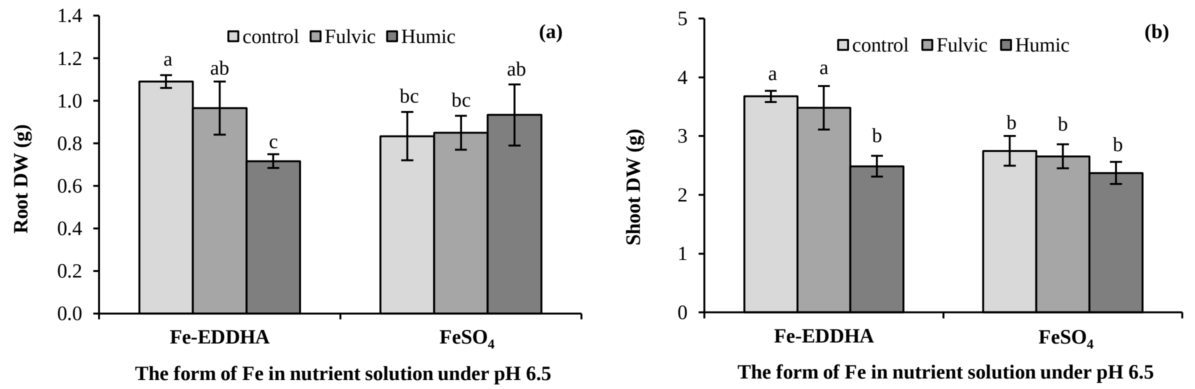

Fig. 1. The effects of fulvic and humic acids on dry weight (DW) of pistachio roots (a) and shoots (b) grown in nutrient solution containing different forms of Fe (as Fe-EDDHA and $FeSO_4$) at acidic pH of 6.5. Values are means ± SD (n = 9). Different letters above bars indicate significant differences in dry weights (Duncan test, P<0.05).

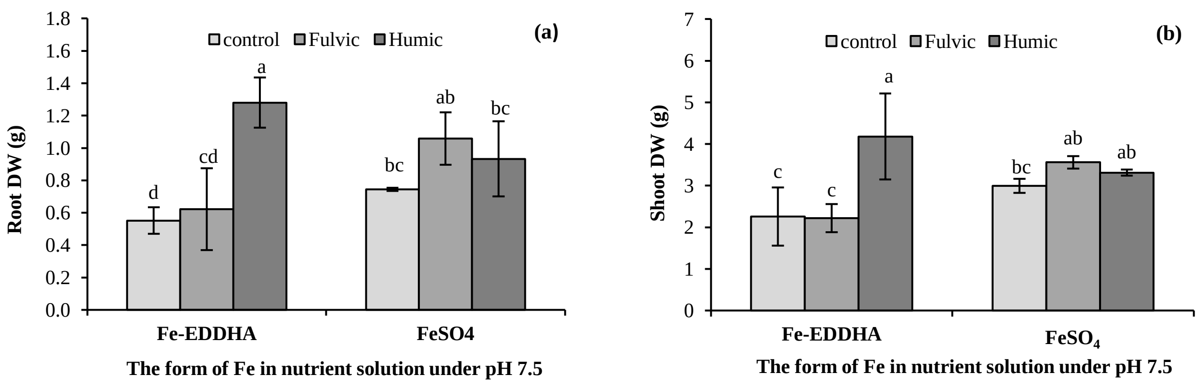

Fig. 2. The effects of fulvic and humic acids on dry weight (DW) of pistachio roots (a) and shoots (b) grown in nutrient solution containing different forms of Fe (as Fe-EDDHA and $FeSO_4$) at pH of 7.5. Values are means ± SD (n = 9). Different letters above bars indicate significant differences in dry weights (Duncan test, P<0.05).

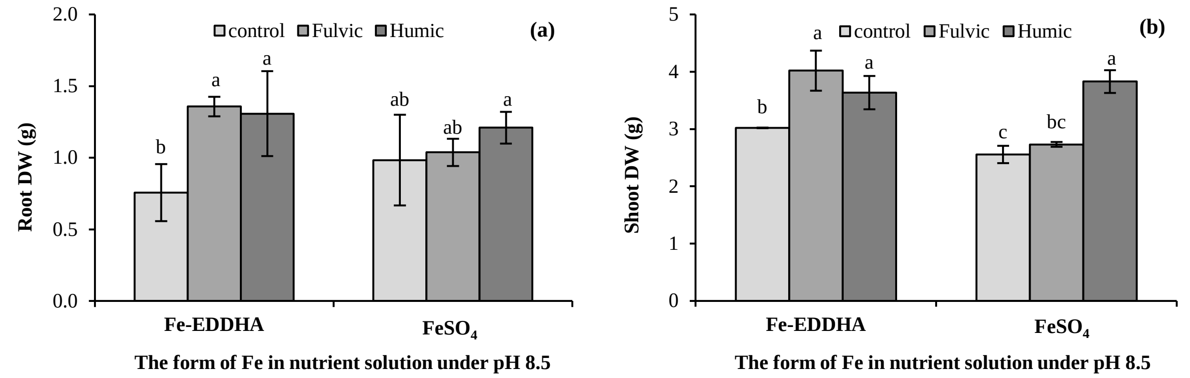

Fig. 3. The effects of fulvic and humic acids on dry weight (DW) of pistachio roots (a) and shoots (b) grown in nutrient solution containing different forms of Fe (as Fe-EDDHA and $FeSO_4$) at pH of 8.5. Values are means ± SD (n = 9). Different letters above bars indicate significant differences in dry weights (Duncan test, P<0.05).

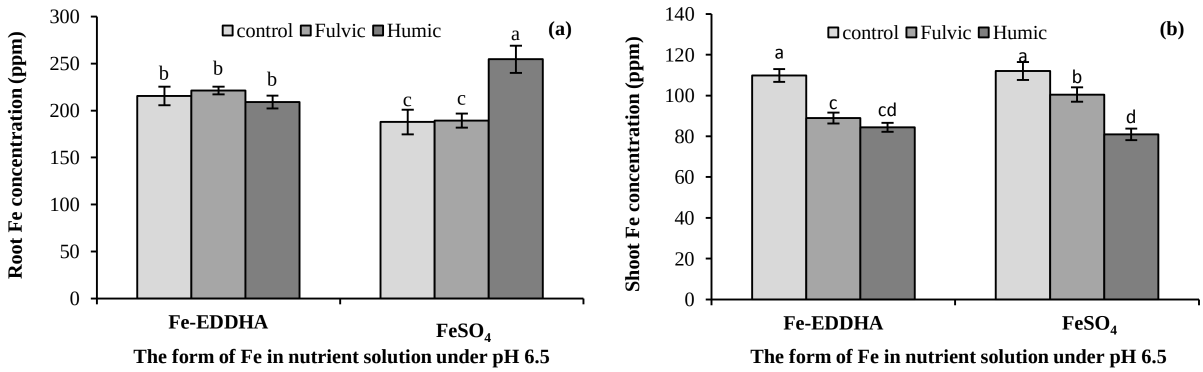

Fig. 4. The effects of fulvic and humic acids on Fe concentration in pistachio roots (a) and shoots (b) grown in nutrient solution containing different forms of Fe (as Fe-EDDHA and $FeSO_4$) at acidic pH of 6.5. Values are means ± SD (n = 9). Different letters above bars indicate significant differences in dry weights (Duncan test, P<0.05).

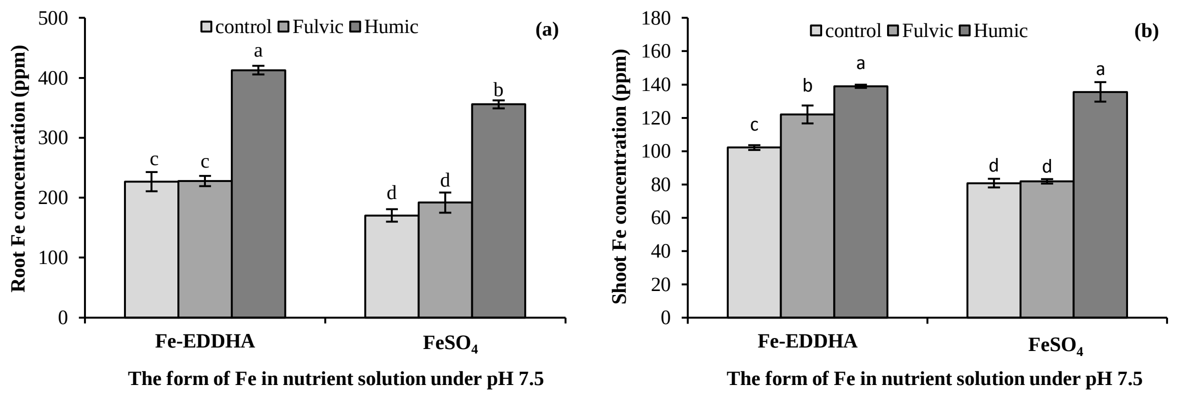

Fig. 5. The effects of fulvic and humic acids on Fe concentration in pistachio roots (a) and shoots (b) grown in nutrient solution containing different forms of Fe (as Fe-EDDHA and $FeSO_4$) at pH of 7.5. Values are means ± SD (n = 9). Different letters above bars indicate significant differences in dry weights (Duncan test, P<0.05).

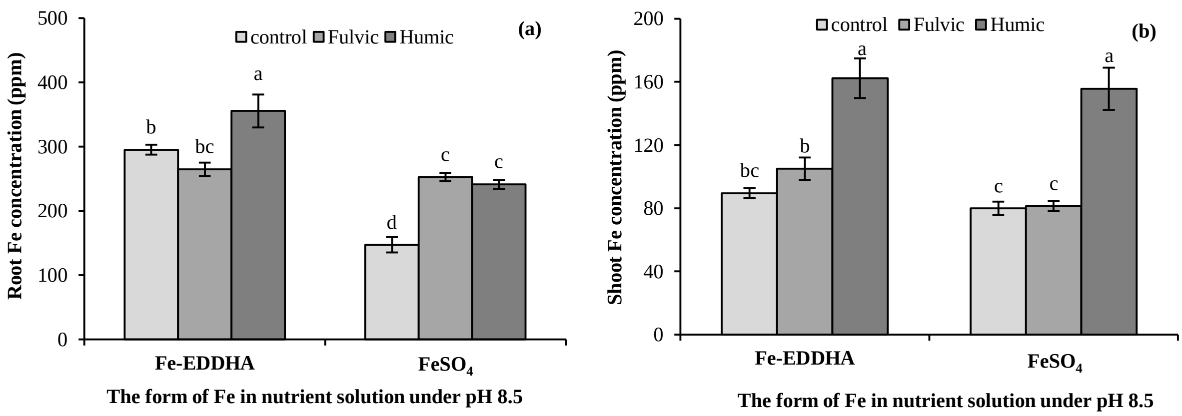

Fig. 6. The effects of fulvic and humic acids on Fe concentration in pistachio roots (a) and shoots (b) grown in nutrient solution containing different forms of Fe (as Fe-EDDHA and $FeSO_4$) at pH of 8.5. Values are means ± SD (n = 9). Different letters above bars indicate significant differences in dry weights (Duncan test, *P*<0.05).

Discussion

Pistachio as one of the most important agricultural product suffers from alkalinity in Iran. The soil of most pistachio orchards are also poor in organic matters and nutritional elements especially Fe (Sheibani, 1998; Talaie and Panahi, 2002; Jalali *et al*., 2018). It is well-known that iron plays different critical roles in plant growth and metabolism and its deficiency affects the physiology and biochemistry of whole plant (Roosta *et al*., 2015). Using iron fertilizers such as inorganic compounds (as $FeSO_4$), synthetic Fe chelators (as Fe-EDDHA) and finally organic complexes (as humic substances) are some solutions to avoid or reduce the detrimental effects of alkalinity on plant growth and production (Abadía *et al*., 2011).

In this research, the effect of humic substances (humic and fulvic acids) was investigated on the plant growth and also Fe content in *P. vera* seedlings grown in a semi-hydroponic medium containing Fe-EDDHA or $FeSO_4$. The pH of modified Hoagland's solutions was adjusted to, 7.5 and 8.5 (as alkaline pHs) versus pH 6.5 as the optimal pH in hydroponic medium. As the results show, any form of iron (including Fe-EDDHA and also $FeSO_4$) would work to supply plant's need in acidic pH (6.5). Organic fertilizers especially humic acid which is insoluble in acidity condition may precipitate some essential elements and keep them out of plant reach (Piccolo, 2012; Fuentes *et al*., 2018). The reducing Fe concentration especially in the shoots of pistachio seedlings treated with humic acids under acidic pH of 6.5 proved this claim. By increasing pH to 7.5 and 8.5 in growth medium, the importance of organic fertilizer (fulvic and humic acids) application was remarkable in this research. Some of the positive effects of organic fertilizers (especially humic acid) might be attributed to improving Fe uptake via pistachio roots under alkalinity. Pandeya *et al*. (1998) demonstrated that the uptake of Fe by paddy seedlings (*Oryza sativa*) and tissue Fe concentration were higher in Fe-fulvic acid treatment in comparison with $FeCl_3$. This finding indicated the superiority of Fe organic fertilizers over inorganic ones. In the other research, Elena *et al*. (2009) investigated the effect of purified humic acid on the transcriptional regulation and also the activity of some principle enzymes involved in iron assimilation and absorption. They proved that the expression and activity of plasma membrane H^+-ATPase, Fe (II) high-affinity transporter and Fe (III) chelate-reductase increased in non-deficient cucumber plants (*Cucumis sativus* L.). All current evidence suggests that beneficial effects of humic substances (humic and fulvic acids) may be related to iron absorption, assimilation and distribution (Fe use efficiency) (Mikkelsen, 2005).

Roosta *et al.* (2015) investigated the effects of different iron sources as Fe-EDDHA and $FeSO_4$ on lettuce (*Lactuca sative*) growth in alkaline hydroponic culture. They showed that leaf Fe content and overall plant growth increased significantly by iron chelate (Fe-EDDHA) application. The lowest content of Fe, chlorophyll, carotenoids and soluble sugars were detected under $FeSO_4$ application. In the current research, it was remarkable that humic substances (fulvic and humic acids) increased plant growth and also Fe content in *P. vera*, (especially in the case of shoots) treated with Fe-EDDHA and also $FeSO_4$ under alkalinity (pHs of 7.5 and 8.5). The effect of humic acid was more detectable in these conditions. This impact is mainly attributed to the complexity properties of humic substances to modify iron solubility in alkaline medium containing $FeSO_4$. They prevent precipitation and following crystallization of iron as well-ordered Fe-(hydro) oxides by forming soluble complexes and then moving toward plant roots (Varanini and Pinton, 2006). So, humic substances (as organic-stimulants) can reduce or compensate some detrimental effects of alkalinity by improving pistachio nutrition condition.

Agricultural intensification through synthetic fertilizers (such as Fe-EDDHA) has been recently expanded with an alarming rate. This practice in addition to being economically costly may be also accompanied with a major reduction in ecological heritage resulted from soil erosion, deforestation, industrial pollution, reduces ground- and surface-water quality and also biodiversity (Altieri, 2002). By contrast, humic substances along with economic benefit and promoting plant growth, may also contribute to the regulation of many crucial environmental and ecological processes. As they regulate both carbon and nitrogen cycling in the soil, the growth of soil microorganisms, heavy metal fate and transport, and the stabilization of soil structure (Piccolo, 2012; Jomhataikool *et al*., 2019).

In conclusion, as pistachio normally encounters alkaline conditions in Iran, it suffers from iron deficiency. The application of some organic-stimulants such as humic substances can reduce or compensate some detrimental effects of alkalinity and thereby improve plant growth. The results of the current research also demonstrated that these organic matters can stimulate Fe uptake by plant and thereby increase the vegetative growth of pistachio seedlings. Therefore due to non-friendly environmental effects and also the unprofitable cost of synthetic fertilizers, humic substances can be introduced as proper substitutes for them in the future.

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