The Effect of Humic and Fulvic Acids as Bio-Fertilizers on the Growth of *Pistacia vera* Seedlings under Alkaline Conditions

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Abstract

Introduction: Pistachio (*Pistacia vera*), as an important agricultural product, normally faces different problems in Iran. Alkalinity is one of the main factors that has detrimental effects on pistachio growth and its production by reducing availability and solubility of essential elements. Using some fertilizers and chelating agents improves the availability and solubility of nutritional elements in the rhizosphere and thereby improves the growth ofplants.

Materials and Methods: The current research investigated the effect of two bio-fertilizers (humic and fulvic acids) on the growth of pistachio seedlings grown in semi-hydroponic cultures containing either Fe-EDDHA or $FeSO_4$. The pH of culture mediums was adjusted to 6.5, 7.5 and 8.5.

Results: The results indicated that using humic substances (humic and fulvic acids) can increase pistachio dry weight under alkalinity conditions. These findings were remarkable, especially in the presence of Fe-EDDHA versus $FeSO_4$.

Conclusion: Humic substances (as bio-stimulants), especially in the presence of EDDHA, can reduce or compensate some detrimental effects of alkalinity and thereby improve plant growth.

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1. Introduction

One of the combined challenges facing the world is feeding a growing population along with environment protection and the production of reversible energy resources. Regarding food, although demand may reach 2-5 folds by 2030, the increase in its production will not exceed 60% in the next decades [1]. In recent years, agricultural intensification has been done using irreversible energy resources as synthetic fertilizers. So, increasing crop yields would be accompanied by a major reduction in ecological heritage at an alarming rate [2]. In this regard, humic-based products may be a potential technology to integrate various biotechnological approaches for environmental intensification in relation to promoting plant growth and also its adaptation to new food production methods.

Humic substances are formed from the degradation of plant and animal residues through microbial metabolism (Fig. 1) [3], and make up the major source of carbon at the surface of earth [4]. It has been demonstrated that humic substances have many beneficial effects on soil and plant growth. Through direct interaction with physiological and metabolism processes, they can stimulate nutrient uptake and increase plant root, shoot and leaf growth and also crop germination [5, 6]. Therefore, they are considered as agriculturally organic fertilizers.

In solutions, humic substances are revealed as a collection of diverse and relatively low molecular weight components which can provide dynamic associations through hydrogen bonds and hydrophobic interactions. The hydrophobic and hydrophilic ratio reveals its environmental activity [7]. Based on solubility, Humic substances are divided into three sections: humin, humic acid and fulvic acid (Fig. 1) [3]. Although, humin is presented as the insoluble residue, humic acid is defined as humus materials which are soluble in alkaline solutions but precipitate in acidic pH. In contrast, fulvic acid remains soluble in alkaline and also acidic solutions [8]. In fact, humic acids are made of a mixture of weak aliphatic and also aromatic molecules with predominantly hydrophobic associations. Because of hydrophobic diffuse forces, they are stabilized at neutral as well as alkaline pH. In acidic pH, inter-molecular hydrogen bonds grow their confirmations progressively until flocculation. However, fulvic acids are smaller hydrophilic molecules with enough functional acidic groups to keep the fulvic cluster dissolved at any pH [4].

Alkalinity is one of the main agricultural problems in Iran, especially in dry areas. The lack of rainfall leads to the accumulation of exchangeable bases as well as carbonate and bicarbonate ions in the soil, which increases pH [9, 10]. Alkalinity detrimentally affects plant growth via reducing availability and solubility of nutritional elements such as Fe, P, etc. [10]. The application of some fertilizers and also suitable elemental sources can compensate or reduce the detrimental effects of alkalinity on plant growth [11-13]. Humic substances as natural chelators of nutritional elements under alkaline conditions can improve their absorption by roots [14]. On the other hand, some researchers have demonstrated that using such synthetic chelators as ethylenediamine-di (o-hydroxy phenyl acetic acid) (EDDHA) can stimulate plant growth [12, 13].

Pistachio (*Pistacia vera*), which is recognized as a strategic agricultural production in Iran, normally encounters alkalinity and elemental deficiencies. Therefore, the aim of this study was to investigate the effects of different humic substances such as fulvic and humic acids on the growth of *P. vera* under different pHs of 6.5, 7.5 and 8.5 in a semi-hydroponic culture. We also used Fe element as Fe-EDDHA or $FeSO_4$ in the culture medium.

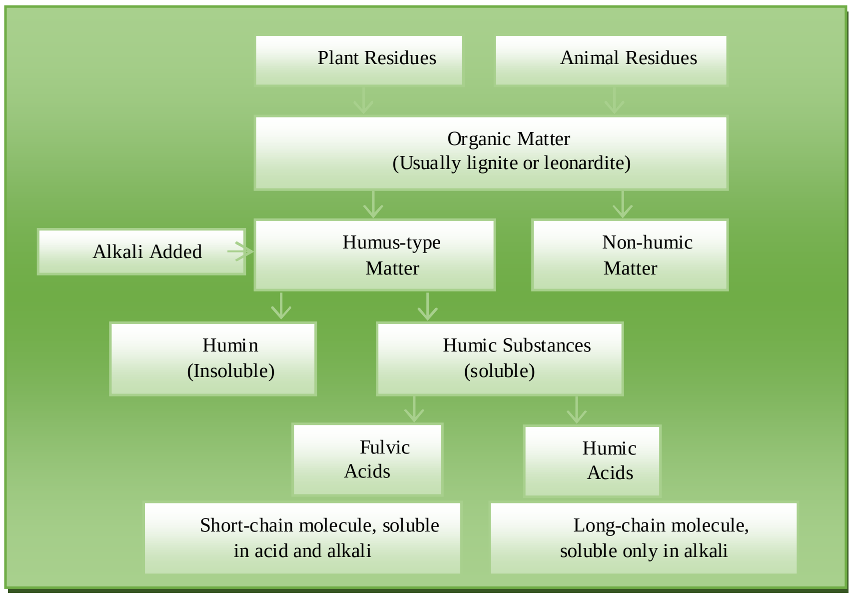

Fig 1. Formation of humic substances and their drivatives from bio-residues [3]

2. Materials and Methods

Seeds of *P. vera* L. cv. "Ghazvini" were prepared from Pistachio Research Center (PRC) in Rafsanjan, Kerman province, Iran. To grow seedlings, the dehisced seeds of pistachio were first pre-chilled for 10 days at 4°C and then soaked for 2 days in water. After covering seeds in wet cotton cloth, the germinated seeds were transferred into black plastic pots (12 cm in diameter) containing water-washed perlite [15].

One week after transferring the seedlings into the pots, they were treated by modified nutrition solutions of Hoagland. The basal nutrition solution 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 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 $CuSO_4$[16]. To treat plants, the basal nutrition solution was modified by adding 0.25 mg/l potassium humate or fulvic acid obtained from Barafza Keshavarz Pars Company, Iran. The pH of each nutrition solutions containing humic or fulvic acids and also the basal solution (as the control) was adjusted to 6.5, 7.5 and 8.5, respectively, by using weak acids or alkalines such as HCl and NaOH.

In the other part of the experiment, SO_4^{-2} was used instead of chelator (EDDHA) for binding Fe^{+2}, as $FeSO_4$ (0.05 mM), in the basal and modified Hoagland's solution.

All nutrition solutions were exchanged every two weeks. Plants were harvested 9 months after the application of the treatment and were then divided into roots and shoots. They were oven-dried for 72 h at 70°C and their dry weights were measured.

Three pots each containing 3 seedlings (totally 9 replicates) were considered for each nutrient treatment at every pH, and they were arranged in a randomized complete block design in a greenhouse. It is notable that the light direction was considered as the block in this design. Statistical analyses were performed using SPSS software, version 21. The data were analyzed using one-way ANOVAs, and means were compared using Duncan's multiple range test (P<0.05).

3. Results

The effect of fulvic and humic acids on the dry weight of *P. vera* roots and shoots grown in nutrient solution containing chelator binding agent (EDDHA) at different pHs of 6.5, 7.5 and 8.5 are shown in Fig. 2. As the results in pH 6.5 indicate, the root dry weight of plants grown in the basal Hoagland's solution (control) was significantly higher than humic acid containing solution (Fig. 2a). There is no significant difference between root dry weight of plants grown in control and fulvic acid containing solutions (Fig. 2a). Humic acid increased root dry weight about 4 times in comparison with the control plants at pH 7.5 (Fig. 2a). There was no significant difference between root dry weights of plants grown in control and fulvic acid containing solutions at this pH. Humic and fulvic acids increased root dry weights significantly in comparison with control plants at alkaline pH 8.5 (Fig. 2a).

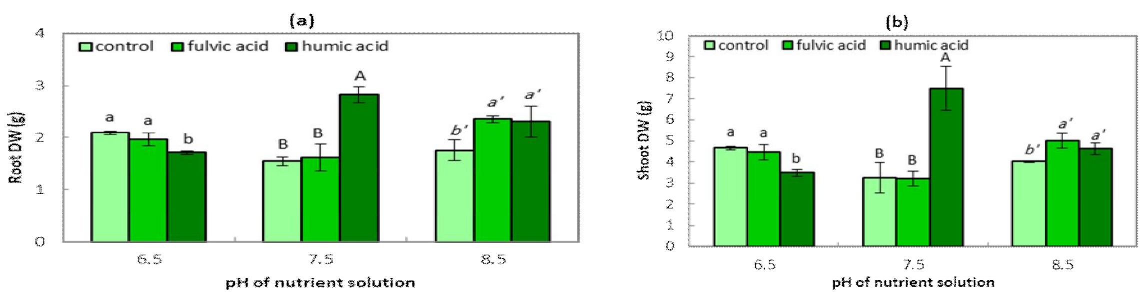

Fig 2. The effect of fulvic and humic acids on dry weight (DW) of *P. vera* roots (a) and shoots (b) grown in nutrient solution containing chelator binding agent (EDDHA) at different pH scales. Values are means ±SD (n= 9). Different letters (small letters for pH 6.5, capital letters for pH 7.5 and small italicized letters with prime sign for pH 8.5) above bars indicate significant differences in dry weights (Duncan test, P< 0.05).

The effect of fulvic and humic acids on the shoot dry weight of *P. vera* was also detectable especially at pHs 7.5 and 8.5. Shoot dry weights of plants grown in control and fulvic acid containing solutions were significantly higher than humic acid containing solution at pH 6.5 (Fig. 2b). At pH 7.5, the highest shoot biomass was seen in the plants treated by humic acid compared with plants grown in control and fulvic acid containing medium (Fig. 2b). At the highest pH (8.5), plants treated with fulvic and humic acids had higher shoot dry weight than the ones grown in the control. There is no significant difference between humic and fulvic acid treated plants at pH 8.5.

The effect of fulvic and humic acids and also pH scales (6.5, 7.5 and 8.5) on root and shoot dry weights of *P. vera* grown in modified Hoagland's solution containing SO_4^{-2} binding agent is shown in Fig. 3. As the results show, fulvic and humic acid treatments had no significant effect on root dry weights at different pH scales (Fig. 3a). Shoot dry weights did not change by being treated with humic and fulvic acids at pH 6.5 (Fig. 3b), but they increased at pH 7.5. There was no significant difference between shoot dry weights of *P. vera* treated with humic and fulvic acids at this pH.

At the alkaline pH (8.5), plants grown in humic acid containing medium had the highest shoot dry weight compared with control and fulvic acid containing solutions (Fig. 3b).

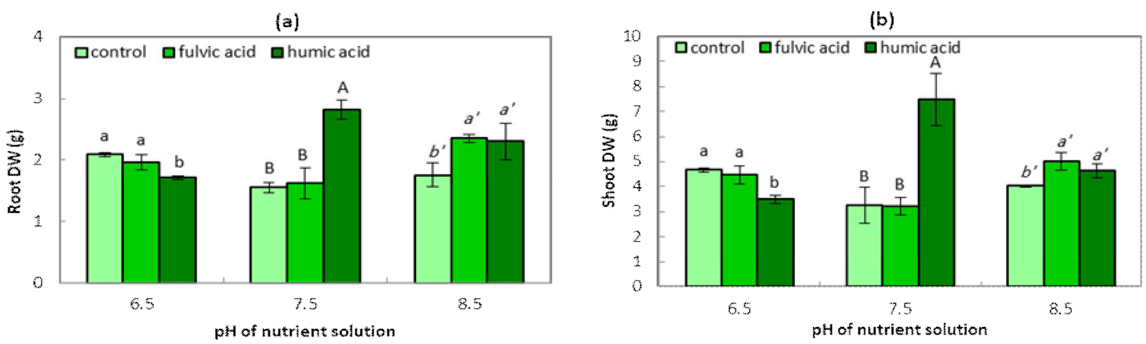

Fig 3. The effect of fulvic and humic acids on dry weight (DW) of *P. vera* roots (a) and shoots (b) grown in nutrient solution containing SO_4^{-2} binding agent at different pH scales. Values are means± SD (n= 9). Different letters (small letters for pH 6.5, capital letters for pH 7.5 and small italicized letters with prime sign for pH 8.5) above bars indicate significant differences in dry weights (Duncan test, *P*<0.05).

4. Discussion

Pistachio, which belongs to the tree group of nuts, is a very important economic product in Iran. The province of Kerman, especially the city of Rafsanjan, is recognized as the main pistachio producer in Iran. In these areas, underground water sources of saline nature are mostly used to irrigate pistachio orchards. The electrical conductivity (EC) of irrigation water is normally between 1.1-16.0 ds/m and the pH is between 7.3-8.9. On the other hand, most of the soil in these areas suffers from alkalinity and salinity. The general soil pH is between 7.2-8.5 in Kerman and Rafsanjan [17]. The alkaline pH (higher than 7) can detrimentally affect plant growth by reducing the availability and solubility of essential elements such as P, Fe, etc. [9, 10, 18]. One solution to avoid or overcome this problem is to use fertilizers such as inorganic compounds (*e.g.*, $FeSO_4$), synthetic elemental chelates (*e.g.* Fe-EDDHA), and also natural elemental complexes (*e.g.* humic substances and amino acids) [19].

In the current research, the growth of pistachio seedlings was evaluated in a semi-hydroponic culture medium by modified Hoagland's solution containing humic or fulvic acids in the presence of $SO4^{2-}$ or -EDDHA binding agents. The pH of culture solutions was adjusted to 6.5 (as the optimal pH in hydroponics), 7.5 and 8.5 (as alkaline pHs). The results in the presence of the chelator agent of EDDHA indicated that humic acid fertilizer can improve plant growth (shoot and root dry weights) under the two higher pHs 7.5 and 8.5 (Fig. 2). The effect of humic acid on plant growth was inverted in acidic pH of 6.5 (Fig. 2). Fulvic acid had also positive effects on plant growth at two higher pHs of 7.5 and 8.5. These findings were in accordance with those obtained by other researchers [3, 20-23]. As humic acids are insoluble in acidic pH, they can precipitate some essential elements and keep them out of root reach [7, 24]. So, the negative effect of humic acid on plant growth at pH 6.5 is reasonable in the current research. Previously, some researchers investigated the effect of root application of purified humic acid on the expression of some genes encoding plasma membrane H^+-ATPase, Fe(II) high-affinity transporter and Fe(III) chelate-reductase in non-deficient cucumber plants (*Cucumis sativus* L.) [21]. They also studied the activity of these enzymes and demonstrated that humic acid increased their expression and activity. Therefore, Fe assimilation and absorption is improved under humic acid treatments. In fact, humic substances act as a natural chelator of nutritional elements under alkaline conditions and improve their absorption by the roots [13]. In sum, all existing evidence suggests that bio-stimulant effects of humic substances, including humic and fulvic acids, characterized through both structural and physiological changes in plant shoots and roots, are related to nutrient absorption, assimilation and distribution (efficiency of nutrient use) [3].

The results of the current research indicated that the growth of pistachio seedlings was improved in the presence of Fe-EDDHA complex in comparison with $FeSO_4$. Humic substances had beneficial effects on the growth of shoots but not roots grown in a hydroponic culture containing $FeSO_4$. These findings are compatible with previous studies. Some researchers investigated the effects of different Fe sources (Fe-EDDHA and $FeSO_4$) on the growth of lettuce (*Lactuca sativa*) in hydroponic systems containing alkaline solutions [12]. They showed that the Fe content of leaves and also the overall plant growth increased significantly due to the application of Fe-chelate (Fe-EDDHA).

5. Conclusions

Pistachio is known as a strategic agricultural product in Iran. It normally suffers from alkalinity and thereby nutritional deficiencies. So, the application of humic substances as bio-stimulants, especially in the presence of some chelates (as EDDHA), can reduce or compensate some detrimental effects of alkalinity and thereby improve plant growth.

Conflict of interest

The authors declare no conflict of interest.

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