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Effects of Humic Acid on Root and Shoot Growth and Leaf Nutrient Contents in Seedlings of *Pistacia vera* cv. Badami-Riz-Zarand

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Abstract

Humic acid is a biochemical constituent of humus and increases root biomass and shoot growth by improving absorption of nutrients. In order to study the effects of humic acid on root and shoot growth and leaf nutrient contents of pistachio seedlings, an experiment was conducted of completely randomized design with five treatments in three replications. In the first step seeds of *Pistacia vera* cv. Badami-Riz-Zarand were planted in plastic pots containing vermiculite in March 2013. The humic acid treatments were of five levels (0, 20, 40, 60 & 80 g) and were applied to the seedlings at the four-leaf stage. Results showed that humic acid had significant effect on the plant length, internode distance, root length and width (root expansion) and root fresh and dry weight. The greatest effect was seen for 60 g of humic acid and the least in control treatment (0 g humic acid). In measuring leaf nutrient content in different humic acid treatments, the greatest amounts of Zn, Cu and Mn were in the 40-g humic acid treated plants. Overall, considering growth characteristics and leaf nutrient content, the best results for pistachio seedlings were obtained with about 60 g humic acid. Presumably, through an increase in root expansion, humic acid causes better absorption of nutrients and increased shoot growth. Therefore, humic acid can be used to increase shoot growth and expansion of root system in pistachio seedlings.

Keywords: Humic acid, Nutrient contents, Pistachio, Root and shoot growth.

Introduction

Pistachio is one of Iran's major horticultural crops and one of the most important non-petroleum exports. One of the varieties of pistachio is Badami-Riz-Zarand, which is used as rootstock for commercial varieties. Its fruit is very tiny, almond-like and of a dark color, and it has its origin in the old gardens of Zarand in Kerman (Panahi *et al*., 2002). Recently, it has become common to apply organic acids to improve the quantity and quality of horticultural products (Samavat and Malakooti, 2005). Very small amounts of organic acids have a considerable effect on improving the physical, chemical and biological characteristics of soil and they have suitable effects in increasing production and quality of agricultural products because of hormone compounds (Samavat and Malakooti, 2005). Soil fertility is heavily dependent upon its content of organic material (Liu and Cooper, 2000). One of the widely used organic fertilizers is humic acid, which is one of the major components of humic substances. Humic matter is formed through the chemical and biological decomposition of plant and animal matter and through the activities of microorganisms (Metzger, 2010). Humic acid forms stable and dissolved complexes and fulvic acid forms soluble complexes with trace elements and increase the absorption of elements, soil fertility and production in plants (Potter, 2001).

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Humic acid also increases herbage from sown grass pastures (Verlinden *et al*., 2010). Ghasemi *et al.* (2012) reported that using humic acid in potato minitubers increases root dry weight of the underground part. It has been reported that application of humic acid increases plant growth and absorption of nutrients (Dursun *et al.*, 2002; Türkmen *et al.*, 2004). Application of humic acid to foliage and to soil increases auxin, cytokinin and gibberellin levels in plants (Abdel-Mawgoud *et al.*, 2007). Studies of Valdrigi *et al.* (1996) showed that humic acid increases mineral absorption through an increase in permeation of root membrane. Root growth is influenced by environmental factors like humidity, temperature and soil nutrients (Huang and Taylor, 1993; Miyasaka and Grunes, 1990). As with shoot organs, the study of roots can identify factors that increase yield, and, because of the complexity of soil different mechanisms and to better investigate the effects of humic acid on pistachio seedlings, vermiculite was used in this study. Vermiculite is a hydrated magnesium-aluminum-iron silicate, with a suggested formula of $(Mg,Fe^{+2},Al)_3(Al,Si)_4O_{10}(OH)_2 \cdot 4H_2O$. Flakes of raw vermiculite concentrate are mica-like in appearance and contain water molecules within their internal structure. Vermiculite can absorb liquids such as fertilizers, herbicides, and insecticides, which can then be transported as free-flowing solids (Karr, 2002).

Because of past reports on the positive effects of humic acid on promoting plant growth and increasing absorption of nutrients in various plants, the present research was initiated to investigate the effects of humic acid on root and shoot growth and leaf nutrient content in pistachio seedlings, and to determine the best humic acid treatment.

Materials and Methods

This experiment carried out in greenhouse conditions in the pistachio Research Institute in Rafsanjan, Iran. The study of humic acid effects on root and shoot growth and leaf nutrient content of pistachio seedlings was done using a completely randomized design with five treatments in three replications. Each treatment included twenty seven plants. Seeds of *Pistacia vera* cv. Badami-Riz-Zarand, which is a common rootstock in pistachio gardens, were planted in plastic pots containing 1000 g vermiculite in March 2013. After germination, at the four-leaf stage, 20, 40, 60 and 80 g humic acid, derived from Leonardite, were applied to the pots while 15 pots were reserved as controls (without adding humic acid). Irrigation of seedlings to four leaf stage was with distilled water once every three days and after feeding the seedlings with Hoagland's complete nutrient solution (which includes $NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, micronutrients: H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $H_2MoO_4.H_2O$ and Fe-EDTA) in distilled water. In August, growth indicators such as plant length, internode distance, root width and length (root expansion) and leaf area was measured. Fresh and dry root weights were measured with milligram accuracy. To measure root dry weight, samples were dried in an oven 80^0C for 48 h. To determine the leaf nutrient (P, K, Zn, Cu and Mn) content, the plant extract was analyzed using the dry ash method (Emami, 1996). For this purpose, after washing leaf samples and drying them in air, they were ground and placed in oven 70 ^{0}C for 24 h. Then 1g of each sample was weighed and burnt in an electric furnace at 550°C for 5 h, which converted the extract to white ash. When the samples were cooled 10 mL of 3N HCl was added to each sample to a volume of 100 mL with distilled water. The resulting extract was analyzed for P, with a UV-VIS Spectrophotometer; K, with a Flame Photometer; and Zn, Cu and Mn, with an Atomic Absorption Spectrometer. Data were analyzed with SAS 9.1 Software and means were compared with the Tukey test at a 5% level.

Results

In this research, the analysis of variance for growth characteristics of pistachio seedlings showed that humic acid had significant effect on the plant length, internode distance, root width and length (root expansion) and root fresh and dry weight but had no significant effect on leaf area. Comparison of the mean values showed the maximum plant length was obtained with the 60 g treatment and the treatments 20, 40 and 80 g had lesser effects, with minimal effect in the control treatment (Fig. 1). With regard to internode distance, the 60 g and 40 g humic acid treatments showed the maximum effect and there was no significant difference between them; again, the minimum effect was seen in the control (Fig. 1). Humic acid increased plant length through an influence on internode distance. Comparison of the means showed the maximum root length with the 60-g humic acid treatment and somewhat lesser effects with the 20, 40, 80 g and control treatments (Fig. 2). In view of the above results, the same amounts of humic acid are recommended for increasing stem and root length. The maximum root width was seen with 60 g of humic acid and the minimum with the 20-g and control treatments (Fig. 2). Comparison of the means showed the maximum root fresh weight with the 60-g and 80-g humic acid treatments, but there was no significant difference between them. This variable was reduced in the 40-g and 20-g treatments and minimal in the control treatment (Fig. 3). The maximum root dry weight was seen for the 60-g humic acid treatment and the minimum for the 20-g and control treatments (Fig. 3). Humic acid appeared to increase root expansion and to improve nutrient absorption and, as a result, increases root fresh and dry weight. Any increase in root weight can provide better access to soil nutrients. In consideration of the above results, the overall maximum of measured effects was greatest for the 60 g humic acid treatment and the minimum for the control (Table 1).

Table 1. Analysis of variance for plant length, internode distance, leaf area, root length, root width and root fresh and dry weight

SOV	df	Mean Square						
		Plant Length	Internode Distance	Leaf Area	Root length	Root Width	Root Fresh Weight	Root Dry Weight
Humic acid	4	846.5**	56.65**	9.93^{ns}	847.5**	24.85**	0.047*	0.059**
Error	5	4.4	4.9	2.83	3.3	1.1	0.005	0.003

^{ns,}* and**: non-significant, significant at 5 and 1% probability levels, respectively.

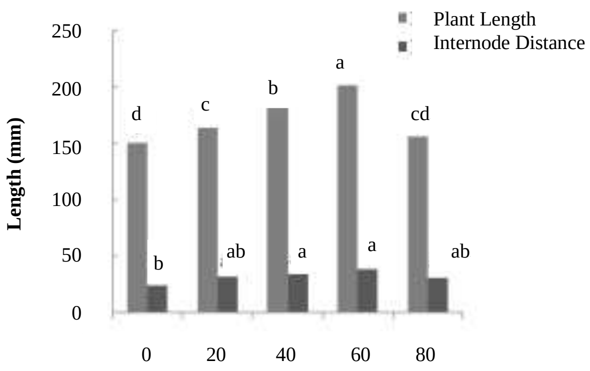

Fig. 1. Effect of different humic acid treatments on plant length and internode distance in pistachio seedlings.

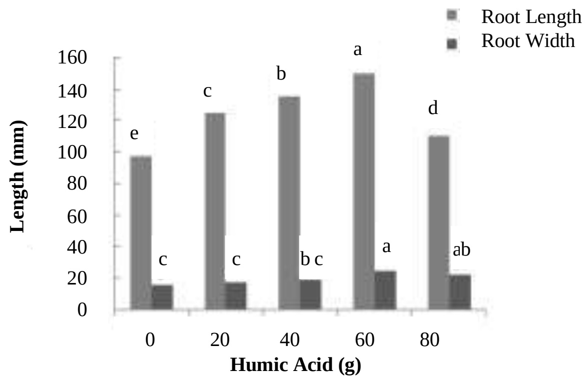

Fig. 2. Effect of different humic acid treatments on root length and width in pistachio seedlings.

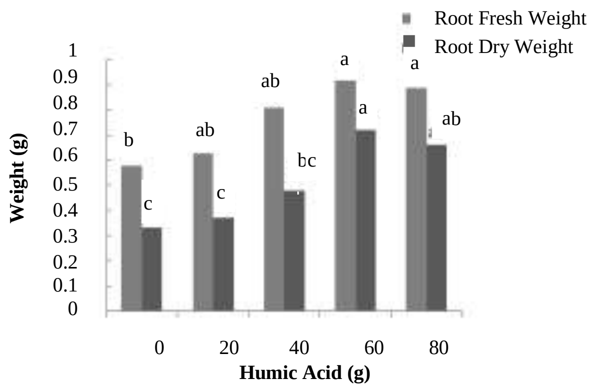

Fig. 3. Effect of different humic acid treatments on fresh and dry weights in pistachio seedling roots.

In this research based on analysis of variance for leaf nutrient contents, humic acid had significant effect on amounts of Zn, Cu and Mn but had no significant effect on the amounts of P and K (Table 2). In comparison of the means, the maximum Zn uptake was seen for the 40-g humic acid treatment; the 60-g and 20-g treatments resulted in less incorporation, and the 80-g treatment and control were further reduced (Fig. 4). The maximum Cu and Mn uptake were seen for the 40-g humic acid treatment and the minimum for control treatment (Figs. 5 and 6).Considering all of the above results, the maximum levels of Zn, Cu and Mn nutrients were seen with the 40-g humic acid treatment.

Table 2. Analysis of variance for leaf nutrient contents of pistachio seedlings

SOV	df	Mean Square				
		P	K	Zn	Cu	Mn
Humic acid	4	0.046^{ns}	0.036^{ns}	585.21**	95.72**	3048.65**
Error	5	0.028	0.031	3.173	7.03	296.6

^{ns},* and**: non-significant, significant at 5 and 1% probability levels, respectively.

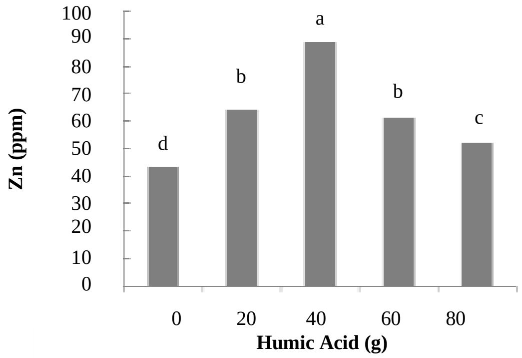

Fig. 4. Amount of Zn in pistachio seedlings leaf as a function of different humic acid treatments.

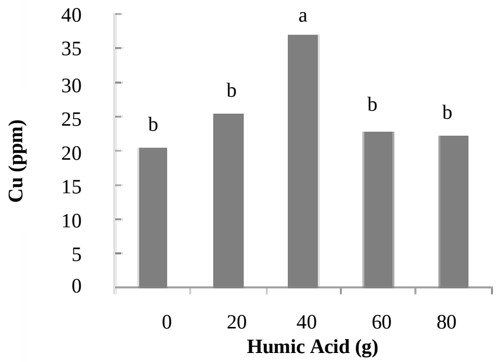

Fig. 5. Amount of Cu in pistachio seedlings leaf as a function of different humic acid treatments.

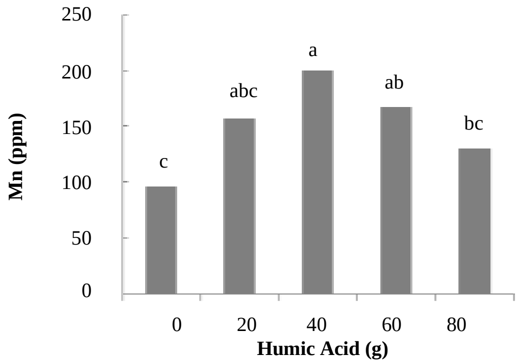

Fig. 6. Amount of Mn in pistachio seedlings leaf as a function of different humic acid treatments.

Discussion

These experiments demonstrated that humic acid had significant effects on root system expansion, shoot and plant length, root width and length, and the fresh and dry weights of roots, in pistachio seedlings. The effect of humic acid on root growth was especially significant. As we know, plants with thick and strong roots will be more healthy and resistant.

The findings of this study are in accordance with results of other researchers on the effects of humic acid on root and aerial parts growth. Humic acid has been shown to increase shoot growth in cucumber considerably (Mora *et al.*, 2010). Studies of Adani *et al.* (1998) showed that humic acid obtained from Leonardite stimulates root and stem growth in tomato. In okra, humic acid increased root size, root dry weight and branch length (Paksoy *et al.*, 2010). Albayrak and Camas (2005) showed that humic acid increases stem length in turnip. Humic acid and fulvic acid increased the root and stem length and resulted in a significant increase in root dry weight of pepper seedlings (Kline and Wilson, 1994). Studies of Rengrudkij and Partida (2003) showed that humic acid increased stem height and relative growth rate in avocado, and that treated trees were larger than untreated ones and had better root systems. Studies have shown that application of humic acid as solution or powder to soil increases length and weight of carrot root and generally increases plant growth (Gillis and Louie, 2004). Cordeiro *et al.* (2011) reported that humic acid has a positive effect on corn physiology and expands root and lateral roots. Studies have shown that humic acid significantly increased root biomass and nutrient content in bentgrass (Liu *et al.*, 1998). Tattini *et al.* (1990, 1991) reported that applying humic acid to olive trees increases the root to stem ratio and also lateral roots. Nikbakht *et al.* (2008) showed that humic acid causes significant increase in Gerbera root grown in nutrient solution. Humic acid increased root length in red maple (Kelting *et al.,* 1998). The use of humic acid in poplar increased root activity and root weight (Zhang *et al*., 2010). Liu and Cooper (2000) showed that any increase in root weight provides better access to soil nutrients and increases soil fertility. An increase in root dry weight after using humic acid may be due to hormone-like effects of humic acid that cause an increase in root growth and root dry weight.

Furthermore, the use of humic acid increases Zn, Cu and Mn absorption in pistachio seedlings. Zn is a catalyst in many plant enzyme systems is involved in protein synthesis and analysis. One of the important roles of Zn is synthesizing the amino acid, tryptophan, which is a precursor for the auxin, indoleacetic acid, which promotes branch length growth. Cu plays a role in the activation of plant oxidases. Mn has an essential role in chlorophyll production in plants.

The results of this study are in accordance with those of Hakan *et al.* (2011) who showed foliar application of humic acid on corn has a significant and positive effect on Zn, Cu, Mn absorption. Other studies have shown that humic acid increases the cation exchange capacity of avocado, thus improving nutrient absorption (Rengrudki and Partida, 2003). The use of humic acid on wheat (*Triticum durum* cv. Salihli) increased Zn and Cu absorption (Bulent Asik *et al.* 2009). And Pinton *et al.* (1999) showed that humic acid significantly increases nitrate absorption and ATPase activity in plasma membrane of root cells in maize. It seems that activation of a membrane proton pump is a primary response to humic acid in absorbing nutrients. In experiments by Paksoy *et al.* (2010) humic acid increased Cu and Mn in okra. Both foliar and soil application of humic acid increases nutrient absorption from soil in tomato (Adani *et al.*, 1998).

Thus our results with pistachio seedlings are in accordance with those of other researchers, who studied other plants, namely that humic acid increased plant length and expanded roots and also increased Zn, Cu and Mn absorption. Presumably humic acid through increase in root expansion causes better absorption of nutrients and increases shoot growth. It appears, from our research that an application of 60 g humic acid per pot containing 1000 g of vermiculite gave the best results, overall. In general, humic acid can be used in the early stages of development to increase the shoot growth and root system expansion of pistachio seedlings.

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