

An Investigation into the Effect of Humic Acid Application on Some Morphological and Physiological Characteristics of China Rose

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In recent years, excessive use of chemical fertilizers and lack of organic fertilizers have caused to reduce the rate of soil organic matter in Iran. The increased use of chemical fertilizers in agriculture causes environmental problems such as soil physical degradation and soil nutrient imbalances. This study was conducted to investigate the effects of foliar and soil application of humic acid on some morphological and physiological characteristics of *Rosa chinensis* 'Baby Masquerade' in a completely randomized design with two factors including four concentrations of humic acid (0, 500, 1,000 and 2000 mg/L) and two application methods (soil and foliar application) for 8 times (every 15 days) with three replications and two observations per replication under controlled greenhouse conditions. The results showed that humic acid had additive effects on anthocyanin and total soluble solids content. It should be noted that the increase in the content of total soluble solids resulted in the increase in anthocyanin. Foliar application of 500 mg/L humic acid significantly increased chlorophyll content and photosynthetic rate. Also, foliar application of 1000 mg/L humic acid improved total soluble solid content more than the other treatments. Humic acid had significant effects on leaf area index and shoot and root fresh and dry weight. Overall, it seems that humic acid can improve morphological and physiological characteristics of *Rosa chinensis* var. 'Minima'.

Abstract

Keywords: Anthocyanin, Chlorophyll, Foliar application, Growth indices, *Rosa chinensis*.

INTRODUCTION

Rose (*Rosa* sp.) belongs to the Rosaceae family and is native to several regions of the northern hemisphere (Anderson, 2006). Miniature rose is one of the important species of this family with many applications, especially in the landscape design. Thus, the production of this plant is very valuable. Humic substances are a major component of soil organic matter that has been subject to study in different fields of agriculture, chemistry, soil fertility, plant physiology, and environmental science because these materials have several beneficial roles to play in plant growth and development (Tan, 1998). Humic acid as a combination of a natural organic polymer can increase the availability of nutrients and improve plant yields (Sharif *et al.*, 2002). It, also, has auxin, cytokinin and gibberellin-like effects on plant metabolism (Zhang and Ervin, 2004; Pizzeghello *et al.*, 2001; Piccolo *et al.*, 1991). Reports have classified the effects of humic acid into two distinct categories: direct effects and indirect hormone-like effects by increasing nutrient uptake, maintaining membrane permeability, increasing metabolism of microorganisms, improving the physical condition of the soil and root and shoot growth (Kafi *et al.*, 2009).

Humic acid increases the population of soil organisms, improves soil physical condition, and adjusts the pH of the media. It, also, has enzymatic and hormonal effects on plant growth. It enhances plant resistance to drought and salinity, improves nutrient absorption, enhances seed germination and root growth, and improves crop quantity and quality (Cangi *et al.*, 2006). Studies show that humic acid has beneficial effects on nutrient uptake in plants, particularly on mobilization and availability of essential micronutrients (Bohme and Thilua, 1997; Nikbakht *et al.*, 2008), photosynthetic activity (Haghighi *et al.*, 2012), cation exchange capacity (Marinari *et al.*, 2000), and root growth (Li and Evens, 2000; Chen and Aviad, 1990), resulting in the increase in the quality of many ornamental plants (Nikbakt *et al.*, 2008). Liu *et al.* (1998) reported that application of 400 mg/L humic acid on bent-grass plants improved plant nutrients, root development, and photosynthesis rate. Adani *et al.* (1998) reported that the incorporation of humic acid into nutrient solution in greenhouse grown tomato helped 18% more development of roots as compared to control.

There are several reports regarding the influence of humic acid on the transport of glucose across cell membranes that increases the amount of carbohydrates and alkaloids in the leaves of grasses (Delfine *et al.*, 2005). Therefore, it is usually suggested to use humic substances as a way to increase agricultural production. This study aims to apply different levels of humic acid as a soil or foliar application and evaluate its effects on plant growth and physiological parameters.

MATERIALS AND METHODS

This research was conducted in the greenhouse of Horticultural Science Department, Faculty of Agriculture, Urmia University (latitude 37.53° N, longitude 45.08° E and 1320 m above sea level) under 24/19°C day/night temperature regime and natural sunlight. In this study, the rooted cuttings of *Rosa chinensis* 'Baby Masquerade' were transferred to 14 cm diameter pots containing a culture media mixture of soil: sand: leaf mold (1:2:2 v/v) (Table 1). This study was conducted as a factorial completely randomized design with two factors of the concentration of humic acid (Sigma-Aldrich) at four levels (0, 500, 1000 and 2000 mg/L) and the application procedure at two levels (soil or foliar application) with three replications and two observations. All treatments were applied a month after the plants were transferred to the pots. The foliar spraying was done every two weeks and soil application of humic acid (chemigation) was also based on plant water requirement. These treatments were applied for ten weeks.

At the end of the experiment, leaf area index (LAI), root and shoot fresh and dry weight and plant height were measured. In order to measure LAI, three leaves from the middle parts of the plants were harvested and measured with a leaf area meter (Leaf Area Meter, model: AM200). For dry weight measurements, samples were oven-dried at 70°C for 48 h.

Table 1. Physical characteristics of soil utilized in the present experiment.

Character	Content
Texture	Clay loam
Clay (%)	12.75
Silt (%)	8.07
Sand (%)	79.17
pH	7.5
EC (ds/m)	1.8

Leaf chlorophyll index was measured using a chlorophyll meter (SPAD-5902) on mature leaves. Three leaves from the middle part of the plants were selected and mean values for the treatments was recorded. Photosynthesis device (Walz, HCM-1000) was used to measure the rate of photosynthesis.

Anthocyanin concentration was determined by the Wagner (1979) protocol. To measure the amount of anthocyanin, 0.1 g fresh leaves were weighed with a precision digital scale, were completely pulverized in a mortar containing 5 ml of acidified methanol. Then, 5 ml of acidified methanol was added. This extract was poured into the test tube and the lid was tightly closed and stayed in the dark at laboratory temperature for about 24 h. After 24 h, the extract was centrifuged at 4000 rpm for 10 minutes. One ml of the supernatant extract was transferred to the cell and its absorption intensity was read at 550 nm wavelength by a spectrophotometer.

Total soluble sugars were extracted from leaf tissues by the method of Irigoyen *et al.* (1992) as follows. 0.5 g of fresh leaves was homogenized with 5 ml of 95% ethanol and the sample was vortexed for 2 min. After centrifugation at 3500 g for 10 min at 4°C, the supernatant was collected and an alcoholic extract was kept in a refrigerator at 4°C. Three ml of freshly prepared anthrone was added to 1 ml of alcoholic extract. The test tube was placed in boiling water bath for 10 min. until the dye was formed. After cooling the sample, the amount of absorption was read at 625 nm with a spectrophotometer.

Statistical analysis of experimental data was performed using SAS 9.1 software and mean comparison was compared with Duncan's multiple range test (DMRT) at 1 and 5% probability level.

RESULTS AND DISCUSSION

Effect of humic acid on photosynthesis rate

Foliar and soil application of humic acid (HA) could increase the rate of photosynthesis. The results showed that the highest rate of photosynthesis (9.533 mol/ms) was observed at 500 mg l⁻¹ HA as spraying, but the soil incorporation of 2000 mg l⁻¹ HA reduced this rate although it was still higher than that of control (Fig. 1). Probably, humic acid increases nutrient (especially nitrogen and phosphorus) uptake, leaf area, plant biomass and permeability of plant tissue through increasing plant roots, thereby enhancing photosynthesis (Atiyeh *et al.*, 2000). Humic materials can facilitate respiration and photosynthesis processes through modifying chloroplast and mitochondria (Orlov and Sadovnikova, 2005). In another research, Chaves *et al.* (2002) reported a positive relationship between photosynthetic rate of leaves and leaf chlorophyll concentration.

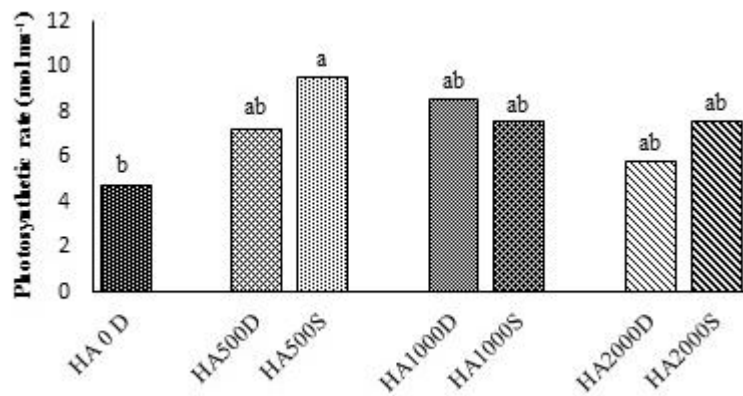


Fig.1. The effect of different concentrations of humic acid on the rate of photosynthesis. A: Humic acid; D: Drench; S: Spray. *In each column, means with the similar letters are not significantly different ($P < 0.05$) using LSD test.

Chlorophyll index

Comparison of mean data indicated that the effect of HA on chlorophyll index was significant (Table 2). Maximum level of chlorophyll was obtained at 500 mg l⁻¹ HA as foliar application and the minimum amount of chlorophyll was observed in control showing insignificant differences with other treatments. Karakurt *et al.* (2008) reported that with foliar application of 20 mg l⁻¹ humic acid, the chlorophyll content of pepper was increased significantly. Humic substances are effective in biological processes such as photosynthesis and chlorophyll content of plants (Salaman *et al.*, 2005). The increase in chlorophyll content may be due to the acceleration of the uptake of N and NO₃, improvement of metabolism of N and proteins synthesis, which eventually increases the chlorophyll content (Haghighi *et al.*, 2012) or due to other functions of humic acid such as increasing the permeability of cell membranes, oxygen uptake, respiration and photosynthesis, phosphate absorption, and root elongation (Russo and Berlyn, 1990). Tejada and Gonzalez (2003) reported that foliar application of humic acid and amino acids in asparagus plants enhanced chlorophyll and carotenoid of edible stems.

Table 2. Effect of different concentrations and application mode of humic acid (HA) on some morphological characteristics of *Rosa chinensis*.

HA application	HA concentration	Chl index (SPAD)	RFW (g)	RDW (g)	SFW (g)	SDW (g)	H (cm)	LAI (mm ²)
Drench	0	30.9c	22.61d*	5.92d	5.61e	2.31e	22c	842.8b
	500	38.1ab	26.36c	7.34c	12.53c	5.84c	35.6ab	1217.6a
	1000	38.5ab	45.5a	17.08a	16.74a	7.50a	38ab	1223.4a
	2000	37.2ab	33.67b	11.52b	15.29b	15.29b	39.3ab	1079.8ab
Spray	0	30.9c	22.61d	5.92d	5.61e	2.31e	22c	842.8b
	500	40.8a	22.89d	6.56c	7.69d	7.69d	34.6b	1098.1ab
	1000	39.3ab	33.39b	11.07b	16.95a	7.48a	45a	1271.3a
	2000	39.6ab	23.92d	7.23c	8.43d	3.51d	38ab	907.1b

RFW= Root fresh weight; RDW= root dry weight; Chl= Chlorophyll; LAI= Leaf area index; SDW=Shoot dry weight; SFW= Shoot fresh weight; H = Plant height. *In each column, means with the similar letters are not significantly different ($P < 0.05$) using LSD test.

Fresh and dry weight of roots

The results of this study showed that all concentrations of humic acid (both as soil and foliar application) increased fresh weight of roots. The highest fresh and dry weight of roots (45.50 and 17.08 g, respectively) was observed with the application of 1000 mg l⁻¹ HA as soil treatment while the lowest rate of this trait was observed in control (Table 2).

Effect of humic acid on root growth is more than on shoots and this effect is more pronounced in root fresh weight than in root dry weight (Liu *et al.*, 1998). The effect of humic acid on root growth improvement is not only due to hormone-like effect of HA but also due to the increase in nutrient absorption, especially phosphorus in the roots. In general, we can say that the root growth was significantly increased with the increase in the concentration of humic acid. This effect was more pronounced in soil application than in foliar spraying, which is in accordance with the results of other researchers on the effect of humic acid on root growth. Application of humic acid as a powder in the soil increased the length and weight of carrot roots and overall full plant growth (Taylor and Cooper, 2004). Canellas *et al.* (2012) reported that humic acid caused the formation of lateral roots and increased the yield of Gerbera flowers because HA increased nutrient uptake and has hormone-like properties. Nikbakht *et al.* (2008) reported that 500 mg l⁻¹ humic acid increased flower yield at 52% in Gerbera (*Gerbera jamesonii*) and 1000 mg l⁻¹ humic acid significantly increased root growth. Lulakis and Petsas (1995) reported that in the presence of HA, higher water and nutrient absorption by the roots occurred. The increase in root dry weight with the application of humic acid may be because of the fact that humic acid increases chlorophyll and photosynthesis rate and subsequently, dry matter was increased.

Shoot height, fresh and dry weight

Results of data analysis showed that soil and foliar applications of humic acid increased the plant height. Foliar application of 1000 mg l⁻¹ humic acid increased the plant height to 45 cm while the height of control was 22 cm. Other treatments were not significantly different to one another, but they significantly increased the plant height as compared to control.

Comparison of the data means showed that various concentrations of humic acid increased the shoot fresh weight compared to control (Table 2). The maximum increase in this trait was seen at HA rate of 1000 mg l⁻¹ both as soil and foliar applications, and the minimum shoot fresh weight was obtained in control.

As shown in Table 2 humic acid has a significant effect at the 5% level on shoot dry weight. Soil drench was more effective than foliar treatments. The maximum dry weight was observed with the application of 1000 mg l⁻¹ HA as a drench.

Recent research has shown that humic acid can act as a growth regulator because it enhances the auxin, cytokinin and gibberellins, thereby elongating the stem and improving plant growth (Muscolo *et al.*, 2007; Albayrak and Camas, 2005). Humic acid increases soil porosity and root growth that lead to an increase in the shoot system (Garcia *et al.*, 2008). By increasing the absorption of nutrients, humic substances affect plant metabolism and increase growth rate (Rengrudkij and Partida, 2003; Muscolo *et al.*, 2007). Turkmen *et al.* (2005) reported that application of humic acid at the rates of 500, 1000 and 2000 mg l⁻¹ increased hypocotyl length, stem diameter, stem length, dry weight, and the amount of nutrients in pepper plants. As well, the application of humic acid in the form of soil drench increased nutrient uptake from the soil and improved plant growth. It was shown that seeds of wheat in humic acid solution showed 38% higher shoot dry weight (Azam and Mauk, 1983). In another study, dry weight of corn plants was significantly increased by the application of 150 mg kg⁻¹ humic acid in soil (Sharif *et al.*, 2002).

Leaf area index

The foliar application of 1000 mg l⁻¹ humic acid resulted in the maximum leaf surface (1271.3 mm²). In addition, 500 and 1000 mg l⁻¹ HA applied as drench significantly increased leaf

area compared to control. Other concentrations did not cause significant differences in comparison to control (Table 2). Humic acid plays an important role in development of leaf area by reducing pH and increasing iron uptake (Hanafy Ahmad *et al.*, 2010). The application of 1200 mg l⁻¹ humic acid caused further development of leaf area in the forage Turpin (Albayrak and Camas, 2005). Humic acid increased LAI probably through increasing N content. Figliolia *et al.* (1994) reported that the use of humic acid significantly increased the leaf surface in plants.

Total soluble sugars

The results of this experiment showed that humic acid significantly increased the leaf sugar. Soluble sugar content was increased with the increase in humic acid concentration, while 500 mg l⁻¹ HA was more effective than 2000 mg l⁻¹. Minimum sugar content was observed in control (without HA). In this research, the concentration of 1000 mg l⁻¹ HA as a foliar application had the strongest effect on increasing the sugar content of plants. Thus, foliar application treatments were more effective in total soluble sugars than soil application (Fig. 2). Chen and Aviad (1990) reported that the addition of humic acid reduced chlorosis, resulting in an increase in photosynthetic pigments and total sugar content of plants. Humic acid increases the transfer of glucose across cell membrane in onions, sugar beets and sunflowers and increases the amount of carbohydrates in potatoes, sugar beets, carrots and tomatoes (Tan, 2003).

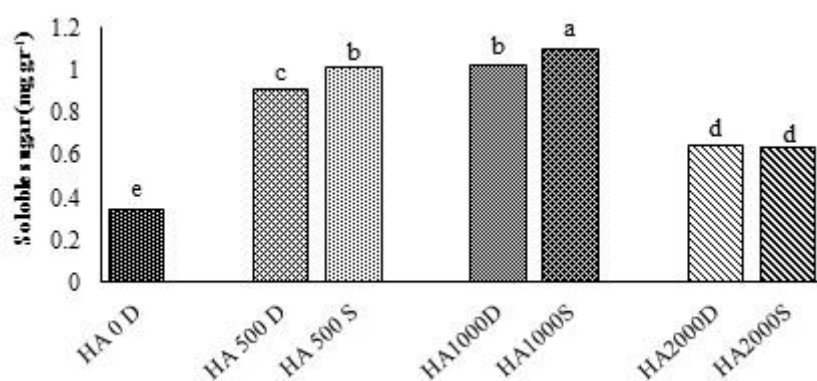


Fig. 2. The effect of different concentrations of humic acid on total soluble sugar content. HA: Humic Acid; D: Drench; S: Spray. *In each column, means with the similar letters are not significantly different ($P < 0.05$) using LSD test.

Anthocyanin

Results of data analysis showed that all humic acid treatments were effective in this trait. The soil application of 1000 mg l⁻¹ HA was most effective in increasing leaf anthocyanin content (Fig. 3). The results showed that soil application was more effective in improving anthocyanin content than foliar application. Gibberellin-like properties of humic substance stimulates α -amylase and other hydrolytic enzymes that are effective in increasing the level of plant carbohydrate and anthocyanin (Edrisi, 2009). Theunissen *et al.* (2010) reported that vermicompost can result in the synthesis of phenolic compounds such as flavonoids and anthocyanin in vegetables due to the fact that it contains a large amount of humic acid. Saeedi (2001) reported that vermicompost increased the anthocyanin content of the *Lilium* flowers.

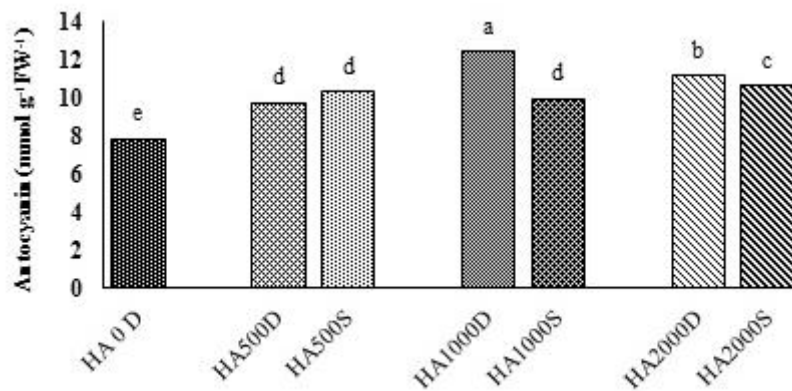


Fig. 3. The effect of different concentrations of humic acid on anthocyanin content. HA: Humic Acid; D: Drench; S: Spray. *In each column, means with the similar letters are not significantly different ($P < 0.05$) using LSD test.

CONCLUSION

The results of this study showed that the use of humic acid has positive effects on the photosynthesis, morphological and physiological traits. These effects can result in hormonal and physiological effects of humic acid. Based on the findings of this study, the concentration of 500 mg l⁻¹ HA in the miniature rose plant can be fitted more efficiency of photosynthesis. The application of 1000 mg l⁻¹ HA was more effective in most indicators than the other treatments. Therefore, it is recommended to use natural fertilizer resources to increase the quantity and quality of the plants.

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