

Impact of Humic Acid on Yield and Quality of Sugar Beet (*Beta vulgaris* L.) Grown on Calcareous Soil

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Abstract

A field experiment was performed to determine the effects of using humic acid with irrigation on quantitative and qualitative yield traits of sugar beet grown on calcareous soils in Esfaraen, located in the North-East of Iran. Treatments consisted of three levels of humic acid concentration (zero or control, 2.5 and 5 L ha⁻¹) and the number of applications (once 45 days after planting; twice, 45 and 75 days after planting; three times, respectively 45, 75 and 105 days after planting). The effect of the two experimental factors on sugar beet yield traits such as the content of sucrose, refined sugar, molasses forming substances, root yield and refined sugar yield were evaluated. The results proved the existence of a significant interaction between humic acid concentration and the number of applications on all parameters under study. The application of humic acid caused a significant increase of sucrose, refined sugar, root yield and refined sugar yield and a reduction in molasses forming substances content, compared to the control. The increased amount of sucrose and refined sugar content in all applications in concentration of 2.5 L ha⁻¹ humic acid was more than with 5 L ha⁻¹. Similarly, the content of molasses forming substances showed more reduction in 2.5 L ha⁻¹ than in 5 L ha⁻¹ treatment. However, the results revealed that the highest root yield and refined sugar yield, as the main qualitative and quantitative parameters of sugar beet yield, were achieved by three times application of 5 L ha⁻¹ that had 24 and 37% increase compared to control.

Keywords: humic acid, root yield, sucrose, sugar beet

Introduction

Availability of nutrients is highly dependent on soil properties. Calcareous soils are among the most important factors limiting the availability of nutrients and agricultural production (Leytem and Mikkelsen, 2005; Çelik *et al.*, 2010; Tahir *et al.*, 2011). Calcareous soils cover more than 30% of the earth's land surface and they are the main soil of most of the arid and semi-arid climates (FAO, 1973). These soils contain different amounts of calcium carbonate (CaCO₃) that affects the physical (such as soil water relations) and chemical (such as fertility, nutrient availability) soils properties related to plant growth (FAO, 1973; Leytem and Mikkelsen, 2005). The excess of calcium carbonate increases soil pH, whereas a pH higher than 8 (up to 8.4) leads to reduced access to micronutrients, emissions of ammonium and reduced solubility and phosphorus uptake (Katkat *et al.*, 2009; Çelik *et al.*, 2010). In addition, calcareous soils in warmer regions are naturally low in organic matter due to high temperature (Leytem and Mikkelsen, 2005; Çelik *et al.*, 2010).

Humus substances (humic and fulvic acids) constitute 65-70% of organic matter in soils and the term *humus* is widely accepted as synonymous for soil organic matter (Chen and

Aviad, 1990). The major functional groups of humic acids include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid (Russo and Berlyn, 1990). Humic compounds have multiple roles which can significantly improve plant growth. It has been shown that the use of humic compounds improves the properties of soil (such as aggregation, aeration, permeability, water-holding capacity), enhance the uptake of macro and microelements, increase cell permeability, has stimulating effect on respiration, photosynthesis, protein and nucleic acid synthesis, modulates the activity of H⁺-ATPase in plasmalemma and tonoplast, increase chlorophyll content, enhance stress tolerance, increase activity of soil microbial populations and hormonal regulation of plant (Visser, 1985; Chen and Aviad, 1990; Russo and Berlyn, 1990; Muscolo *et al.*, 1993; Nardi *et al.*, 2002; Tan, 2003). The positive effects of humic acid on the growth and yield of some plants such as wheat (Katkat *et al.*, 2009), corn (Eyheraguibel *et al.*, 2008), barely (Ayuso *et al.*, 1996) and tomato (Lulakis and Petsas, 1995) have been reported. There are studies that also looked at the effect of humic acid on the qualitative and quantitative yield of sugar beet. However, the effect of humic acid on the growth and yield of sugar beet grown in calcareous soils is less studied.

It is assumed that under calcareous soils, humic acid improves the growth and yield of sugar beet and increase the

Table 1. Particle size distribution and physical-chemical properties of the soil at the experimental field site

Texture	Loam Clay Values
Sand (%)	18
Clay (%)	24
Silt (%)	58
pH	8.05
EC (ds.m ⁻¹)	3.56
Total CaCO ₃ (%)	28
Organic matter (%)	1.11
Total N (%)	0.053
P (mg kg ⁻¹)	5.45
K (mg kg ⁻¹)	415
Fe (mg kg ⁻¹)	2.15
Zn (mg kg ⁻¹)	0.23
Cu (mg kg ⁻¹)	0.88
Mn (mg kg ⁻¹)	5.45

absorption of nutrients as a result. Therefore, this study was conducted to investigate the effect of humic acid on the yield and quality of sugar beet grown in calcareous soils.

Materials and Methods

The field experiment was conducted in Esfaraen in North-Eastern Iran. The area has a dry climate (with warm and dry summers) with an average annual rainfall of 270 mm and the mean temperature of 15 °C. The meteorological data were collected from weather stations near the experiment field. The soil is Aridisol according to U.S. soil taxonomy. The soil in the area is characterized by large amounts of calcium carbonate (containing 28%). The existence of this amount of calcium carbonate limits the growth of crops in the area. Other soil properties are presented in Table 1.

The experiment was organized following a factorial randomized complete-block design, with three replications. The factors were: different concentrations of humic acid (0, 2.5, 5 L ha⁻¹) and the number of applications (once at 45 days after planting; twice at 45 and 75 days after planting; three times at 45, 75 and 105 days after planting respectively). Humic acid was used along with irrigation in specified concentrations and time.

The sugar beet seeds were sown by hand at the density of 120,000 plants ha⁻¹ on 24 June 2013. Each plot consisted of 6 rows of 4 m at 50 cm spacing in between. Based on the soil test results, nitrogen, phosphorus and potassium fertilizers in the recommended amounts were added to the ground before planting. Weeds, diseases and insects were controlled continuously throughout the growing season.

Harvesting was done on 18 November 2013. A representative sample from each plot (selecting 4 rows of 3 m, by excluding plants along the plot edges) was used to measure the qualitative and quantitative parameters.

Parameters include the content of sucrose, refined sugar, molasses forming substances (Na, K and amino-N), root yield and refined sugar yield.

Data were analyzed by analysis of variance using the SAS computer program (SAS Institute, 1988). Multiple comparisons of variables were made using the least significant differences (LSD) at P < 0.05.

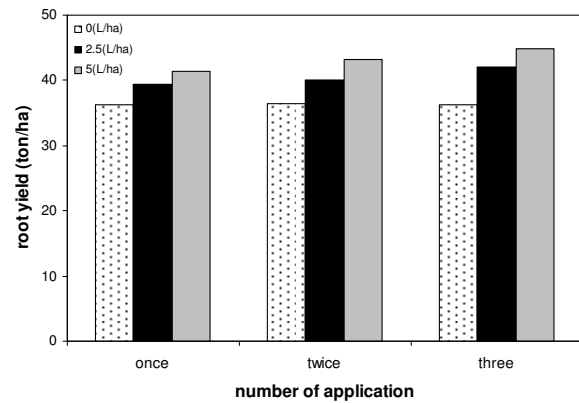


Fig. 1. Effect of humic acid concentration and the number of application(s) on root yield

Results and Discussion

The results of variance analysis showed that the effect of different concentrations, number of applications and the interaction effect of concentration and the number of applications of humic acid were statistically significant on all qualitative and quantitative parameters studied for sugar beet grown on calcareous soil (Table 2).

Root yield

The application of humic acid increased sugar beet root yield compared to control (Fig. 1). For any number of applications, the greatest increase in the root yield compared to control was witnessed when 5 L ha⁻¹ humic acid were used. Also, with the increasing number of applications of 5 L ha⁻¹ humic acid, the root yield increased by 14, 19 and 24% compared to control in one, two and three times of use respectively. There are many reasons to justify the increase of the root yield caused by humic acid application especially in calcareous soils. Katkat *et al.* (2009) stated that in calcareous soils, due to the high pH, the solubility of micronutrients is reduced and the plants grown in such soils encounter micronutrient deficiency. Other researchers have also reported plant growth simulation and nutrient uptake by adding humic acid (Chen and Schnitzer, 1978; Fagbenro and Agboda, 1993; David *et al.*, 1994). In addition, positive effects of humic acid on the growth and production of plants are attributed to the hormone-like activity through its involvement in cell respiration, photosynthesis, protein synthesis, various enzymatic reactions and antioxidant effect (Vaughan, 1974; Muscolo *et al.*, 1993; Zhang and Schmidt, 1999; Türkmen *et al.*, 2004). Feckova *et al.* (2013) have reported that humic acid application increased the sugar beet root yield up to 20% compared to control (without application); the reason was due to the fact that the humate chelates complexes with microelements can get easier to the plant cell than the common ions. Sadeghi-Shoae *et al.* (2013) concluded that the sugar beet root yield was 25% higher in the plots containing humic acid than in plots without any added humic acid. The positive effects of humic acid in calcareous soils on other crops such as wheat (Katkat *et al.*, 2009; Tahir *et al.*, 2011) and maize (Çelik *et al.*, 2010) have also been proven.

Table 2. Variance analysis of the humic acid concentration and the number of application(s) on yield and quality of sugar beet

Refined sugar yield	Root yield	F-values					df	Var. Source
		Amino N	K	Na	Refined sugar content	Sucrose content		
0.12	1.72	1.31	5.43	0.08	0.2	0.06	2	Replication
787.14**	8874**	20.32**	30.46**	12.4**	195.74**	207.24**	2	Humic acid concentration (HA)
15.08**	674.1**	3.49*	16.37**	2.99*	20.1**	20.54**	2	Number of application (NA)
41.19**	243.4**	11.55**	13.95**	3.48*	28.77**	25.79**	4	HA × NA
							16	Error

** significant at $P < 0.05$ and $P < 0.01$

Table 3. Effect of humic acid concentration and the number of application(s) on sucrose content, refined sugar content and refined sugar yield

Rel.%	Refined sugar yield (t ha ⁻¹)	Rel.%	Refined sugar content (%)	Rel.%	Sucrose content (%)	Concentrations (L ha ⁻¹)	Number of application
100	4.63b	100	12.77c	100	14.80c	0	
130	5.97a	118	15.13a	114	16.86a	2.5	Once
131	6.02a	114	14.51b	110	16.3b	5	
100	4.61b	100	12.76c	100	14.82c	0	
131	6.03a	118	15.05a	114	16.81a	2.5	Twice
131	6.08a	110	14.10b	108	15.97b	5	
100	4.61c	100	12.80b	100	14.83b	0	
126	5.79b	110	14.06a	107	15.87a	2.5	Thrice
138	6.35a	110	14.02a	107	15.78a	5	

In any number of application(s) and in each column, means with the same letter were not significant different (LSD test, $P < 0.05$)

The content of sucrose and refined sugar

Applying humic acid was accompanied by increased percentage of sucrose and refined sugar compared with the control (Table 3). Nevertheless, the trend of increasing content of sucrose and refined sugar was not associated with humic acid concentration, so that in all applications the increased concentration of humic acid from 2.5 to 5 L ha⁻¹ was associated with a reduction in refined sugar and sucrose percentage. Similarly, an increase in the frequency of application of each of the concentrations reduced the content of refined sugar and sucrose. Accordingly, the content of sucrose in once application of 2.5 and 5 L ha⁻¹ humic acid was reduced from 16.86% and 16.35% to 15.87% and 15.78% in three applications and the content of refined sugar was reduced from 15.13% and 14.51% in once application of 2.5 and 5 L ha⁻¹ to 14.06% and 14.02% in three applications. It seems that an increase in humic acid application in terms of amount and frequency is negatively related with the content of sucrose and refined sugar in the root. Lee and Bartlett (1976) and David *et al.* (1994) reported that too much humic acid reduced its beneficial effects. Research on tomatoes grown in saline soils showed that 1 g/kg humic acid improved some parameters, but the increased humic acid concentration to 29 g/kg led to the reduction of the parameters under study (Türkmen *et al.*, 2004). Researchers have reported different levels of desirable humic acid concentration to achieve maximum qualitative and quantitative yield. For example, Tan (2003) noted the amount of 400-600 mg L⁻¹, while Chen and Avid (1990) concluded that 50-300 mg L⁻¹ represents desired quantities of humic acid.

Content of molasses forming substances

The refined sugar content is dependent on many root features: morphological- size and shape, physical- tissue

elasticity, physiological- intensity of constituent roots' respiration on piles before processing, chemical- saccharose content, and content of melassigenic substance impeding sugar extraction (Rychcik and Zawislak, 2002). The main components that make it difficult to extract sugar are: potassium, sodium and alpha-amino nitrogen in the form of amino-acids (glutamic acid and aspartic acid) and amides (glutamines, asparagines). The content of molasses forming substance in roots depends mainly on the quality of soil, mineral fertilization (mostly nitrogen and potassium) and meteorological condition during the vegetation period (Rychcik and Zawislak, 2002). High content of sodium, potassium and nitrogen prevents sucrose crystallization and reduces the white sugar extraction.

Data analysis of molasses forming substances showed that they were reduced by applying humic acid (Table 4). These results are consistent with those reported by Feckova *et al.* (2005). Although humic acid application reduced molasses forming substances, however, the reduction percentage was lower in 5 L ha⁻¹ application compared to 2.5 L ha⁻¹ application in any number of applications. This is supported also by the data obtained by Vaughan and McDonald (1976) who stated that humic acid application in large amounts may increase the absorption capacity of the elements such as Na⁺ in sugar beet. Changes in molasses forming substances compared with the control indicated that potassium content has the lowest variation in different concentrations and number of applications tested.

Refined sugar yield

The refined sugar yield is the most important economic parameter in sugar beet industry. The results showed that the control with 4.61 t ha⁻¹ had the lowest

Table 4. Effect of humic acid concentrations and the number of application(s) on content of molasses forming substances

Rel.%	AminoN (meq. 100g ⁻¹ beet)	Rel.%	K (meq. 100g ⁻¹ beet)	Rel.%	NA (meq. 100g ⁻¹ beet)	Concentrations (L ha ⁻¹)	Number of application
100	2.31a	100	5.90a	100	2.42a	0	Once
73	1.68b	92	5.43b	69	1.66b	2.5	
81	1.86b	94	5.56c	84	2.03c	5	
100	2.33a	100	5.88a	100	2.40a	0	Twice
75	1.74b	88	5.22c	67	1.62b	2.5	
77	1.79b	96	5.66b	96	2.31a	5	
100	2.29a	100	5.90a	100	2.41a	0	Thrice
96	2.22a	98	5.76a	86	2.08a	2.5	
98	2.27b	99	5.82a	76	2.11a	5	

In any number of application(s) and in each column, means with the same letter were not significant different (LSD test, P < 0.05)

refined sugar yield, while applying humic acid increased refined sugar yield (Table 3). Accordingly, the maximum refined sugar yield (6.35 t ha⁻¹) was achieved by 3 times application of 5 L ha⁻¹ humic acid. Similarly, Feckova *et al.* (2005) reported that the refined sugar yield was 25-36% higher than the control by applying humic acid, depending on the variety. Sadeghi-Shoae *et al.* (2013) also reported 27% increase of refined sugar yield in the plots containing humic acid.

The yield difference of refined sugar was not significant between the concentrations of 2.5 and 5 L ha⁻¹ of humic acid applied once or twice. The refined sugar yield is the result of root yield multiplied by the percentage of refined sugar and the increase of each content leads to the increased amount of white sugar. Accordingly, the yield decline of root in the treatment with 2.5 L ha⁻¹ concentration (39.45 and 40.1 t ha⁻¹ in once and twice application(s)) compared to 5 L ha⁻¹ (41.45 and 43.84 t ha⁻¹ in once and twice application(s)), with increased refined sugar percentage in 2.5 L ha⁻¹ concentration (15.3% and 15.05% in once and twice application(s)) compared to 5 L ha⁻¹ concentration (14.51% and 14.10% in once and twice application(s)) was compensated and the concentration differences were not significant in once and twice application(s). However, low increased of refined sugar percentage in three times application of 2.5 L ha⁻¹ (14.06%) compared to 5 L ha⁻¹ (14.02%) cannot compensate root yield decline. Thus, the sugar yield in three times application of 5 L ha⁻¹ humic acid had a significant difference compared with three times application of 2.5 L ha⁻¹ humic acid.

Conclusions

The experiment results showed that the use of humic acid can improve the quantity and quality of sugar beet yield, even grown on calcareous soils. The increased amount of sucrose and refined sugar content in all applications in concentration of 2.5 L ha⁻¹ humic acid was more than with 5 L ha⁻¹. Similarly, the content of molasses forming substances showed more reduction in 2.5 L ha⁻¹ than in 5 L ha⁻¹ treatment. Nevertheless, the trend of increasing content of sucrose and refined sugar was no associated with humic acid concentration. The maximum refined sugar yield (6.35 t ha⁻¹), as the most important economic parameter of the yield, was achieved by 3 times application of 5 L ha⁻¹ humic acid.

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