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Effect of humic acid foliar application on growth and quantity of corn in irrigation withholding at different growth stages

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Abstract

In order to study effect of humic acid foliar application and limited irrigation on growth and quantitative characteristics of corn an experiment was conducted in research field of Varamin in Iran during 2012 growing season. The experimental design was laid out in a randomized complete block with a split plots arrangement of treatments in three replications. Main plots included three different levels of irrigation (complete irrigation, irrigation withholding at 8-leaf stage and irrigation withholding at staminate inflorescence) and four different concentration of humic acid foliar application (0, 150,300 and 450 ppm) was allocated to subplots were. The results showed that irrigation withholding conditions in different growth stages significantly decreased plant height, yield components, seed yield, biological yield and harvest index. Humic acid foliar application in irrigation with holding in different growth stages had positive effect on all attributes in this experiment. In general, the results of the present study indicate that usage of humic acid reduces the harmful effects of water deficit stress and increases resistance to drought stress in corn plant.

Keywords: humic acid, corn, irrigation withholding, seed yield, yield components

Introduction

Across the globe today, maize is a direct staple food for millions of individuals and, through indirect consumption as a feed crop, is an essential component of global food security (Campos et al, 2004). In Iran water is a scarce resource due to the high variability of rainfall. The effects of water stress depend on the timing, duration and magnitude of the deficits (Pandey et al, 2001). It causes stress in plants and is not only caused by the reduction of rainfalls and great heat, but in the cases where there is moisture in the soil, this moisture cannot be used for plants for some reasons such as excessive soil salinity or soil frost, and plants will be stressed (Baydar and Erbas, 2005; Borrell et al, 2008). On the other hand, humic acid is an organically charged bio-stimulant that significantly affects plant growth and development and increases crop yield. It has been extensively investigated (Nardi et al, 2004) that humic acid improves physical (Varanini et al, 1995), chemical and biological properties of soils (Keeling et al, 2003; Mikkelsen, 2005). The role of humic acid is well known in controlling, soil-borne diseases and improving soil health and nutrient uptake by plants, mineral availability, fruit quality, etc (Mauromicale et al, 2011). Humic acid based fertilizers increase crop yield (Mohamed et al, 2009), stimulate plant enzymes/hormones and improve soil fertility in an ecologically and environmentally benign manner (Mart, 2007; Sarir et al, 2005). Several research workers highlighted the positive benefits of humic acid application on higher plants (Ashraf et al, 2005; Susilawati et al, 2009). Other researcher have reported that HA increase crop growth and productivity, and help in moisture retention and mitigation of salinity (Yoon-Ha Kim et al, 2012). Hence in this field experiment, an attempt was made to investigate the effect of humic acid foliar application on the growth, yield and yield components of corn plants under complete irrigation and irrigation withholding at different growth stages.

Materials and Methods

In order to study effect of humic acid foliar application and limited irrigation, on quantitative and qualitative characteristics of corn an experiment was conducted in research field of Varamin in Iran during 2012 growing season. Site of study was situated at 31°51'E and 20°35'N and 1,050 m above sea level. Before beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. A composite soil sample was collected at a depth of 0-30 cm. It was air dried, crushed, and tested for physical and chemical properties. The research field had a clay loam soil. Details of soil properties are shown in Table 1. After plow and disk, plots were prepared. The experimental design was carried out in a randomized complete block with a split plot arrangement of treatments in three replications. Main plots included three different levels of irrigation (complete irrigation, irrigation withholding at 8-leaf stage and irrigation withholding at staminate inflorescence appearance stage) and four different concentration of humic acid foliar application (0, 150, 300, and 450

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Table 1 - Soil properties of the experimental site

Depth	EC (ds m^{-1})	pH	O.C (%)	T.N.V (%)	K (ppm)	P (ppm)	Total N (%)	Texture
0-30 cm	4.1	7.4	0.71	<10	368	25.9	0.079	Clay loam

ppm) was allocated to subplots were. The 18.75 m^2 plots were prepared with 5 m long and consisted of five rows, 0.75 m apart. Between all main plots, 2 m alley was kept to eliminate all influence of lateral water movement. Polyethylene pipeline was performed for control of irrigation as dropping irrigation. Treflan and gallant super were applied to control weeds. According to soil analysis, phosphorus (150 kg ha^{-1} P) and potassium (200 kg ha^{-1} K) fertilizers were applied into the soil. Nitrogen was supplied from ammonium nitrate source (300 kg ha^{-1}) at three stages; seed sowing, 8-leaf stage and before flowering stage. The plots were sown with corn seeds (NS 640) with 75 cm row to row distance and 20 cm between plants. Corn was planted manually in May 2012. Seeds were sown 3-4 cm deep. Two seeds were sown in each position and the plots thinned to the desired plant population (67,000 plant ha^{-1}). After seed sowing, irrigation was applied as required during the growing season. HA were obtained from cattle-manure vermicompost after 70 days of ripening. The procedure provided by the International Humic Substances Society (IHSS) (Swift, 1996) was followed, with some modifications described in a technical bulletin of the Brazilian Ministry of Agriculture, Livestock and Commodities (Benites et al, 2003). Total acidity was determined by titrating the HA solution with excess $Ba(OH)_2$ under an N_2 atmosphere. The HA solution was then back-titrated with HCl (0.1 M). Carboxyl groups was determined by chemical titration, adding an excess amount of $Ca(CH_3COO)_2$ in the prepared solution of AH, this solution was then stirred for 24 h and CH_3COOH released was titrated with NaOH solution (0.1 M) (Schnitzer and Gupta, 1965). The content of phenolic groups was determined by the mathematical difference between the total acid group content (total acidity) and the content of carboxylic acid groups (carboxylic acid). The humic acid foliar application was applied with a pressurized backpack sprayer (12 l capacity) calibrated to deliver 1,000 l ha^{-1} of spray solution. Sprayer was equipped with a spiral solid cone spray nozzle. At the end of growing season crop were harvested and agronomic traits such as plant height, row number in ear, seed number in row, total seed number in ear, 1,000 seed weight, seed yield, biological yield and harvest index were assayed. All data were analyzed from analysis of variance (ANOVA) using the GLM procedure in SAS (SAS Institute, 2002). The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. Duncan's multiple range tests was used to measure statistical differences between treatment methods and controls.

Results and Discussion

Analysis of variance showed that the effect of irrigation withholding in different growth stages was significant on all traits experiment. Also the effect of humic acid foliar application was significant on all measured traits experiment except harvest index (Table 2). Interaction of experimental factors (irrigation withholding in different growth stages × humic acid foliar application) was not significant on all measured traits experiment except plant height, total seed number in ear and 1,000 seed weight. As can be seen from Table 3, the highest plant height was obtained from complete irrigation. Irrigation withholding at 8-leaf stage decreased plant height. The decrease in plant height, under drought conditions, may be due to suppression of cell expansion and cell growth that is in response to low turgor pressure (Jaleel et al, 2008; Ogbonnaya et al, 2003). Irrigation withholding at staminate inflorescence appearance less decreased plant height compared to irrigation withholding at 8-leaf stage. Water stress induction after flowering stage does not decrease plant growth and elongation. Humic acid foliar application with 450 ppm concentration improved plant height both complete irrigation and irrigation withholding at different growth stages conditions. The increase in the plant height in the HA-amended treatments most probably was due to the improvement of growth of the root zone. Also the results showed that row number in ear

Table 2 - Analysis of variance on corn attributes affected by irrigation withholding in different growth stages and humic acid foliar application.

S.O.V	d.f.	Plant height	Row number in ear	Seed number in row	Total seed number in ear	1000 seed weight	Seed yield	Biological yield	Harvest index
Replication	2	3825.33**	0.66**	121.10**	10476.66**	2025.89**	2153296.42**	29945437.59ns	4.36ns
Irrigation	2	1174.33**	0.40**	226.57**	65657.16**	708.51**	21749189.37**	24256804.34**	152.02**
Error (a)	4	67.79	0.001	1.05	243.02	6.97	22996.70	165861.30	3.36
Humic acid foliar application	3	888.91**	0.76**	49.56**	22869.77**	298.15**	946422.91**	849253.41*	6.11ns
Interaction	6	19.88**	0.01ns	0.40ns	238.47**	60.88**	62466.50ns	410667.23ns	3.36ns
Error (b)	18	18.34	0.01	0.45	26.14	3.61	39650.63	195065.30	3.36
C.V		1.43	0.59	1.56	10.05	7.08	14.49	10.78	4.33

*,** and ns significant at 0.05, 0.01 and no significant.

affected by irrigation withholding in different growth stages. Decrease of ear diameter due to water stress at flowering stage can be due to negative effect of water stress on row number in ear. Also the result showed that the main effect of humic acid and interaction between humic acid foliar application and irrigation withholding in different growth stages were significant on row number in ear corn plants (Table 2 and 3). As can be seen from Table 3, under complete irrigation and withholding in different growth stages conditions, humic acid foliar application with 450 ppm increased seed number per row although there was no significant difference between this treatment and humic acid foliar applications with 300 ppm concentration (Table 2 and 3). Concerning the positive effect of HA on seed number per row, Shuixiu and Ruizhen (2001) mentioned that HA used as a soil treatment at the seeding stage significantly increased the seeds per plant in soybean plants. Also Saruhan et al (2011) have reported that the highest grain number per bunch was obtained from HA treatment. Total seed number decreased as result of irrigation withholding at 8-leaf and staminate inflorescence appearance at by 5.39 and 18.58%, respectively with compared complete irrigation treatment conditions. Irrigation withholding during flowering and pollination affect on metabolism, physiology and morphology of plants. It seems that decrease in seed number is due to lack of fertilization. In addition, water stress leads to reduction in nutrient uptake and photosynthesis rate and thus reproductive organs will damage (Table 3). There is direct relation between seed number and seed yield. Our results are in agreement with findings of Hirisch et al (2007). Increase of seed number per ear can be due to positive effect of normal irrigation on improving of productivity potential and increase of anther area. In addition, water deficit stresses affects on source and sink relations and assimilate transportation. Humic acid treatment with 450 ppm improved total seed number under complete irrigation and irrigation withholding in different growth stages. Similarly, Albayrak (2005) reported that humic acid significantly affected most of the yield components of *Brassica raya*. Also the results showed that 1000 seed weight decreased as result of irrigation withholding at staminate inflorescence appearance at by 7.80 with compared complete irrigation treatment condition. Drought stress reduced the capacity of assimilate production due to a small green leaf area and leaf greenness. Thus, reduced current and reserve carbohydrates production during reproductive and/or vegetative water deficit may have limited the 1,000 seed weight in our study. Humic acid treatment with 450 ppm improved 1,000 seed weight under complete irrigation and irrigation withholding in different growth stages. These results are in line with Delfine et al (2005), who reported that application of humic acid caused a transitional production of plant dry matter with respect to the unfertilized control. Also Saruhan et al (2011) have reported that the highest 1000 seed weight was obtained from HA treatment. As can be seen from table 3, seed yield decreased as result of irrigation withholding at 8-leaf and irrigation withholding at staminate inflorescence stage at by 12.91 % and 25.95%, respectively with compared complete irrigation treatment conditions. Similar findings have been reported in faba bean (*Vicia faba* L) by Mwanamwenge et al (1999). Acceleration of flowering and/or maturity probably contributed to reduce the impact of drought stress in corn plants. However humic acid treatment with high concentration (300 and 450 ppm) improved seed yield under complete irrigation and irrigation withholding in different growth stages. It seems that HA maintain soil nutrients supply, help in moisture retention and mitigation of salinity. Our results are supported by Suganya and Sivasamy (2006), Selim et al (2009), Buyukkeskin and Akinci (2011), Çelik et al (2011), Tahir et al (2011), and Yoon-Ha Kim et al (2012) who have reported that HA increase crop growth and productivity, and help in moisture retention and mitigation of salinity. Biological yield decreased as result of irrigation withholding at 8-leaf and irrigation withholding at staminate inflorescence stage at by 8.68% and 14.17%, respectively with compared complete irrigation treatment conditions. Anyia and Herzog (2004) indicated

Table 3 - Interaction between irrigation withholding in different growth stages and humic acid foliar application on some attributes of corn.

Treatments	Humic acid foliar application	Plant height (cm)	Row number in ear	Seed number in row	Total seed number in ear	1000 seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Irrigation									
Complete Irrigation	Untreated (0 ppm)	293.33 de	17.50 d	43.43 d	779.35 d	295.23 cde	11779.2 bc	26779.6 a	44.00 ab
	Treated (150 ppm)	301.66 cd	17.63 cd	45.36 c	814.43 c	297.70 bcd	11570.3 c	25722.9 b	45.00 ab
	Treated (300 ppm)	315.66 a	17.93 a	47.52 ab	859.38 a	299.96 ab	12011.9 ab	26135.5 ab	46.00 a
	Treated (450 ppm)	315.69 a	17.95 a	48.16 a	869.33 a	302.00 a	12160.4 a	26466.6 ab	46.00 a
Irrigation withholding at 8-leaf stage	Untreated (0 ppm)	278.00 g	17.23 e	41.28 e	737.27 ef	292.16 e	10258.6 e	24454.2 cde	42.00 bcd
	Treated (150 ppm)	281.00 fg	17.53 d	43.78 d	783.01 d	293.03 e	10548.2 e	24551.6 cd	43.00 abc
	Treated (300 ppm)	294.33 de	17.83 ab	46.49 bc	844.01 b	298.96 abc	10921.3 d	24849.0 cd	44.00 ab
	Treated (450 ppm)	297.66 cde	17.93 a	47.15 ab	861.50 a	298.66 abc	10945.7 d	24902.8 c	44.00 ab
Irrigation withholding at staminate inflorescence appearance	Untreated (0 ppm)	289.66 ef	17.03 f	35.53 g	634.56 h	272.23 g	8722.8 g	22984.8 f	38.00 ef
	Treated (150 ppm)	301.33 cd	17.16 ef	36.73 g	660.96 g	277.76 f	8946.6 g	22972.9 f	39.00 def
	Treated (300 ppm)	306.00 bc	17.63 cd	39.43 f	728.49 f	291.93 e	9480.5 f	23724.3 ef	40.00 cde
	Treated (450 ppm)	312.66 ab	17.73 bc	40.21 ef	744.54 e	294.73 de	9619.0 f	24075.2 de	36.33 f

Treatment means followed by the same letter within each common are not significantly different ($P < 0.05$) according to Duncan's Multiple Range test that water deficit caused between 11 and more than 40% reduction of biomass across the genotypes of cowpea (*Vigna unguiculata* L) due to decline in leaf gas exchange and leaf area. However humic acid foliar application with high concentration (300 and 450 ppm) could improve biological yield. Our results are in agreement with findings Yoon-Ha et al (2012) who have reported that HA increase crop growth and productivity. Also the result showed that Harvest index decreased as result of irrigation withholding in different growth stages. Singh and Saxena (1998) showed that seed yield has positive correlation with harvest index while it has negative with plant growth. Under water deficit stress, economical yield and biological yield affect by different factors such as plant growth rate, leaf size, root hydrolytic resistance and evaporation and then harvest index changes. Small leaves decrease transpiration rate and conserve more water into the soil, this water will consume during seed setting and seed filling stage. One of the most important physiological processes which affect by water deficit stress is assimilate transport. The main effect of humic acid foliar application and interaction between humic acid foliar application and irrigation withholding at different growth stages were not significant on harvest index.

References

Albayrak SC, 2005. Effects of different levels and application times of humic acid on root and leaf yield and yield components of forage turnip (*Brassica rapa* L). J Agron 4(2): 130-133

Anyia AO, Herzog H, 2004. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur J Agron 20: 327-339

Ashraf MW, Saqib N, Sarfraz TB, 2005. Biological effect of bio-fertilizer humic acid on mung beans (*Vigna radiate* L). J Bio & Biotech 2(3): 737-739

Baydar H, Erbas S, 2005. Influence of seed development and seed position on oil, fatty acids and total tocophorol contents in sunflower (*Helianthus annuus* L). Turk J Agric 29: 179-186

Benites VM, Madari B, Machado PL,2003. Quantitative extraction and fractionation of humic substances in soils: a simplified procedure for low cost, pp. 1-7. Technical communicators. Ministry of Agriculture. Livestock and Supply

Borrell AK, Jordan D, Mullet J, Klein P, Klein R, Nguyen H, Rosenow D, Hammer G, Douglas A, Henzell B, 2008. Discovering stay-green drought tolerance genes in sorghum: a multidisciplinary approach. 14th Australian Agronomy Conference, Adelaide, SA

Buyukkeskin T, Akinci S, 2011. The effects of humic acid on above-ground parts of broad bean (*Vicia faba* L) seedlings under $Al^{(3+)}$ toxicity. Fresenius Env Bull 20(3): 539-548

Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR, 2004. Improving drought tolerance in maize: a view from industry. Field Crops Res 90: 19-34

Çelik H, Vahap KA, Bulent AB, Turan MA, 2011. Effect of foliar-applied humic acid to dry weight and mineral nutrient uptake of maize under calcareous soil conditions. Comm Soil Sci Plant Anal 42(1): 29-38

Delfine S, Tognetti R, Desiderio E, Alvino A, 2005. Effect of foliar application of N and humic acids on growth and yield of durum wheat. Agron Sustain Dev 25(2): 183-191

Hirisch AM, Fang Y, Asad S, Kapulnik Y, 2007. The role of phytohormones in plant-microbe symbioses. Plant and Soil 94: 171-184

Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R, 2008. Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. Colloid Surf B Biointerf 61: 298-303

Keeling AA, McCallum KR, Beckwith CP, 2003. Bioresource Tech 90 (2): 127-137

Mart I, 2007. Fertilizers, organic fertilizers, plant and agricultural fertilizers. Agro and Food Business Newsletter

Mauromicale G, Angela MGL, Monaco AL, 2011. The effect of organic supplementation of solarized soil on the quality of tomato. Scientia Hort 129(2): 189-196

Mikkelsen RL, 2005. Humic materials for agriculture. Better Crops with Plant Food 89(3): 6-7

Mohamed A, Bakry A, Soliman YRA, Moussa SAM, 2009. Importance of micronutrients, organic manure and bio-fertilizer for improving maize yield and its components grown in desert sandy soil. Res J Agric & Bio Sci 5(1): 16-23

Mwanamwenge J, Loss SP, Siddique KHM, Cocks PS, 1999. Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (*Vicia faba* L). Eur J Agron 11: 1-11

Nardi S, Pizzeghello D, Pandalai SG, 2004. Rhizosphere: A communication between plant and soil. Rec Res Dev Crop Sci 1(2): 349-360

Ogbonnaya CI, Sarr B, Brou C, Diouf O, Diop NN, Macauley HR, 2003. Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance. Crop Sci 43: 1114-1120

Pandey RK, Maranville JW, Admou A, 2001. Tropical wheat response to irrigation and nitrogen in a Sahelian environment. I. Grain yield, yield components and water use efficiency. Eur J Agron 15: 93-105

Sarir MS, Sharif M, Ahmed Z, Akhlaq M, 2005. Influence of different levels of humic acid application by various methods on the yield and yield components of maize. Sarhad J Agric 21(1): 75-81

SAS Institute Inc. 2002. The SAS System for Windows, Release 9.0. Statistical Analysis Systems

Institute, Cary, NC, USA

Saruhan V, Kusvuran A, Babat S, 2011. The effect of different humic acid fertilization on yield and yield components performances of common millet (*Panicum miliaceum* L). Sci Res & Essays 6(3): 663-669

Schnitzer M, Gupta UC, 1965. Determination of acidity in soil organic matter. Soil Sci Soc Am J. 27: 274-277

Selim EM, Mosa AA, El-Ghamry AM, 2009. Evaluation of humic substances fertigation through surface and subsurface drip irrigation systems on potato grown under Egyptian sandy soil conditions. Agr Water Manage 96: 1218-1222

Shuixiu H, Ruizhen W, 2001. A study on the effect of KOMIX, humic acid containing organic fertilizer on spring soybean. Acta Agric Uni Jiang 23: 463-466

Suganya S, Sivasamy R, 2006. Moisture retention and cation exchange capacity of sandy soil as influenced by soil additives. J App Sci Res 2: 949-951

Susilawati K, Ahmed OH, Nik Muhammad AM, Khanif MY, 2009. Effect of organic based N fertilizer on dry matter (*Zea mays* L), ammonium and nitrate recovery in an acid soil of Sarawak, Malaysia. Am J App Sci 6(7): 1282-1287

Swift R,1996. Organic matter characterization, pp. 1011-1069. Soils Science Society of America, Bock Ser 5, SSSA, Madison

Tahir MM, Khurshid M, Khan MZ, Abbasi MK, Kazmi MH, 2011. Lignite-derived humic acid effect on growth of wheat plants in different soils. Pedosphere 21(1): 124-131

Varanini Z, Pinton R, Behnke HD, Luttge U, Esser K, Kadereit JW, Runge M, 1995. Humic substances and plant nutrition. Progress in Botany: Structural botany, physiology, genetics and taxonomy. Geobotany 56: 97-117

Yoon-Ha Kim YH, Khan AL, Shinwari ZK, Kim DH, Waqas M, Kamran M, In-J L, 2012. Silicon treatment to rice (*Oryza sativa* L cv 'Gopumbyeo') plants during different growth periods and its effects on growth and grain yield. Pak J Bot 44(3): 891-897