

The Effects of Humic Substances on Some Physiological Properties of *Citrus sinensis* cv. *Thomson navel* under Lime Condition

Mehran Mahmoudi, Hoda Barzegar zoghalchali, Zarrintaj alipour, and Peyman Aryaee

Abstract—The effects of soil application of humic acid and fulvic acid on some Physiological properties of *Citrus sinensis* cv. *Thomson plants* grown at various lime conditions were examined. Lime concentrations were 0, 15 and 30%. The liquid humic acid and fulvic acid were put into the soil pots three times on 15, 30 and 45th day after bud emerged. The application doses of humic acid and fulvic acid were 0, 50 and 100 mg/l. Each application consists of four replications. The experiment was conducted in Pot experiment under controlled conditions in completely randomized factorial design in Mazandaran. The interaction effect between lime & spray for N and RWC was not found statistically significant ($P < 0.05$) and the interaction effects between lime & spray for chlorophyll a, chlorophyll b was found statistically significant ($P < 0.01$) (table 1). According to the analysis results, the most measurement of chlorophyll a, chlorophyll b and RWC in the level of lime (0%) and the least measurement of chlorophyll a, chlorophyll b and RWC by the level of lime (30%) obtained (table 2).

Keywords—Citrus, Humic Substances, Lime, Physiological properties.

I. INTRODUCTION

IN many studies, humic acids were reported to increase the growth and development of plants, therefore improving the nutritional status of the plant (Maggioni et al. 1987; Mackowiak et al. 2001, Rauthan and Schnitzer, 1982). Humic acids may also reduce plant uptake of certain toxic metal ions, adsorbing them from the soil solution (Strickland et al., 1979). On the other hand, foliar and root application of commercial fulvic acids led to a severe plant growth depression (Cerdán et al., 2006). High amounts of lime in soils have negative effects on the plant growth. The plant growth and yield are reduced in lime-affected soils because of the excess uptake of potentially toxic ions (Grattan & Grieve 1999). A high lime content decreases the osmotic potential of the soil water and, consequently, this reduces the availability of the soil water for plants. One negative effect of increasing

lime concentration is inhibited uptake of essential micronutrients such as N, P, and K (Ahmad, et al., 1981; Peacock et al., 1993). Briefly, high lime concentrations in the soil reduce the absorption of nutrients by plants which negatively affects the fertility of the soil (Khaled and Hassan, 2011). Many laboratory and glasshouse studies have shown that high amounts of lime can reduce total nitrogen accumulation (Alam 1994), P concentrations and the uptake of K in plants (Lopez & Satti 1996). In lime soil, the content of water is particularly low and the plants grown in these soils often show deficiencies of RWC (Relative Water Content) (Page et al. 1990). Humic substances may enhance uptake of chlorophyll a and chlorophyll b, one might reason that application of HS could improve plant response to lime. However, there is a lack of research regarding HS and application and its impacts on *Citrus sinensis* lime tolerance. Therefore, The aim of this study was to compare the effect of humic acid and fulvic acid on the physiological properties of *Citrus sinensis* cv. *Thomson* under lime condition.

II. MATERIAL AND METHODS

The soil used in this study was collected from 0–30 cm depth of the field located in Mazandaran. The planting dates were on 15th March, 2012. Some physical and chemical properties of the soil were determined and as a basal fertilization, the fertilizer crystalon comprised of nitrogen, phosphorus, potassium, Fe, Mn and Cu were applied to the pots before planting. The experiment was conducted in Pot experiment under controlled conditions in completely randomized factorial design with three soil application doses of humic Acid 0, 50, and 100 mg/l, three soil application doses of fulvic Acid 0, 50, and 100 mg/l, and three lime doses 0, 15, and 30%. Each application consists of four replications. All pots were irrigated with distilled water during the experiment. The soil applied humic acid and fulvic acid were prepared from humax company (United states). Air-dried soil samples were passed through 4 mm sieve. For soil applications, humic acid and fulvic acid were solved into distilled water according to the application doses. The plants into polyethylene covered plastic pots sprayed based on the application doses. Lime were added to the pots according to the application doses. Fulvic acid was sprayed three times (15, 30 and 45 days) after bud emerged (Beginning in late

Mehran Mahmoudi, Department of Agronomy, College of Agriculture, Ayatollah Amoli Science and Research Branch, Islamic Azad University, Amol, Iran. *Corresponding Author email: mehran.mahmoudi@yahoo.com

Hoda Barzegar zoghalchali, and Zarrintaj alipour, are with Department of Soil science, College of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran.

Peyman Aryaee, Department of food science, College of Agriculture, Ayatollah Amoli Science and Research Branch, Islamic Azad University, Amol, Iran.

march), with the soil application doses of humic acid treatment into the pots at the same times. After two months vegetation period, the plants were discarded and dried at 60°C and samples were wet digested by using HNO₃ + HClO₄ (4:1) and RWC was determined by comparing wet and dry weight, N by kjeldahl and the manner of titration and chlorophyll a and b by spectrophotometre (Hanlon, 1998). The statistical analysis was performed using EXCEL, SAS, MSTATC statistical soft wares, and mean values were grouped with Duncan multiple range test (P < 0.05)..

III. RESULTS AND DISCUSSION

According to the analysis results, the most measurement of RWC in T0h50 treatment, and the least measurement by the treatment of T30blank obtained (table2). These Results corroborate findings of Chen & Aviad (1990), Fagbenro & Agboda (1993) & David et al., (1994) regarding RWC of plants. According to the analysis results, the measurement of N in every treatment was the same statistically, and the differences between treatments were Nonsignificant (table2). These results corroborate findings of Chen & Aviad (1990), Fagbenro & Agboda (1993) & David et al.,(1994) Regarding N uptake of plants. According to the analysis results, the most measurement of chlorophyll a in T0f100 treatment, and the least measurement by the treatment of T30f100 obtained (table2). According to the analysis results, the most measurement of chlorophyll b in T0f100 and T30h100 treatment, and the least measurement by the treatment of T30f100 obtained (table2). These Results approve findings of Chen & Aviad (1990), Fagbenro & Agboda (1993) & David et al.,(1994) regarding chlorophyll content of plants. Also Khaled and Fawy (2011) reported that application in 0.1% humic acid treatment increased N amounts in plants which 30% lime treatment when compared with the control and 0.2% humic acid treatment. Liu and Cooper (2002) in creeping bentgrass reported that lime did not affect the content of N and other elements. Neeraja et al., (2005) found that foliar and root application of organic acids product from animal origin led to a severe plant growth depression. Anjum et al., (2008) in citrus reported that lime condition increased the accumulation of chlorophyll b, but decreased chlorophyll a and RWC. Abdalhamid et al., (2003) reported that the application of exogenous organic acid on desert plants stimulated the growth and water content by neutralizing destructive effects of lime.

IV. CONCLUSION

Humic substances can ameliorate negative soil properties and improve physiological properties. They may be used in the case of the negative effect of lime that would inhibit growth and development of plants. Regarding our finding, humic acids and fulvic acid are ineffective on N uptake. This study indicated that the application doses are important for deriving

benefit from humic substances under lime conditions. However, there are not many researches into humic substances application and their effects on plant lime tolerance. Therefore, more research is necessary to explain the positive impacts of humic acid and fulvic acid on citrus plants under lime condition..

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REFERENCES

- [1] A.O.A.C.1990. Official methods of analysis. Association of official analytical chemists, Incorporated. Virginia.
- [2] Abdelhamid A, Khedr M, Amal A, Abdelwahid W, Paul MA. 2003. Proline induces the expression of salt stress responsive proteins and may improve the adaptation of pancreatium to salt stress. *J exp bot.* 392:2553-2562.
- [3] Ahmad I, Wainwright SJ, Stewart GR. 1981. The solute and water relations of *Agrostis stolonifera* ecotypes differing in their salt tolerance. *New Phytol.* 87:615-629
<http://dx.doi.org/10.1111/j.1469-8137.1981.tb03232.x>.
- [4] Alam SM. 1994. Nutrient by plant under stress condition. *Hand book of Plant and Crop Stress.* MarcelDekker, New York. 227-246.
- [5] Alloway BJ. 2003. Zinc deficiency in crops. Reading RG66DW, United Kingdom. 1-14.
- [6] Arancon NQ, Edwards CA, Bierman P, Welch C, Metzger JD. 2004. Influences of vermicomposts on field strawberries. *Bioresource Technol.* 93(2):145-153.
<http://dx.doi.org/10.1016/j.biortech.2003.10.014>
- [7] Cerdán M, Sánchez SA, Oliver M, Juárez M, Sánchez AJJ. 2006. Effect of foliar and root application of organic acids on iron uptake by tomato plants. *Acta Hort.* 830: 481-488.
- [8] Chen Y, Aviad T. 1990. Effects of Humic Substances on Plant Growth. *American Soc of Agro, Madison WI.* p. 161-186.
- [9] Cooper RI, Liu C, Fisher DS. 1998. Influence of humic substances on rooting and nutrient content of creeping bentgrass. *Crop Sci.* 38: 1639-1644.
<http://dx.doi.org/10.2135/cropsci1998.0011183X003800060037x>
- [10] David PP, Nelson PV, Sanders DC. 1994. A humic acid improves growth of tomato seedling in solution culture. *JofPlantNutr.* 17:173-184.
- [11] Dudeck AE, Peacock CH. 1985. Effects of salinity on seashore paspalum turfgrasses. *Agron J.* 77:47-50.
<http://dx.doi.org/10.2134/agronj1985.00021962007700010012x>
- [12] Fagbenro JA, Agboda AA. 1993. Effect of different levels of humic acid on the growth and nutrient uptake of teak seedlings. *JofPlantNutr.* 16:1465-1483.
- [13] Grattan SR, Grieve CM. 1999. Salinity mineral nutrient relations in horticultural crops. *Sci Hort.* 78: 127-157.
[http://dx.doi.org/10.1016/S0304-4238\(98\)00192-7](http://dx.doi.org/10.1016/S0304-4238(98)00192-7)
- [14] Hanlon EA. 1998. Elemental determination by Atlantic absorption spectrophotometry. In: Karla Y P (ed.). *Handbook of Reference Methods for Plant Analysis.* CRC Press, Washington, 157-164.
- [15] Khaled H, FaWy H. 2011. Effect of Different Levels of Humic Acids on the Nutrient Content, Plant Growth, and Soil Properties under Conditions of Salinity. *Soil & Water Res.* 1: 21-29.
- [16] Jensen A. 1978. Chlorophylls and carotenoids. In *Handbook of physiological methods. physio and biochem methods.* Cambridge Univ Press.
- [17] Linchan DJ. 1978. Humic acid and nutrient uptake by plants. *Plant and Soil.* 50: 663-670.
<http://dx.doi.org/10.1007/BF02107217>
- [18] Mackowiak CL, Grossl PR, Bugbee BG. 2001. Beneficial effects of humic acid on micronutrient availability to wheat. *Soil Sci Soc of Amer J.* 56: 1744-1750.
<http://dx.doi.org/10.2136/sssaj2001.1744>
- [19] Maggioni A, Varanini Z, Nardi S, Pinton R. 1987. Action of soil humic matter on plant roots: Stimulation of ion uptake and effects on (Mg²⁺, K⁺) ATPase activity. *Sci of the Total Environ.* 62:355-363.
[http://dx.doi.org/10.1016/0048-9697\(87\)90522-5](http://dx.doi.org/10.1016/0048-9697(87)90522-5)

- [20] Marcum KB, Murdoch CL.1994. Salinity tolerance mechanism of six C4 turfgrasses. *J. Amer Soc Hort Sci.*119:779–784.
- [21] Nasir Khan M, Manzer H, Seddiqui F, Msroor M.2007. Salinity Induced Changes in growth,EnzymeActivities,Photosynthesis,Proline accumulation and yield in Linseed Genotypes.*World J of Agr Sci.*3(5):685-695.
- [22] Neeraja GIP, Reddy BG.2005. Effect of growth promoters on growth and yield of tomato cv. Marutham. *J Res.*33(3):68-70.
- [23] Neumann PW , Volkenburgh EV, Cleland RE.1988.Salinity stress inhibits bean leaf expansion by reducing turgor,notwallextensibility.*PlantPhysiol.*88:233–237.
<http://dx.doi.org/10.1104/pp.88.1.233>
- [24] Olsen SRC, Cole V, Watanabe FS, Dean LA. 1954.Estimation of available phosphorus by extraction with sodium bicarbonate, USDA. Cir.939.US Govern Printing Office,Washington,DC.
- [25] Page AL, Miller RH, Keeney DR.1990. Methods of soil analysis. Part 2:Chemical and microbiological properties. *Agr J.* 9: 2.
- [26] Peacock CH , Dudeck AE , Wildmon JC.1993.Growth and mineral content of St. Augustine cultivars in responsetosalinity.*JAmerSocHortSci.*118:464-469
- [27] Rauthan BS, Schnitzer M.1981. Effects of soil fulvic acid on the growth and nutrient content of *Cucumis sativus*.*PlantSoil.*63:491–495.
<http://dx.doi.org/10.1007/BF02370049>
- [28] Schnitzer M.1982. Organic matter characterization. In: Page A L , Miller R H , Keeney D R (eds): *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties.* 2nd Ed. Soil Sci Soc of Amer . 581–594.
- [29] Strickland RC , Chaney WR, Lamoreaux RJ. 1979. Organic matter influences phytotoxicity of cadmium to soybeans.*PlantandSoil.*52:393–402

TABLE I
THE ANALYSIS OF VARIANCE OF PHYSIOLOGICAL PROPERTIES

	N	CHLb	CHLa	RWC
Lime	2.83**	0.082**	0.00121**	371.33**
Spray	2.78**	0.003**	0.000017**	602.64**
Spray*Lime	0.108ns	0.004**	0.000011**	11.20ns
Error	0.166	0.00000067	0.00000001	16.66
CV	14.74	1.08	1.15	5.00

Ns,*,**Nonsignificant or significant at P = 0.05, 0.01 respectively.

TABLE II
THE MEAN VALUES OF EFFECTS OF HUMIC SUBSTANCES
ON PHYSIOLOGICAL PROPERTIES

Lime*Spray	N	CHLb	CHLa	RWC
T0blank	2.18a	0.112e	0.0112e	72.44ef
T0f100	3.60a	0.196a	0.0188a	85.66b
T0f50	3.00a	0.158b	0.0160c	91.80a
T0h100	3.84a	0.143c	0.0183b	86.16b
T0h50	3.16a	0.128d	0.0155d	92.24a
T15blank	2.02a	0.096f	0.0029g	72.66ef
T15f100	2.96a	0.048i	0.0049f	81.00d
T15f50	2.74a	0.064h	0.0036f	88.40ab
T15h100	3.06a	0.039j	0.0042f	80.28de
T15h50	2.96a	0.028k	0.0032g	88.00ab
T30blank	1.80a	0.088g	0.0013i	68.14f
T30f100	2.60a	0.009l	0.0009j	74.60e
T30f50	2.30a	0.112e	0.0021h	84.00bc
T30h100	2.88a	0.196a	0.0009j	72.44ef
T30h50	2.44a	0.158b	0.0018i	85.66b

H50 & H100 = humic acid 50 & 100 mg/l, F50 & F100 = fulvic acid 50 & 100 mg/l, T0, T15 & T30 = lime %, respectively, chlorophylls= (ppm).N&RWC=%.