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Effects of Humic Substances on Cotton (*Gossypium hirsutum* L.)

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Soil organic matter is a fundamental, but dynamic component of soils that influences many chemical, physical and biological properties that regulate soil productivity. An important objective for using humic substances in cotton is to balance vegetative and reproductive growth as well as to improve lint yield and fiber quality. Field study was conducted in a randomized complete block design with 3 replications under irrigated conditions at Diyarbakir in 2004, to determine the effects of different humic acid treatments (seed soaking, foliar spray, seed soaking + foliar spray) on yield and fiber technological properties of cotton. The comparison with the untreated control, application of humic acid significantly affected fiber length, fiber uniformity (variety × treatment interaction) and micronaire (treatment) parameters, whereas there were no significant differences in lint turnout, seed-cotton yield and fiber strength parametres.

Key Words: Cotton, Fiber quality, Humic acid, Plant growth regulator.

INTRODUCTION

Changes in fiber-quality requirements and increases in economic competition on the domestic and international levels have resulted badly fiber quality becoming a value determinant equal to fiber yield. Cotton provides a useful system to investigate the effects of fertilizer on yield and fiber development. To improve the organic contents of soils for growing industrial crops there are some applications such as planting rotation, various plough techniques, green fertilizer application and animal fertilizer application. In addition to these practices, utilization of organic-mineral fertilizers in agriculture increased in recent years[1]. One of the used organic-mineral fertilizers is humic acid. Humic acid is one of the major components of humic substances. The effects of humic substances on plant growth depend on the source and concentration[2], as well as on the molecular fraction weight of humus. Lower molecular size fraction easily reaches the plasmalemma of plant cells, determining a positive effect on plant growth, as well as a later effect at the level of plasma membrane, *i.e.*, the nutrient uptake, especially nitrate. The effects on intermediary metabolism are less understood, but it seems that humic substances may influence both respiration and photosynthesis[2].

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Effects of humic substances on various plants have been reported, *i.e.*, stimulate growth and nutrient uptake in barley plants[3] and olive plants[4]; enhance maize seed germination and growth[5]; increase dry weight of shoot, root growth, plant height and macronutrient uptake in oat plants[6]; increase grapevine berry diameter[7]. Humic substances have been reported to influence plant growth both directly and indirectly. The indirect effects of humic compounds on soil fertility include (i) Increase in the soil microbial population including beneficial microorganisms, (ii) Improved soil structure and (iii) Increase in the cation exchange capacity and the pH buffering capacity of the soil. Directly, humic acid compounds may have various biochemical effects either at cell wall, membrane level or in the cytoplasm, including increased photosynthesis and respiration rates in plants, enhanced protein synthesis and plant hormone like activity[8]. Humic substances may possibly enhance the uptake of minerals through the stimulation of microbiological activity[9]. When adequate humic substances are present within the soil, the requirement for nitrogen, phosphorus and potassium fertilizer applications may be reduced[10]. Humic substances are major components of organic matter, often constituting 60 to 70 % of the total organic matter[11]. Soil organic matter are mainly consists of humic and fulvic acids which called humic materials[12,13]. They are mainly produced from nitrogenous compounds containing decomposed amino acids and aromatic complexes[13]. Those organic complexes affect soil properties and physiological properties of plant due to carboxyl (-COOH) and phenolic (-OH) groups[14,15]. It was reported that humic acid affects physical and chemical properties of soils[15-17]. Humic acids have been shown to improve phosphate availability and uptake by crop plants[18]. The mechanism by which humic and fulvic acids stimulate plant growth are not fully clear, although there are some theories which probably work together. Maggioni *et al.*[19] indicated that humic and fulvic acids can influence the nutrient absorption, due to their effect on the K^+ and Mg^{2+} dependent ATPase. Humic acids have the ability to transform solid phase forms of micronutrient cations such as Fe^{3+}, Fe^{2+}, Mn^{2+} and Zn^{2+} into soluble metal complexes, which are available to plants[20]. Pinton *et al.*[21] demonstrated that the humic substances affect the activity of microssomal and tonoplast. Samson and Visser[22] demonstrated that cellular membrane permeability can be altered by these acids. These substances also present auxin and gibberellin-like effects on *Raphanus sativus*[23]. Humic substances have profound influence on the growth of plant roots. When humic acids and/or fulvic acids are applied to soil, enhancement of root initiation and increased root growth may be observed[10]. The stimulatory effects of humic substances have been directly correlated with enhanced uptake of macronutrients, such as nitrogen, phosphorus and sulfur[8] and micronutrients, *i.e.* Fe, Zn, Cu and Mn[24]. Humic substances also increased copper and manganese uptake and by enhancing root development, also improved nitrogen uptake and biomass yield[25]. The aim of this study is to determine the effects of humic acid (given to cotton through different application methods) on yield and technological traits.

EXPERIMENTAL

This study was carried out in Diyarbakir (lat 37° 54' N, long 40° 14' E and altitude of about 660 m). The soils of the experimental area were thinly structured alluvial material or limestone. The soil is low in organic material and phosphorus and has adequate calcium and high clay content (49-67 %) in the 0-150 cm profile. Water permeability of the soil is good and salt levels are suitable for cotton production.

Treatment material used in this study was liquid humic acid (humic acid 15 %, fulvic acid 2.4 % and organic matter 16 % and carbon 9.8 %). Humic acid was applied by 3 different treatments (seed soaking, foliar spray, seed soaking + foliar spray with humic acid) and a control. Treatments were applied with 150 mL humic acid/100 kg seed + 1000 mL water for seed before sowing and 200 mL humic acid/m^2 + 2000 mL water for foliar spray in initial flowering. In control plots, only the water was sprayed to the seeds and plants. The experiment was carried out with GW Teks, DP Opal, DP Diamond, Stoneville 453, BA 119 and Sahin 2000 cotton varieties sown (7 May 2004) in the field according to randomized complete block design with split plot arrangement with 3 replications. The harvest was made with hand and at 3 different times. Fiber quality was determined from the hand-picked seed cotton samples by High Volume Instrument, after the crop was harvested and ginned by a mini laboratory rollergin.

Data obtained from the various analyses and measurements were subjected to analysis of variance in MSTAT-C statistical program[26] and the least significant difference (p: 0.05) was used to verify the significance of differences among treatment means to determine the effects of humic acid.

RESULTS AND DISCUSSION

Significant effects for humic acid treatments were observed for fiber length, fiber uniformity (variety × treatment interaction) and micronaire (treatment) parameters except for lint turnout, seed-cotton yield and fiber strength parameters.

There were no significant differences (p: 0.05) between treatments for lint turnout, seed-cotton yield and fiber strength, although there was a trend for humic acid application to increase lint turnout, seed-cotton yield and fiber strength properties (Tables 1-3). However, humic acid treatments increased lint turnout, seed-cotton yield and fiber strength compared with the untreated control. Especially, humic acid applied through seed soaking tended to more increase the lint turnout, seed-cotton yield and fiber strength than the others. These results indicated that humic acid application affected the lint turnout, seed-cotton yield and fiber strength. This may be due to the increased chlorophyll content and enhanced rate of photosynthesis in response to the humic acid treatments. Effects of humic substances on plants have been reported, *i.e.*, enhance phosphorus uptake and dry matter production in corn[27]; increase leaf area and photosynthesis in cacao[28]; stimulate growth and leaf N and chlorophyll content in wild olive[29]. Studies of the effects of humic substances on plant growth, under conditions of adequate mineral nutrition, consistently show

TABLE-1
EFFECT OF HUMIC ACID ON LINT TURNOUT

Humic acid treatments	Lint turnout (%)						
	DP Opal	GW Teks	Stoneville 453	DP diamond	BA 119	Sahin 2000	Means
Foliar spray	40.27	41.20	40.93	43.33	43.73	42.13	41.93
Seed soaking	40.80	42.27	41.73	44.67	44.00	41.47	42.49
Seed soaking + Foliar spray	40.40	43.47	40.00	43.07	44.27	42.13	42.22
Control	39.33	41.47	40.40	44.40	43.33	42.00	41.82
Means	40.20 c	42.10 b	40.77 c	43.87 a	43.83 a	41.93 b	
LSD (%)	Variety × treatment int: n.s.				Variety: 1.167 **		Treatment: n.s.

*,**Significant at the 0.05 and 0.01 levels of probability, respectively. n.s. = non-significant.

TABLE-2
EFFECT OF HUMIC ACID ON SEED-COTTON YIELD

Humic acid treatments	Seed-cotton yield (kg ha^{-1})						
	DP Opal	GW Teks	Stoneville 453	DP diamond	BA 119	Sahin 2000	Means
Foliar spray	4088.90	3847.23	4536.10	4169.47	4411.53	4295.63	4224.81
Seed soaking	4168.63	3907.17	4730.93	4501.57	5019.87	4565.87	4482.34
Seed soaking+Foliar spray	4148.80	3741.27	5373.00	3800.77	4847.63	4238.90	4358.39
Control	3915.54	3653.17	4502.80	4046.43	4626.57	4235.33	4162.29
Means	4080.45	3787.21	4785.71	4129.56	4726.40	4333.93	
LSD (%)	Variety × treatment int: n.s.				Variety: n.s.		Treatment: n.s.

*,**Significant at the 0.05 and 0.01 levels of probability, respectively. n.s. = non-significant.

TABLE-3
EFFECT OF HUMIC ACID ON FIBER STRENGTH

Humic acid treatments	Fiber strength (g/tex)						
	DP Opal	GW Teks	Stoneville 453	DP diamond	BA 119	Sahin 2000	Means
Foliar spray	34.17	35.27	30.80	32.67	32.87	28.90	32.44
Seed soaking	33.63	35.13	32.13	31.17	31.30	31.67	32.51
Seed soaking+Foliar spray	32.70	35.53	31.93	33.87	31.30	27.67	32.17
Control	33.10	35.00	31.07	32.23	32.27	28.90	32.09
Means	33.40 ab	35.23 a	31.48 bc	32.48 b	31.93 bc	29.28 c	
LSD (%)	Variety × treatment int: n.s.				Variety: 2.668 **		Treatment: n.s.

*,**Significant at the 0.05 and 0.01 levels of probability, respectively. n.s. = non-significant.

TABLE-4
EFFECT OF HUMIC ACID ON FIBER LENGTH

Humic acid treatments	Fiber length (mm)						
	DP Opal	GW Teks	Stoneville 453	DP diamond	BA 119	Sahin 2000	Means
Foliar spray	29.33 cdef	30.50 ab	29.27 cdef	30.07 abc	28.73 f	28.60 f	29.42
Seed soaking	28.87 ef	30.60 a	29.23 cdef	29.53 abcdef	28.90 def	29.97 abcd	29.52
Seed soaking+Foliar spray	29.50 bcdef	29.87 abcde	28.80 ef	29.83 abcde	29.43 bcdef	28.60 f	29.34
Control	29.30 cdef	28.63 f	28.80 ef	29.47 bcdef	29.50 bcdef	28.87 ef	29.09
Means	29.25	29.90	29.03	29.73	29.14	29.01	
LSD (%)	Variety × treatment int: 1.085 *				Variety: n.s.		Treatment: n.s.

*,**Significant at the 0.05 and 0.01 levels of probability, respectively. n.s. = non-significant.

TABLE-5
EFFECT OF HUMIC ACID ON FIBER UNIFORMITY

Humic acid treatments	Fiber uniformity (%)						
	DP Opal	GW Teks	Stoneville 453	DP diamond	BA 119	Sahin 2000	Means
Foliar spray	84.13 bcde	86.60 a	83.87 bcde	84.27 bcde	84.03 bcde	80.67 g	83.93
Seed soaking	83.63 bcde	85.43 abc	84.07 bcde	83.47 cde	84.20 bcde	83.17 def	83.99
Seed soaking+Foliar spray	84.83 abcd	83.93 bcde	83.40 de	84.97 abcd	84.37 bcde	82.63 efg	84.02
Control	84.27 bcde	83.10 def	82.50 efg	84.97 abcd	85.57 ab	81.37 fg	83.63
Means	84.22 a	84.77 a	83.46 ab	84.42 a	84.54 a	81.96 b	
LSD (%)	Variety × treatment int: 1.986 *				Variety: 1.520 *		Treatment: n.s.

*,**Significant at the 0.05 and 0.01 levels of probability, respectively. n.s. = non-significant.

TABLE-6
EFFECT OF HUMIC ACID ON MICRONAIRE

Humic acid treatments	Micronaire (mic)						
	DP Opal	GW Teks	Stoneville 453	DP diamond	BA 119	Sahin 2000	Means
Foliar spray	3.82	4.09	4.49	4.29	3.65	3.93	4.05 b
Seed soaking	3.96	4.15	4.80	4.36	4.22	4.08	4.26 a
Seed soaking+Foliar spray	4.12	4.33	4.72	4.28	3.98	3.84	4.21 ab
Control	3.96	3.80	4.62	4.29	3.69	4.04	4.07 b
Means	3.97 bc	4.09 bc	4.66 a	4.31 b	3.89 c	3.97 bc	
LSD (%)	Variety × treatment int: n.s.				Variety: 0.3520 **		Treatment: 0.1642 *

*,**Significant at the 0.05 and 0.01 levels of probability, respectively. n.s. = non-significant.

positive effects on plant biomass[8]. Thus, it can be concluded that photosynthesis and correspondingly, formation of carbohydrates such as glucose, saccharose, *etc.* increased due to chlorophyll content in response to humic acid treatments compared with the untreated control. Differences among varieties were significant and DP Diamond and BA 119 had higher lint turnout (43.87 and 43.83 %, respectively) and GW Teks had higher fiber strength (35.23 g/tex). The present study confirms the findings of Basbag[30] reported that humic acid application had no significant effect on ginning percentage and fiber strength, but is in contrast for seed-cotton yield.

There were significant differences (p: 0.05) between variety × treatment interaction for fiber length and fiber uniformity traits and treatment for micronaire (Tables 4-6). Application of humic acid increased both fiber length and fiber uniformity between variety × treatment interaction and micronaire between treatment compared with the untreated control. These results indicated that cellulose is very important for fiber development and derived from photosynthetic rate. Thus, any factor that influences photosynthetic efficiency ultimately influences the fiber properties. Increased chlorophyll content in response to humic acid treatments may substantially contribute for enhanced photosynthetic efficiency because higher chlorophyll content is one of the main factors stimulating the rate of photosynthesis and biological productivity[31]. Differences among varieties were not significant for fiber length and were significant for fiber uniformity and micronaire. DP Opal, GW Teks, DP Diamond and BA 119 had higher fiber uniformity (84.22, 84.77, 84.42 and 84.54 %, respectively) and Stoneville 453 had higher micronaire (4.66 mic). The present study is in contrast to the findings of Basbag[30] reported that humic acid application had no significant effect on both fiber length and fiber fineness.

REFERENCES

1. I. Doran, C. Akinci and M. Yildirim, Effects of Delta Humate Applied with Different Doses and Methods on Yield and Yield Components of Diyarbakir-81 Wheat Cultivar, 5th Field Crops Cong, Diyarbakir, Turkey, Vol. 2, pp. 530-534 (2003).
2. S. Nardi, D. Pizzeghello, A. Muscolo and A. Vianello, *Soil Biol. Biochem.*, **34**, 1527 (2002).
3. M. Ayuso, T. Hérnandez, C. Garcia and J.A. Pascual, *Bioresour. Technol., Kidlington*, **57**, 251 (1996).
4. R. Fernández-Escobar, M. Benlloch, D. Barranco, D. Duenas and J.A.G. Ganán, *Sci. Horticult., (Amsterdam)*, **66**, 191 (1996).
5. B. Eyheraguibel, J. Silvestre and P. Morard, Physiological Effects of Humic Like Substances on Maize. In: Humic Substances and Soil and Water Environment, São Pedro. Proceedings São Pedro: Embrapa Instrumentação, pp. 200-202 (2004).
6. C.M. Rosa, R.M.V. Castilhos, L.C. Vahl and P.F.P. Costa, Effect of Fulvic Acids on Plant Growth, Root Morphology and Macronutrient Uptake by Oats. In: Humic Substances and Soil and Water Environment, São Pedro, Proceedings... São Pedro: Embrapa Instrumentação, pp. 207-210 (2004).
7. L.H. Bassoi, S.T. Silva and A.V. Silva Filho, Efeito da aplicação de ácidos orgânicos na produção de uva cv. Itália no Vale do São Francisco. In: Encontro Brasileiro De Substâncias Húmicas, 6, 2005, Rio de Janeiro. Anais Rio de Janeiro, Embrapa Solos, pp. 1-3 (2005).
8. Y. Chen and T. Aviad, in eds.: C.E. Clapp, R.L. Malcolm and P.R. Bloom, Effects of Humic Substance on Plant Growth, In MacCarthy, Humic Substances in soil and Crop Sciences: Selected Readings. Soil Sci. Society of America, Madison, Wisconsin, pp. 161-186 (1990).

9. L. Mayhew, Humic substances in biological agriculture [Online]. Available at www.acresusa.com/toolbox/reprints/Jan04_Humic%20Substances.pdf (2004).
10. R.E. Pettit, Organic Matter, Humus, Humate, Humic Acid, Fulvic Acid and Humin: Their Importance in Soil Fertility and Plant health [Online]. Available at www.humate.info/mainpage.htm (2004).
11. M. Schnitzer and S.U. Khan, Humic Substances in the Environment, Dekker Publ., New York (1972).
12. M. Schnitzer, in eds.: A.L.Page, R.H. Miller and D.R. Keeney, Organic Matter Characterization, In: Methods of Soil analysis, Part 2, Soil Science Society of America, Madison, WI, pp. 581-594 (1982).
13. J.P. Andriesse, *FAO Soils Bull.*, **59**, 165 (1988).
14. Y.S. Lee and R.J. Bartlett, *Soil Sci. Soc. Am. J.*, **40**, 876 (1976).
15. M. Schnitzer, Significance of Soil Organic Matter in Soil Formation, Transport Processes in Soils and in the Formation of Soil Structure, Soil Utilization and Soil Fertility, Vol. 4, Humus budget, 206, pp. 63-81 (1992).
16. D. Vaughan and D.J. Linehan, *Plant Soil*, **44**, 445 (1976).
17. M. Boyle, W.T. Frakenburger Jr. and L.H. Stolyz, *J. Prod. Agric.*, **2**, 290 (1989).
18. K.S. Day, R. Thornton and H. Kreeft, in ed.: E.A. Ghabbour, Humic Acid Products for Improved Phosphorus Fertilizer Management, In Humic Substances, Versatile Components of Plants, Soil and Water, Royal Society of Chemistry, pp. 321-325 (2000).
19. A. Maggioni, Z. Varanini and S. Nardi, *Sci. Total Environ., Madison*, **62**, 355 (1987).
20. M. Raviv, Chen and Y. Yand Inbar, in eds.: Y. Chen and Y. Avnimelech, Peat and Peat Substitutes as Growth Media for Container Grown Plants, The Role of Organic Matter in Modern Agriculture. Martinus Nijhoff, Boston, pp. 257-287 (1986).
21. R. Pinton, Z. Varanini and G. Vizzotto, *Plant Soil*, **42**, 203 (1992).
22. G. Samson and S.A. Visser, *Soil Biol. Biochem.*, **21**, 343 (1989).
23. P. Petrovic, D. Vitorovic and M. Jablanovic, *Acta Biolog. Med. Experim., Budapest*, **7**, 21 (1982).
24. Y. Chen, C.E. Clapp, H. Magen and V.W. Cline, in eds.: E.A. Ghabbour and G. Davies, Stimulation of Plant Growth by Humic Substances: Effects on Iron Availability, Understanding Humic Substances: Advanced Methods, Properties and Applications, Royal Society of Chemistry, Cambridge, UK, pp. 255-263 (1999).
25. R.A. Bidegain, M. Kaemmerer, M. Guiresse, M. Hafidi, F. Rey, P. Morard and J.C. Revel, *J. Agric. Sci.*, **134**, 259 (2000).
26. MSTAT-C, A Microcomputer Program for Design Management and Analysis of Agronomic Research Experiments, Michigan State Uni, East Lansing, MI, USA (1991).
27. F.V. Andrade, E.S. Mendonça, I.R. Silva and R.F. Mateus, Low Molecular Weight and Humic Acids Increase Phosphorus Uptake and Corn Growth in Oxisoils, In: Humic Substances and Soil and Water Environment, São Pedro, Proceedings... São Pedro: Embrapa Instrumentação, pp. 211-214 (2004).
28. D.F. Calima, R.O. Paneto, M.A.G. Aguilar, F.B. Folli, C.A.S. Souza and S. Sonegheti, Crescimento e fotossíntese de clones de cacau (*Theobroma cacao* L.) com resistência diferencial a Verticillium dahliae submetidos diferentes doses de Turfa Líquida®. In: Encontro Brasileiro De Substâncias Húmicas, 6., 2005, Rio de Janeiro. Anais... Rio de Janeiro: Embrapa Solos, pp. 9-11 (2005).
29. J.M. Murillo, E. Madejón, P. Madejón and F. Cabrera, *J. Arid Environ.*, **63**, 284 (2005).
30. S. Basbag, *Asian J. Chem.*, **20**, 1961 (2008).
31. M. Berova and Z. Zlatev, *Plant Growth Regul.*, **30**, 117 (2000).

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