Response of tomato plants grown in limited nutrient solution to applied humic acids.[1]

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ABSTRACT The effects of humic acid(HA) on nutrient accumulation and growth of tomato seedlings was evaluated in a solution of limited nutrient availability in a greenhouse. HA additions were made to the nutrient solution at rates of 640, 1280 or 2569 mg/liter. The addition of 1280 mg/liter HA produced significant increases in shoot accumulation of P,K,Ca,Mg,Fe,Mn,and Zn as well as, fresh and dry weights of roots, and increased accumulation of N,Ca,Fe,Mn,Zn and Cu in roots. However, on comparing nutrient accumulation in plants treated with 1280 mg/liter HA and those given an additional supply of nutrients equivalent to those supplied by HA at the 1280 mg/liter rate, shoots accumulated more N,P,K,Fe and Cu, while roots accumulated more K,Ca and Mg. Therefore these increases were not due to nutrients contained in HA. Root volume and

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electrolyte leakage, as an indication of membrane permeability, did not differ as a consequence of HA additions. However, there was a clear trend toward increases in both with HA additions. Also, electrolyte leakage correlated positively with HA rate. There were no effects of a shift in solution pH from 5.8 to 7.0 nor of the interaction of pH and HA on nutrient accumulation or growth of tomato seedlings.

The beneficial effect of humic acid (HA) on plant growth has long been recognized (Lee and Bartlette,1976). HA is believed to increase nutrient uptake (Stevenson,1972; Tyler and McBride,1982; Tan and Nopamornbodi,1979) and cell permeability (Gawronski,1969; Guminski et al.,1983), and to accelerate the respiratory processes of plants (Smidova,1960). It also stimulates germination of different varieties of crops (O'Donnell,1973; Sanders et al.,1990) and possibly affects fresh and dry weights of plants grown in nutrient solutions (Linehan,1978; Pal and Sengupta,1985).

Root exudation of plants in solution often lowers the pH of the nutrient medium, resulting in precipitation of HA; hence, HA activity is reduced (Vaughn and Malcolm,1988). Stevenson (1982) found that when trivalent ions such as Fe^{3+} and Al^{3+} are added to HA, precipitation of HA will occur at low concentration, but the precipitate will dissolved as pH rises above 7.0 during titration.

Most studies in nutrient culture have not explained whether HA influences plant growth under limited nutrient conditions or if its beneficial effect is due to the additional nutrients it contains. Currently, little information is available as to the optimum amount of HA needed for beneficial effects on plants grown under nutrient stress, or if pH affects HA activity. This study was initiated to examine the effects of a commercial preparation of HA (Enersol, American Colloid Co.), made from leonardite shale on tomato seedlings, grown in nutrient solutions at different pH levels.

Seedlings of tomato (*Lycopersicon esculentum* Mill. cv. Mountain Pride) germinated in quartz sand, were rinsed and transferred to a 42 L tank containing aerated nutrient solution for 10 days after germination. The nutrient solution was prepared in distilled water as follows: 5mM $Ca(NO_3)_2$; 1.5mM $NH_4H_2PO_4$; 0.5mM NH_4NO_3; 2.5 mM KNO3; 5.2 mM KCl, 2mM $MgSO_4$; 3 mg/liter FeDTPA; 0.175 mg/liter H_3BO_4; 0.06 mg/liter $NaMoO_4$; 0.2 mg/L MNCl; 0.025 mg/L $ZNCl_2$ and 0.05 mg/L $CuCl_2$. Macronutrient concentrations in this formulation were typical of those commonly used for tomato culture, while the micronutrients levels were half of the customary levels.

Then plants were transferred to opaque plastic 3.8 liter container (one plant per container) containing 3.6 liter of aerated treatment solutions. To obtain limited nutrient availability, solutions were allowed to be depleted through plant uptake to half the initial concentration for macronutrient or total depletion for micronutrients before elements were replaced. To verify this condition, samples of nutrient solutions were analyzed twice weekly by atomic absorption spectroscopy.

Containers were maintained at 3.6 liter of solution by adding measured amounts of distilled water daily. Plants were harvested after 22 days in treatment solutions. Fresh weights of shoots and roots were determined after which plants were dried at 70C for dry weight determinations. Samples were then ground in a Wiley mill to a particle size of 1mm and finer. N content of shoots and roots were determined separately by a Kjeldahl procedure (Fleck,1974). Levels of K,Ca,Mg,Fe,Mn,Zn and Cu were analyzed by atomic absorption spectroscopy using a Perkin Elmer Atomic Absorption Spectrophotometer, Model 373 (Christian et al., 1970). Phosphorous was determined colormetrically by the molybdate vanadate method (Jackson,1958).

Analysis of variance was conducted and where F test warranted, least significant differences at the 5% level of probability were calculated.

In the first experiment treatments consisted of three levels of HA (Enersol): 0, 640 and 1280 mg/liter as well as 2 pH levels: 5.8 and 7.0. To maintain a pH, NaOH was added to the nutrient solution as needed. Estimates on the amount of NaOH to be added were based on titration curves of HA with NaOH. Treatments were arranged factorially using a randomized complete block design with four one plant replicates.

In the second experiment HA was applied to solutions identical in composition and handling to those used in experiment I at rates 0, 1280 and 2560 mg/liter. To determine whether HA effect on nutrient accumulation was due to other factors rather than the nutrients it contained, a fourth treatment was prepared similar to the control, but nutrients equivalent to those supplied by the HA at 1280 mg/liter were added. Nutrient analysis of HA was determined similarly to that for plant tissue. The HA contained 0.48% ash and the following quantities: 30 P, 12,000 K, 400 Mg, 900 Ca, 453 Fe, 9.1 Mn, 0.5 Zn and 0.7 Cu mg/liter.

To obtain root volume, roots were washed with distilled water and blotted dry. Volume displacement was obtained by submerging roots in water and recording, in milliters, the amount of water displaced into a graduated cylinder.

Electrolyte leakage(EL) was used to determine if HA had affected membrane permeability. Procedures used were those of Buesher (1973). Leaf disc tissues from five randomly selected leaves (most recent fully expanded leaf) were obtained with a 8mm diameter cork borer. Approximately 1gm of tissue was weighed, rinsed three times in distilled water, brought to 70 ml volume with distilled water and incubated 30 min at 30C in a water bath. Conductivity of the solution was read (Fisher Scientific Co. Model 360) and the solution containing the leaf discs was then boiled for 10-15 mins in a water bath, cooled to 30C and adjusted to 70 ml with distilled water. The conductivity of total electrolyte leakage was determined using the formula:

$$\% \ EL = (A/B) \times 100$$

where: A= Initial conductivity after 30 min incubation

B= Final conductivity after 10-15 min boiling

There was no HA x pH interaction with respect to shoot or root fresh and dry weights in the first experiment, and HA had no effect on fresh weight of shoots (Table 1). At 1280 mg/liter HA there were significant increases in shoot dry weights, as well as, root fresh and dry weights. There were no effects of pH on fresh or dry weight of shoot or root and no effect of HA or pH on the shoot/root ratio.

Again, nutrient solution pH had no effect on nutrient accumulation in tomato shoot or root and there were no HA x pH interactions. Applications of 1280 mg/liter HA increased accumulation of P, K, Ca, Mg, Fe, Mn, and Zn in shoots. (Table 2). Accumulation of N, Ca, Fe, Mn, Zn, and Cu was greater in roots of plants treated with 1280 mg/liter HA than in plants in the control and 640 mg/liter HA treatments (Table 3).

There were no effects of HA or pH on water consumption of tomato seedlings. Mean quantity of consumption over 22 days was 10.81 liter per plant.

In the second experiment fresh and dry weights of shoots were greatest in the HA treatments (Table 4) with root dry weight greatest in the 1280 mg/liter HA treatment compared to plants supplied additional nutrients alone.

Applications of 1280 mg/liter HA yielded increases in nutrient accumulation of N,P,K,Fe and Cu in shoots(table 5). Roots accumulated more K,Ca and Mg with HA applications (Table 6). There was increased Fe accumulation in roots treated with additional nutrients over all other treatments.

Root volume of tomato plants was unaffected by HA applications, but there was an apparent trend for increased volume with HA applications(Table 7). Electrolyte leakage, as an indication of membrane permeability, was not affected by HA applications however, a significant correlation was found between the two parameters.

Results presented here confirm earlier findings that HA can increase nutrient uptake of certain elements and stimulate total dry matter production of shoots and roots (Tan and Nopamornbodi, 1979; Mylonas and McCants, 1980).

At 1280 mg/liter HA, roots were highly branched and rich in hairs. This may have resulted in an increase in surface area, which could have facilitated more efficient nutrient accumulation, or HA could be acting as a chelating agent, as previously reported (DeKock,1955; Linehan,1978; Stevenson,1972). The simultaneous increases in Fe and P accumulation in tomato shoots suggest that HA may enhance the uptake of P indirectly by complexing with Fe. Evidence supporting this view has been shown by DeKock (1955), who found that at low pH, HA complexes with iron in solution, making more P available to the plant. He further proposed that without HA, Fe would form insoluble precipitates with P. Increased levels of Fe in untreated plant roots, with a concomitant decrease in shoot tissue possibly indicates that without HA, translocation of Fe to shoots maybe reduced.

On comparing nutrient accumulation in plants treated

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with 1280 mg/liter HA and those given an additional supply of nutrients, it was evident that HA increased nutrient accumulation in roots and shoots. It is therefore clear that this increase is not due to the additional nutrients contained in HA. Although nutrient levels were low, visible deficiency symptoms were not observed on treated or untreated plants in experiment I. This may be a result of plants remaining in treated solution for only 22 days. If plants had remained longer in the solution, deficiency symptoms would have eventually occurred. However, in experiment II, plants showed Mg deficiency throughout the growing period and this could have reduced plant weights and nutrient levels.

Under limited nutrient conditions, applications of 1280 mg/liter HA can stimulate growth of tomato seedlings by increasing nutrient uptake. Also, solution pH ranging from 5.8-7.0 will not affect HA activity.

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TOMATO RESPONSE TO FOLIAR NUTRIENT AND BIOSTIMULANT APPLICATIONS

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Abstract. **The effects of nutrient elements and biostimulants applied as foliar sprays were evaluated on 'Sunny' tomatoes (Lycopersicon esculentum Mill.) in two studies in the spring of 1988. Treatments were three applications 20-8.7-16.6 N-P-K soluble fertilizer with and without micronutrients, chelated micronutrients, amino acids plus micronutrients, and humic acid. Application of the two 20-8.7-16.6 (N-P-K) fertilizers at 10 lb./acre, resulted in moderate foliage tip burn following the first application to tomatoes at the early bloom stage (one month old transplanting). Marketable yields with these two macronutrient treatments were approximately 40% less than that with the control. At one location, humic acid (1 qt/acre at each application) treated plants resulted in 17% higher yield of extra large fruit than that obtained with the check treatments. Plants treated with chelated micronutrients (1 qt/acre) or amino acid plus micronutrients (1 qt/acre) had similar fruit yield to that of control plants. Three days following final treatments, P and K leaf concentrations were higher for plants that received foliar application of macronutrients.**

Commercial fresh market tomato profit is increased by earlier harvest, larger fruit size, and higher yield. In recent

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Table 5. Effect of applied humic acid on root volume and electrolyte leakage of tomato after twenty-two days of treatment in experiment II.

Treatment	Root volume (cm^3/plt)	Electrolyte leakage (%)
Humic acid (mg/liter)		
0	35.3a[y]	35.7a
0+[z]	23.3a	35.3a
1280	51.8a	38.2a
2560	51.8a	36.0a

[z]Control treatment plus nutrients equivalent to those contained in 1280 mg/liter HA treatment.
[y]Means with the same letter within a column are not significantly different at the 5% probability level according to the LSD test.

Table 4. Influence of humic acid levels in nutrient solution on nutrient accumulation in tomato shoots and roots over twenty-two days in experiment II.

Treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	gms/plt					mg/plt			
Humic acid(mg/liter)									
				Shoots					
0	45.9b[y]	10.6b	96.0a	32.0a	9.45a	2121a	635a	704a	149a
0+[z]	37.0a	8.70a	96.0a	27.0a	9.28a	1768a	606a	649a	111a
1280	95.0c	18.9c	125b	33.0a	9.60a	4379b	696a	710a	346b
2560	71.9bc	14.4bc	129b	28.0a	10.2a	3698b	692a	773a	267b
				Roots					
0	5.29a[x]	1.28a	4.27a	1.89a	0.91a	1486a	147a	136a	35.8a
0+[z]	4.36a	1.30a	4.15a	1.84a	0.87a	2866b	141a	143a	41.7a
1280	5.87a	1.70a	10.0b	2.29b	1.29ab	1153a	57a	188a	42.1a
2560	5.34a	1.48a	9.56b	2.46b	1.51b	1011a	98a	188a	54.7a

[z] Control treatment plus nutrients equivalent to those contained in HA in treatment 3.
[y] Means with the same letter within a column are not significantly different at $P<.05$ using the LSD test.

Table 3. Effect of humic acid on fresh and dry weights of tomato plants at twenty-two days in experiment II.

Treatment	Shoot		Root		Shoot/Root Ratio	
	Fr.wt.	Dry wt.	Fr. wt.	Dry wt.	Fr.wt.	Dy wt
	gms/plt					
Humic acid (mg/liter)						
0	219a[y]	19.7b	48.2a	2.03ab	4.54a	9.70a
0+[z]	204a	16.5a	50.3a	1.78a	4.05a	9.27a
1280	242b	25.8c	59.5a	2.72b	4.06a	9.49a
2560	247b	24.2bc	59.5a	2.51ab	4.15a	9.64a

[z] Control treatment plus nutrients equivalent to those contained in HA in treatment 3.
[y] Means with the same letter within a column are not significantly different at the 5% probability level according to the LSD test.

Table 2. Influence of humic acid levels in nutrient solution on nutrient accumulation in tomato shoots and roots over twenty-two days in experiment I.

Treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	gms/plt					mg/plt			
Shoots									
Humic acid(mg/liter)									
0	95.6a[z]	20.8a	177a	68.7a	19.6a	5750a	959a	1299a	279a
640	96.8a	23.1a	183b	66.2a	17.5a	5405a	944a	1309a	235a
1280	98.7a	26.4b	219b	78.4b	22.0b	7576b	1338b	1693b	302a
pH									
5.8	96.2a	23.8a	191a	71.2a	18.9a	6262a	1063a	1415a	265a
7.0	96.2a	23.1a	195a	71.1a	20.4a	6226a	1097a	1452a	279a
Roots									
Humic acid(mg/liter)									
0	10.5a[z]	2.35a	14.4a	2.69a	2.25a	1797a	167b	217a	28.3a
640	10.1a	2.28a	13.5a	2.36a	2.00a	1654a	126a	182a	30.1a
1280	12.5b	2.32a	15.7a	3.51b	2.38a	2709b	199b	259b	47.1b
pH									
5.8	10.8a	2.39a	13.8a	2.60a	2.09a	2149a	167a	216a	33.2a
7.0	11.3a	2.24a	15.1a	3.11a	2.32a	1959a	161a	222a	37.2a

[z] Means with the same letter within a column are not significantly different at $P<.05$ using the LSD test.

Table 1. Effect of humic acid on fresh and dry weights of tomato plants at twenty-two days in experiment I.

Treatment	Shoot Fr wt	Shoot Dry wt	Root Fr wt	Root Dry wt	Shoot/Root Ratio Fr wt	Shoot/Root Ratio Dry wt
	gms					
Humic acid (mg/liter)						
0	368 a[z]	38.6 ab	110 a	4.15 a	3.33 a	9.32 a
640	355 a	36.1 a	134 a	3.71 a	2.65 a	9.47 a
1280	399 a	42.1 b	195 b	5.68 b	2.05 a	7.42 a
pH						
5.8	374 a	37.5 a	140 a	4.34 a	2.67 a	8.65 a
7.0	374 a	40.1 a	152 a	4.69 a	2.46 a	8.56 a

[z]Means with the same letter within a column are not significantly different at the 5% probability level according to the LSD test.