

Low concentrations of humic substances significantly enhanced plant growth

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Abstract

Seedlings of lettuce (*Lactuca sativa* L.) variety Grand Rapids were grown in deep water culture growing units. The units contained growing solution with limited amount of nutrients and different concentrations of humic substances. Six treatments and one control, 12 replications each, were evaluated in a two month period. Only one plant perished from a total of 84, indicating that the growing units were effective. A liquid product containing 13,800 mg L⁻¹ humic substances of small particulate sizing ($1.1 \pm 0.64 \mu\text{m}$ in mean diameter) was utilized as a source of humic substances. Significant plant (root and leaf) growth was observed at low product rates between 70 and 500 mg L⁻¹, corresponding to 1 and 7 mg L⁻¹ humic substances. At increased rates, the growth was reduced. At much higher rates, plant growth was again observed, that was likely caused by the presence of nutrients in the product. These experiments demonstrated the efficacy of humic substances on plant growth, a critical finding in the context of sustainable horticulture, in which maximum yields from minimum input would be desired.

Key words: *Lactuca sativa* L., deep water culture growing units, humic substances, plant, root, leaf, growth.

Introduction

Benefits of humic substances (humic acids and fulvic acids) on the growth of plants and seedlings had been documented for decades. O'Donnell (1973) reported that liquid humic products at a rate as low as 500 mg L⁻¹ promoted root initiation of geranium cuttings grown in solution. Rauthan and Schnitzer (1981) observed that a dry purified humic product enhanced the growth of cucumber plants grown in solution. Optimum rates were reported between 100 to 300 mg kg⁻¹. Reynolds *et al.* (1995) showed that 4,000 mg kg⁻¹ rate of a dry humic product enhanced the growth of vineyards planted on sand. Hartwigsen and Evans (2000) observed that geranium seedlings experienced significant root growth when substrate-drenched with a liquid humic product at a rate as low as 2,500 mg L⁻¹. Evans and Li (2003) found that a liquid humic product was capable of promoting growth of various ornamental plant seedlings grown on germination papers. The optimum rate was recorded at 2,500 mg kg⁻¹. As reported by Dudley *et al.* (2004), 31,250 mg kg⁻¹ rate of a dry humic product promoted the growth of zinnia and marigold seedlings and transplants grown on sand and peat media. Asik *et al.* (2009) found that wheat planted on sandy clayey soil grew well with the addition of either a dry humic product to soil (at 1,000 mg kg⁻¹ rate) or a liquid humic product (at 1,000 mg L⁻¹ rate) to the leaves. Shafeek *et al.* (2013) observed a growth increase of broad bean planted on sandy soil when a liquid humic product was foliar applied at a rate of 4,000 mg L⁻¹.

The results showed that the optimum rates of humic products varied widely from one trial to another. When short term results were desired, liquid (extracted) products would generally be more effective than dry (raw) products. Still, other factors needed to be taken into considerations, such as types and conditions of the growing media, application methods, as well as types of plants

and their growing stages (Chen and Aviad, 1990). Canadian Humalite International Inc. of Edmonton, Alberta, Canada therefore completed laboratory experiments to investigate the efficacy of humic substances, in which significant plant growth would be observed at their lowest possible concentration.

In these experiments, the following conditions were set: 1) the work was completed in a laboratory of controlled environment, 2) lettuce was chosen as the tested plant due to its robustness and simplicity in analyses (on roots and leaves only), 3) the plants were grown in solution, in which nutrients were directly applied to roots to maximize their uptake, 4) the solution contained limited amount of nutrients, as well as negligible organic matter and microbial activities, and 5) a liquid (extracted) humic product was applied to the growing solution, which was manufactured to contain small particulates (*i.e.* humic substances) to maximize their absorption by the roots.

Materials and Methods

The experiments were completed in a laboratory of controlled temperature ($24 \pm 0.5 \text{ }^\circ\text{C}$) and humidity ($76 \pm 1\%$).

Seeds of lettuce (*L. sativa* L.) of Grand Rapids variety were cleaned using deionized water and planted on growing media of 50% perlite+ 50% sphagnum containing 1,000 mg kg⁻¹ N, 500 mg kg⁻¹ P, and 1,000 mg kg⁻¹ K nutrients, and moisture contents of the media were maintained between 34 to 37%. Their survival rates after 3 weeks were recorded as 85%. The seedlings were removed from the media and washed using deionized water to remove as much as soil matter as possible, then transferred to 7 deep water culture growing units.

Each growing unit had dimensions of 50 cm long x 40 cm wide x 13 cm deep, representing one treatment. Each had a cover on top

containing 12 holes, filled with foam, in which one seedling was suspended in it. The seedlings were randomly placed in one unit to another. All units were submerged with growing solution up to 10 cm depth from bottom. All roots were submerged in the solution.

Control was evaluated in one unit with 12 replications. The plants were grown in a control solution. As shown in Table 1, the solution contained limited amount of nutrients as well as negligible content of organic matter (*i.e.* total carbon) and microbial activities (*i.e.* heterotrophic bacteria). This would make the plants grew under stressed conditions.

Table 1. Characteristics of control solution and Product HA

Parameter	Control solution	Product HA
N	224 mgL ⁻¹	110 mgL ⁻¹
P	448 mgL ⁻¹	49 mgL ⁻¹
K	288 mgL ⁻¹	7,600 mgL ⁻¹
S	16 mgL ⁻¹	41 mgL ⁻¹
Ca	44 mgL ⁻¹	107 mgL ⁻¹
Mg	12 mgL ⁻¹	7 mgL ⁻¹
B	< 1 mgL ⁻¹	3 mgL ⁻¹
Cu	< 1 mgL ⁻¹	< 1 mgL ⁻¹
Fe	< 1 mgL ⁻¹	< 1 mgL ⁻¹
Mn	< 1 mgL ⁻¹	< 1 mgL ⁻¹
Mo	< 1 mgL ⁻¹	< 1 mgL ⁻¹
Zn	< 1 mgL ⁻¹	< 1 mgL ⁻¹
Cl	6 mgL ⁻¹	36 mgL ⁻¹
Heterotrophic bacteria	0/100 mL	0/100 mL
Total carbon	2 mgL ⁻¹	4,100 mgL ⁻¹
Humic acids	<100 mgL ⁻¹	5,500 mgL ⁻¹
Fulvic acids	< 100 mgL ⁻¹	8,300mgL ⁻¹
Humic substances	< 100 mgL ⁻¹	13,800mgL ⁻¹
Particulate diameter, maximum		2.4µm
Particulate diameter, mean		1.1µm
Particulate diameter, minimum		0.4µm
Particulate diameter, σ		0.64µm
pH	6.4	10.2

Note: N was analyzed using EN 12260 EU standard method; P, K, S, Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn using APHA 3120 B-ICP-OES; Cl using APHA 4110 B-IC; heterotrophic plate count bacteria using APHA 9215D-MS; total carbon using APHA 5310 B-instrument; humic acid, fulvic acid, and humic acids using HPTA/IHSS method as per Swift (1996); and particulate diameter using Beckman Coulter LC particle size analyzer.

A liquid humic product (Product HA) was manufactured by solubilizing low-energy coal with potassium hydroxide in water. Other than potassium, this product also contained small amount of other nutrients and negligible content of organic matter (*i.e.* total carbon) originating in the raw material, and no microorganisms (*i.e.* heterotrophic bacteria). Product HA contained 13,800 mgL⁻¹ humic substances (5,500 mg L⁻¹ humic acids and 8,300 mgL⁻¹ fulvic acids), analyzed using the HPTA method (Mayhew, 2014), which were based on Swift (1996). Humic acids were defined as components of humic substances that were soluble in aqueous alkali solution and insoluble in aqueous acid solution, while fulvic acids were soluble in both aqueous alkali and acid solutions (Stevenson, 1965). The hydrophobic fulvic acids were isolated by means of the DAX-8 resin (Stevenson, 1994). This product was specifically processed to contain small particulates (*i.e.* humic substances) in the solution, with a mean diameter of $1.1 \pm 0.64 \mu\text{m}$, to maximize their absorption by the roots (Table 1). This liquid humic product was added to the growing solution at different rates from 70 (Treatment 1) to 42,000 mgL⁻¹ (Treatment

6) providing 1 to 580 mgL⁻¹ humic substances in the growing solution (calculated as Product HA rate x 13,800 mgL⁻¹). Each of Treatment 1 to Treatment 6 was evaluated in one growing unit; each treatment had 12 replications.

Four Sun Blaster® growing lamps of 5,022 l mm⁻¹ each were suspended over the growing units. A timer controlled their operations at 10 hours ON during the day and 14 hours OFF in the evening. One air pump of 5.0 L/min capacity was connected to 0.3 cm diameter flexible air tubing, which was branched out and connected to each unit. Using control valves, fresh air was equally distributed among the units, continuously bubbled within the solution to ensure its freshness.

After 2 months, all plants were harvested. Perished plants were separated and recorded as 0 measurements in the analyses. Roots, leaves, and plants (roots and leaves) from each replicate of one treatment were carefully removed from the units and separately measured within 0.1 cm tolerance. Leaves from each treatment were counted. Roots and leaves from each treatment were carefully dried using paper towel and placed in a desiccator at a room temperature for 24 hours to remove all surface moisture. They were aggregated and weighed within 0.01 g tolerance to provide one measurement on plant weight. The plants were oven dried for 72 hours at $80 \pm 0.5 \text{ }^\circ\text{C}$, and weighed within 0.01 g tolerance.

Survival rates for all treatments were tabulated. Root, leaf, and plant (root + leaf) length measurements were analyzed using Minitab® for means, standard deviations (σ), ANOVA ($P < 0.05$), and Fisher's least significant difference ($\alpha = 0.05$). Leaf counts and plant weight measurements were tabulated.

Results and discussion

As shown in Table 2, there was only one perished plant, occurred under Treatment 5. This indicated that the water culture growing units worked effectively, in which limited amount of nutrients and different concentrations of humic substances were directly fed to the roots.

As shown in Table 3 (as well as Fig. 1), control had significantly the shortest roots. Treatment 6 had the longest, followed by Treatment 2, Treatments 1 and 5, and Treatments 3 and 4.

Concentrations of humic substances between 1 and 7 mg L⁻¹ (or 70 and 500 mg L⁻¹ of Product HA) were found to be optimum, resulting in up to 91% increase over control. These low concentrations were likely caused by the following factors: 1) all plant roots were submerged in the growing solution; all nutrients and humic substances were efficiently absorbed by the plants, 2) the control solution contained limited amount of nutrients and different concentrations of humic acids; benefits of humic acids

Table 2. Plant survival rate (based on 12 replications)

Treatment	Product HA (mgL ⁻¹)	Humic acids (mgL ⁻¹)	Survival rate(%)
Control	0	0	100
1	70	1	100
2	500	7	100
3	4,000	55	100
4	6,000	83	100
5	14,000	193	92
6	42,000	580	100

Table 3. Length measurements

Treatment	Root (cm)			Leaf (cm)			Plant (cm)		
	Mean	σ	Confidence Interval (95%)	Mean	σ	Confidence Interval (95%)	Mean	σ	Confidence Interval (95%)
Control	8.8d	3.53	6.46-11.03	9.6c	1.75	8.33-10.93	18.4d	4.99	15.09-21.74
1	15.2bc	4.55	12.91-17.56	10.7c	1.79	9.41-12.01	25.9bc	6.08	22.62-29.28
2	16.8b	4.28	14.50-19.15	10.8c	1.65	9.28-11.88	27.6b	5.67	24.08-30.74
3	12.7c	4.81	10.42-15.06	9.5c	2.89	8.18-10.78	22.2cd	7.07	18.89-25.55
4	12.8c	3.96	10.53-15.17	10.6c	2.53	9.31-11.90	23.4bc	6.01	20.12-26.78
5	13.8bc	3.78	11.44-16.08	12.9b	2.89	11.56-14.17	26.7bc	5.85	23.29-29.95
6	21.7a	4.52	19.33-23.98	17.0a	1.89	15.74-18.34	38.7a	4.53	35.36-21.74

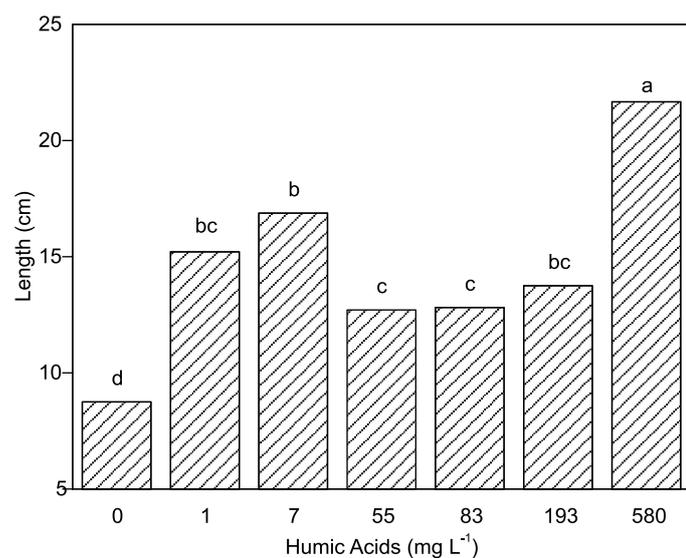


Fig. 1. Fresh root length

would mostly be observed when plants were grown under stressed conditions, and 3) Product HA contained humic substances of small particulate sizing; their absorption by the plant roots would be maximized.

While many efforts have been made to compare the efficacy of humic acids versus fulvic acids, the use of Product HA in these experiments provided a different view on how commercial humic products could be labeled. As shown by Wang *et al.* (1995) and Thurman *et al.* (1982), fulvic acids, in most cases, happened to be smaller in sizing (*i.e.* having smaller molecular weights) than that of humic acids. However, there should be some portions of humic acids (components of humic substances soluble only in aqueous alkali solution) which were small enough in sizing to be absorbed by root pores, which would still contribute to their efficacy.

The results showed that the roots were shorter at higher rates of Product HA. As stated by Whitehead (1963), excessive amount of organic matters (that would also be true in the case of humic substances) could become phytotoxic to plants. Rauthan and Schnitzer (1981), Dudley *et al.* (2004) and Asik *et al.* (2009) confirmed this statement, showing reduced plant growth at relatively high concentrations of humic substances. Interestingly, when the rates were further increased, positive results were observed. Although detailed mechanisms were not investigated, it is suggested that this anomaly may be due to nutrients (*i.e.* potassium) present in the product. At lower rates, its effects were negligible, and therefore the increased (or decreased) root lengths was attributed to humic substances. At higher rates the effects of potassium became more and more dominant (Table 4). This finding was important when comparing different commercial

Table 4. Estimated addition of nutrients in control solution

Treatment	Estimated addition of potassium (mgL ⁻¹)	Estimated increase of NPK nutrients (%)
Control	0	0
1	< 1	< 1
2	4	< 1
3	30	3
4	46	5
5	106	11
6	319	33

Table 5. Leaf counts and plant weight measurements

Treatment	Leaf counts	Plant fresh weight (g)	Plant dried weight (g)
Control	77	22.34	1.49
1	74	25.19	1.87
2	98	36.21	1.89
3	74	25.08	1.71
4	87	32.20	2.00
6	103	60.54	3.69
7	108	85.49	5.44

humic products. Research on commercial humic products should therefore separate the effects of humic substances from nutrients in the products.

As shown in Table 3, control and treatment 3 had the shortest leaves. Treatments 1, 2, and 4 had slightly longer measurements. All of them, however, were not significantly different. Treatment 5 resulted in significantly longer leaves, while the longest were recorded for treatment 6. Although not as obvious as in the case of the roots, the same pattern was also observed here. Optimum concentrations of humic substances were found between 1 and 7 mg L⁻¹, in which up to 13% increase was observed over control. The length decreased at higher rates of Product HA, then increased at much higher rates as in the case of the roots. These findings also showed that direct benefits of humic substances were more clearly observed on the root structure.

As shown in Table 3, control had significantly the shortest plants (roots + leaves), followed by treatment 3. Treatments 1, 4, and 5 resulted in significantly longer plants, followed by treatments 2 and 6. The pattern was the same as that of the roots, in which up to 50% growth increase was observed at concentrations of humic substances between 1 and 7 mg L⁻¹.

Leaf counts as shown in Table 5 also showed the same pattern. The best result was found between 1 and 7 mg L⁻¹ humic substances, resulting in up to 27% more counts than control.

Based on the plant weight measurements (Table 5), between 1 and 7 mg L⁻¹ humic substances were found to be optimum. At these rates, up to 62% fresh weight (or up to 27% dried weight) increase

over control were observed. Better results were recorded at much higher rates of Product HA, in which the presence of potassium in the growing solution became more dominant.

These experiments demonstrated the efficacy of humic substances on the growth of plants. Significant growth was observed on lettuce (*L. sativa* L.) grown in deep water growing units containing limited amount of nutrients and low concentrations of humic substances between 1 to 7 mg L⁻¹. This finding was critical in a context of sustainable horticulture, in which maximum yields from minimum input would be desired.

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