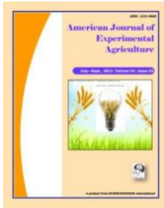

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Low Concentrations of Humic Substances Enhanced the Growth of *Lactuca sativa* L.

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Authors' contributions

This work was carried out in collaboration between all authors. All authors designed and evaluate the experiments, analyzed the results and prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The experiments were completed to evaluate the efficacy of humic substances at low concentrations on the growth of lettuce (*Lactuca sativa* L.).
Methodology: Lettuce (*Lactuca sativa* L.) of Grand Rapids variety seedlings were grown in seven deep water culture growing units. Each unit represented one treatment, in which twelve plants were grown in solution containing limited amount of nutrients and different rates of a liquid (extracted) humic product at 0, 70, 500, 4,000, 6,000, 14,000 and 42,000 mgL^{-1}, corresponding to 0, 1, 7, 55, 83, 193 and 580 mgL^{-1} humic substances, respectively. All plants were harvested and evaluated in a two month period.
Results: Only one plant perished from a total of 84, indicating that the growing units were effective. Plant length measurements were analyzed for means, standard deviations, ANOVA (P=.05) and Fisher's least significant difference (α=.05). Leaf counts and plant weight measurements were recorded. Significant plant growth was observed at low product ratesbetween70 and 500 mgL^{-1},

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corresponding to 1 and 7 mgL^{-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.

Conclusion: These experiments demonstrated the efficacy of humic substances at low concentrations on the growth of lettuce (*Lactuca sativa* L.), a critical finding in the context of sustainable horticulture, in which maximum yields from minimum input would be desired.

Keywords: Lactuca sativa L.; deep water culture growing units; humic substances; plant; root; leaf; growth.

1. INTRODUCTION

Benefits of humic substances (humic acids and fulvic acids) on the growth of plants and seedlings had been documented for decades. It was reported that liquid humic products at a rate as low as 500 mgL^{-1}promoted root initiation of geranium cuttings grown in solution [1]. Laboratory experiments showed that a dry purified humic product enhanced the growth of cucumber plants grown in solution with optimum rates between 100 to 300 $mgkg^{-1}$ [2]. It was found that 4,000 $mgkg^{-1}$ rate of a dry humic product enhanced the growth of vineyards planted on sand [3]. It was observed that geranium seedlings experienced significant root growth when substrate-drenched with a liquid humic product at a rate as low as 2,500 mgL^{-1} [4]. Laboratory experiments 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 $mgkg^{-1}$ [5]. As reported, 31, 250 $mgkg^{-1}$ rate of a dry humic product promoted the growth of zinnia and marigold seedlings and transplants grown on sand and peat media [6]. Greenhouse experiments found that wheat planted on sandy clayey soil grew well with the addition of either a dry humic product to soil (at 1,000 $mgkg^{-1}$ rate) or a liquid humic product (at 1,000 mgL^{-1} rate) to the leaves [7].Field experiments 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 mgL^{-1} [8].

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 [9].

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 the lowest possible concentration.

2. MATERIALS AND METHODS

The experiments were completed to evaluate the efficacy of humic substances at low concentrations on the growth of lettuce (*Lactuca sativa* L.). Lettuce was chosen as the tested plant due to its robustness and simplicity in analyses (on roots and leaves only). All work was completed in a laboratory of controlled temperature (24±0.5°C) and humidity (76±1%). The plants were grown in solution, containing limited amount of nutrients, as well as negligible organic matter and microbial activities. A liquid (extracted) humic product (Product HA), manufactured to contain small particulates (i.e. humic substances) to maximize their absorption by roots, was added to the solution at different rates. The experiments were designed that optimum conditions due to the application of humic substances could be observed.

Two hundred eighty-eight seeds of lettuce (*Lactuca sativa* L.) of Grand Rapids variety were cleaned using deionized water and planted on growing media of 50% perlite+ 50% sphagnum containing 1,000 $mgkg^{-1}$ nitrogen(N), 500 $mgkg^{-1}$ phosphorous (P) and 1,000 $mgkg^{-1}$ potassium (K) nutrients. Moisture contents of the media were maintained between 34 to 37%. Their survival rates after 3 weeks were recorded at 85%. Eighty four surviving seedlings were randomly selected from the media and washed using deionized water to remove as much as soil matter as possible, then transferred to seven deep water culture growing units.

Each unit had dimensions of 50 cm long x 40 cm wide x 13 cm deep. Each had a cover on top containing 12 holes, in which one seedling was suspended on it. The seedlings were randomly placed in one unit to another. Twelve plants were grown in one unit.

One unit was randomly selected from the seven available units. This unit was filled with control solution up to 10 cm depth from bottom. All plant roots were submerged in the solution. This treatment, identified as control, was evaluated on 4 plants, replicated 3 times (12 plants in this unit). As shown in Table 1, the control 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.

Product HA was manufactured by solubilizing low-energy coal with potassium hydroxide in water. Other than potassium, this product 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 mgL^{-1} humic acids and 8,300 mgL^{-1} fulvic acids), analyzed using the HPTA method [10] which were based on the Methods of Soil Analysis [11]. 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 [12]. The hydrophobic fulvic acids were isolated by means of the DAX-8 resin [13]. This product was processed to contain small particulates (i.e. humic substances) in the solution, with a mean diameter of 1.1±0.64 μm, to maximize their absorption by the roots.

Six units were randomly selected from the seven available units. Each unit was filled with control solution mixed with different rates of Product A up to 10 cm depth from bottom. All plant roots were submerged in the solution. Rates of Product A were 70, 500, 4,000, 6,000, 14,000 and 42,000 mgL^{-1} providing 1, 7, 55, 83, 193 and 580 mgL^{-1} of humic substances (calculated as Product HA rate x 13,800 mgL^{-1}, or 1.38%), respectively. One rate corresponded to one treatment, identified as treatments 1 to 6. Each treatment was evaluated on 4 plants, replicated 3 times (12 plants in one unit).

Four Sun Blaster® growing lamps of 5,022 lmm^{-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 solution's freshness and to provide oxygen to the roots.

After 2 months, each plant was harvested. Roots and leaves were measured for length within 0.1 cm tolerance. Leaves were counted. Roots and leaves 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 75±0.5°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 (SDs) and ANOVA (P=.05). The analyses were completed both in the replication and treatment levels. Only results in the treatment level were presented. Fisher's least significant difference (α=.05) analysis was completed among all treatments. Leaf counts and plant weight measurements were tabulated.

3. RESULTS AND DISCUSSION

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

As shown in Table 3, control (0 mgL^{-1} humic substances) had significantly the shortest roots. Treatment 6(580 mgL^{-1} humic substances) had the longest, followed by treatment 2 (7 mgL^{-1} humic substances), treatments 1 and 5 (1 and 193 mgL^{-1} humic substances) and treatments 3 and 4 (55 and 83 mgL^{-1} humic substances).

Concentrations of humic substances between 1 and 7 mgL^{-1} (or 70 and 500 mgL^{-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, therefore all nutrients and humic substances were efficiently absorbed by the plants, 2) the control solution contained limited amount of nutrients, in which benefits of humic acids would mostly be observed when plants were grown under stressed conditions, and 3) Product HA contained humic substances of small particulate sizing, to maximize their absorption by the plant roots.

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
Electrical conductivity	421.1mS/cm	23.1mS/cm
Total carbon	2 mgL^{-1}	4,100 mgL^{-1}
Humic acids	< 100 mgL^{-1}	5,500 mgL^{-1}
Fulvic acids	< 100 mgL^{-1}	8,300 mgL^{-1}
Humic substances	< 100 mgL^{-1}	13,800 mgL^{-1}
Particulate diameter, maximum		2.4 μm
Particulate diameter, mean		1.1 μm
Particulate diameter, minimum		0.4 μm
Particulate diameter, σ		0.64 μm
pH	6.4	10.2

Notes: 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 method; particulate diameter using Beckman Coulter LC particle size analyzer

Table 2. Plant survival rates and study design

Treatment	Product HA (mgL^{-1})	Humic substances (mgL^{-1})	Plants per treatment	Survival rate (%)
Control	0	0	12	100
1	70	1	12	100
2	500	7	12	100
3	4,000	55	12	100
4	6,000	83	12	100
5	14,000	193	12	92
6	42,000	580	12	100

While many efforts had 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. Fulvic acids, in most cases, happened to be smaller in sizing (i.e. having smaller molecular weights) than that of humic acids [14,15]. 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 hairs, which would still contribute to their efficacy.

The results showed that the roots were shorter at higher rates of Product HA. Excessive amount of organic matters (that would also be true in the case of humic substances) could become phytotoxic to plants [16]. Both laboratory and greenhouse experiments confirmed this statement, showing reduced plant growth at relatively high concentrations of humic substances [2,6,7]. Interestingly, when the rates were further increased, positive results were observed. Although detailed mechanisms were not investigated, it suggested that this anomaly be caused by nutrients (i.e. potassium) present in the product. At lower rates, its effects were negligible, and therefore the increased (or decreased) of root lengths was attributed to humic substances. At higher rates, the effects of potassium became more and more dominant (see Table 4).

As shown in Table 3, control and treatment 3 (0 and 55 mgL^{-1} humic substances) had the shortest leaves. Treatments 1, 2, and 4 (1, 7 and 83 mgL^{-1} humic substances) had slightly longer measurements. All of them, however, were not significantly different. Treatment 5 (193 mgL^{-1} humic substances) resulted in significantly longer leaves, while the longest were recorded for treatment 6 (580 mgL^{-1} humic substances). 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 mgL^{-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 showed that direct benefits of humic substances were more clearly observed on the root structure.

As shown in Table 3, control (0 mgL^{-1} humic substances) had significantly the shortest plants (roots + leaves), followed by treatment 3 (55 mgL^{-1} humic substances). Treatments 1, 4, and 5 (1, 83, and 193 mgL^{-1} humic substances) resulted in significantly longer plants, followed by treatment 2 (7 mgL^{-1} humic substances). Treatment 6(580 mgL^{-1} humic substances) resulted in the longest plants. 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 mgL^{-1}.

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

Table 3. Length measurements

Treatment	Root (cm)			Leaf (cm)			Plant (cm)		
	Mean	**SD**	**ANOVA**	**Mean**	**SD**	**ANOVA**	**Mean**	**SD**	**ANOVA**
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

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
5	103	60.54	3.69
6	108	85.49	5.44

Based on the plant weight measurements (also shown in Table 5), between 1 and 7mgL^{-1} humic substances were found to be optimum. At these rates, up to 62% fresh weight (or up to 27% dried weight) increases 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.

4. CONCLUSION

These experiments demonstrated the efficacy of humic substances at low concentrations on the growth of lettuce (*Lactuca sativa* L.). The plants were grown in deep water growing units containing limited amount of nutrients. Their growth was significantly enhanced with the addition of humic substances at low rates between 1 and 7 mgL^{-1}. This finding was criticalin a context of sustainable horticulture, in which maximum yields from minimum input would be desired.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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