Stimulation of Plant Growth by Humic Substances[1]

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ABSTRACT

Humic substances prepared by different techniques of extraction and from different sources of organic materials were tested for their effects on growth of corn seedlings and algae. Stimulating effects were confirmed with optimum concentrations about 5 ppm C as Na-humate for corn and 60 ppm for algae. With corn, the increase was 30 to 50% in nutrient solution or low organic matter soil; with algae, about 100%. Variation of effects among humic acids derived from different organic materials was not great.

The concentrations of elements in corn seedlings did not show any correlation with yield or humic acid level except for P and Fe. Phosphorus concentration was increased with increasing levels of humic acid regardless of the yield response. Higher Fe concentration in the plant tops and lower in roots was observed in the treatments with humic acid.

The application of humic acid to a soil low in organic matter or to nutrient solution gave the greatest growth response. Application to a high organic matter soil gave little growth response, or even a slightly negative response, indicating that the natural soil, without extraction, supplied optimum amount of humic substances to the plants. It is suggested that a test be developed to predict whether a given soil can furnish an optimum level of humic substances.

Different humic substances showed different degrees of stimulation of algal growth, with the most stimulation from a mixture of humic and fulvic acids. Humic substances in paddy soils may affect the growth of beneficial algae and of the rice (*Oryza sativa* L.) itself. As pollutants, humic substances may stimulate undesirable algal growth in bodies of water.

Additional Index Words: **humic acids, growth regulator, stimulating effect, organic matter.**

THE IMPORTANCE OF HUMUS in soil was recognized early in the 18th century. Its beneficial effects can be grouped according to effects on physical, chemical, and microbiological properties of the soil, and effects on physiological properties of the plant. The effects on the soil have been studied extensively (Broadbent, 1953; Bremner, 1954; Whitehead, 1963; Stevenson, 1972). The physiological effects, however, have not received as much attention. The purposes of this study were (i) to examine the magnitude of stimulating effects of humic acids on plant growth, if any, (ii) to define optimum concentrations of humic acids for plants, and (iii) to relate effects to sources of organic materials used.

METHODS AND MATERIALS

General

Sodium-humate solution was prepared by the method described by Kononova (1961). Humic acids were extracted with 0.1*N* NaOH using a soil/solution ratio of 1 to 5 and an extraction time of 24 hours with shaking. Soluble salts were removed by dialysis against running tap water, and then distilled water, until the pH was approximately neutral. Nutrient solution was prepared as follows: 5 m*M* $Ca(NO_3)_2$, 5 m*M* KCl, 2 m*M* $MgSO_4$, 1 m*M* KH_2PO_4, 2 m*M* NH_4NO_3, 0.04 m*M* Fe-citrate (equal molar solution of $FeCl_3$, Na-citrate, and NaOH), 2.5 ppm H_3BO_3, 1.5 ppm $MnCl_2 \cdot 4H_2O$, 0.1 ppm $ZnCl_2$, 0.05 ppm $CuCl_2 \cdot 2H_2O$, and 0.05 ppm MoO_3.

Seed of corn *(Zea mays L.)*, single cross variety 902 JX from Old Fox Agricultural Sales Inc., R. I., was germinated in expanded perlite moistened with 0.01*M* $CaSO_4$ solution. Plants were transplanted after the first leaf appeared. Harvested plants were weighed separately, tops and roots, before and after drying at 70°C. Plant tops and roots were ground and analyzed separately. Nitrogen was determined by the micro-Kjeldahl method, carbon by wet combustion, using sulfuric acid and potassium dichromate, P by the molybdate stanous chloride method, and K, Ca, Mg, Fe, Mn, Zn, and Cu by a Perkin Elmer 403 atomic absorption spectrophotometer after wet digestion with HNO_3 and $HClO_3$.

Experiment 1

The soil used to prepare Na-humate was Calais loam Ap horizon (Typic Fragiorthod). Corn seedlings were transplanted after 4 days into 1.8-liter jars, two per jar, supported by cotton in holes of rubber stoppers. Levels of Na-humate tested were 0, 2.5, 5.0, 10.0, and 50.0 ppm as C. There were three replications.

The plants were grown under fluorescent lights (Westinghouse Daylight) with 6 hours of dark and 18 hours of light per day, at room temperature (around 25°C). The nutrient solution was replaced every 4 days. Air was bubbled into the jar through 1-mm glass tubes every 20 min for 15 min. Corn seedlings were harvested in 12 days.

Experiment 2

Sodium-humates were prepared as described from (i) rye straw compost, (ii) sphagnum moss, (iii) Adams loamy fine sand B2ir horizon, (Typic Haplorthod), (iv) Adams 02 horizon, (v) Calais Ap, (vi) fresh cow manure, and (vii) composted cow manure. The carbon content was adjusted to 1 mg/ml and humic acids were analyzed as described (Table 1). Treatments were nutrient solution only (NS) or nutrient solution plus 5 ppm of Na-humate derived from (i) rye straw compost (RSC), (ii) sphagnum moss (SM), (iii) Adams B2ir (AdB), (iv) Adams 02 (Ad02), (v) Calais Ap (CS), (vi) fresh cow manure (CMF), and (vii) composted cow manure (CMC).

Corn was germinated and transplanted into 1.8-liter jars after 1 week. Jars were set up in a growth chamber in which temperature (30°C day and 20°C night), humidity (about 80%), and light intensity (about 32,300 lux) were controlled. The light period was 15 hours out of 24. Air from a compressor was bubbled by way of 1-mm capillary tubes through the solution 15 out of every 20 min. Nutrient solutions were replaced every 4 days throughout the experiment. Corn plants were harvested 12 days after transplanting.

Experiment 3

The following soils were studied: a Hinesburg loamy fine sand (Entic Haplorthod) Ap horizon, used for several years as a vegetable garden (high organic matter soil); and a Windsor loamy sand (Entic Haplorthod) C horizon (low organic matter soil). Characteristics of Hinesburg and Windsor soils, respectively, were as follows: pH, 6.53 and 5.25; organic matter, 5.05 and 0.65%; total N, 0.184 and 0.024%; available P, 55.5 and 8.0 ppm; exchangeable K, 160 and 44 ppm; exchangeable Ca, 990 and 8 ppm; and exchangeable Mg, 105 and 3 ppm. Available P and exchangeable cations were determined in a 5:1 solution/soil equilibrium extract with NH_4OAc (pH 4.8, 1.25*N* acetate, 15 min shaking). Both soils were fertilized with 10 μM/g KH_2PO_4, 10 μM/g NH_4NO_3, and 2.5

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Table 1—Mineral composition of Na-humates.

Na-humates†	C	N	P	K	Ca	Mg	Al	Fe	Mn	Zn	Cu	Na
	mg/ml	ppm										
CS	1.0	96.2	20.3	14.5	125	20.6	188.0	140.6	6.94	1.33	0.85	1.6
WSC	1.0	44.9	2.8	0.7	71	8.3	0.9	13.8	0.16	0.76	0.53	0.8
AdB	1.0	43.3	4.8	2.6	171	21.0	214.0	235.7	0.28	1.76	1.19	3.1
AdO_2	1.0	38.6	1.4	0.9	107	11.4	6.9	5.2	0.09	3.38	0.95	1.6
CMF	1.0	84.0	12.9	0.9	48	7.1	1.4	3.1	0.10	1.58	0.93	1.2
CMC	1.0	87.0	10.2	0.6	52	7.6	1.5	2.4	0.11	1.16	0.52	1.0
SM	1.0	52.1	1.4	1.4	135	11.4	6.3	4.2	0.23	1.58	1.88	3.0

† CS indicates humic acid extracted by 0.1*N* NaOH from Calais soil; WSC, from composted rye straw; AdB, from Adams B2ir horizon; AdO2, from Adams O2 horizon; CMF, from fresh cow manure; CMC, from composted cow manure; SM, from sphagnum moss.

Table 2—Yield of corn as affected by concentration of humic acids (experiment 1).

Treatments†	0	2.5	5.0	10.0	50.0	*F*-value
Plant tops	0.78	1.50	1.50	1.17	0.84	7.93**
Plant roots	0.19	0.30	0.31	0.26	0.16	7.36**

** Significant at the 0.01 level.
† ppm C as Na-humate in nutrient solution. Each value is the average of 6 plants.

Table 3—Yield of corn as affected by humic substances from different organic materials (experiment 2).

Treatments†	NS	RSC	SM	AdB	AdO_2	CS	CMF	CMC	*F*-value
Plant tops	2.72	4.23	3.70	3.73	3.93	3.09	3.56	3.40	3.30*
Plant roots	0.86	1.25	1.20	1.18	1.36	1.05	1.25	1.13	2.77*

* Significant at the 0.05 level.
† 5 ppm C as Na-humate in nutrient solution. NS indicates nutrient solution; RSC, nutrient solution plus 5 ppm Na-humate derived from composted rye straw; SM, sphagnum moss; AdB, Adams B2ir horizon; AdO2, Adams O2 horizon; CS, Calais Ap horizon; CMF, fresh cow manure; CMC, composted cow manure. Each value is the average of 3 replications.

μM/g $MgSO_4$. The low organic matter soil received 4 meq/100 g $CaCO_3$.

Corn seedlings were transplanted into 10-cm plastic pots containing soil or perlite in the greenhouse. The pots were fastened together in pairs so that the roots in each pair could be divided so that one-half would grow into each pot. Treatments consisted of the following: NS-Sl, NS-Sh, HA-HA, HA-Sl, HA-Sh, Sl-Sl, Sl-Sh, Sl-SlHA, SlHA-SlHA, Sh-Sh, ShHA-ShHA——where NS indicates nutrient solution; HA, Na-humate solution; Sh, high organic matter soil; Sl, low organic matter soil; and the dash represents the split roots. There were 12 treatments and 4 replications. Perlite was used for NS and HA treatments. The pots were moved every day to equalize light supplied. Distilled water or nutrient solution was supplied to the pots, which contained soil or perlite, respectively. Plants were harvested 1 month after transplanting. Diameters of stems and heights of tops were also measured in addition to the analyses already described.

Experiment 4

Two sets of 24 jars were prepared (Table 5). The first set contained eight treatments of nutrient solution, two of which included Na-humate. The second set contained H_2O, $FeCl_3$, Fe-citrate, Na-humate, Na-humate + $FeCl_3$, Na-humate + Fe-citrate, each paired with a nutrient solution treatment in the first set, and $FeCl_3$ and Fe-citrate each paired with the Na-humate treatment in the first set (Table 5). The Fe treatments consisted of either $10^{-4}M$ $FeCl_3$ or Fe-citrate (equal molar solution of Na-citrate, $FeCl_3$ and NaOH). Except for these Fe treatments, the nutrient solution was as previously described. Sodium-humate, prepared from composted rye straw, was added at 5 ppm C.

Corn was germinated, transplanted into the first set of jars, and set up in the growth chamber as in Experiment 2. Plants were transfered to the second set of jars after 2 days, then back to the first set after 1 day in the second set. Following this pattern the plants were transfered back and forth five times before harvesting. The solutions were replaced with fresh solution after three transfers, and the pH was measured in both sets before and after transfer of plants.

Table 4—Dry matter yield of corn grown in high and low organic matter soil (experiment 3).

Treatments†	Total mass		Plant tops		Plant roots	
	g/plant	% yield	g/plant	% yield	g/plant	% yield
NS-NS	0.93	100	0.63	100	0.30	100
NS-Sl	1.19	128	0.80	127	0.38	127
NS-Sh	2.27	244	1.60	254	0.67	223
HA-HA	1.69	182	1.19	189	0.50	167
HA-Sl	1.61	173	1.13	179	0.49	163
HA-Sh	2.23	240	1.59	252	0.64	213
Sl-Sl	1.10	118	0.73	116	0.37	123
Sl-Sh	2.14	230	1.51	240	0.63	210
Sl-SlHA	2.01	216	1.31	208	0.70	233
SlHA-SlHA	1.78	191	1.20	190	0.59	197
Sh-Sh	2.34	252	1.74	276	0.60	200
ShHA-ShHA	1.86	200	1.39	221	0.47	157
	F = 7.57**		*F* = 8.27**		*F* = 6.29**	

** Significant at the 0.01 level.
† NS indicates nutrient solution; HA, Na-humate solution; Sh, high organic matter soil; Sl, low organic matter soil; and the dash represents a split root between the two treatments. Each value is average of 4 replications.

Experiment 5

Nutrient solution for algae was prepared as follows: 5 m*M* $(NH_4)_2SO_4$, 2 m*M* $Ca(NO_3)_2$, 2 m*M* KH_2PO_4, 1 m*M* K_2SO_4, 1 m*M* $MgSO_4$, 0.5 m*M* $NaHCO_3$, 0.12 m*M* Fe-citrate (equal molar solution of $FeCl_3$, Na-citrate, and NaOH), 2.5 ppm H_3BO_3, 1.5 ppm $MnCl_2$, 0.1 ppm $ZnCl_2$, 0.05 ppm $CuCl_2$, and 0.05 ppm MoO_3.

The concentrations of humic substances were 0, 5, 10, 20, 40, 50, 60, 80, 100, and 125 ppm as C in nutrient solution and the different humic substances were: (i) HA+FA dial, (ii) HA+FA resin, (iii) HA, (iv) FA, (v) HA(.01*N* KOH), (vi) FA(.01*N* KOH), (vii) FA(H_2O), (viii) Sh, (ix) Sh without HA, (described in Table 6). Humic substances were extracted with 0.1*N* KOH in place of NaOH. Humic substances were placed into beakers in amounts calculated to provide equal C contents, 50 ml of nutrient solution was added, and a pinch of $CaCO_3$. Then the volume was brought to 100 ml with nutrient solution. Each beaker was inoculated with 0.2 ml of an algae suspension *(Botrydium sp.)* before placing it in the greenhouse for 5 days. The beakers were swirled occasionally.

At harvest time, chlorophyll content of each beaker was determined as follows. The suspensions were centrifuged, and 95% commercial ethanol was added to the residue and left overnight. The ethanol suspensions were then centrifuged and the supernatants and one washing were collected in 50-ml volumetric-flasks. The optical densities were measured with a spectrophotometer at 660 mn with red filter.

RESULTS AND DISCUSSION

Application of small amounts of humic substances increased dry matter yield of corn seedlings (Table 2, 3, and 4). The optimum concentration of humic substances for maximum stimulating effect was 5 ppm C as Na-humate (Table 2). This result is in agreement with those of Fernan-

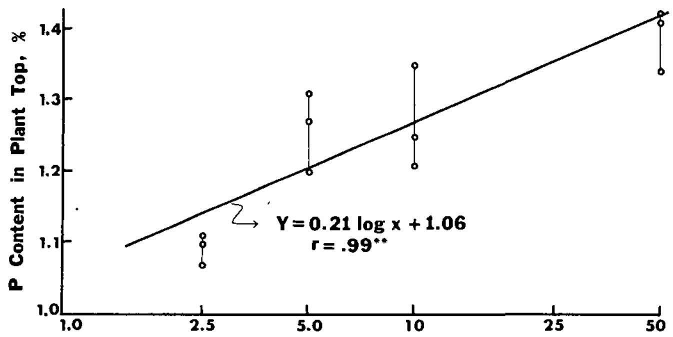

Fig. 1—Relationship between amount of humic acid added and concentration of P in plant (experiment 1).

dez (1968) and Kononova (1961). Plant growth tended to be less stimulated by high concentrations of Na-humates (Table 2). Yield was decreased from 187% of the control at 5 ppm to 103% at 50 ppm (Table 2). Kononova (1961) and Poapst et al. (1970, 1971) observed similar results with wheat (*Triticum aestivum* L.) and Alaska pea (*Pisum sativum* L. cv. Alaska) respectively.

Phosphorus concentrations in plant tops were increased by the increasing concentrations of humic acid (Fig. 1), even though the higher rates of humic acid decreased yields. Increased uptake of P with higher levels of humic acid also was found in Experiment 3. Increased concentrations of P in plants resulting from addtitions of humic substances also were observed by several other investigators (Hajdukovic and Ulrich, 1965, Jelenic et. al, 1966, Hashimoto, 1965).

In Experiment 2, the yields of corn did not differ significantly from different humic substances derived from different sources of organic materials, except that the yield with rye straw compost was slightly higher than the others (Table 3). However, the influence of humic acid application was apparent in all humic acid treatments. The highest increase was with rye straw compost (52% over control) and the lowest was 113% with the Calais soil (Table 3).

There were no clear relationships among yields of plants and the elemental contents of the organic materials used as sources of humic acids (Table 1). No correlation was found between yield and elemental composition of plant tissues. It is quite unlikely that the increased growth resulted from the additional supply of nutrient elements in the Na-humate added because the amounts of these elements contained in humic acids were negligible compared to the amounts in the nutrient solution (Table 1).

Table 5—Effects of humic acids on yields and Fe contents of corn grown with different forms of Fe (experiment 4).

Treatments†	Plant tops		Plant roots	
	Yield	Fe	Yield	Fe
	g/pot	ppm	g/pot	ppm
NS to H_2O	3.30	37	1.60	75
NS to $FeCl_3$	3.41	133	1.08	6963
NS to Fe-citrate	3.44	209	1.48	3073
NS to HA	3.21	60	1.51	108
NS to HA+$FeCl_3$	3.58	193	1.20	5913
NS to HA+Fe-citrate	3.74	321	1.71	2303
NS+HA to $FeCl_3$	3.33	151	1.34	5955
NS+HA to Fe-citrate	3.81	306	1.48	2975
F Value	0.30	8.3**	2.08	1991**

** Significant at the 0.01 level.
† Plants transfered back and forth between nutrient solutions without and with humic acids added and different forms of Fe. Each value is average of 3 replications.

Roots were highly branched and proliferant root hair development was observed in the treatments with humic acids (Table 2). This proliferant root growth seems likely to be one of the reasons why humic acid treatments gave higher yield. Increased surface area from branches and root hairs should increase the efficiency of the root system. O'Donnell (1973) felt that the growth promoting activity of humic substances was caused by plant hormone-like material contained in the humic substances. However, Poapst et. al. (1970, 1971) demonstrated that the activities of indole acetic acid (IAA) and of humic substances were independent. Treatments with humic substances gave additional effects over IAA treatments.

The dry matter yields of corn in experiment 3 were increased by application of humic acids only in the cases of nutrient solution or low organic matter soil. With high organic matter soil, however, no increase was observed with the application of humic acids (Table 4). This result is in agreement with the results obtained in experiment 1.

Apparently the concentration of humic acid available to the plant was below optimum in the low organic matter soil. Hence increased growth resulted from its application. The decreased production in the high organic matter soil upon the addition of humic acid may have resulted from concen-

Table 6—Chlorophyll content representing growth of algae affected by different treatments of humic substances (experiment 5).

Treatments†	Concentrations of C									
	0	5	10	20	40	50	60	80	100	125
	mg/liter									
HA+FA dial	0.286‡	0.382	0.407	0.457	0.524	0.519	0.515	0.515	0.456	0.390
HA+FA resin	0.265	0.491	0.518	0.565	0.585	0.595	0.598	0.563	0.511	0.495
HA	0.275	0.388	0.423	0.456	0.476	0.509	0.512	0.504	0.504	0.466
FA	0.278	0.390	0.428	0.434	0.460	0.489	0.534	0.539	0.502	0.491
HA (0.01*N* KOH)	0.272	0.443	0.504	0.520	0.532	0.565	0.577	0.510	0.478	0.461
FA (0.01*N* KOH)	0.273	0.426	0.452	0.464	0.470	0.467	0.473	0.479	0.460	0.448
FA (H_2O)	0.272	0.426	0.496	0.507	0.537	0.567	0.600	0.553	0.222	0.210
Sh	0.274	0.334	0.404	0.419	0.357	0.369	0.362	0.340	0.333	0.328
Sh without HA	0.275	0.282	0.315	0.312	0.297	0.352	0.348	0.358	0.381	0.359

F value for concentrations: 47.49**
F value for treatments: 272.35**

** Significant at the 0.01 level.
† HA+FA dial indicates humic and fulvic acid mixture prepared by dialization after extraction of 0.1*N* KOH solution; HA+FA resin, by resin treatment instead of dialization; HA, humic acid; FA, fulvic acid; HA (0.01*N* KOH), humic acid extracted with 0.01*N* KOH; FA (0.01*N* KOH), fulvic acid; FA (H_2O), fulvic acid extracted with H_2O; Sh, high organic matter soil; Sh without HA, high organic matter soil residue after extraction of humic substances with 0.1*N* KOH.
‡ Optical densities: average of three replications.

trations of humic acid that were too high (Table 4). Yet, in a soil very high in organic matter, there is no evidence of natural levels of humic acid being too high.

Poapst and Schnitzer (1971) and Schnitzer and Poapst (1967) observed that root initiation of hypocotyl segments of bean (*Phaseolus vulgaris* L.) plants were stimulated by treatment with low concentrations of soil fulvic acid. With high concentrations of fulvic acid, however, stem elongation of Alaska pea stems was inhibited (Poapst et al., 1970). Fernandez (1968) also found that low concentrations of humic acid increased the dry matter production of corn plants. With high concentrations, decreased yield resulted. These results support the idea that more than one mechanism may be involved in effects of humic substances on plant growth.

The amount of extractable organic matter in a given soil may indicate whether or not the soil needs additions of organic materials such as manure or plant residues. It is generally accepted that application of organic materials gives beneficial effects to most soils, particularly those low in organic matter. With high organic matter soils, perhaps beneficial effects from application of organic matter should not always be expected. Therefore, development of an adequate soil test to predict the response of plant growth to application of organic materials to a given soil would be worthwhile. Determination of amounts of soluble humic acids could serve as a basis for such a test. The structure of soil and kinds of organic materials also should be considered.

With foliar application of humic substances, the increase of dry matter yield was not spectacular (data not shown). There was approximately a 20% yield increase at 20 ppm C as Na-humate.

The Fe content of plant tops was much higher in treatments receiving humic acid application (Table 5). DeKock (1955) reported that humic substances prevented immobilization of Fe and P, and facilitated the translocation of these elements from roots to shoots in sunflower. He hypothesized that humic acids served as carriers of Fe by making low molecular weight complexes with Fe. These complexes increased the mobility of Fe by passing through the cell membranes easily. Without humic acids, Fe would form insoluble precipitates with P under acid conditions. By preventing precipitation, humic acids could increase the availability of P.

Humic substances also stimulated the growth of algae. The optimum concentration, however, was much higher (60 ppm C) than in the case of corn (5 ppm C). It is hard to explain the reasons behind the differing optimum concentrations. But, the results of Fernandez (1968), Kononova (1961), and Poapst et. al. (1970) demonstrated differences among plants in optimum concentrations of humic acid for the highest stimulating effects.

Different humic substances also produced different effects on the growth of algae. Fulvic acids were expected to show greater effects on algae growth than humic acids because of the higher concentration of functional groups in fulvic acids (Schnitzer and Khan, 1972). But, the results were similar. The mixture of high and low molecular weight humic substances seemed to be the best for stimulation of algal growth. More investigation is needed to explain the differing effects of different humic substances.

It is interesting that the effects of soil treatments were much less than the treatments with humic substances (Table 6). This suggests that it might be possible, under natural conditions, to increase algae populations by the applications of humic extracts. Increases in populations of N-fixing algae in paddy fields could increase the N content of the soil. Direct stimulation effects of humic acids on rice plants also would be expected. On the other hand, humic substances from polluting sewage, manure, or from erosion of top soil, may stimulate undesirable algal growth in streams, lakes, and other bodies of water.

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