agronomy

MDPI

Article

Comparative Study of Biostimulant Properties of Industrially and Experimentally Produced Humic Substances

Maris Klavins [1,*], Santa Grandovska [1], Vaira Obuka [1] and Gederts Ievinsh [2]

[1] Department of Environmental Science, University of Latvia, Raina bulv. 19, LV-1586 Riga, Latvia; santa.grandovska@gmail.com (S.G.); vaira.obuka@lu.lv (V.O.)
[2] Faculty of Biology, University of Latvia, Raina bulv. 19, LV-1586 Riga, Latvia; gederts.ievins@lu.lv
* Correspondence: maris.klavins@lu.lv; Tel.: +371-294-794-30

Abstract: Demand for new biological technologies in agriculture is ever growing, particularly with respect to the need of restoring the soil organic matter and soil fertility. Products of natural origin are developed to stimulate plant growth and productivity. Humic substances (HS) are the decay products of living matter, with high molecular weight and complex structure. Nowadays, HS are industrially produced from various materials like peat, lignin, soil and compost. The objective of this study is to make a comparison of the impact of HS of different origin on plant development. In total, eight different HS were used; four were extracted from various materials and four were commercially available products. To evaluate the stimulating effect, three different species of plants were used (*Triticum aestivum*, *Sinapis alba*, *Lepidum sativum*). The tests were carried out on Phytotestkit plates, germinating the seeds in different solutions of HS in various concentrations in dark, with or without added nutrient solution. Then, the growth parameters were measured. All tested products showed increase in at least some concentrations compared with the control sample. Significant differences in the stimulating effect of HS depending on their origin were found.

Keywords: humic substances; germination; agriculture; humates; biostimulants

Citation: Klavins, M.; Grandovska, S.; Obuka, V.; Ievinsh, G. Comparative Study of Biostimulant Properties of Industrially and Experimentally Produced Humic Substances. *Agronomy* **2021**, *11*, 1250. https://doi.org/10.3390/agronomy11061250

Academic Editor: Maria Roulia

Received: 10 May 2021
Accepted: 16 June 2021
Published: 20 June 2021

1. Introduction

Humic substances (HS), being a refractory part of natural organic matter, are compounds with a high molecular mass (from <1000 up to several millions Da) and acidic nature attributed by the presence of carboxylic and phenolic hydroxyl groups [1]. HS represent a diverse group of large molecular compounds of organic origin that can be found in every biome of the planet, occurring naturally in the soil, sediments, hydrosphere and atmosphere. HS are products of decay of plant, animal and microbial residues [2] formed in their degradation reactions and can provisionally be classified as humic acids (HA), fulvic acids (FA) and humin, depending on their solubility [2,3]. Both degradation and synthetic processes in decaying organic matter are described as humification. Generally, the term refers to the transformation of numerous groups of substances and individual molecules present in living organic matter into groups of substances with similar properties and, finally, into mineral carbon compounds [4]. Typically, HS are extracted from low-rank coal (lignite, leonardite and others), weathered coal, sediments of waterbodies, peat, and also from soils, composts and other organic matter degradation products [1].

Owing to an increasing demand for food production, the negative impact of agriculture on the environment is rising, especially affecting water and soil resources. The major problems encountered are soil erosion and decrease in organic matter, decline in groundwater and surface water quality, eutrophication, and over-use of chemicals and pesticides produced from non-renewable sources. HS have been observed to influence plant physiology [5], act as biostimulants [6], demonstrate biological activity [7] as well as form complexes with nutrients and clay particles, increase soil cation exchange capacity, prevent nutrient leaching, as well as bind with heavy metals [8] and other soil pollutants.

Owing to the plant nutrient absorption ability of HS, slow-release complexes are formed in soil, protecting against loss of nutrients through infiltration and leaching into groundwater and ensuring longer availability for plants [7]. This results in more efficient use of fertilisers and less environmental pollution.

To justify the application of HS and related products with no fertiliser properties in agriculture [6], a recently approved term "biostimulants" can be used for denoting substances of natural origin and microorganisms that enhance plant growth [9]. The ability of HS and different products containing them to promote plant growth has been well documented [10,11]. Nutrient uptake enhancement in plants and hormone-like activity are mostly considered in explaining the growth-promoting mechanism of HS [6]. The ability of HS to stimulate plant growth is highly variable, depending on many factors, including the properties of HS themselves (their origin, degree of humification, age, molecular size), plant species used, cultivation conditions (inert substance, hydroponics, soil), as well as method of application [9,11,12]. As a result, practical benefits from application of HS-containing products are highly unpredictable due to inconsistent effect, especially when compared with the use of conventional fertilisers.

The role of HS in soil environments and their possible use in agriculture have been examined in reviews [12,13]. Currently, HS are marketed for use in agriculture to enhance soil health and crop productivity worldwide. Different HS-containing products are made on industrial scale in quantities of thousands of tonnes yearly. Potassium and sodium humates are especially popular because of their high solubility in water. Owing to increasing demand, studies on isolation, characterisation and testing of humic material are performed in many laboratories worldwide. Still, there is a lack of broader comparative studies aimed at understanding the functional relationship between physico-chemical characteristics of HS and their biological effects.

The objective of the present study was to evaluate the biostimulant effect of various industrially and experimentally produced HS-containing products depending on their properties by comparing the growth-stimulating activity. Tests were conducted with different plant species cultivated in hydroponic conditions.

2. Materials and Methods

2.1. The Studied Humic Samples

HS produced in China, USA, Russia and Latvia (Table 1) were used in the study. The source materials of industrially produced HS included low-rank coal (such as leonardite and lignite), peat and, in some cases, waste products of cellulose production (lignosulphonates).

Table 1. Description of the samples.

Abbreviation	Source	Description	Producer, Country
RBP	peat	raised bog peat	Saukas Bog, Latvia
LMP	peat	low moor peat	Latvia
VC	vermicompost	organic waste vermicompost made using redworms (*Eisenia fetida*)	Daga, Latvia
SC	soil compost	garden compost	Latvia
HT	leonardite	growth stimulator and soil conditioner	*Humintech GmbH*, Germany
HGS	leonardite	fertiliser supplement	*Humic Growth Solutions*, USA
LH	lignite	humic additive to mineral fertilisers	*Lignohumate*, Russia
JT	coal	FA	Jin Tai, China

For comparison, HA and potassium humate were isolated from well characterised samples of raised bog peat, soil compost and vermicompost. HA were extracted and

purified using the procedures recommended by the International Humic Substances Society (IHSS) [1].

2.2. Potassium Humate Extraction

Air-dried and finely ground peat and compost samples were treated with 2% potassium hydroxide solution in a ratio of 1:3 and heated to 60 °C, stirring the extract periodically. After heating, the extract was left to settle overnight. On the next day, the supernatant was separated, another portion of potassium hydroxide was added and the process was repeated, three times in total. The obtained supernatant was filtered using paper filters, and the pH was neutralised with phosphoric acid (using 20 mL L^{-1} 50% H_3PO_4). The humate solution was then evaporated until about 80% of the water was lost, and the samples were air-dried and ground into fine powder.

2.3. Characterisation of HS

Elemental analysis (C, H, N, S) was carried out using a Model EA-1108 Elemental Analyzer (Carlo Erba Instruments, Italy). Elemental composition was corrected considering the ash content, and the oxygen amount was calculated as a difference. UV/Vis spectra were recorded on a Thermospectronic Helios γ UV (Thermo Electron Co, Beberly, MA, USA) spectrophotometer in a 1-cm quartz cuvette. The E_4/E_6 ratio [14], i.e., the ratio of absorbances at 465 and 665 nm, was determined for a solution of 5 mg of a humic sample in 10 mL of 0.05 N $NaHCO_3$. The total organic carbon (TOC) content was measured using a catalytic oxidation method with a Shimadzu (Kyoto, Japan) TOC-V_{CSN} carbon content analyser. First, the TOC values of the sample were obtained, then FA and HA were separated using 6 M HCl, and the value of FA TOC was obtained. Subtracting this value from that of the entire sample gives the value of HA. Metal content was determined by acid digestion using Atomic Absorption Spectrometry (AAS). Acid digestion was carried out using 25 mL 50% HNO_3 and 5 mL 30% H_2O_2 on 0.250 g of oven-dried HA and FA samples. Each sample was mixed up with the acid solution and left for 24 h; mixtures were then boiled at 150 °C until half of the liquid evaporates, and then another 25 mL of 50% HNO_3 were added and mixture boiled until first vibration. Digested samples were filtered and diluted with Millipore water up to 50 mL of the total volume and transferred into tubes and further used in AAS. Metal concentrations were measured with the acetylene-air flame and acetylene-N_2O flame with the background correction.

2.4. Plant Growth Tests

To evaluate the growth-stimulating activity of the studied HS, plant growth tests were performed, considering methods of other studies [15]. The tests had two parts: the first part included tests with HS only (testing their ability to stimulate growth through hormone-like and fertiliser-like activities), while the second part included tests with HS with added nutrient solution (testing only the hormone-like activity). For plant growth tests, three plant species were used: winter wheat (*Triticum aestivum*), white mustard (*Sinapis alba*) and watercress (*Lepidium sativum*). Seeds were obtained from local suppliers. The tests were performed using five different concentrations for each solution of HS (50, 250, 500, 1000 and 1500 mg L^{-1}). Deionised water was used as a control. The growth tests were carried out on Phytotestkit plates, using polyester cloth and filter paper as a substrate replacement. Before planting wheat (*Triticum aestivum*) seeds on the Phytotestkit plates, they were previously surface-sterilised in chlorine-containing bleach (Ace, Procter and Gamble) diluted with deionised water in a ratio of 1:1 for 7 min, then washed with at least 2 L of deionised water, put between filter papers, dampened with deionised water, placed in Petri dishes and kept in dark at room temperature (21 °C) for two days to germinate. Only the seeds that had begun to germinate (about 50% of the seeds) were further used for testing. For tests with white mustard (*Sinapis alba*) and watercress (*Lepidium sativum*) seeds, they were soaked in deionised water for half an hour prior to placing on plates. Before adding the seeds, the Phytotestkit plates were covered first with polyester cloth

dampened with 7 mL of the appropriate solution and then with filter paper. After that 10 seeds of each sample were put on the plate, covering it with a transparent plastic film. Each treatment was replicated twice. In testing HS combined with nutrient solution, 2 mL macronutrient solution (Vito, Spodrība, a product containing 3.5% nitrogen, 2.3% water-soluble phosphorous as P_2O_5, water-soluble potassium as K_2O, 0.08% iron, 0.01% manganese and 0.003% zinc; the latter three can be found as OEDF chelates; the product may also contain trace amounts of magnesium, boron, molybdenum and cuprum) in a concentration of 20 mL L^{-1} was used for dampening the polyester cloth. The plates were put in a vertical position in a growth chamber in dark at 25 °C temperature. The white mustard (*Sinapis alba*) and watercress (*Lepidium sativum*) seeds were grown for three days, the wheat (*Triticum aestivum*) seeds—for five days. After the growth test, both root and shoot lengths were obtained by measuring, and then the samples were separated and dried at a 50 °C temperature for 12 h in a laboratory oven. After that, dry mass of the sample was obtained.

For comparison, the concentration-response dependence was calculated from the initial measurements (shoot or root length) as an increase in percentage of the parameter over the respective values of untreated controls. The data obtained were subject to one-way analysis of variance tests (at a 95% confidence level), with a post-hoc least significant difference (LSD) test to separate different treatments using SPSS and Excel software, as well as to correlation analysis for testing the relationship between the biological activity of HS and their physico-chemical characteristics.

3. Results and Discussion

3.1. Characterisation of HS

To describe the analysed samples, the elemental characterisation was performed first. Table 2 shows the elemental composition of the samples, as well as their ash content. HS are carbon-based compounds, as seen from their composition. Carbon values vary from 31.71% (Jin Tai FA) and 34.36% (Lignohumate HS) to 55.46% (low moor peat). Oxygen values for HS tend to be higher for samples with lower carbon content, varying from 36.08% (low moor peat, highest C) to 61.03% (Jin Tai FA, lowest C) and 61.97% (Lignohumate HS, lowest C). Nitrogen content in samples varies from 0.44% (Lignohumate HS) to 3.69% (vermicompost) and 3.94% (soil compost). Hydrogen values, in turn, vary from around 3% (Humintech, Humic Growth Solutions, Lignohumate HS products) to 5.92% (low moor peat). H/C ratio shows the aromaticity of samples: the higher the value, the lower is the aromaticity. This ratio in the studied samples varies from 0.76 (the Humic Growth Solutions product) to 1.60 (raised bog peat). As the aromaticity of HS increases, their persistence in the environment increases as well. H/C ratio, which describes the level of aromaticity, has the smallest value (which means the highest aromaticity) in the samples of Humic Growth Solutions and Humintech products. O/C ratio, which shows the quantity of oxygen-containing functional groups and the level of humification in a sample, has the highest values in the samples of Lignohumate and Jin Tai products. Ash content is expressed as percentage in a sample that consists of inorganic substances. The purest HS samples with the lowest ash content are those of vermicompost (0.39%), Jin Tai (0.79%) and low moor peat (0.89%). These samples predominantly consist of organic matter. The highest ash content is presented in the Humintech (18.69%) and Lignohumate (35.23%) products. These samples have high content of inorganic compounds.

Analysis of metal content was done to further characterise the studied HS (Table 3). Some of these elements are plant nutrients and are vital for plant growth and development (K, Ca, Mg, P, S, Fe, Zn), while others have been included to determine the degree of contamination (if any) in samples (Na, Al, Pb). All samples have high potassium content, especially the raised bog peat sample and Lignohumate and Jin Tai products. This could be explained by the use of potassium hydroxide extracts. Calcium content in samples has a wide range of variation, from almost none (less than 0.5 μg g^{-1}) in vermicompost HS to 19.85 μg g^{-1} in Jin Tai FA. Magnesium content is high in Jin Tai FA (6750 μg g^{-1}),

which is at least 15 times more than that in the rest of the samples. Phosphorus content is extremely high (126,574 μg g^{-1}) in raised bog peat, whereas Jin Tai FA contains less than 2 μg g^{-1}. Elevated content of sulphur is detected in the Lignohumate (52,850 μg g^{-1}) and Jin Tai (11,370 μg g^{-1}) samples. The samples containing the least amounts of iron, such as those of raised bog peat and Lignohumate product, also have the smallest aluminium concentrations, while the samples containing the highest amounts of iron, such as those of Humintech, Humic Growth Solutions and low moor peat, also have the highest aluminium content. The Lignohumate product has a very high concentration of zinc (862 μg g^{-1}), while the rest of the samples have an average of 9.6 μg g^{-1} of zinc. Sodium concentrations are extremely high in the Lignohumate (43.57 μg g^{-1}) and Jin Tai (21.50 μg g^{-1}) products. The highest concentration of lead (37.9 μg g^{-1}) is detected in the low moor peat sample, while the rest of the samples have concentrations lower than 5 μg g^{-1}.

Table 2. Elemental composition and ash content in the samples. (See Table 1 for sample name abbreviation definitions.)

Sample	N, %	C, %	H, %	O, %	Ash Content, %	H/C	O/C
RBP	0.83	42.71	5.65	50.3	3.61	1.60	0.84
LMP	2.54	55.46	5.92	36.08	0.89	1.27	0.49
VC	3.69	50.64	4.91	40.76	0.39	1.16	0.60
SC	3.94	50.00	4.77	41.29	8.65	1.14	0.62
HT	1.01	47.74	3.28	47.97	18.69	0.82	0.75
HGS	1.50	52.67	3.35	42.48	5.77	0.76	0.61
LH	0.44	34.36	3.23	61.97	35.23	1.12	1.35
JT	3.23	31.71	4.03	61.03	0.79	1.51	1.44

Table 3. Minor and trace elements in the studied humic matter samples (μg g^{-1}). (See Table 1 for sample name abbreviation definitions.)

Sample	$W_{FA, TOC}$, %	$W_{HA, TOC}$, %
RBP	57.2	42.8
LMP	22.7	77.3
VC	7.4	92.6
SC	10.9	89.1
HT	46.1	53.9
HGS	78.9	21.1
LH	60.2	39.8
JT	89.3	10.7

Total organic carbon content in the samples varies from 31.1 mg g^{-1} (Jin Tai FA) to 188.2 mg g^{-1} (Humintech product). The highest content of HA is detected in the vermicompost (92.6%) and soil compost (89.1%) samples, which are almost pure HA. The Jin Tai-produced FA product contains only 89.3% of FA. As seen in Table 4, commercial products obtained from lignite or leonardite generally have a higher FA content, while HS obtained from peat and compost in a laboratory mostly have a higher HA content. At the same time, the two samples (raised bog peat and the Humintech product) contain equal amounts of both FA and HA.

Table 4. Composition of HS in the studied samples (W = mass fraction). (See Table 1 for sample name abbreviation definitions.)

Sample	K, μg g^{-1}	Ca, μg g^{-1}	Mg, μg g^{-1}	P, μg g^{-1}	S, μg g^{-1}	Fe, μg g^{-1}	Zn, μg g^{-1}	Na, μg g^{-1}	Al, μg g^{-1}	Pb, μg g^{-1}
RBP	246.97	290	56	126.57	297	29	3.7	1.31	51	<1.1
LMP	3.54	229	20	2.07	2.04	3.89	26	33	3.46	37.9
VC	1.45	<0.50	28	294	5.26	489	6.5	<11	729	<1
SC	2.66	215	126	641	5.69	1.04	13	16	1.31	2.4
HT	2.67	197	448	107	3.76	7.44	13	78	31.55	<1
HGS	8.50	1.816	210	16	4.66	3.50	4.4	203	3.46	4.2
LH	102.20	1.10	131	561	52.85	66	862	43.57	10	<0.9
JT	27.22	19.85	6750	<2	11.37	792	1	21.50	882	1

3.2. Germination Tests in Presence of HS

Shoot length tests of white mustard without added nutrients (Figure 1) show significant increases for some products (LMP, SC, RBP, VC), while the values of root length have high variability. Statistically significant shoot elongation occurs in the treatments with RBP at 250 to 1500 mg L^{-1}, VC and LMP at all concentrations, SC at 250 to 1500 mg L^{-1}, HGS at 250, 500 and 1500 mg L^{-1}, JT at 1000 mg L^{-1} and LH at 1000 mg L^{-1} concentrations, while treatments with HGS at 50 mg L^{-1} and JT at 500 mg L^{-1} concentrations lead to significant decreases. The root length shows significant increases only in the treatments with LMP at concentrations of 50 and 250 mg L^{-1} and with HGS at 500 mg L^{-1}. Most treatments show increase in measurements, although some concentrations have lower values than the control. When comparing the tests with and without added nutrients, the latter show a more enhanced growth-stimulating activity and higher results, especially for shoot length, while data on the tests with added nutrients is more fluctuating and shows greater decreases in values.

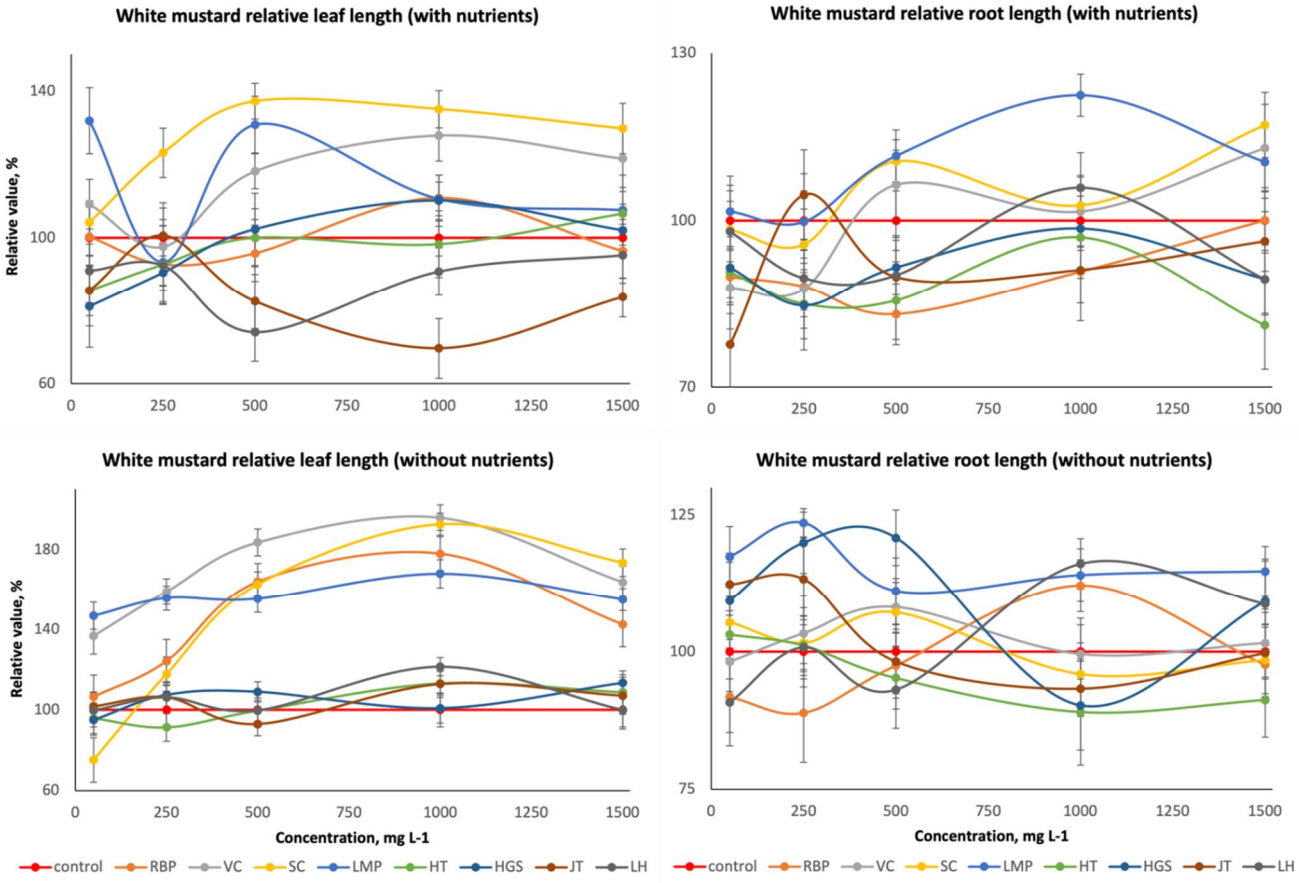

Figure 1. Relative changes in white mustard (*Sinapis alba*) shoot and root length over a concentration range of potassium humate solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate HS.

After measuring the length of shoots and roots, the samples were dried and the dry weight was obtained. The results were calculated relative to untreated control samples. Germination tests with white mustard seeds (Figure 2) show some stimulating effect, mostly for root weight, although some of the obtained results show decreases compared with control values. Shoot weight with added nutrients is significantly lower in the treatment with SC at 250 and 1000 mg L^{-1} concentrations, while the results are significantly higher in the treatment with LH at 1000 and 1500 mg L^{-1} concentrations. Significantly lower results

for root weight are obtained from treatment with VC at all concentrations, as these values show a considerable reduction in sample weight, as well as from treatments with RBP and HT at a 1500 mg L^{-1} concentration. Significant increases in root weight occur in treatments with SC at 250 mg L^{-1}, LMP at 250 and 1500 mg L^{-1}, HT at 50 mg L^{-1}, JT at 250, 500 and 1500 mg L^{-1} and LH at 50, 500, 1000 and 1500 mg L^{-1} concentrations.

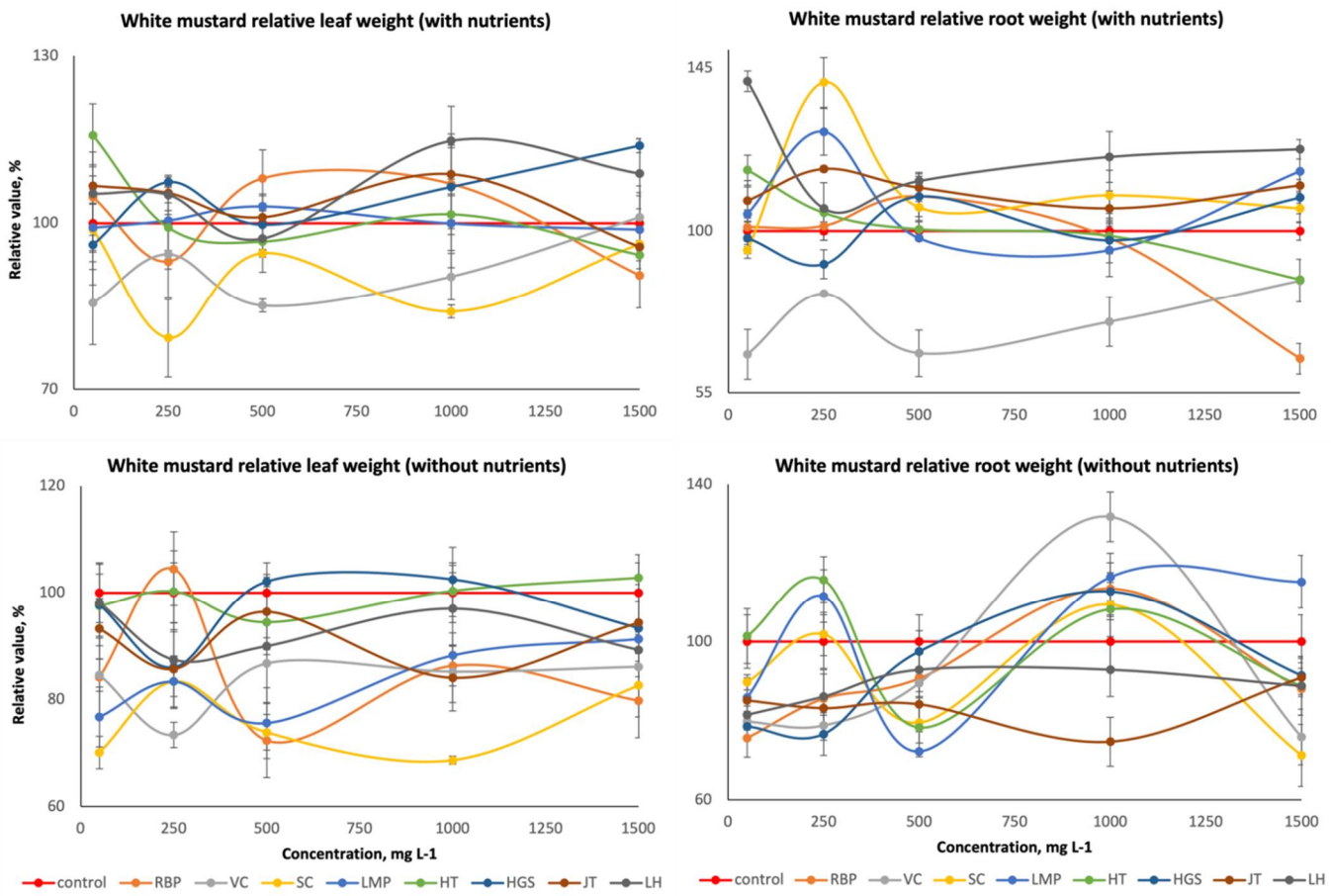

Figure 2. Relative changes in white mustard (*Sinapis alba*) shoot and root weight over a concentration range of potassium humate solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate HS.

White mustard tests without added nutrients, however, show even more significant reductions in weight, especially for shoots. Significant reductions are obtained in the treatments with RBP at 500 mg L^{-1}, VC at 250 mg L^{-1} and SC at 50, 500 and 1000 mg L^{-1} concentrations. For root weight, there are significant decreases in the treatments with RBP at 50 mg L^{-1}, VC and SC at 1500 mg L^{-1}, LMP at 500 mg L^{-1}, HGS at 250 mg L^{-1} and JT at 1000 mg L^{-1} concentrations; at the same time, a significant increase in the results occurs only in the treatment with VC at a 1000 mg L^{-1} concentration. When comparing the tests with and without added nutrients, it is evident that the former shows a greater growth-stimulating activity, although differences among the treatments occur. That is to say, the treatments with VC have a significant positive impact on root weight with the use of HS alone, while all of the obtained results are negative in the tests with nutrients added to the solution. In addition, the treatment with LH demonstrates the stimulating activity with nutrients present, and the impact is negative only with the use of HS alone. Comparing the effects on changes in the length and weight of samples, it can be observed that increases in the length of shoots are proportional to decreases in the dry weight values.

In the tests with watercress (*Lepidium sativum*) seeds (Figure 3), an increase in shoot and root length is detected in all tested solutions in at least some of the concentrations used. Upon examining test results for the samples with added mineral nutrients, the values of shoot length show an increase in case of all tested products in all concentrations, except for the treatment with RBP at a concentration of 50 mg L^{-1}. Statistically significant increases are found in the treatments with RBP at 1500 mg L^{-1}, VC at 250 and 1000 mg L^{-1}, SC at 500 to 1500 mg L^{-1}, LMP at 250 mg L^{-1}, HGS at 1000 and 1500 mg L^{-1} and JT at 1500 mg L^{-1} concentrations. Root length shows the optimal values between the concentrations of 500 and 1000 mg L^{-1}, where all treatments yield higher results. Significant increases are also found in the treatment with RBP at 1000 and 1500 mg L^{-1} concentrations, while the treatments with LMP and HT show a significant decrease at a 1500 mg L^{-1} concentration.

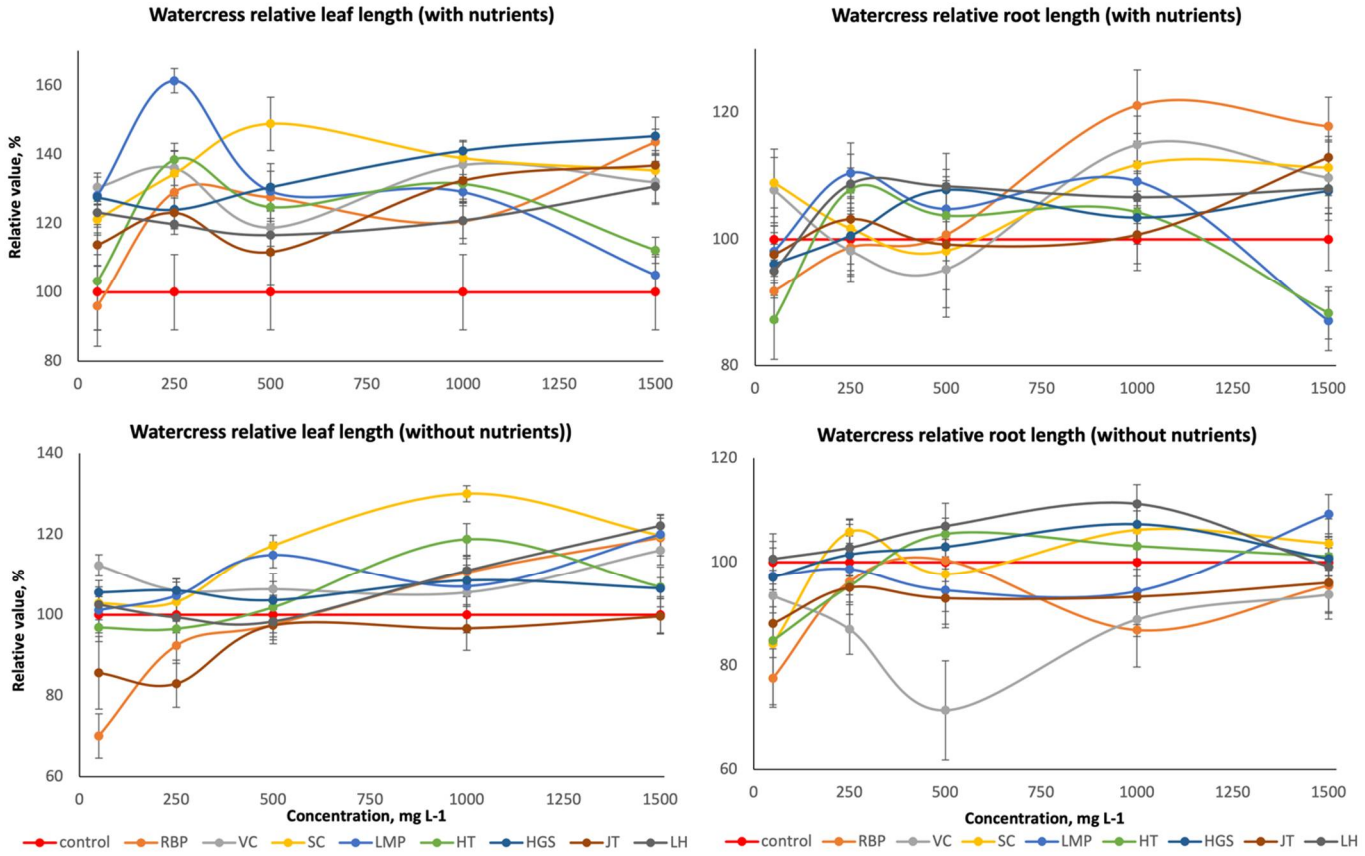

Figure 3. Relative changes in watercress (*Lepidium sativum*) shoot and root length over a concentration range of potassium humate solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate HS.

The tests without added nutrients show an increase in shoot length results in the majority of treatments, especially at highest concentrations; still, some treatments also show decreasing results. Significantly lower values of shoot length are obtained in the treatments with RBP at 50 mg L^{-1} and JT at 50 and 500 mg L^{-1} concentrations. Significant increases are observed in the treatments with RBP at 1500 mg L^{-1}, VC at 50, 250 and 1500 mg L^{-1}, SC at 500 to 1500 mg L^{-1}, LMP at 500 and 1500 mg L^{-1} and LH at 1000 and 1500 mg L^{-1} concentrations. Root length variations demonstrate significantly lower values relative to the control in the treatments with SC and HT at 50 mg L^{-1}, RBP at 1000 mg L^{-1} and VC at 1000 mg L^{-1} concentrations, whereas a significant increase in the results is seen in the treatment with LH at a 1000 mg L^{-1} concentration. Unlike the tests with white mustard, watercress tests show greater increases for both root and shoot lengths in the

samples with added nutrients, while the tests with HS alone have greater decreases in results, especially for root length.

Results for the dry weight of watercress plants (Figure 4) exhibit a stimulating activity in all of the treatments in at least some concentrations, except for shoot weight. In the tests with added nutrient solution, significant decrease in shoot weight takes place in the treatments with VC at 1000 mg L^{-1}, HT at 1500 mg L^{-1}, HGS at 50 and 500 mg L^{-1}, JT at 50, 1000 and 1500 mg L^{-1} and LH at 50 and 1000 mg L^{-1} concentrations. One case of a significant increase is presented in the treatment with HT at a 250 mg L^{-1} concentration. These results are opposite of the increases in the length of shoots found in the watercress tests with added nutrients. Both positive and negative results are obtained for root weight. Significantly decreased values are obtained in the treatments with JT at 500 mg L^{-1} and LH at 50, 250 and 1000 mg L^{-1} concentrations, while significant increases in root weight are found in the treatments with RBP at 50, 1000 and 1500 mg L^{-1}, SC at 50 mg L^{-1}, LMP at 1000 mg L^{-1} and HGS at 50 mg L^{-1} concentrations.

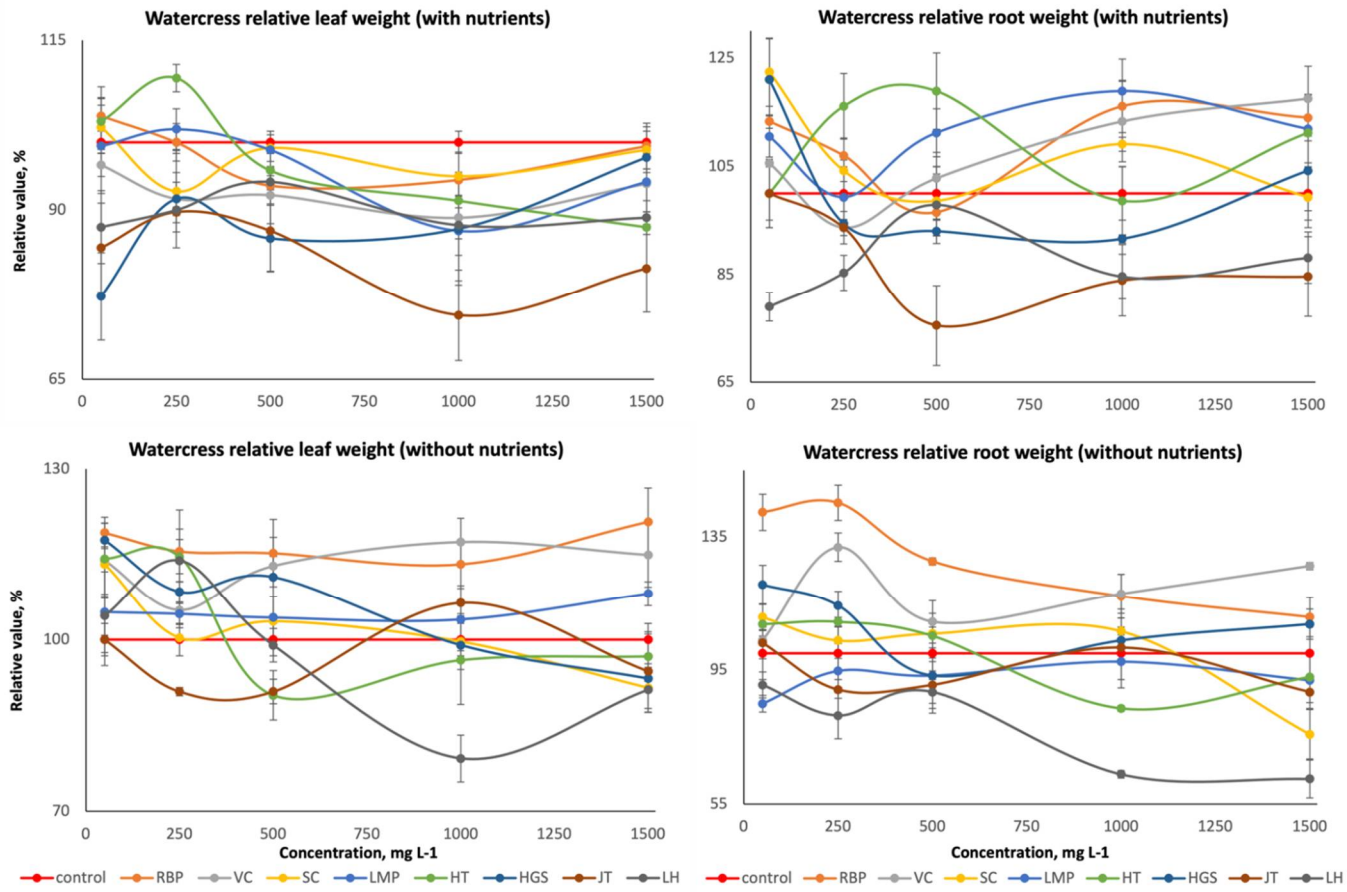

Figure 4. Relative changes in watercress (*Lepidium sativum*) shoot and root weight over a concentration range of potassium humate solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate HS.

Watercress tests without added nutrients show increase in results for some of the treatments used for both shoots and roots. Treatment with LH at a 1000 mg L^{-1} concentration produced one case of significant reduction in shoot weight. In contrast to that, significant increases in results are found in the treatments with RBP in nearly all concentrations (except for 1000 mg L^{-1}), VC at 50, 1000 and 1500 mg L^{-1}, SC at 50 mg L^{-1}, HGS at 50 and 500 mg L^{-1} and LH at 250 mg L^{-1} concentrations. Dependence of the optimum results on concentration is apparently associated with the treatment applied: two peaks of effectivity

can be observed at the concentrations of 250 and 1000 mg L^{-1}. Results for root weight show a significant decrease in results in case of the treatment with SC at 1500 mg L^{-1} and LH at 1000 and 1500 mg L^{-1} concentrations. Significant increases are obtained in the treatments with RBP at 50 to 500 mg L^{-1} and VC at 250 and 1500 mg L^{-1} concentrations.

When looking at changes in both length and weight in the watercress seed tests, the optimum results for both values occur at a concentration range between 50 and 250 mg L^{-1}, although some of the treatments also show significant increases at higher concentrations.

Germination test results (Figure 5) for winter wheat (*Triticum aestivum*) with added nutrient solution show an increase in shoot length in all treatments in most of the concentrations used. For root elongation, in turn, some treatments are more efficient. Treatment with RBP shows a significant increase in shoot length at 1500 mg L^{-1}; treatment with HT—a significant increase at 500 mg L^{-1} and a significant decrease at 1000 mg L^{-1}; and treatment with LH—a significant increase at 1000 mg L^{-1}. Root length changes show significantly decreasing results in the treatments with RBP at 250 and 1000 mg L^{-1} and LH at 50 and 250 mg L^{-1} concentrations, while significantly increasing changes transpire in the treatments with SC at 50 to 500 mg L^{-1}, LMP at 1000 mg L^{-1} and LH at 1000 mg L^{-1} concentrations.

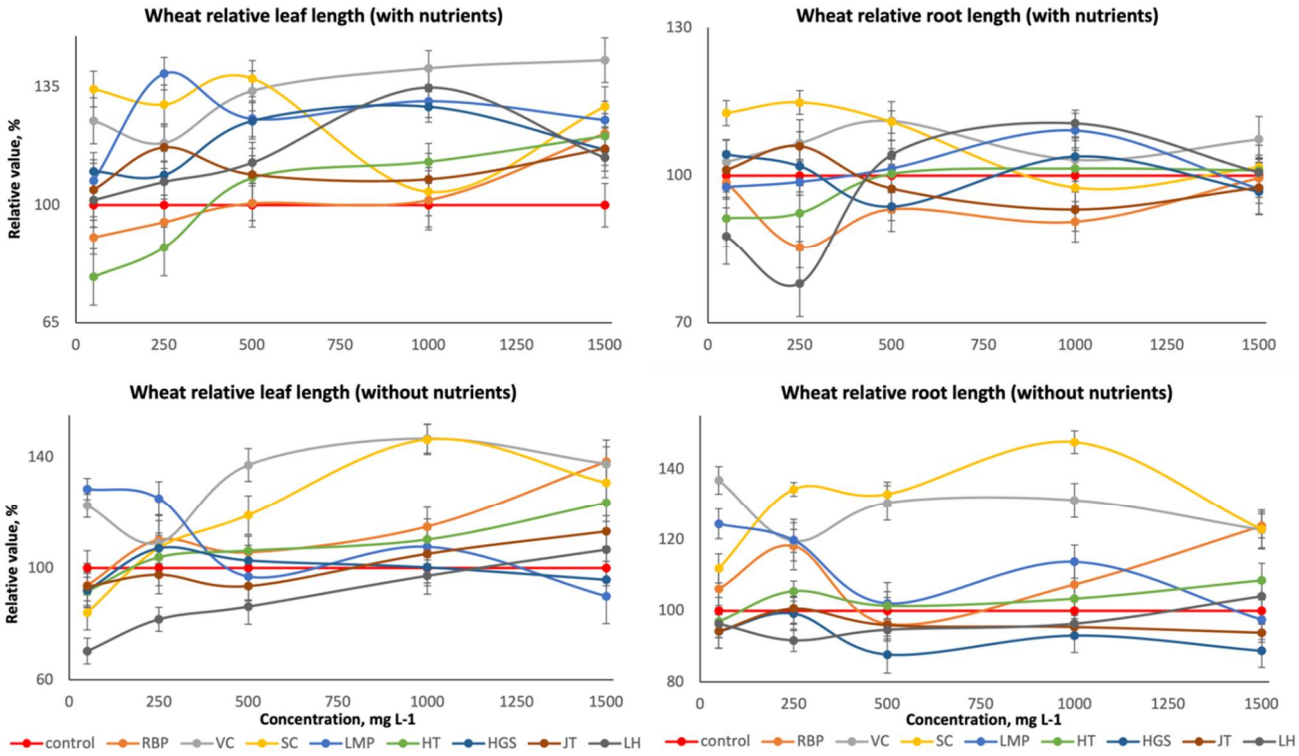

Figure 5. Relative changes in winter wheat (*Triticum aestivum*) shoot and root length over a concentration range of HS solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate HS.

Wheat germination tests without added nutrients produce a wider range of values than tests with nutrients. Significantly lower results are obtained in the treatments with SC and LH at a 50 mg L^{-1} concentration, and significantly higher results—in the treatments with RBP at 1500 mg L^{-1}, VC at 500, 1000 and 1500 mg L^{-1}, SC at 1000 and 1500 mg L^{-1}, LMP at 50 mg L^{-1}, HT at 500 to 1500 mg L^{-1}, HGS at 250 mg L^{-1}, JT at 1000 and 1500 mg L^{-1} and LH at 1500 mg L^{-1} concentrations. Root elongation changes demonstrate a significant negative impact in samples with HGS treatment at 500 and 1500 mg L^{-1} concentrations and a significant increase—in the treatments with VC at 50, 500 and 1000 mg L^{-1}, SC at 250 to 1500 mg L^{-1} and HT at 250 and 1500 mg L^{-1} concentrations.

When using HS alone, treatments with VC and SC have significantly higher rates of elongation than most of the other treatments. However, when nutrients are added, these treatments lose effectivity at increased concentrations, while other treatments show better results.

The wheat test results for changes in shoot and root weight (Figure 6) show a positive impact on the observed parameters, except in the results of root weight without added nutrients, where decreases are obtained. Tests with added nutrients, however, produced great results in shoot elongation relative to the control—as high as 207% in the treatment with HGS at a 500 mg L^{-1} concentration. All the obtained increases in shoot weight are statistically significant, except for treatments with LMP at 50 and 1500 mg L^{-1}, JT at 1000 mg L^{-1} and LH at 50 mg L^{-1} concentrations. There are practically no decreases in the dry weight (except for LH at 50 mg L^{-1}) of wheat shoots. The roots, however, do show some decreasing results, but these values are not significant. Increases in root weight are significant in the treatments with RBP at 50 and 1000 mg L^{-1}, VC at 250 mg L^{-1}, HT at 50 and 250 mg L^{-1}, HGS at 250 and 500 mg L^{-1}, JT at 1500 mg L^{-1} and LH at 250, 1000 and 1500 mg L^{-1} concentrations. Although the increases in root weight are not as high as in shoots, the treatments with HGS and HT still reach as much as an approximately 50% increase.

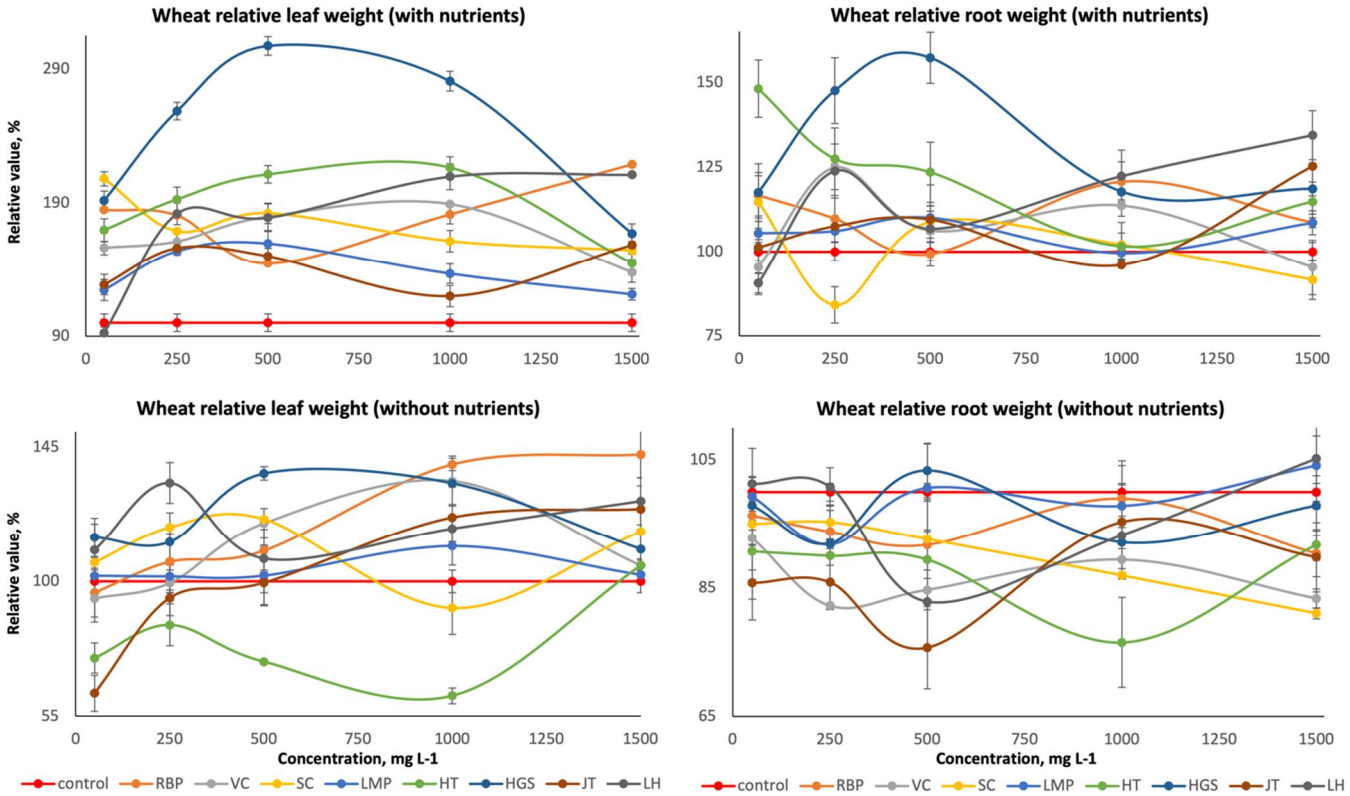

Figure 6. Relative changes in winter wheat (*Triticum aestivum*) shoot and root weight over a concentration range of HS solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate HS.

Wheat tests without added nutrients show increases in shoot weights in all treatments, except for HT, which has a significant negative impact at all concentrations except 1500 mg L^{-1}, and also JT at a 50 mg L^{-1} concentration. All other treatments show a mostly positive impact on shoot weight. However, significantly higher values are obtained in the treatments with RBP at 1000 and 1500 mg L^{-1}, VC at 500 and 1000 mg L^{-1}, SC at 250 and 500 mg L^{-1}, HGS at 500 and 1000 mg L^{-1}, JT at 1000 and 1500 mg L^{-1} and LH at

250 and 1500 mg L^{-1} concentrations. As already mentioned, all the treatments without added nutrients show negative influence on root weight, and the values are significant in the treatments with RBP at 1500 mg L^{-1}, VC at all concentrations except 50 mg L^{-1}, SC at 1000 and 1500 mg L^{-1}, HT 500 and 1000 mg L^{-1}, JT at 50, 250 and 500 mg L^{-1} and LH at 500 mg L^{-1} concentrations. Tests with added nutrients show significantly higher results than tests with HS alone. When looking at changes in both length and weight, we found that HS with nutrients have a stimulating impact on early wheat seedling development, whereas HS used alone may cause reductions in root weight, although increases in length are also observed.

These results are in agreement with reports of other authors who also have found that HS from sources like peat [11,16], composts [17] and leonardite [12] can have a stimulating effect on plant growth. A review by Rose et al. (2014), analysing and comparing response ratios of plants to tests with differently sourced HS, found that HS from composts and soil had higher results in terms of a positive impact on shoot length than peat. Moreover, the latter was also less potent than brown-coal-derived HS [18]. Conversely, when looking at the impact on root length, soil-based HS had a sudden decrease in effectiveness, although still being higher than peat and brown coal-based HS. Notably, the tests performed in soil gave the best results, not the tests in hydroponic or hybrid systems.

In order to eliminate the species-specific variability in the assessed effects of various HS on seed germination, an aggregate stimulating and inhibiting activity of different treatments was calculated (Figure 7) for all concentrations using all measurements. The optimum results for plant stimulation occur between the concentrations of 500 and 1000 mg L^{-1}, and the greatest reductions for most treatments occur between the concentrations of 50 and 500 mg L^{-1}. In the samples with added nutrients, the stimulating activity reaches as high as 339% in the treatment with HGS at 500 mg L^{-1}, while the lowest values of 64% and 71% belong to JT and LH at a 50 mg L^{-1} concentration, respectively. From the tests without added nutrients, the maximum stimulation takes place in the naturally-occurring treatments—i.e., VC, SC, RBP and LMP—at the concentrations of 1000 and 250 mg L^{-1}. The effect of commercially available products ranged between a maximum stimulation of 92% in the treatment with HGS and a minimum of 0% in the treatment with JT at 500 mg L^{-1}.

Looking at the aggregate decreases in the results of tests with added nutrients, the scores of JT at higher concentrations stand out as being significantly lower, reaching the lowest point at 1000 mg L^{-1} with −91% changes, whereas all other treatments have minimal decreases in results (e.g., −16% in the treatment with HT). The smallest percentage of decrease (−3%) is seen in the treatment with LMP at 500 mg L^{-1}. At smaller concentrations, on the contrary, the treatment with JT showed minimal decreases (−16%), while all treatments except JT and LMP display increased rates of inhibition. The aggregate relative decreases in tests without added nutrients show a different tendency in the treatment with JT, where the maximum inhibition (−112%) is attained with lower concentrations. In addition, the inhibition subsided with increasing concentrations. The same was the case in the treatment with RBP, reaching the maximum inhibition among all treatments, which is −114% at a 50 mg L^{-1} concentration. Treatment with VC also had a peak negative effect at 250 mg L^{-1}. On the other hand, other treatments, like SC and HGS with −21%, showed minimal decreases in results at 250 mg L^{-1} and 500 mg L^{-1} concentrations, respectively. Treatments with RBP (−27%), VC (−37%) and LMP (−22%) show minimal decreases at a concentration of 1000 mg L^{-1}. Treatment with LH caused about −72% changes for all concentrations used. Comparing both stimulating and inhibiting effects of treatments with and without nutrients, for most treatments the addition of nutrients increase the efficiency, as the total stimulation rates are higher in these tests. Moreover, the maximum efficiency rates begin to appear at lower product concentrations.

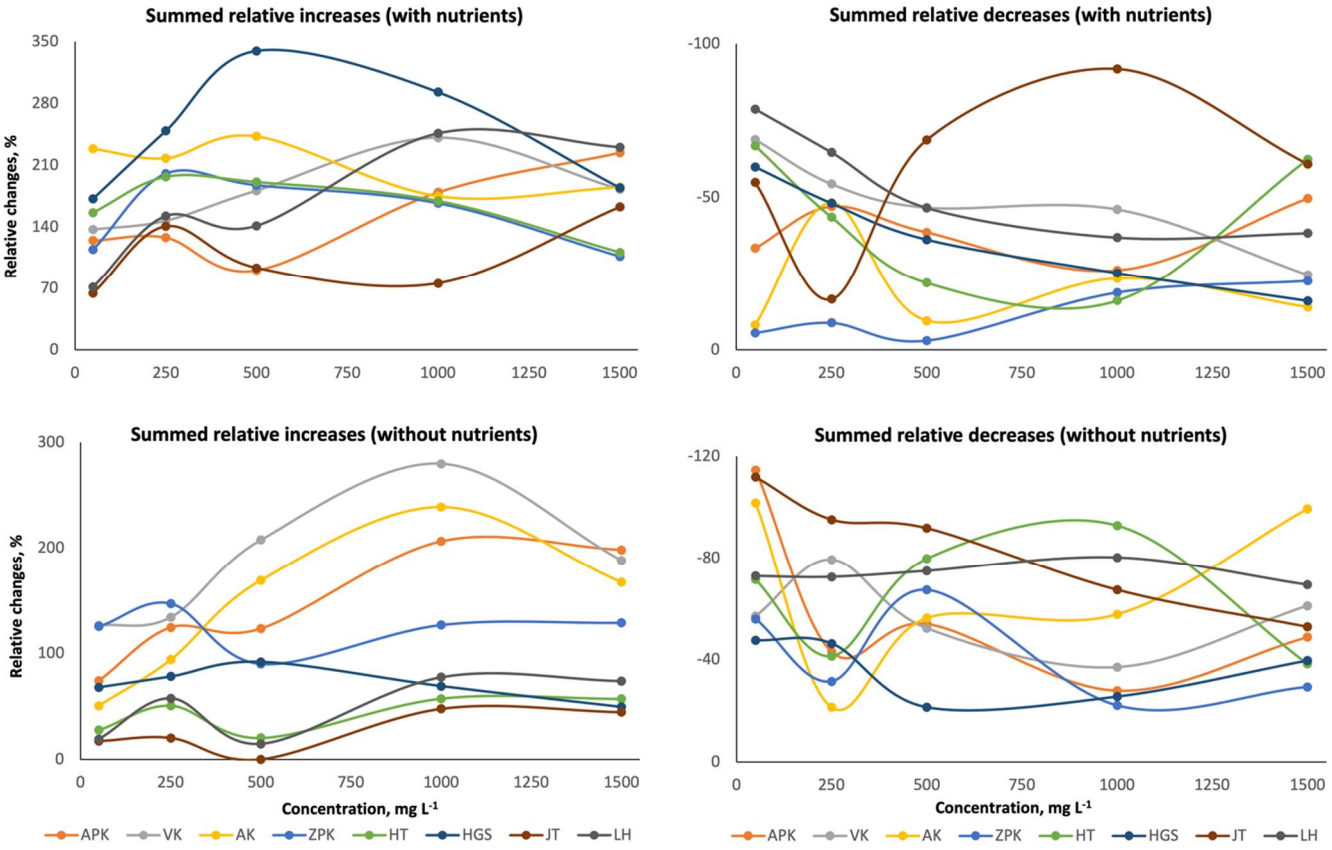

Figure 7. Aggregate relative increases and decreases in results yielded by the tested products by all parameters over a concentration range of HS solutions of different origins, with and without added nutrient solution. RBP = raised bog peat, VC = vermicompost, SC = soil compost, LMP = low moor peat, HT = Humin Tech potassium humate, HGS = Humic Growth Solutions potassium humate, JT = Jin Tai FA, LH = Lignohumate humic acid.

3.3. Activity-Properties Relationships

In order to evaluate the relationships that could influence the effectivity of HS, the correlation coefficients were calculated between positive and negative variations caused by the various HS samples for all parameters at two of the concentrations used in tests (250 mg L^{-1} and 1000 mg L^{-1}), with respect to control, and the HS properties examined. For germination tests at 250 mg L^{-1} concentration negative correlation ($p < 0.001$) was found for increases in results (without added nutrients) and Ca and Mg content, oxygen (%), O/C values and FA content, but positive correlation between carbon (%), hydrogen (%) and HA content. Decreases in results (without nutrients) showed negative correlation with Ca and Mg content and O/C values, but positive correlation with carbon (%). The levels of increases obtained (with added nutrients) showed positive correlation with carbon content but decreases showed correlation with nitrogen (%).

At the concentration of 500 mg L^{-1} negative correlation between summed stimulation of samples (without nutrients) and fulvic acid content. The summed inhibiting activity levels showed negative correlation with ash content. In the presence of nutrients, the values of enhancement showed negative correlations with Ca and Mg content and H/C values, but inhibiting activity also showed negative correlations with Ca and Mg content and H/C values, but a positive correlation was found for carbon (%).

4. Conclusions

All of the tested products demonstrated at least some growth-stimulating activity at certain concentrations at least for some of the tested species, showing sensitivity of different plants to different HS-containing products in the concentrations used. The stimulating

effects did not increase linearly but rather were fluctuating. As a result, for some samples, small concentrations of HS proved to be more effective, while others needed a higher dose. When evaluating the application potential of a product for agronomical purposes, it is important to consider the composition of the product, since excessive impurities or contamination can modify the activity of the product and thereby have a reverse effect on plant stimulation.

Author Contributions: Methodology, supervision, project administration, funding acquisition, writing, M.K.; germination test, writing, S.G.; conceptualization, editing, V.O.; germination test experiment design and validation, writing, G.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Latvia Science Council project "Properties and structure of peat humic substances and possibilities of their modification" lzp-2018/1-0009.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tan, K.H. *Humic Matter in Soil and the Environment: Principles and Controversies*, 2nd ed.; Taylor & Francis Group: Boca Raton, FL, USA, 2014.
2. Huang, P.M.; Hardie, A.G. Formation Mechanisms of Humic Substances in the Environment. In *Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems*; Senesi, N., Xing, B., Huang, P.M., Eds.; Wiley: New York, NY, USA, 2009; pp. 41–109.
3. Stevenson, F.J.; Olsen, R.A. A simplified representation of the chemical nature and reactions of soil humus. *J. Agron. Educ.* **1989**, *18*, 84–88. [CrossRef]
4. Zech, W.; Senesi, N.; Guggenberger, G.; Kaiser, K.; Lehmann, J.; Miano, T.; Miltner, A.; Schroth, G. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma* **1997**, *79*, 117–161. [CrossRef]
5. Zandonadi, D.B.; Santos, M.P.; Busato, J.G.; Peres, L.E.P.; Façanha, A.R. Plant physiology as affected by humified organic matter. *Theor. Exp. Plant Physiol.* **2013**, *25*, 13–25. [CrossRef]
6. Canellas, L.P.; Olivares, F.L.; Aguiar, N.O.; Jones, D.L.; Nebbioso, A.; Mazzei, P.; Piccolo, A. Humic and fulvic acids as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 15–27. [CrossRef]
7. Trevisan, S.; Francioso, O.; Quaggiotti, S.; Nardi, S. Humic substances biological activity at the plant-soil interface. *Plant Signal. Behav.* **2010**, *5*, 635–643. [CrossRef] [PubMed]
8. Senesi, N.; Loffredo, E. Metal Ion Complexation by Soil Humic Substances. *Chem. Process. Soils* **2018**, *8*, 563–617. [CrossRef]
9. Ricci, M.; Tilbury, L.; Daridon, B.; Sukalac, K. General Principles to Justify Plant Biostimulant Claims. *Front. Plant Sci.* **2019**, *10*, 1–8. [CrossRef] [PubMed]
10. Marenych, M.M.; Hanhur, V.V.; Len, O.I.; Hangur, Y.M.; Zhornyk, I.I.; Kalinichenko, A.V. The Efficiency of Humic Growth Stimulators in Pre-Sowing Seed Treatment and Foliar Additional Fertilizing of Sown Areas of Grain and Industrial Crops. *Agron. Res.* **2019**, *17*, 194–205. [CrossRef]
11. Rose, M.T.; Patti, A.F.; Little, K.R.; Brown, A.L.; Jackson, W.R.; Cavagnaro, T.R. A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. *Adv. Agron.* **2014**, *124*, 37–89. [CrossRef]
12. Conselvan, G.B.; Pizzeghello, D.; Francioso, O.; Di Foggia, M.; Nardi, S.; Carletti, P. Biostimulant activity of humic substances extracted from leonardites. *Plant Soil* **2017**, *420*, 119–134. [CrossRef]
13. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
14. Chen, Y.; Senesi, N.; Schnitzer, M. Information Provided on Humic Substances by E4/E6 Ratios. *Soil Sci. Soc. Am. J.* **1977**, *41*, 352–358. [CrossRef]
15. Ali, H.; Akbar, Y.; Razaq, A.; Muhammad, D. Effect of Humic Acid on Root Elongation and Percent Seed Germination of Wheat Seeds. *Int. J. Agric. Crop Sci.* **2014**, *7*, 196–201.
16. Vieira, J.H.; dos Santos Silva, L.K.; de Oliveira, L.C.; do Carmo, J.B.; Rosa, L.M.T.; Botero, W.G. Evaluation of Germination of Chilli Pepper Using Humic Substances And Humic Acids. *IOSR J. Environ. Sci. Toxicol. Food Technol.* **2018**, *12*, 33–39.
17. Traversa, A.; Loffredo, E.; Gattullo, C.E.; Palazzo, A.J.; Bashore, T.L.; Senesi, N. Comparative evaluation of compost humic acids and their effects on the germination of switchgrass (*Panicum vigatum* L.). *J. Soils Sediments* **2013**, *14*, 432–440. [CrossRef]
18. Da Silva, R.R.; Botero, W.G.; de Lima, R.S.N.; Leite, J.; dos Santos, C.L.; de Oliveira, L.C. Evaluation of Root Growth of Coriander (*Coriandrum sativum* L.) by Humic Substances. *IOSR J. Environ. Sci. Toxicol. Food Technol.* **2019**, *13*, 15–19.