

University of Latvia biostimulant report

Laboratory characterisation of one peat-and-mushroom sample and seven-day seedling observations in oats and peas.

Full text and tables in the selected language. Automatic translation with terminology and numerical checks; not a certified translation. Author statements, including disputed claims, are retained. Editorial notes are separate.

Editorial note

The report is not a field-yield trial; the supplied protocol does not establish a replicated spinach/nitrate result. Values below a laboratory detection or quantification limit do not establish that a substance is absent. The report's wording about heavy metals must be read together with the measured values, units and limits in its tables.

[NanoImport — Editorial note ↗](#)

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Report

Chemical evaluation of peat and mushroom biostimulant LIAA Voucher No.

9.2-29-V-2025/64

Riga, 2026

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The tasks of the job:

To carry out a comprehensive physico-chemical and agronomic characterization of peat and fungi biostimulant according to the order of JIKO Ent Ltd. 9.2-29-V-2025/64. The characteristics include:

1. Germination tests on three (3) crops (peas, durum wheat, oats) assessing the efficacy of biostimulant at different concentrations;
2. To evaluate the effect of biostimulant on germination by seed pretreatment seeds;
3. Humic and fulvic acid content of the product by spectrophotometric and TOC methods;
4. Protein, carbohydrate and chitin contents, which characterize the profile of bioactive components of the biostimulant;
5. Quantitative microelement and macroelement content, using ICP-OES and the Kjeldahl method;
6. Physicochemical characterisation of humic acids using FTIR, UV-Vis and FLR 3D-EEM spectroscopy.

Materials and methods:

Sample. In August 2025, the biostimulant was delivered in a 5 L can, designated as SK-04-S01-N.

Chitin secretion. 100 mL of biostimulant centrifuged for 30 min at 4500 rpm. After centrifugation, the centrifuged solid residue was collected and resuspended in 0.1 M NaOH and stirred for 60 min. After stirring, the sample was centrifuged again at 4500 rpm for 30 minutes. The process was repeated 3 times until the presence of humus was no longer detected in the sample. The solid residue is washed with deionized water to pH<9. 1 M NaOH was added and heated at 70 °C for 2 hours while stirring and quaternised. The solid residue was rinsed with deionized water to a neutral pH medium. 0.5 M HCl was added and centrifuged again. The sample was rinsed to neutral pH with deionized water and 1% NaClO was added and stirred at room temperature for 60 min. The sample was then washed with 70% ethanol and dried in an oven at 40 °C for 3h.

Release of humic acids. 200 mL of biostimulant was filtered through filter paper. After filtration, the solution was centrifuged with a laboratory centrifuge at 4500 rpm. After centrifugation, the solution was acidified with HCl to pH<1. Sample filled and precipitated

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humic acids were rinsed with 0.1 M CaCl₂ solution. The samples were then rinsed with deionized water to neutral pH and dried in a drying chamber for 2 hours at 105 °C.

Quantitative determination of fulvic and humic acids. The biostimulant was centrifuged at 4500 rpm and the liquid fraction was collected. The sample was diluted 50 times and measured spectrophotometrically at 410 nm wavelength. The concentration was calculated from the calibration chart obtained from the extracted humic acids. After the extraction of humic acids, the fulvic acid solution was separately collected from the biostimulant, diluted 10 times and measured spectrophotometrically at 410 nm using the fulvic acid standard as a reference.

With a 1000 µL automatic pipette, 500 µL of biostimulant solution was quantitatively transferred to a 100 mL target flask and 500 µL of the separated fulvic acid solution was transferred to a separate 100 mL target flask and diluted to the mark with deionized water. The diluted solutions were analyzed with a total carbon analyzer (Shimadzu TOCV CSN) and the results were expressed as milligrams of organic carbon (TOC) per litre of biostimulant.

Molecular absorption spectra (UV-Vis) capture. 20 mg of the isolated and purified humic acid was weighed on analytical scales and quantitatively transferred into a 1 L measuring flask and dissolved in 0.5 M NaHCO₃ solution.

The solutions were measured in a 1 cm quartz cuvette with a UV-VIS spectrometer UV-1800 in the range of 220 to 700 nm with a step length of 1 nm. Absorption ratios at wavelengths 254 nm (E2) were used for characterization of humic acids; 365 nm (E3), 465 nm (E4) and 665 nm (E6) and expressed as E2/E3; E2/E4; E2/E6 and E4/E6.

Fourier transform infrared spectroscopy (FTIR). Before analysis, KBr was dried at 105 °C for 2 hours, then weighed 200 mg and added 2 mg of humic acid, and mixed. The mixture was compressed into a capsule and measured with an FTIR spectrometer (Shimadzu IR-Tracer 100). Measurements were made in the range from 4000 cm⁻¹ to 400 cm⁻¹ with a resolution of 8 cm⁻¹ and 16 scans were performed.

Fluorescence 3D induction/emission matrices (FLR 3D-EEM) were obtained using HORIBA Jobin Yvon Aqualog fluorescence spectrophotometer. The synthetic humic acids were prepared in 0.5 M NaHCO₃ medium at a concentration of 20 mg/L. The samples were measured using 1 cm quartz cuvettes without ground sides. Excitation wavelengths: 240-600 nm; emission wavelengths: 260-620 nm. Measuring step length – 3 nm. After the measurements were made, the first-order Rayleigh scattering was masked in the output data. The obtained fluorescence spectra data were processed, visualized

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and prepared for rendering using the RStudio (version 2024.12.1.563) environment and the R (version 4.5.0) language libraries readxl, dplyr, akima and ggplot2. The data were interpolated by linear two-dimensional method (akima::interp) depending on the excitation and emission wavelengths.

Analysis of total carbohydrates. For analysis, 1 mL of sample was transferred into a glass tube, 1 mL 5% phenol solution was added and incubated for 10 minutes. 4 mL of conc. H₂SO₄, incubated for 20 minutes and measured spectrophotometrically at 490 nm wavelength. Samples were measured in 3 repetitions using glucose as a standard.

Analysis of microelements. Approximately 5 mL of biostimulant was weighed in PTFE capsules, dried to dry and sampled and 10 mL of concentrated HNO₃ was added. The capsules were placed in pressure-resistant frames, sealed and wet mineralized using a microwave mineralization machine (Milestone Ethos EASY). The sample was heated to 200 °C for 15 minutes at a maximum power of 1800 W, held at this temperature for another 15 minutes and after mineralization cooled to room temperature. The sample was quantitatively transferred into a 50 mL polypropylene tube and diluted to 50 mL using deionized water (0.055 µS/cm). Samples prepared in 2 repetitions.

The sample was diluted another 10 times before analysis and analyzed with an inductively coupled plasma spectrometer equipped with optical emission detection (Thermo-Scientific iCAP 7200 series). ICP analysis parameters: radio frequency power – 1150 W; plasma gas flow (argon 5.0) – 12 L/min; sample sprayer gas flow: 0,5 L/min; supplementary gas (nitrogen 5.0) flow – 0.15 L/min; sample flow rate 1 mL/min. In total, 29 elements were identified: Al (396.152 nm, radial); As (189,042 nm, axial); B (249,773 nm, radial); Ba (455,403 nm, axial); Be (313,042 nm, radial); Ca (422,673 nm, radial); Cd (228,802 nm, axial); Co (228,616 nm, axial); Cr (267,716 nm, axial); Cu (324,754 nm, axial); Fe (240,488 nm, radial); K (766,490 nm, radial); Li (670,784 nm, radial); Mg (285,213 nm, radial); Mn (257,610 nm, radial); Mo (202.030 nm; axial); Na (589,592 nm, radial); Ni (231,604 nm, axial); P (177,495 nm, axial); Pb (220,353 nm, axial); S (180,731 nm, axial); Sb (206,833 nm, axial); Se (196,090 nm, axial); Si (251,611 nm, radial); Sr (407,771 nm, radial); Ti (334,941 nm, radial); Tl (190,856 nm, axial); V (292,402 nm, axial); Zn (213,856 nm, axial). Standardized reference materials are used to create calibration tables.

Determination of total nitrogen. Weighing 0.5 g of completely dried unfiltered biostimulant sample on analytical scales and transferred to a Kjeldal flask, 10 mL of conc. H₂SO₄ and

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in a hot Kjeldal mineralisation unit (Buchi SpeedDigester K-436 equipped with Buchi Scrubber K-415 acid neutralisation module), heated for 2 hours until the entire matrix has been demineralised. After mineralization, the sample was distilled with Buchi Kjelflex K-360 and the distillate was titrated with 0.05 M H₂SO₄.

Seeds to be tested. Durum wheat (*Triticum durum*) and oats (*Avena sativa*) were harvested in the Aknīste parish in late August 2025. Peas (*Pisum sativum*) were purchased in October 2025 in the retail store Depo, Riga.

Germination tests. Four biostimulant test concentrations were selected: 1:500; 1:350; 1:200 and 1:100, obtained by diluting the biostimulant 500, 350, 200 and 100 times, respectively. To assess the effect of seed pretreatment, seeds were treated with 1% NaOH and labelled with the relevant dilution and 'kod'. Seeds were stirred in NaOH solution for 2 minutes and rinsed with deionised water. Before placing them on germination plates, seeds were kept overnight in the respective biostimulant dilutions. Each germination plate received 10 mL of diluted biostimulant and 10 seeds. The plates were closed and placed in an incubator at 22 °C for 7 days, with 12 h light and 12 h darkness for all seed varieties. Deionised water served as the control.

Results:

For the evaluation of the biostimulant composition, the content of humic acids, fulvic acids, total protein, total carbohydrates, total nitrogen, total organic carbon in the biostimulant and the extracted fulvic acid solution were determined, and the results are shown in Table 1.

Table 1

Results of the overall indicators of the biostimulant

Table 1

Parameter	Result	Unit
Dry matter content	12,19 ± 0,17	%
Humic acids	624 ± 12	mg/L
Fulvic acids	712 ± 21	mg/L
Total protein	1,30 ± 0,07	%
Total carbohydrates	198 ± 6	mg/L
Total nitrogen	0,21 ± 0,04	%
TOC — biostimulant	1150 ± 40	mg/L

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Table 2

TOC — fulvic acid solution	790 ± 30	mg/L
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The results in Table 1 reflect the total composition of the biostimulant. A high dry matter content was observed, determined at $12,19 \pm 0,17$ %, mainly composed of colloidal particles in the solution. It should be noted that the product is predominantly composed of fulvic acids with a content of 712 ± 21 mg/L and humic acids with a content 624 ± 12 mg/L. Attention should be paid that the product composition contains a significant amount with various soluble by-products, so the humic acid content after organic carbon analysis is not unequivocal. However, the analysis of the solution separated from the humic acids shows a tendency that the removal of carbon containing humic acids from the product shows a loss of 360 mg/L C, which can be approximated to ~ 720 mg/L of humic acids, taking into account that the average carbon content in humic acids is 50 %, but the truth of such a conclusion must be critically evaluated, thus considering spectrophotometric analysis as the truest value.

By performing sequential chitin release purification from the biostimulant sample as described in the work, first by separating humus, then separated proteins, followed by demineralization process, then bleaching process of the material, where other organic pigments were removed and, finally, after lipophilic compounds were removed. removal step and drying, obtained gray-white chitin with a total content of ~ 235 mg/L. The purified chitin is shown in Figure 1.

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Original experimental graphics are preserved. Labels are translated in the accompanying text.

Figure 1. Chitin separated and purified from the biostimulant.

The biostimulant was analysed for microelements and macroelements using ICP-OES. In total, 29 elements were determined: Al; As; B; Ba; Be; Ca; Cd; Co; Cr; Cu; Fe; K; Li; Mg; Mn; Mo; Na; Ni; P; Pb; S; Sb; Se; Si; Sr; Ti; Tl; V; Zn. The results are shown in Table 2.

Table 2

Composition of micro- and macroelements of biostimulant

Table 3							
Al, µg/L	As, µg/L	B, µg/L	Ba, µg/L	Be, µg/L	Ca, µg/L	Cd, µg/L	Co, µg/L
143 ± 5	<1,77	1,057 ± 0,114	0,28 ± 0,09	<0,014	348 ± 12	<0,15	<0,25

Table 4						
Cr, µg/L	Cu, µg/L	Fe, µg/L	K, µg/L	Li, µg/L	Mg, µg/L	Mn, µg/L

0,462 ± 0,007	1,82 ± 0,16	36,3 ± 0,4	34955 ± 1880	<0,65	45,83 ± 0,02	0,57 ± 0,05
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Table 5

Mo, µg/L	Na, µg/L	Ni, µg/L	P, µg/L	Pb, µg/L	S, µg/L	Sb, µg/L
<0,80	150 ± 3	0,381 ± 0,008	17370 ± 740	<1,12	104 ± 5	<2,44

Table 6

Se, µg/L	Si, µg/L	Sr, µg/L	Ti, µg/L	Tl, µg/L	V, µg/L	Zn, µg/L
<2,00	23 ± 4	1,053 ± 0,013	0,57 ± 0,19	<2,89	<0,12	2,19 ± 0,13

According to the elemental analysis data (Table 2), potassium and phosphorus clearly dominate the biostimulant prototype (K 34955 ± 1880; P 17370 ± 740), at concentrations several orders of magnitude higher than other elements. Thus, the formulation is oriented toward a K/P-driven physiological effect: energy metabolism, root development, osmoregulation and stress tolerance. The next concentration level includes Ca 348 ± 12; Na 150 ± 3; Al 143 ± 5; S 104 ± 5, while Mg is relatively low (Mg 45.83 ± 0.02). Among microelements, Fe is most prominent (Fe 36.3 ± 0.4); the following are also present: Si 23 ± 4; Zn 2.19 ± 0.13; Cu 1.82 ± 0.16; B 1.057 ± 0.114; Sr 1.053 ± 0.013; Mn 0.57 ± 0.05. The remaining elements occur at low concentrations (Cr 0.462 ± 0.007; Ni 0.381 ± 0.008; Ba 0.28 ± 0.09; Ti 0.57 ± 0.19). Positively, several potentially toxic elements are below the detection limit (As < 1.77; Cd < 0.15; Pb < 1.12; V < 0.12; Co < 0.25; Mo < 0.80; Se < 2.00; Sb < 2.44; Tl < 2.89; Be < 0.014), which indicates the absence of heavy metals in the product.

Several spectroscopic analyses were used to characterize the humic acids of the biostimulant – FTIR spectroscopy, UV-Vis molecular absorption spectroscopy and fluorescence 3D-EEM spectroscopy. The results of the analyses are shown in Figures 2-4.

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Original experimental graphics are preserved. Labels are translated in the accompanying text.

Relative transmittance. Wavenumber (cm^{-1}). O-H; $\equiv\text{C-H}$; $=\text{C-H}$; C-H (aliphatic); CO_2 ; C=O; C=C; N-H bending; C-H bending; C-O/C-N; C-Hal / C-H (aromatic).

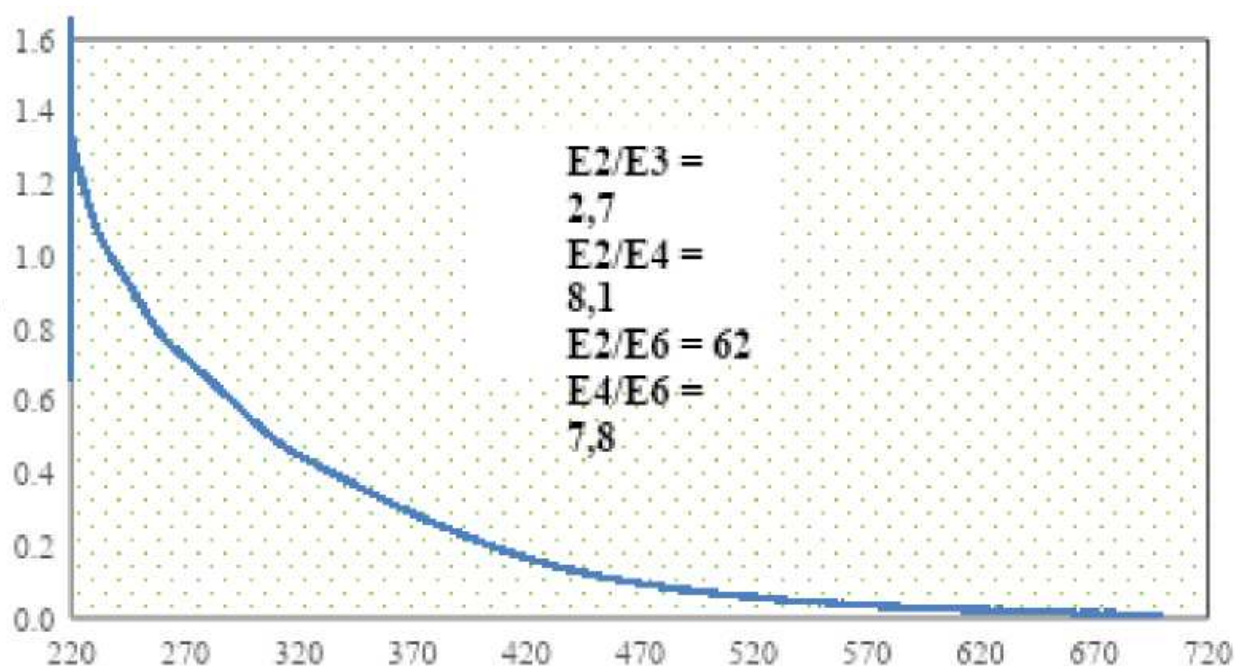
Figure 2. FTIR spectrum of humic acids released from biostimulant

The FTIR spectrum of natural peat humic acids shown in Figure 2 is typical of a heterogeneous humic acid matrix with many polar functional groups and an aromatic-aliphatic structure. The broad, pronounced band around 3600-3200 cm^{-1} corresponds to O-H stretch fluctuations (phenols, alcohols, carboxylic acids) and usually also indicates strong hydrogen bonds. The weaker bands at $\sim 3100\text{-}3000\text{ cm}^{-1}$ indicate aromatic or olephinic structures, while the bands at $\sim 2920\text{-}1$ and $\sim 2850\text{ cm}^{-1}$ characterize aliphatic C-H (CH_2/CH_3) stretching, i.e., side chain and lignin fragments. The core of the spectrum for humic acids is usually a pronounced absorption around $\sim 1720\text{-}1700\text{ cm}^{-1}$, which is seen here as a significant signal and corresponds to C=O stretching (mainly carboxylic groups, but possibly also esters/ketones) - this is an important indicator of acidity of humic acids and their ability to complex with metals. The region $\sim 1620\text{-}1580\text{ cm}^{-1}$ is dominated by the contribution of aromatic C=C and/or conjugate carbonyl groups, indicating an aromatic skeleton (lignin/humidification products). At $\sim 1550\text{-}1500\text{ cm}^{-1}$, N-H bending amide signs may appear, but the $\sim 1450\text{-}1380\text{ cm}^{-1}$ bands are usually associated with C-H bending and possible COO-symmetric oscillations. In the "fingerprint" zone at $\sim 1250\text{-}1000\text{ cm}^{-1}$ C-O and C-O-C (phenols, alcohols, ethers, polysaccharide residues) and partly also C-N are visible.

Relative transmittance. Wavenumber (cm^{-1}). O-H; $\equiv\text{C-H}$; $=\text{C-H}$; C-H (aliphatic); CO_2 ; C=O; C=C; N-H bending; C-H bending; C-O/C-N; C-Hal / C-H (aromatic).

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fluctuations inherent in the diversity of humus. At $\sim 900\text{-}700\text{ cm}^{-1}$, the aromatic C-H bends off the plane, while the band around $\sim 2350\text{ cm}^{-1}$ is a typical CO_2 (adsorbed from the atmosphere) signal and is not usually an indicator of the structure of the sample itself. Overall, the spectrum confirms that the humic acids present in the biostimulant are dominated by carboxylic and phenolic groups on an aromatic basis with a mixture of aliphatic chains, which is in accordance with their high reactivity and complexing/biostimulating potential. To further characterize the structure of humic acids, UV-Vis molecular absorption spectra were performed, as shown in Figure 3.



Original experimental graphics are preserved. Labels are translated in the accompanying text.

Absorption, a.u. $E2/E3 = 2.7$; $E2/E4 = 8.1$; $E2/E6 = 62$; $E4/E6 = 7.8$.

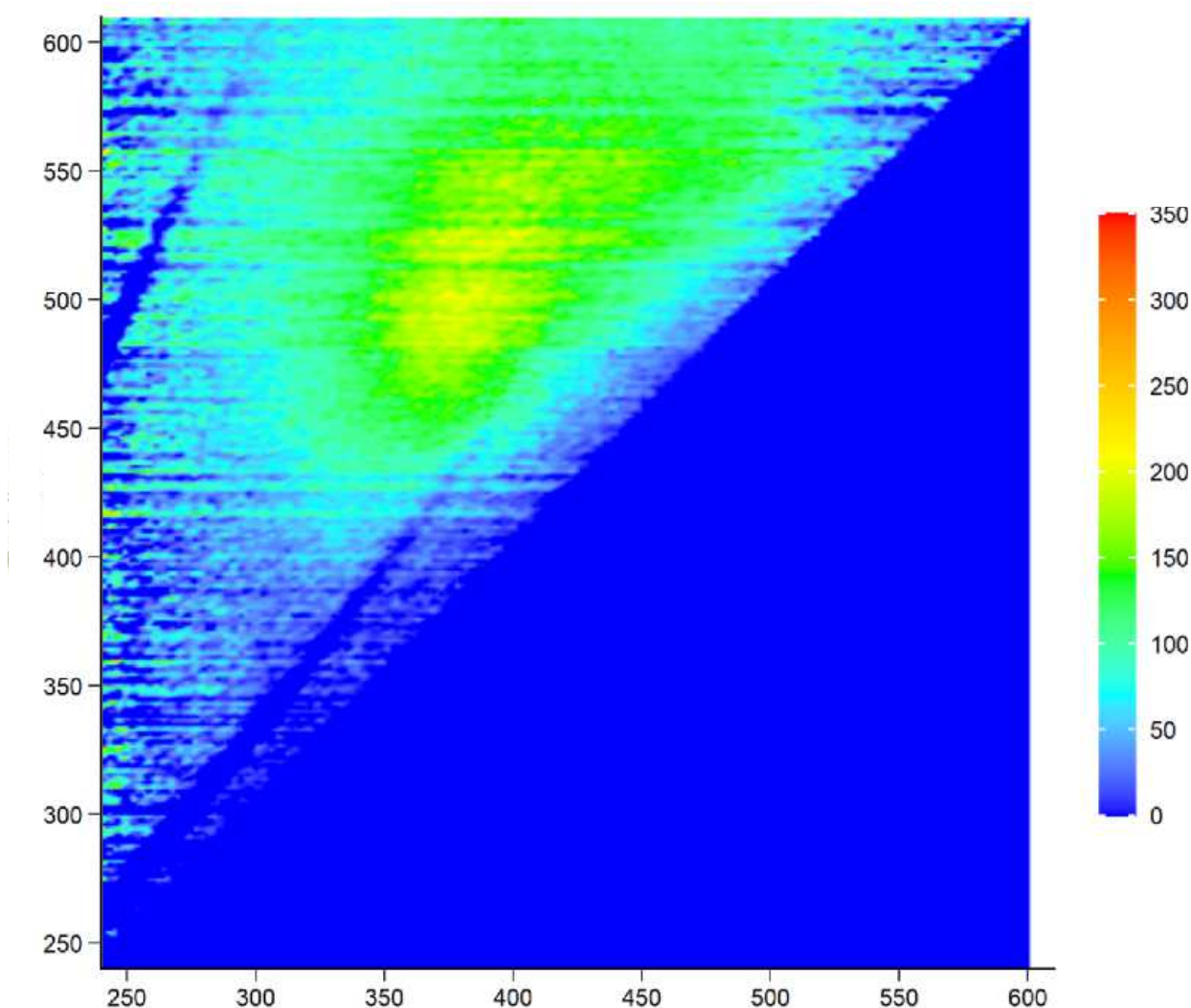
3. fig. UV-Vis molecular absorption spectrum of humic acids released from biostimulant

The UV-Vis spectrum in Figure 3 shows a uniformly decreasing absorption characteristic of humic acids without narrowly expressed signals, indicating a wide mixture of chromophores (aromatic, phenolic, carbonyl groups, etc.) and structures. The calculated $E4/E6 = 7.8$ is higher than the typical humic acids (<5), therefore it indicates a higher degree of humification, higher average molecular weight and more condensed more aromatic structures, while $E2/E3 = 2.7$ (lower than the often cited 3-5 interval) indicates a comparatively higher 365 nm absorbing, more the proportion of conjugated/more humified components and the transition to more humified organic matter. $E2/E4 = 8.1$ indicates the presence of pronounced lignin-derived functional groups and aromatic

Absorption, a.u. $E2/E3 = 2.7$; $E2/E4 = 8.1$; $E2/E6 = 62$; $E4/E6 = 7.8$.

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$E2/E6 = 62$ reflects a shift in the balance in favor of less humified and less condensed fraction with smaller aggregates, so it can be concluded that the biostimulant-excreted peat humic acids are a heterogeneous mixture, where there are both aromatic/lignyl fragments and a significant proportion of lighter, less concentrated humic acids. humified fractions, which are usually associated with improved solubility and availability of functional groups. In order to fully understand the structure of humic acids, fluorescence 3D-EEM spectra were performed, shown in Figure 4.



Original experimental graphics are preserved. Labels are translated in the accompanying text.

Emission, nm Excitation, nm Intensity, au.

Figure 4. FLR 3D-EEM spectrum of humic acids released from biostimulant

This 3D-EEM matrix (Figure 4) shows a broad humic acid-specific fluorescence zone with a peak at approximately $Ex \sim 390-430$ nm and $Em \sim 520-560$ nm (yellow field), indicating strongly conjugated, aromatic and relatively more condensed structures (more humified fraction, typical of peat).

Emission, nm Excitation, nm Intensity, au.

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Additionally, a “shoulder” is visible around Ex ~320-360 nm; Em ~440-500 nm, typical of humic acids and a weaker component around Ex ~250-280 nm; Em ~420-460 nm, which is more typical for lignin components. The spectrum generally shows that the sample is a heterogeneous mixture with chromophores of different degrees of humification. At the same time, the protein-like fluorescence region (tyrosine/tryptophan-type signals around Ex ~275 nm and Em ~300-350 nm) is practically not expressed, which indicates a low proportion of easily degradable impurities and a more stable overall dominance of humus. To fully characterize the effectiveness of the biostimulant in terms of its effect on crop germination, germination tests were carried out. An example of a germination plate on wheat is shown in Figure 5.

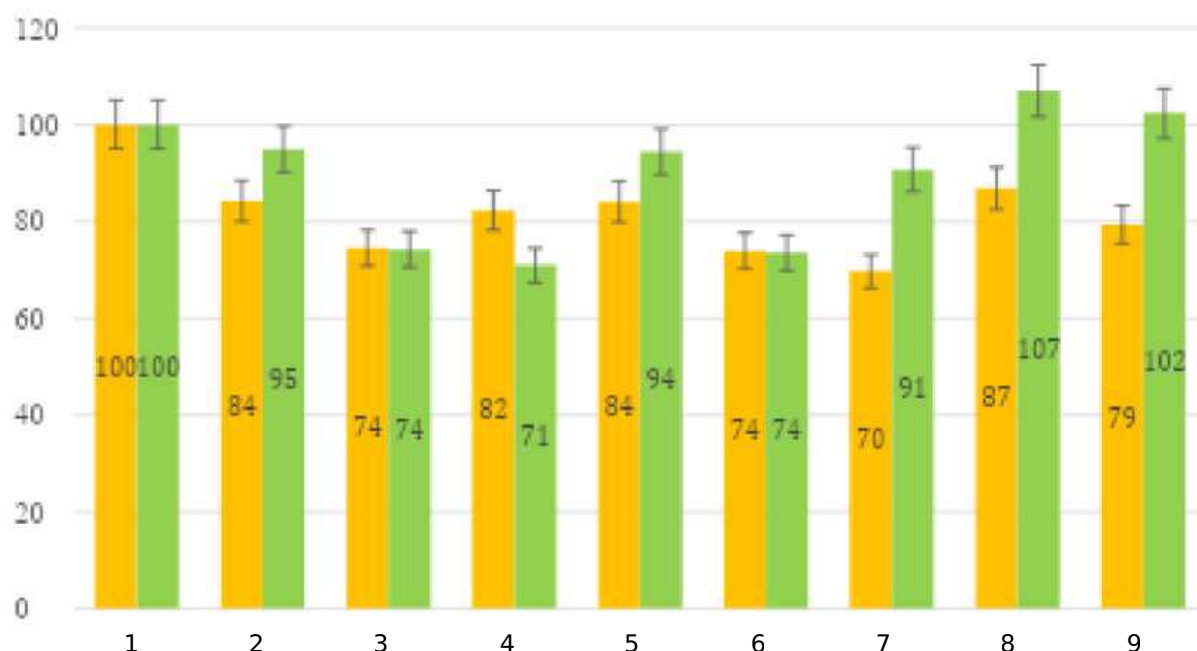


Original experimental graphics are preserved. Labels are translated in the accompanying text.

Figure 5. Germination plate with sprouted wheat for control sample

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Figure 5 shows a germination plate in which wheat germinated after 7 days of germination using deionized water, while the effect of biostimulant on oats germination is shown in Figure 6 (size) and Figure 7 (weight).



Original experimental graphics are preserved. Labels are translated in the accompanying text.

Yellow: roots. Green: shoots. Groups 1–9 follow the treatment order listed below.

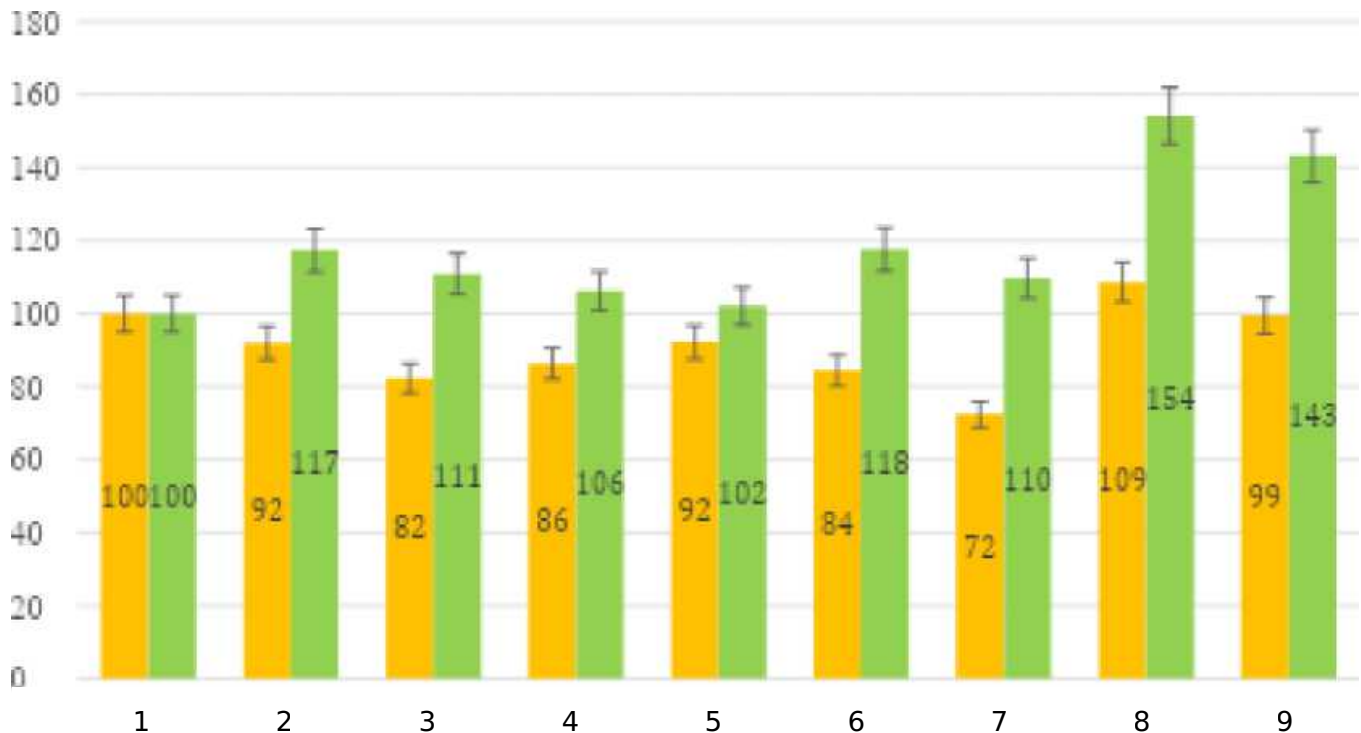
Plant-part length relative to the control, %. Root length. Shoot length. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

6th ed. Oat germination test results for root (yellow bars) and sprout (green bars) length after 7 days of germination, normalized to control

Plant-part length relative to the control, %. Root length. Shoot length. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

Plant-part mass relative to the control, %. Root mass. Shoot mass. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

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Original experimental graphics are preserved. Labels are translated in the accompanying text.

Yellow: roots. Green: shoots. Groups 1–9 follow the treatment order listed below.

Plant-part mass relative to the control, %. Root mass. Shoot mass. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

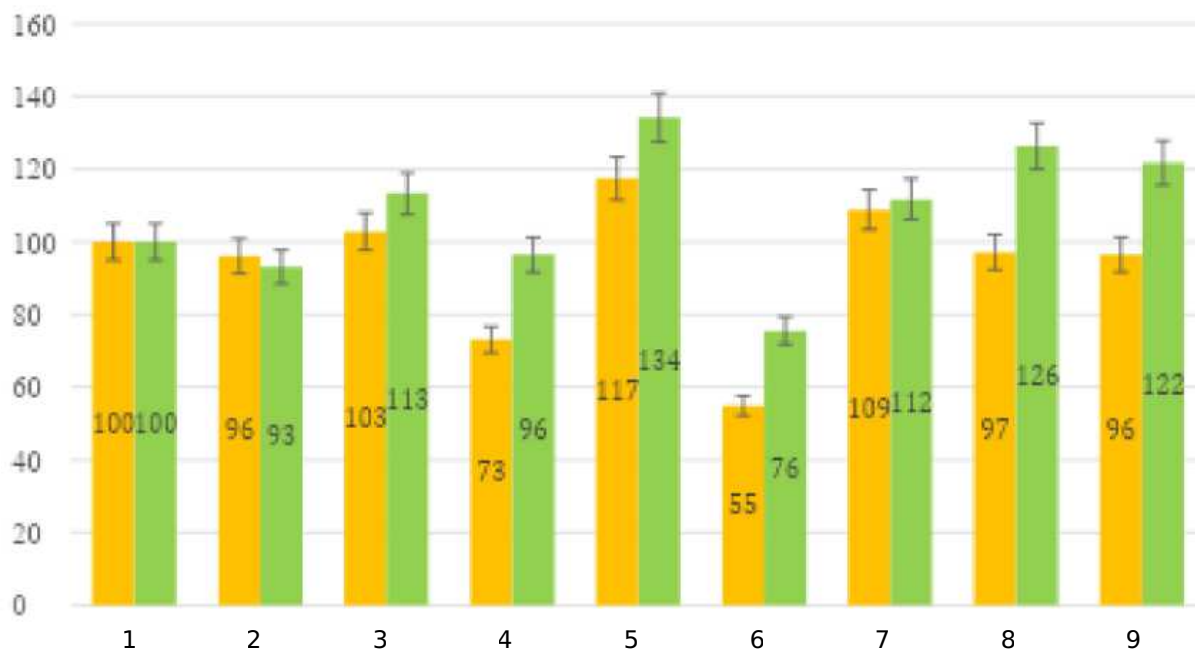
7th ed. Results of oat germination tests for root (yellow bars) and sprout (green bars) mass after 7 days of germination, normalized to controls

6. The figure shows the results of oat germination tests for plant-part length, normalised to the control in which oats were germinated using deionised water. At biostimulant dilutions 1:500; 1:350 and 1:200, both pretreated and untreated seeds showed lower germination performance in terms of root and shoot length. At dilution 1:100, untreated and pretreated seeds showed higher germination performance in terms of shoot length (+7 and +2%, respectively), although root length remained reduced.

Figure 7 shows the result of oat germination tests on plant part mass normalized against control where oats were germinated using deionised water. It was observed that seed pretreatment of seeds in all biostimulant dilutions positively affected the actual mass of sprouts compared to control, especially for biostimulant dilution 1:100 for both untreated and picked seeds (+54 and +43 % respectively). However, root mass was reduced in almost all tests, except for 1:100 uncanned seeds, which had a +9% mass increase. It should be noted that in terms of germination mass, seed pretreatment of seeds has a negative effect on untreated seeds (4-11 % reduction).

Based on the results of oat germination tests, it was concluded that only the mass of plant roots and sprouts will be used for further studies, which more accurately represents the germination capacity of the plant. The test results for durum wheat are shown in Figure 8.

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Original experimental graphics are preserved. Labels are translated in the accompanying text.

Yellow: roots. Green: shoots. Groups 1–9 follow the treatment order listed below.

Plant-part mass relative to the control, %. Root mass. Shoot mass. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

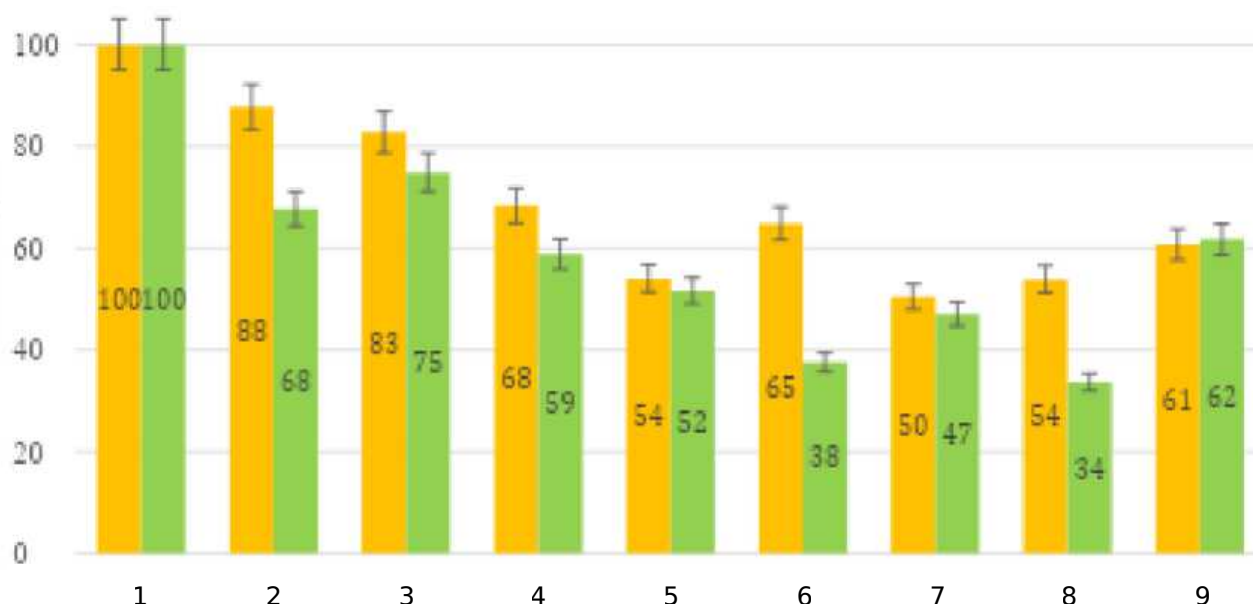
8th ed. Results of germination tests for root (yellow bars) and sprout (green bars) mass after 7 days of germination of durum wheat, normalized to controls

In the durum wheat germination test (Figure 8), the biostimulant increased shoot mass most at 1:100 (+26%) for untreated seeds, while 1:500 produced a small decrease (-7%) and 1:350 and especially 1:200 caused a pronounced mass decrease (-4% and -24%, respectively). Root mass of untreated seeds generally decreased, particularly at 1:350 (-27%) and 1:200 (-45%); 1:500 and 1:100 were close to the control (-4% and -3%). Seed pretreatment generally improved results: shoot mass exceeded the control at every dilution (1:500 kod +13%; 1:350 kod +34%; 1:200 kod +12%; 1:100 kod +22%). Root mass also exceeded the control at 1:500, 1:350 and 1:200 (+3%, +17% and +9%, respectively), but pretreatment slightly reduced root mass at 1:100. Comparing pretreated and untreated seeds, pretreatment had a positive effect at dilutions 1:500–1:200, while a small decrease was observed at 1:100, which may indicate no additional benefit from pretreatment at the higher concentration. Finally, germination was carried out

Plant-part mass relative to the control, %. Root mass. Shoot mass. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

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the results of the tests on peas and the germination tests on pea plant parts are shown in Figure 9.



Original experimental graphics are preserved. Labels are translated in the accompanying text.

Yellow: roots. Green: shoots. Groups 1-9 follow the treatment order listed below.

Plant-part mass relative to the control, %. Root mass. Shoot mass. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

9th ed. Pea germination test results for root (yellow bars) and sprout (green bars) mass after 7 days of germination, normalized to control

In the pea germination test, the overall picture is unambiguous - the biostimulant reduces both root and sprout mass in all dilutions compared to the control, and the most pronounced decrease in sprout mass occurs with decreased dilution. For unfermented seeds, the drop in the most diluted solution 1:500 is more moderate, but when switching to more concentrated solutions, the effect is intensified - at 1:200 and especially 1:100 the mass of sprouts decreases most markedly (to about one third of the control). Seed pretreatment in general does not give a stable improvement. In some dilutions it even deteriorates the root mass (e.g. 1:350), however, in the case of 1:100, seed pretreatment significantly affects the sprout mass, as the sprout mass increases from 34% to 62% (about $\sim 1/3$) to about $\sim 2/3$ of the control. So, for peas in this test, the biostimulant mostly acts inhibitory.

After germination tests it is clear that the prototype of biostimulant has an adverse effect on peas and possibly other legumes, while a significant positive effect has been observed on cereals – durum wheat in dilution 1:350 with pretreated seeds with root mass

Plant-part mass relative to the control, %. Root mass. Shoot mass. Control; 1:500; 1:500 with seed pretreatment; 1:350; 1:350 with seed pretreatment; 1:200; 1:200 with seed pretreatment; 1:100; 1:100 with seed pretreatment.

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increase of +17 % and increase of sprout mass of +34 %, whereas for oats diluted 1:100 with unseeded seeds with an increase of root mass of +9 % and an increase of sprout mass by 54 %.