Report for the tests of plant growth-affecting activity with two liquid products

Objective:	to test plant growth-affecting activity of two different products with four crop species.
Products:	L1 1001 (product A) and LP-2012 (product B).
Model crops:	rapeseed, corn, barley, radish.
Test system:	filter paper roll hydroponics (for all crops except corn) or peat hydroponics (for corn) without mineral nutrients (–MIN) and with mineral nutrients (+MIN), control + 5 concentrations in 10 biological replicates for each product and crop species, 10 plants per replicate.
Concentrations:	working concentrations (0, 2, 5, 10, 50, 100 mg L^{-1}) of tested products were prepared as based on humic substance concentration (10% for product A and 2.5% for product B).
Other conditions:	rolls placed in 800 mL plant tissue culture containers in 48 L closed plastic boxes to maintain stable concentration, laboratory plant growth cabinet, temprerature 22 ± 2 °C, photoperiod 16 h, photon flux density of photosynthetically active radition 100 μmol m^{-2} s^{-1}.
Implementation:	Prof., Dr. habil. biol. Gederts Ievinsh, Department of Plant Physiology, Faculty of Biology, University of Latvia, Rīga.
Time:	March – April 2020.

Summary

Two humic-based products were tested in a hydroponics model system for their ability to stimulate growth of a number of agricultural crop species (rapseed, corn, barley, radish) with and without added mineral nutrients. Due to significant stimulative effect on plant growth, it can be concluded that both products correspond to the category of plant biostimulants, according to the recent definition of biostimulants as "mixture of products applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content". Product A (L1 1001) showed characteristics of plant hormonelike substance-containing products with pronounced stimulative effect at a low concentration range (up to 50 mg L^{-1} corresponding to 5 mg L^{-1} of humic substances). Small differences between the two types of tests (without minerals and with minerals) clearly indicated that the stimulative effect was due to presence of substances with growth-promoting activity. Product B (LP-2012) was relatively weak stimulator within a low concentration range, with more pronounced stimulative effect at a higher concentration range (above 400 mg L^{-1} coresponding to 10 mg L^{-1} of humic substances). Pronounced difference between the both test types indicated that stimulative effect by this product is mostly due to chemical constituents with mineral nutrient characteristics. In conclusion, product A can be efficiently used at 10 to 50 mg L^{-1} dose but product B above 400 mg L^{-1} dose for their biostimulant effect.

1. Methods

The purpose of the test is to distinguish between two types of putative biological activity: (i) total activity with both hormone-like and fertilizer-like components (test without mineral nutrients, –MIN), (ii) only hormone-like activity, excluding fertilzer effect (test with mineral nutrients, +MIN). Seeds from local commercial suppliers were used. Seeds were surface sterilized in KMnO4 solution for 15 min, rinsed with water 5 times and left in deionized water for no longer than 1 h. Partially imbibed seeds were individually planted in filter paper rolls (or in neutralized peat substrate with no added mineral nutrients). Rolls were placed in containers with appropriate concentration (prepared with deionized water) of the product and cultivated in closed 48 L plastic boxes in light (photoperiod 16 h, photosynthetically active radiation with a photon flux density 100 μmol m^{-2} s^{-1}) at room temperature for 7 to 9 days. In a test without mineral nutrients (–MIN), deionized water was used as a cultivation media, in a test with mineral nutrients (+MIN), commercial fertilizer Kristalon Blue with CalciNit (both produced by Yara, Norway) at concentration 1.0 g L^{-1} (full macronutrient and micronutrient composition in deionized water) was used as a cultivation media.

Rolls (in batches by 5) were placed in 800 mL plastic containers containing a test solution (deionized water or nutrient solution plus tested product, in a total volume 200 mL per container). The following parameters were measured at termination of each test: height of shoot (individual seedlings), length of root (individual seedlings), fresh mass of shoot (per replicate), fresh mass of root (per replicate).

All measurement data were recalculated and reported on individual seedling basis. For comparison of growth-affecting effects, relative data were calculated and shown in a graphical form as % from the respective control. Summary positive plant growth- stimulating effect of each product was calculated as summed % increase at particular concentration with all model crrop species, separately for –MIN, +MIN and total stimulation, and was shown as a concentration dependence graph. Statistical significance of differences between control and each treatment concnetration were calculated according to *t* test (GraphPad Prism v. 8, San Diego, USA).

2.1. Tests with rapeseed seedlings

Table 1. Test 1: changes in morphological parameters of rapeseed seedlings at different concentrations of product A (L1 1001), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	30.9 ± 2.6	34.5 ± 2.3	16.9 ± 2.3
2	33.4 ± 3.0	39.3 ± 4.2	20.6 ± 1.9
5	33.5 ± 2.6	39.0 ± 3.8	19.0 ± 2.5
10	31.3 ± 1.3	39.3 ± 2.6	18.9 ± 1.6
50	**35.1 ± 2.2**	**45.6 ± 2.5**	**23.1 ± 2.2**
100	**35.4 ± 1.7**	**42.1 ± 1.4**	**20.9 ± 0.6**

Table 2. Test 2: changes in morphological parameters of rapeseed seedlings at different concentrations of product A (L1 1001), with minerals (+MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	42.9 ± 4.1	37.5 ± 3.4	9.0 ± 1.0
2	43.4 ± 4.4	38.8 ± 8.5	9.1 ± 1.7
5	41.0 ± 3.8	31.3 ± 6.9	8.6 ± 0.7
10	35.2 ± 4.8	31.0 ± 5.4	7.3 ± 1.0
50	39.0 ± 2.6	35.1 ± 3.1	10.3 ± 1.6
100	43.5 ± 4.1	44.0 ± 5.7	**14.5 ± 2.8**

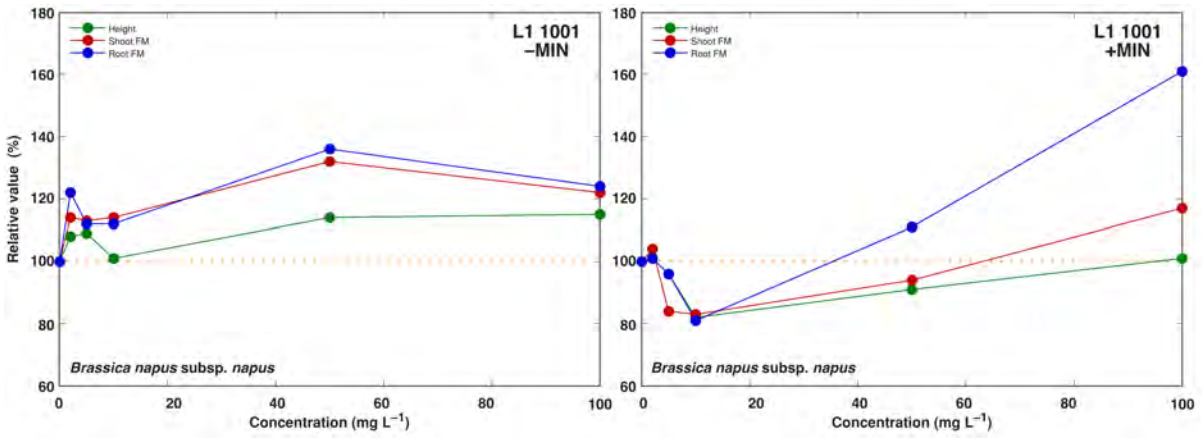


Fig. 1. Relative changes in morphological parameters of rapeseed seedlings with product A (L1 1001) without minerals (–MIN, Test 1) and with minerals (+MIN, Test 2).

Table 3. Test 3: changes in morphological parameters of rapeseed seedlings at different concentrations of product B (LP-2012), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	32.3 ± 2.1	38.8 ± 3.1	17.3 ± 2.0
2	31.5 ± 4.0	33.2 ± 4.3	18.3 ± 2.2
5	29.0 ± 2.4	35.5 ± 4.0	**13.7 ± 1.8**
10	35.3 ± 2.9	42.5 ± 3.1	17.9 ± 3.0
50	32.8 ± 2.9	39.8 ± 2.8	16.1 ± 1.8
100	**46.7 ± 3.0**	**61.9 ± 5.3**	20.4 ± 2.7

Table 4. Test 4: changes in morphological parameters of rapeseed seedlings at different concentrations of product B (LP-2012), with minerals (+MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	72.1 ± 2.6	106 ± 7	21.8 ± 2.7
2	**64.7 ± 1.6**	**92 ± 6**	**16.5 ± 1.3**
5	71.3 ± 3.3	104 ± 8	21.7 ± 2.4
10	72.7 ± 2.4	105 ± 5	22.7 ± 1.9
50	68.5 ± 2.2	103 ± 7	22.2 ± 1.4
100	69.9 ± 2.1	95 ± 5	23.4 ± 1.6

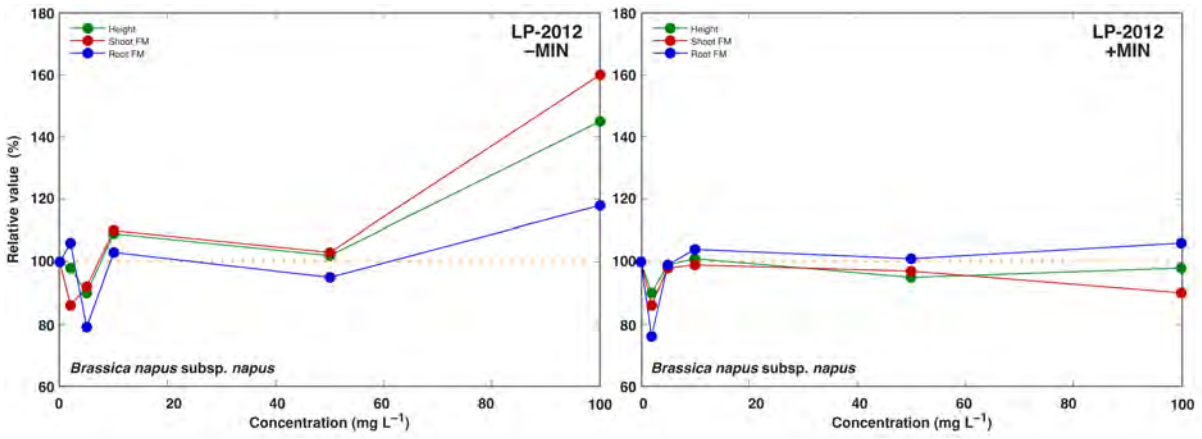


Fig. 2. Relative changes in morphological parameters of rapeseed seedlings with product B (LP-2012) without minerals (–MIN, Test 3) and with minerals (+MIN, Test 4).

In general, tests with rapeseed seedlings showed relatively low effect of both products, with better activity of product A.

Convincing positive effect of product A was evident within a range 50 to 100 mg L^{-1} for all parameters in a test without minerals (Table 1), but statistically significant effect in a test with minerals was only at the highest concentration (Table 2). There was a tendency that without minerals product A had some stimulative activity also in a low concentration range (2 to 10 mg L^{-1}, Fig. 1).

In a test without minerals, product B showed significant stimulative effect only at the highest concentration, but root growth was inhibited at 5 mg L^{-1} (Table 3). There was no positive effect of product B in a test with minerals, with significantly negative effect at 2 mg L^{-1} (Table 4). Tendency of growth inhibition in a low concentration range for product B in both types of tests was rather convincing (Fig. 2).

Test 4 with rapeseed seedlings

2.2. Tests with corn seedlings

Table 5. Test 5: changes in morphological parameters of corn seedlings at different concentrations of product A (L1 1001), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	210 ± 15	632 ± 72	591 ± 87
2	215 ± 2	618 ± 34	566 ± 24
5	**186 ± 5**	**480 ± 34**	**440 ± 42**
10	214 ± 10	615 ± 47	543 ± 49
50	200 ± 17	607 ± 128	552 ± 102
100	**172 ± 15**	**471 ± 34**	**403 ± 37**

Table 6. Test 6: changes in morphological parameters of corn seedlings at different concentrations of product A (L1 1001), with minerals (+MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	310 ± 5	1114 ± 48	593 ± 57
2	**340 ± 13**	1221 ± 131	539 ± 23
5	327 ± 19	1083 ± 83	**426 ± 45**
10	318 ± 16	1212 ± 171	548 ± 74
50	**359 ± 2**	**1414 ± 129**	543 ± 24
100	**335 ± 12**	1246 ± 113	513 ± 39

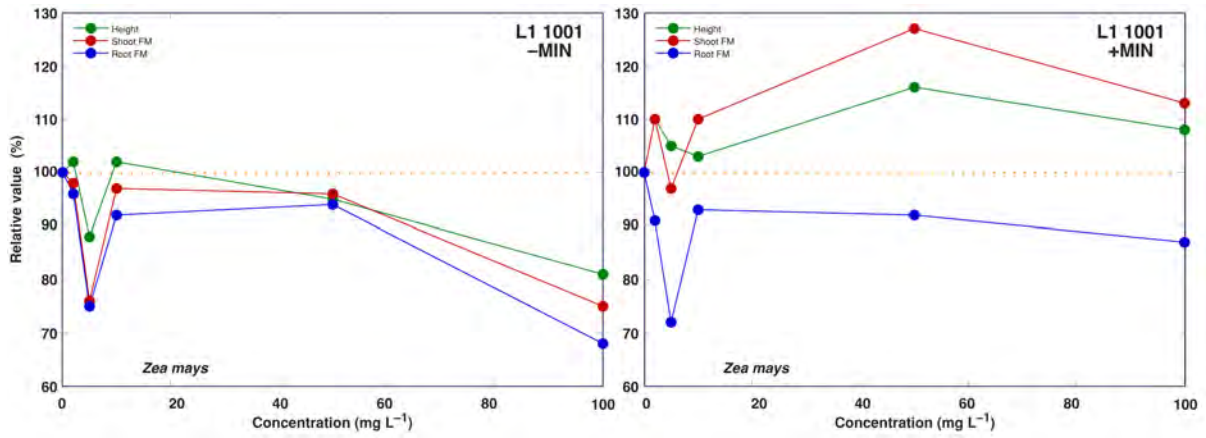


Fig. 3. Relative changes in morphological parameters of corn seedlings with product A (L1 1001) without minerals (–MIN, Test 5) and with minerals (+MIN, Test 6).

Table 7. Test 7: changes in morphological parameters of corn seedlings at different concentrations of product B (LP-2012), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	237 ± 9	628 ± 31	465 ± 26
2	222 ± 5	645 ± 33	**331 ± 27**
5	223 ± 2	565 ± 6	**334 ± 22**
10	227 ± 3	558 ± 64	449 ± 168
50	232 ± 3	624 ± 46	**364 ± 41**
100	241 ± 3	**699 ± 12**	478 ± 17

Table 8. Test 8: changes in morphological parameters of corn seedlings at different concentrations of productB (LP-2012), with minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	255 ± 6	884 ± 41	538 ± 18
2	245 ± 3	823 ± 56	**451 ± 29**
5	244 ± 13	866 ± 40	494 ± 37
10	235 ± 7	771 ± 65	**399 ± 33**
50	236 ± 28	831 ± 201	**412 ± 99**
100	253 ± 13	919 ± 104	503 ± 65

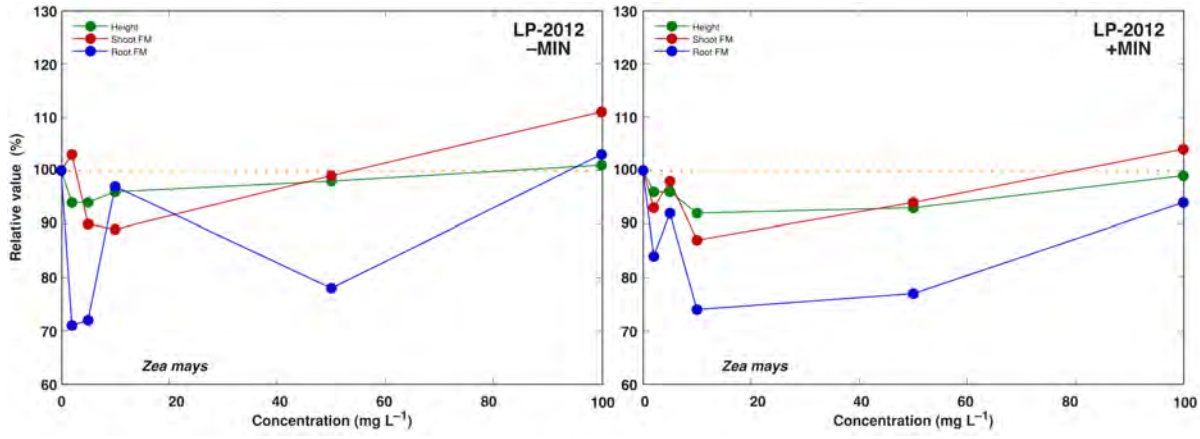
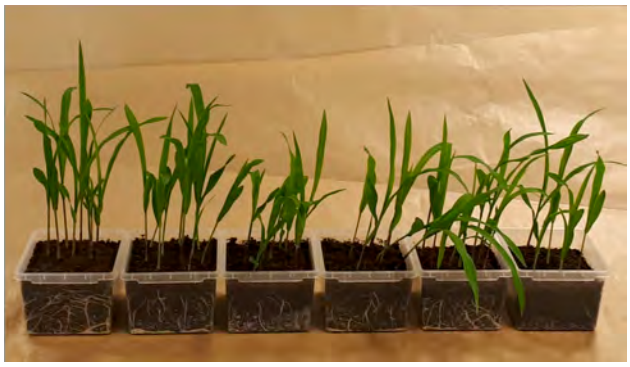


Fig. 4. Relative changes in morphological parameters of corn seedlings with product B (LP–2012) without minerals (–MIN, Test 7) and with minerals (+MIN, Test 8).

With product A, corn seedlings responded rather negatively in the case of a test without minerals (Table 5), but the effect on shot growth was positive in a test with minerals (Table 6). In particular, 5 and 100 mg L^{-1} had statistically significant inhibitory effect, but 2, 50 and 50 mg L^{-1} had significantly stimulative effect. In addition, in a test with minerals, root growth was generally inhibited over a wide range of concentration (Fig. 3).

In contrast, product B had very little effect on shoot growth of corn seedlings, with only significantly stimulative effect at the highest concentration for shoot mass in a test without minerals (Table 7), but root growth was significantly inhibited in both test systems by a product B at concentration 2, 10 and 50 mg L^{-1} (Table 7, Table 8).



Test 5 Test 8

Test 7 with corn seedlings

2.3. Tests with barley seedlings

Table 9. Test 9: changes in morphological parameters of barley seedlings at different concentrations of product A (L1 1001), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	128 ± 4	118 ± 5	91 ± 5
2	134 ± 3	**132 ± 6**	100 ± 4
5	**140 ± 4**	128 ± 3	99 ± 4
10	134 ± 4	129 ± 4	96 ± 3
50	**142 ± 4**	129 ± 5	96 ± 4
100	131 ± 2	**133 ± 6**	**104 ± 4**

Table 10. Test 10: changes in morphological parameters of barley seedlings at different concentrations of product A (L1 1001), with minerals (+MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	113 ± 5	97 ± 4	69 ± 5
2	**147 ± 5**	**134 ± 7**	**98 ± 9**
5	**143 ± 3**	**121 ± 3**	**83 ± 4**
10	**129 ± 3**	**119 ± 3**	**78 ± 3**
50	**127 ± 6**	105 ± 6	75 ± 6
100	123 ± 5	**114 ± 5**	76 ± 3

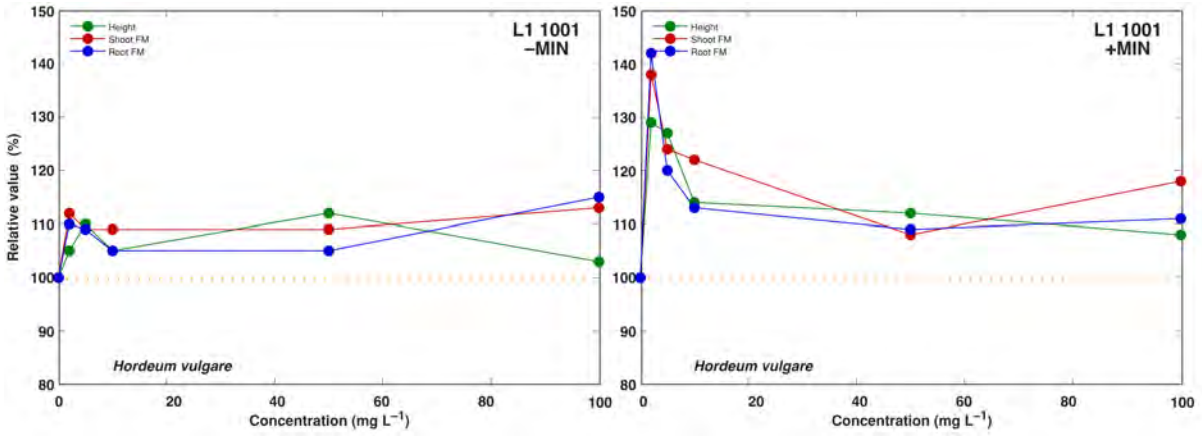


Fig. 5. Relative changes in morphological parameters of barley seedlings with product A (L1 1001) without minerals (–MIN, Test 9) and with minerals (+MIN, Test 10).

Table 11. Test 11: changes in morphological parameters of barley seedlings at different concentrations of product B (LP-2012), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	146 ± 7	124 ± 7	204 ± 17
2	143 ± 5	**144 ± 5**	214 ± 11
5	153 ± 4	**151 ± 7**	216 ± 11
10	155 ± 5	**150 ± 8**	199 ± 13
50	**157 ± 5**	**151 ± 6**	185 ± 10
100	**160 ± 5**	**163 ± 10**	**165 ± 14**

Table 12. Test 12: changes in morphological parameters of barley seedlings at different concentrations of productB (LP-2012), with minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	123 ± 5	119 ± 7	96 ± 10
2	134 ± 7	124 ± 9	104 ± 8
5	130 ± 4	129 ± 5	98 ± 4
10	130 ± 8	**136 ± 9**	102 ± 9
50	**145 ± 6**	**140 ± 8**	96 ± 8
100	126 ± 7	**134 ± 8**	**82 ± 6**

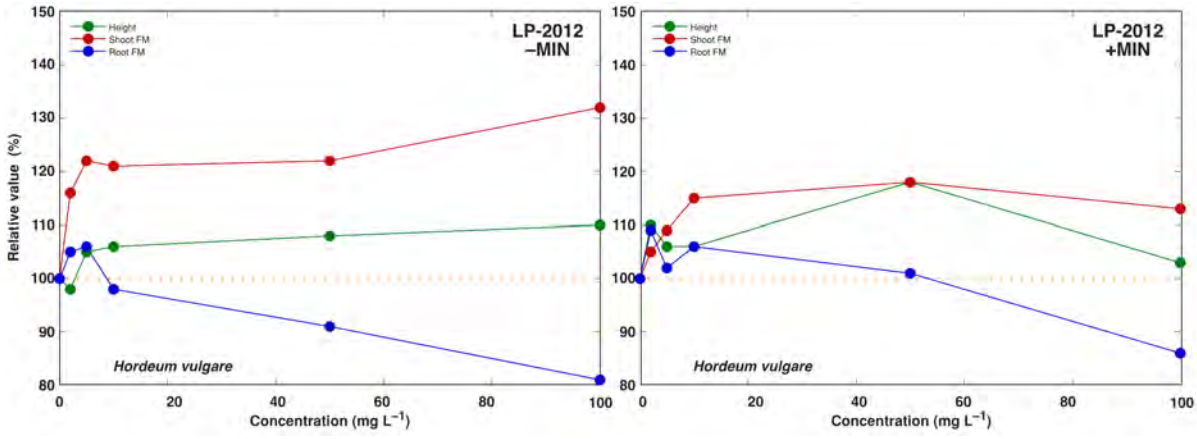


Fig. 6. Relative changes in morphological parameters of barley seedlings with product B (LP–2012) without minerals (–MIN, Test 11) and with minerals (+MIN, Test 12).

Tests with barley seedlings showed predominantly positive effect of both tested products on plant growth.

For product A, there was growth stimulation of seedlings at all concentrations in both test systems (without minerals, Table 9; with minerals, Table 10), but the effect was more pronounced in the case of a test with minerals (Fig. 5). Statistically significant stimulation in a test without minerals was at 5 and 50 mg L^{-1} for shoot height, 2 and 100 mg L^{-1} for shoot mass, and 100 mg L^{-1} for root mass (Table 9). In a test with minerals, statistically significant stimulation was from 2 to 50 mg L^{-1} for shoot height, 2 to 10 and 100 mg L^{-1} for shoot mass, and 2 to 10 mg L^{-1} for root mass (Table 10).

For product B, the effect was positive over the whole range of concentration for shoot growth in both test systems (without minerals, Table 11; with minerals, Table 12), but root growth was stimulated only at low concentration, with pronounced inhibition at the highest concentration (Fig. 6). Statistically significant shoot growth stimulation was at 50 mg L^{-1} for shoot height and 10 to 100 mg L^{-1} for shoot mass (Table 12).

Test 11 with barley seedlings

2.4. Tests with radish seedlings

Table 13. Test 13. changes in morphological parameters of radish seedlings at different concentrations of product A (L1 1001), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	42.4 ± 2.4	114 ± 7	31 ± 5
2	40.1 ± 2.3	105 ± 10	**42 ± 8**
5	41.9 ± 2.5	101 ± 8	35 ± 4
10	43.1 ± 1.5	110 ± 4	**40 ± 3**
50	43.2 ± 1.6	106 ± 9	**41 ± 6**
100	43.9 ± 1.5	112 ± 5	**38 ± 5**

Table 14. Test 14: changes in morphological parameters of radish seedlings at different concentrations of product A (L1 1001), with minerals (+MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	63.5 ± 1.9	131 ± 8	35 ± 2
2	64.9 ± 3.1	134 ± 9	37 ± 3
5	61.3 ± 1.9	118 ± 6	31 ± 1
10	58.9 ± 2.8	**107 ± 7**	31 ± 2
50	**67.6 ± 1.9**	127 ± 8	**40 ± 2**
100	67.9 ± 3.8	136 ± 9	**44 ± 3**

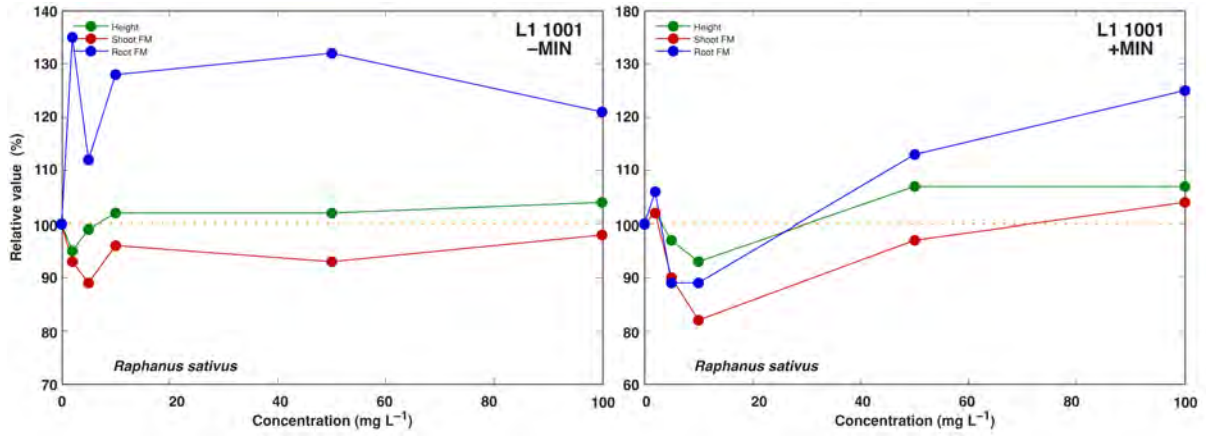


Fig. 7. Relative changes in morphological parameters of radish seedlings with product A (L1 1001) without minerals (–MIN, Test13) and with minerals (+MIN, Test 14).

Table 15. Test 15: changes in morphological parameters of radish seedlings at different concentrations of product B (LP-2012), without minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	37.0 ± 2.4	92 ± 9	25 ± 5
2	40.3 ± 2.4	94 ± 7	25 ± 6
5	40.9 ± 1.9	105 ± 8	23 ± 2
10	41.4 ± 2.0	99 ± 11	**19 ± 2**
50	**46.0 ± 2.1**	**111 ± 9**	22 ± 4
100	**42.0 ± 1.9**	104 ± 7	**19 ± 3**

Table 16. Test 16. changes in morphological parameters of radish seedlings at different concentrations of productB (LP-2012), with minerals (–MIN). Statistically significant increase is shown in green, statistically significant decrease is shown in blue, in comparison to control

Concentration (mg L^{-1})	Shoot height (mm)	Shoot mass of single plant (mg)	Root mass of single plant (mg)
0	43.6 ± 1.2	98 ± 4	39 ± 2
2	39.4 ± 1.9	96 ± 7	35 ± 2
5	41.0 ± 1.8	86 ± 3	**33 ± 2**
10	40.9 ± 1.1	86 ± 4	**32 ± 2**
50	41.8 ± 1.8	87 ± 5	38 ± 2
100	**47.7 ± 1.2**	101 ± 4	41 ± 2

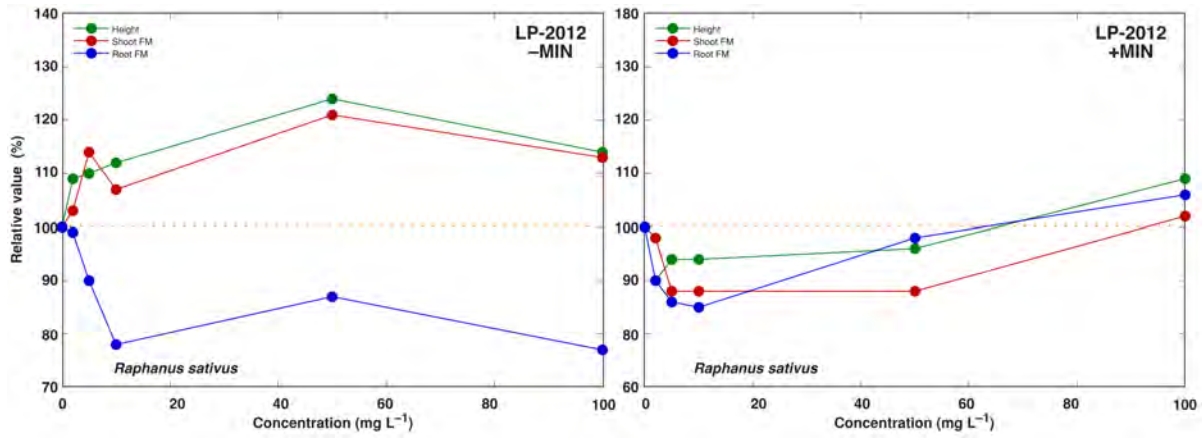


Fig. 8. Relative changes in morphological parameters of radish seedlings with product B (LP–2012) without minerals (–MIN, Test 15) and with minerals (+MIN, Test 16).

Product A had no effect on shoot growth of radish seedlings in a test without minerals (Table 13), with some positive effect in a test with minerals (Table 14). In contrast, root growth of radish seedlings was very positively affected by product B over an entire range of concentration in a test without minerals (Table 13) and at 50 to 100 mg L^{-1} in a test with minerals (Table 14). Statistically significant differences were for 2, 10. 50 and 100 mg L^{-1} in a test without minerals and 50 to 100 mg L^{-1} in a test with minerals.

Product B had positive effect on shoot growth of radish seedlings over a whole range of concentration in a test without minerals, but with statistically significant differences only for 50 to 100 mg L^{-1} (Table 15). In a test with minerals shoot growth tended to be inhibited, but statistically significant stimulation was evident at 100 mg L^{-1} (Table 16). In contrast to product A, product B had pronounced negative effect on growth of roots of radish seedlings in a test without minerals in both test systems, with some improvement at the highest concentration in the case of a test with minerals.

Test 16 with radish seedlings

3. Summed effect of the tested products

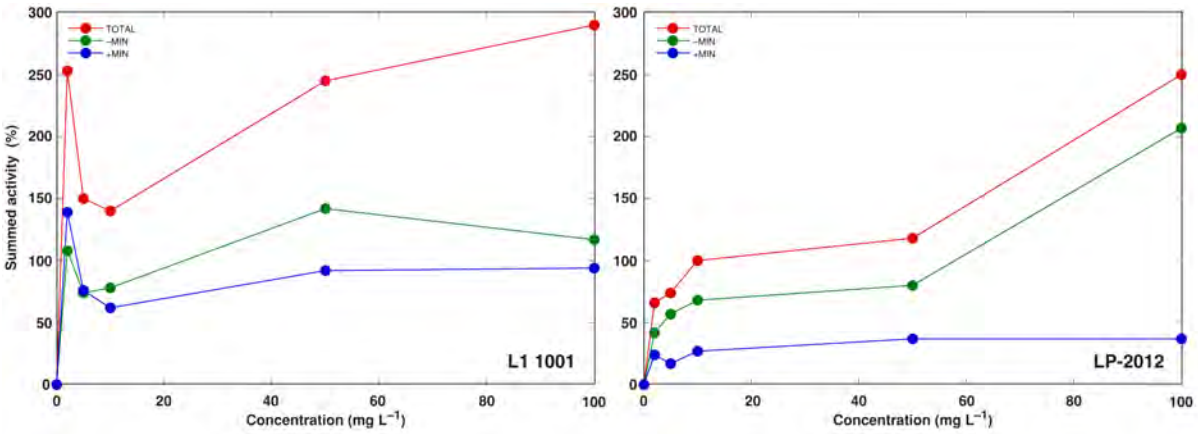


Fig. 9. Summed concentration-dependent positive plant growth-affecting effect of product A (L1 1001) and product B (LP-2012).

The aim of the performed test series was to measure plant growth-affecting activity of two different products with four crop species using complex of tests to distinguish between plant growth-affecting activity resulting either from general action of mineral nutrients or specific activity of plant hormonelike substances.

The purpose of this complex test is not to characterize response of each single crop species to various products with putative growth-stimulating activity, but rather to show general tendencies of growth responses in a genotype-independent way. This method allows eliminating any effects caused by genotype specificity and stochastic variation of the experimental system. Therefore, summed effect of both products was compared as based on the performed 16 individual tests (Fig. 9). It is important to note that the absolute level of summed stimulating activity of the particular product in the respective test indicates not only the intensity of possible stimulative effect, but also the degree of specificity of the effect, as not all species showed identical concentration-dependent stimulative response.

Product A (L1 1001) showed dose-response curve characteristic for plant hormonelike substance-containing products with pronounced stimulative effect at a low concentration range. Moreover, only small differences were seen between the two types of tests (without

minerals and with minerals), clearly indicating that the stimulative effect was due to presence of substances with growth-promoting activity. In contrast, product B (LP-2012) was relatively weak stimulator within a low concentration range, with more pronounced stimulative effect at a higher concentration range. Also, extremely pronounced difference between the both test types indicated that stimulative effect by this product is mostly due to chemical constituents with mineral nutrient characteristics.