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Cladophora glomerata EXTRACT AND STATIC MAGNETIC FIELD INFLUENCES THE GERMINATION OF SEEDS AND MULTIELEMENTAL COMPOSITION OF CARROT

Abstract: As carrot seeds are notoriously slow to germinate and are often irregular in breaking dormancy, new methods of stimulation are still sought. This study examines for the first time the effect of an algal extract and static magnetic field (SMF) and their synergistic effect on carrot seeds germination. The algal extract, produced from freshwater macroalgae - *Cladophora glomerata*, was used directly to the paper substrate at a dose of 20, 40, 60, 80 and 100 %. The exposure of seeds to the magnetic field (500 mT and 1 T) was applied for 3, 6 and 12 min. The highest germination ability of carrot seedlings was observed for 20 and 80 % algal extract. The weakest germination was observed for the highest concentration of algal extract causing the highest amount of abnormal and dead seedlings. Parallel use of seeds stimulated with magnetic field and algal extract did not increase the number of germinated seeds significantly. Carrot's seeds treated with algal extract showed increased content of elements - macro- Ca, K, Mg, S and microelements Cu, Fe, Mn and Zn. Future experiments are required to confirm the stimulation effect of algal extract (optimal concentration) and magnetic field (various induction values) on seeds germination.

Keywords: static magnetic field, algal extract, carrot, germination, multielemental composition

Introduction

Nowadays, novel chemical and non-chemical methods are used in agriculture. Among them, biostimulants of plant growth are especially popular. These bioproducts enhance nutrition efficiency, tolerance to the abiotic stress and crop quality, regardless of their nutrients content [1]. Seven main categories of biostimulants can be distinguished: humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds and beneficial fungi

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and bacteria [1, 2]. Taking into account cultivation of carrot (*Daucus carata* L.), the following commercial biostimulants of plant growth are used, for example Acadian™ (seaweed extract from *Ascophyllum nodosum*) [3], biostimulants - Kelpak SL (seaweed extract from *Ecklonia maxima*) [4, 5], Asahi SL (mixture of nitrophenols) [4, 5], Agro-Lig, Enersol (humic acids) and Ergostim (folic acid) [6], Aminoplant (known also as Siapton®, solution of amino acids derived from hydrolyzed animal proteins) [7], Asahi SL, Bio-algeen S 90 (extract from marine seaweeds) and Tytanit (mineral biostimulant) [8, 9], Actiwave® (seaweed extract) [10]. Most of them is produced using chemical methods and currently environmentally friendly methods are being sought. In the present study, algal extract used as a biostimulant of plant growth was produced from naturally occurring freshwater macroalga - *Cladophora glomerata* using Ultrasound Assisted Extraction which is recognized as a novel extraction technique.

In the case of carrot, the final yield depends on germinations of seeds which notoriously slowly germinate. In order to increase the germination of seeds and plants growth and development, also non-chemical methods are used, for example stimulation of seeds before sowing with magnetic field (MF). Stimulation with a magnetic field depends on many parameters such as type (static/variable) and intensity of the magnetic field, exposure time and stimulated plant species [11-19].

Research, which relates to the biological effects of using MF has been carried out for many years. It showed that MFs can generate or modify many different phenomena. The explanation of these effects is a serious problem and is a discussion in the content of many scientific publications [11, 15, 20-22].

Among the physical processes or models that underlie the hypothetical basic mechanisms of interaction for magnetic field effects in biological systems are (selected): (a) models of classical and quantum oscillators; (b) a cyclotron resonance model; (c) interference of quantum states of bound ions and electrons; (d) coherent quantum excitations; (e) biological effects of torsion fields accompanying MF; (f) biologically active metastable states of liquid water; (g) free radical reactions and other “spin” mechanisms; (h) a parametric resonance model; (i) stochastic resonance as a strengthening mechanism in magnetobiology and other random processes; (j) phase transitions in biophysical systems displaying the order of liquid crystals; (k) bifurcation behavior of solutions of nonlinear chemical kinetics equations; (l) radiotechnical models, in which biological structures and tissues are presented as equivalent electrical circuits; (m) charged macroscopic vortices in the cytoplasm [23-26]. Many authors of scientific publications indicate that these concepts and models can be combined [14, 27-29].

The results presented in many research, partially confirm the stimulating effect of magnetic fields on the germination and efficiency of seedlings of many plants [11, 30-38]. However, this is closely related to the time of exposure, type of magnetic field, frequency (for alternating field) and plant species.

In the literature, there are many papers showing the effect of algal extracts and physical factors on the plant growth and development, but they are not tested in combination. The aim of the present study was to examine the effect of the algal extract produced from freshwater *Cladophora glomerata* and the stimulation of seeds with magnetic field on the germination of carrot seeds and the multielemental composition of seedlings. Both factors were applied independently, as well as in combination. Synergistic effects of such action have been tested. For the production of extract, novel extraction technique - Ultrasound Assisted Extraction with water as a solvent was used.

Materials and methods

Production of algal extract

Algal extract (E) was produced from a freshwater macroalga - *Cladophora glomerata*, which was collected by hand from the surface of the pond in a village Tomaszowek (Lodz Province, Poland) in October 2016. Before extraction, the biomass was air-dried, milled using a grinding mill (Retsch GM300, Haan, Germany) and sieved. The particles with the size lower than 400 µm were used for extraction. Briefly, dry algae (4 g), suspended in an aqueous solution (100 cm^3) were subjected to the ultrasound homogenizer working at parameters 50 watts, ultrasonic frequency 30 kHz, amplitude 100 % (UP 50H; Hielscher Ultrasonics, Teltow, Germany). After 30 min, the mixture was centrifuged at a speed rate 4000 rpm for 10 min. The obtained supernatant was treated as 100 % algal extract [39]. As the algal extract contained organic matter, a fresh portion was produced before germination tests and stored in a refrigerator. For germination tests on carrot, different concentrations of algal extracts were used: 20, 40, 60, 80 and 100 %.

The application of the static magnetic field (SMF) on carrot seeds

Seeds of carrots were exposed to the static magnetic field. For stimulation, carrot seeds were grouped by 100. Next the seeds were exposed to the static magnetic field. Halbach array (HA) was used in the experiment. This is a special arrangement of permanent magnets. Magnets have different magnetizing directions, i.e. they are arranged in the appropriate configuration. The Halbach array ideal concept and stimulation system were shown in Figure 1. Seeds were placed in Eppendorf test tube. Then they were subjected to a static magnetic field (SMF). Two doses of the magnetic field were used - 500 mT and 1 T. The exposure time of seeds to the static magnetic field was 3, 6 and 12 min [18, 39-41]. After stimulations seeds were delivered to the laboratory for sowing.

Germination tests

The germination test of carrot seeds (cv. 'Berlikumer') was assessed according to the International Seed Testing Association (ISTA). The following groups were examined in the present study:

1. Carrot - Control group
2. Carrot + SMF 500 mT + 3 min
3. Carrot + SMF 500 mT + 6 min
4. Carrot + SMF 500 mT + 12 min
5. Carrot + SMF 1 T + 3 min
6. Carrot + SMF 1 T + 6 min
7. Carrot + SMF 1 T + 12 min
8. Carrot + SMF 500 mT + 3 min + 20 % algal extract
9. Carrot + SMF 500 mT + 6 min + 20 % algal extract
10. Carrot + SMF 500 mT + 12 min + 20 % algal extract
11. Carrot + SMF 1 T + 3 min + 20 % algal extract
12. Carrot + SMF 1 T + 6 min + 20 % algal extract
13. Carrot + SMF 1 T + 12 min + 20 % algal extract
14. Carrot + 20 % algal extract

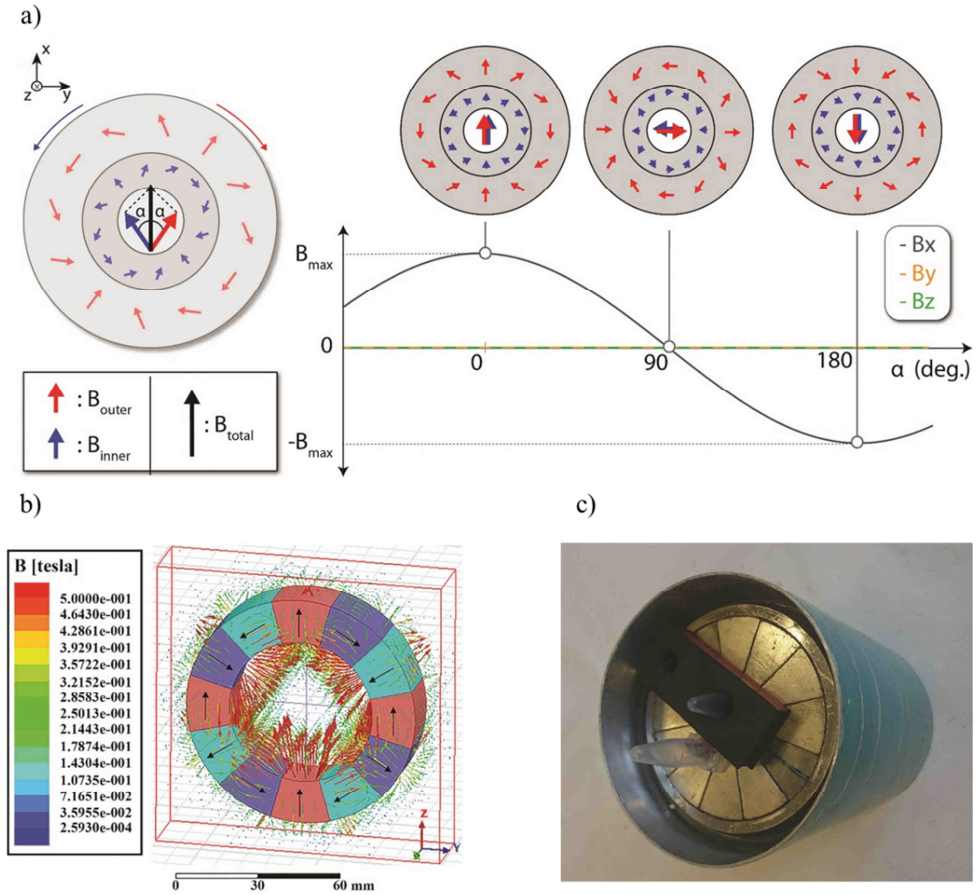


Fig. 1. a) Schematic illustration of the magnetic field generated within the nested cylindrical ideal Halbach array (HA) dipoles as a function of their mutual angle, b) simulated magnetic field of circular HA in Maxwell 3-D, c) Halbach array and Eppendorf test tubes that were used in the experiment. Source: [42, 43]. Photo: Katarzyna Niemczyk

In the preliminary tests, different concentrations of algal extracts (20, 40, 60, 80 and 100 %) were examined in order to choose the best concentration, providing the highest number of germinated seedlings and the content of macronutrients such as carbon and nitrogen in seedlings (Fig. 2). Seeds treated with distilled water were used as a control group. For preliminary research, 50 seeds were utilized for each of 4 repetitions (n = 4). Proper germination tests were carried out for 100 seeds in each repetition using 20 % algal extract (chosen on the basis of preliminary tests) according to the regulations established by ISTA. The first counting of seedlings was conducted after 7 days and the second one after 14 days. The seedlings were stored in a growth chamber at a constant temperature of 25 °C.

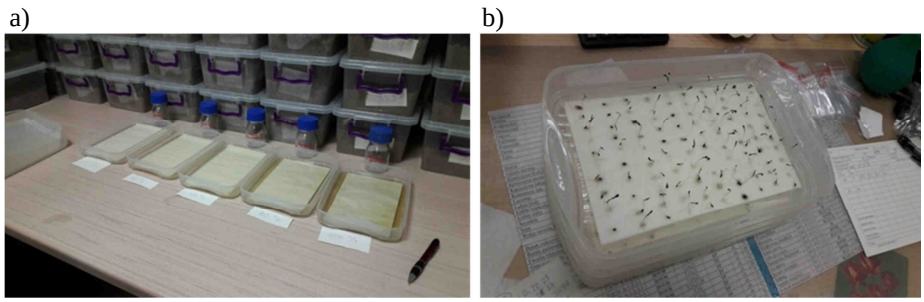


Fig. 2. a) Soaking of seeds in different concentrations of algal extracts, b) first carrot seedlings after 7 days (germination energy). Photo: Sylwia Lewandowska

Multielemental analysis of carrot's seedlings

ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry) technique was applied to determine multielemental composition of carrot seedlings. Analyses were performed using Varian VISTA-MPX ICP-OES apparatus (Victoria, Australia) in the Chemical Laboratory of Multielemental Analysis at Wroclaw University of Science and Technology (accreditation number No AB 696 of PCA - Polish Centre for Accreditation). Before analysis, dry carrot's seedlings were digested with 69 % nitric acid (Suprapur, Merck KGaA, Darmstadt, Germany) in a microwave oven - StartD (Milestone MLS-1200 MEGA, Bergamo, Italy). The analysis of the content of nitrogen (N) and carbon (C) in carrot's seedlings was performed using CN analyser - Vario Macro Cube (Elementar Analysensysteme GmbH, Germany).

Statistical analysis

Statistica software (ver. 12.0; TIBCO Software Inc., Tulsa, OK, USA) was used to elaborate the obtained results. The normality of the distribution of experimental results was assessed by the Shapiro-Wilk test and the homogeneity of variances by the Brown and Forsythe test. The differences between groups were determined with the one-way analysis of variance (ANOVA) using the Tukey multiple comparison test (for normal distribution and the homogeneity of variances). If the conditions about normal distribution and the homogeneity of variances were not met, Kruskal-Wallis test (nonparametric test) was used. The results were considered significantly different when $p < 0.05$.

Results and discussion

Literature data confirm that biostimulants used in the cultivation of carrot promote plant growth [3], root yield [3-8], macroelements content in storage roots of carrot [4, 9], carrot emergence [6], chemical composition of the roots: dry matter, soluble sugars, carotenoids [7], content of L-ascorbic acid and total sugars in carrot roots [8].

In the present study, preliminary experiments were performed on carrot seeds in order to establish the best concentration of algal extract among examined 20, 40, 60, 80 and 100 %. Table 1 presents results for the number of germinated seeds (normal seedlings), abnormal seedlings, dead seeds and the content of nitrogen and carbon in the normal seedlings.

Table 1
The effect of different concentrations of algal extracts on germination of carrot seeds and the content of nitrogen and carbon in the biomass (for 50 seeds in each repetition; mean ± *SD*; $n = 4$)

Group	Normal seedlings	Abnormal seedlings	Dead seedlings	N [% d.m.]	C [% d.m.]
Control	33 ±1(c)	9 ±1	8 ±1	10.1 ±2.5	41.73 ±0.45
E-20 %	31 ±5(a)	10 ±1	9 ±5	13.4 ±2.1	43.2 ±2.6
E-40 %	23 ±2	15 ±2	13 ±2	13.1 ±3.8	40.45 ±0.49
E-60 %	29 ±10	11 ±6	10 ±4	11.7 ±3.1	39.1 ±2.3
E-80 %	32 ±4(b)	9 ±2(*a*)	9 ±4	5.97 ±0.62	40.6 ±4.5
E-100 %	18 ±5(abc)	20 ±3(*a*)	12 ±3	13.0 ±3.3	40.7 ±3.3

(a, b, c) statistically significant difference ($p < 0.05$; Tukey test - explanations in the text)
(*a*) statistically significant difference ($p < 0.05$; Kruskal-Wallis test)

Taking into account the number of germinated seeds, the highest value was for the control group, and then E-20 % and E-80 %. These values are comparable. There is no clear relationship between the extract concentration and the number of germinated carrot seeds. But the weakest germination of carrot seeds was observed for the highest concentration of algal extract. Statistically significant differences were observed between E-100 % and E-20 % - a, between E-100 % and E-80 % - b and between E-100 % and the control group - c (Table 1). The results obtained in the present study are comparable with our previous research where it was also shown that higher concentrations of *C. glomerata* extract (50 and 100 %) inhibited the germination of another plant specious - soybean [44]. The literature depicts that there are some papers concerned with the effects of the concentration of algal extract on seeds germination and parameters of plant growth. For example, Rathore et al. tested different algal concentrations, starting from 2.5, 5, 7.5, 10, 12.5 until 15 % [45]. The highest soybean yield was recorded for 15 % seaweed (*Kappaphycus alvarezii*) extract. Also Lodhi et al. [46] in their research avoided higher concentrations of algal extracts than 15 % in the cultivation of soybean. The biomass of algae is known to be rich in biologically active compounds that can positively affect the metabolism of plants, their growth and development of seedlings. Used in the present study freshwater *Cladophora glomerata* contains micro- and macroelements [47], vitamins including vitamin C (21.7 mg/100 g d.m.) and α-tocopherol (2.5 mg/100 g d.m.), polyphenols (196 mg/100 g d.m.) and a wide range of amino acids [48]. However, still further research is needed to determine the precise concentration of these compounds in order to obtain higher number of well-developed seedlings.

Concerning the content of nitrogen and carbon in germinated carrot, no statistically significant differences ($p < 0.05$) were noted between experimental groups. The best results concerning the content of nitrogen and carbon were determined for the group treated with 20 % algal extract. The content of nitrogen was by 33 % higher than in the control group and carbon by 3.5 %. The value of 20 % algal extract was selected for further research. These results are consistent with literature data - Rathore et al. [45] also found that the application of 15 % seaweed (*Kappaphycus alvarezii*) extract increased significantly N, P and K uptake by soybean grains. Extract from green macroalga - *Ulva lactuca* increased significantly the nitrogen content in lettuce and mustard. In the work of Jannin et al. [49] it was shown that the extract from brown seaweed (*Ascophyllum nodosum*) caused the activation of genes associated with the metabolic pathway of nitrogen. It is suggested that the initial nutrient content in algae and algal-based products would have an impact on

nutrient concentrations in the soil and consequently in the plant biomass [50]. In our recent study it was shown that the raw biomass of *Cladophora glomerata*, as well as obtained 100 % extract contain numerous micro- and macroelements that can influence not only plant growth, but also multielemental composition of cultivated plant [41, 44]. In the case of *C. glomerata* extract, concentrations of these elements were as follows: Ca = 185.6 ±3.4, K = 829.7 ±7.9, Mg = 28.92 ±0.87, S = 220.2 ±0.8, P = 19.87 ±0.08, Cu = 0.1726 ±0.0026, Fe = 4.285 ±0.097, Mn = 1.314 ±0.0074, Zn = 0.2614 ±0.0442, Na = 23.06 ±1.23 mg/dm^3 [41]. Algal extracts applied as biostimulants of plant growth may enhance root growth of plant to increase the nutrient uptake, as well as can enhance the effectiveness of nutrient utilization [45].

In the next step, we examined the effect of 20 % algal extract (chosen on the basis of preliminary research) on the germination of carrot seeds exposed additionally before sowing to the static magnetic field for 3, 6 and 12 minutes. The obtained results are presented in Table 2. The number of germinated seeds was comparable in all experimental groups. The differences between tested groups were not statistically significant (for $p < 0.05$). The same was observed for the number of abnormal seedlings with lack of root or primary infection and dead seeds.

Table 2

The number of normal and abnormal seedlings and dead seeds in germination tests (for 100 seeds in each repetition; mean ±*SD*; *n* = 4)

Group	Normal seedlings	Abnormal seedlings	Dead seeds
1	89 ±3	2 ±1	9 ±3
2	88 ±3	4 ±2	8 ±3
3	87 ±2	3 ±2	10 ±3
4	88 ±4	3 ±1	9 ±5
5	88 ±1	2 ±1	10 ±2
6	88 ±3	2 ±1	10 ±3
7	86 ±3	4 ±1	10 ±3
8	85 ±1	3 ±1	12 ±1
9	85 ±3	2 ±1	13 ±4
10	87 ±2	2 ±1	11 ±2
11	85 ±1	3 ±1	12 ±2
12	84 ±5	3 ±1	13 ±4
13	88 ±3	2 ±1	10 ±2
14	86 ±3	2 ±1	12 ±2

Additionally, we examined the multielemental composition of germinated carrot's seedlings, results of which are presented in Table 3. Generally, the content of nitrogen and carbon was comparable in all experimental groups (2-13), but slightly smaller than in the control group (1 - only water). Carrot seedlings obtained in the group with the algal extract (14) had the highest nitrogen content when compared with other groups (1-13). It was by 25 % higher than in the control group (1). It confirms the results obtained in the experiments testing different concentrations of *Cladophora glomerata* extract. Generally, the level of most elements (macroelements: Ca, K, Mg, S and microelements: Cu, Fe, Mn and Zn) was the highest in the group with algal extract applied alone. Seedlings of carrot whose seeds were exposed to SMF and algal extract also exhibited increased content especially of K, Mg, S and Zn, when compared with the control group (1). These results are in accordance with literature data. In the work of Szczepanek et al. [4] the application of

seaweed extract from *Ecklonia maxima* (Kelpak SL - extract containing phytohormones) in field experiments resulted in the increase in the Mg, P, Na and K content in storage roots of carrot. Potassium is recognized as the most important macroelement in carrot [51]. In this study it was shown that the content of K in the carrot biomass was 3.5 times higher than in the control group.

Table 3

Multielemental composition of germinated carrot's seedlings (n = 4)

Element	N	C	Ca	Cu	Fe	K
Group	[% d.m.] ±*SD*		[mg/kg d.m.] ±*SD*			
1	4.74 ±0.060	45.59 ±0.50	14 511 ±2 902	29.1 ±4.4	148 ±22	17 141 ±3 428
2	4.65 ±0.15	45.51 ±0.94	13 234 ±2 647	20.1 ±3.0	136 ±20	17 893 ±3 579
3	4.65 ±0.050	45.52 ±0.35	12 433 ±2 487	20.7 ±3.1	143 ±21	18 392 ±3 678
4	4.73 ±0.070	45.40 ±0.13	11 862 ±2 372	30.6 ±4.6	165 ±25	20 573 ±4 115
5	4.71 ±0.070	45.49 ±0.29	8 529 ±1 706	13.9 ±2.1	80 ±12	10 942 ±2 188
6	4.68 ±0.30	**46.25 ±0.46**	14 690 ±2 938	18.3 ±3.6	128 ±19	15 579 ±3 116
7	4.79 ±0.10	45.93 ±0.36	14 726 ±2 945	17.3 ±2.6	119 ±18	16 458 ±3 292
8	4.66 ±0.20	45.15 ±0.67	14 010 ±2 892	16.8 ±2.5	125 ±19	27 333 ±5 467
9	4.66 ±0.13	44.92 ±0.24	8 196 ±1 638	12.4 ±1.9	76 ±11	18 078 ±3 616
10	4.78 ±0.17	44.58 ±0.70	12 945 ±2 589	19.0 ±2.8	157 ±24	33 240 ±6 648
11	4.54 ±0.22	45.28 ±0.44	13 030 ±2 606	16.8 ±2.5	127 ±19	28 279 ±5 656
12	4.58 ±0.19	44.7 ±2.5	15 131 ±3 026	21.2 ±3.2	142 ±21	32 060 ±6 412
13	4.64 ±0.19	44.62 ±0.65	13 405 ±2 681	16.8 ±2.5	121 ±18	31 185 ±6 237
14	**5.93 ±0.30**	40.18 ±0.69	**24 576 ±4 915**	**41.8 ±6.3**	**180 ±27**	**61 332 ±12 266**

Element	Mg	Mn	Na	P	S	Zn
Group	[mg/kg d.m.] ±*SD*					
1	5 224 ±1 045	67 ±10	2 271 ±454	9 408 ±1882	3 639 ±728	100 ±15
2	5 313 ±1 063	63.2 ±9.5	2 699 ±540	9 002 ±1800	3 659 ±732	105 ±16
3	5 306 ±1 061	58.7 ±8.8	2 902 ±580	9 108 ±1822	3 634 ±727	94 ±14
4	5 441 ±1 088	61.8 ±9.3	**3 273 ±655**	9 531 ±1906	3 713 ±743	107 ±16
5	3 147 ±629	38.0 ±5.7	1 569 ±314	5 458 ±1092	2 211 ±442	63.2 ±9.5
6	5 108 ±1 022	59.1 ±8.9	1 953 ±391	8 849 ±1770	3 474 ±695	90 ±13
7	5 143 ±1 029	61.6 ±9.2	2 182 ±436	8 982 ±1796	3 574 ±715	87 ±13
8	5 368 ±1 074	52.9 ±7.9	2 065 ±413	8 904 ±1781	4 147 ±829	92 ±14
9	3 378 ±676	32.3 ±4.8	1 458 ±292	5 582 ±1116	2 628 ±526	55.6 ±8.3
10	5 563 ±1 113	48.9 ±7.3	2 645 ±529	9 389 ±1878	4 501 ±900	106 ±16
11	5 316 ±1 063	53.2 ±8.0	2 109 ±422	8 747 ±1749	4 091 ±818	93 ±14
12	6 112 ±1 222	63.7 ±9.6	1 956 ±391	**10 456 ±2091**	4 945 ±989	108 ±16
13	5 484 ±1 097	51.3 ±7.7	2 510 ±502	9 292 ±1858	4 397 ±879	96 ±14
14	**6 629 ±1 326**	**147 ±22**	2 070 ±414	8 263 ±1653	**5 099 ±1 020**	**223 ±33**

Bold - the highest values in a given experimental group; *SD* - standard deviation

Many researchers indicate that magnetic field stimulation causes changes in the course of some biochemical and physiological processes in seeds and plants that have a positive effect on plant development and productivity [33, 52-61]. As a result of the external magnetic field, the plants intensify gas exchange, and as a result, photosynthesis and enzyme activity increase. The effects of MF use in relation to chlorophyll content have been documented for many plant species [35, 61-63]. Pre-sowing plant stimulation with a constant or alternating magnetic field resulted in the increase of chlorophyll *a* (Chl *a*). Polyphasic Chl *a* fluorescence transients from the samples that was treated with a magnetic

field caused a higher fluorescence yield [54, 63-67]. This in turn results in better plant growth and ultimately increase crop yields [11, 21, 28, 40, 69-77].

Conclusion

In the present study, algal extracts and stimulation of seeds by the magnetic field were examined as factors that could increase the number of germinated seeds. Individual and combined application of these operations did not increase the germination of seeds, but influenced the chemical composition of carrot's seedlings - its multielemental composition (Ca, K, Mg, S, Cu, Fe, Mn and Zn), especially when algal extract was applied alone. The number of germinated seeds was comparable in all experimental groups and higher than 84 %. The results also showed that lower concentrations of *Cladophora* extract (20 %) increased the content of nitrogen and carbon in germinated seedlings. The use of the static magnetic field (SMF) at higher doses (induction value/time) increases the carbon and sodium content. The combination of SMF with algae extract causes a significant increase the phosphorus content in germinated seedlings. Definitely, further research is needed to determine the presence and concentration of biologically active compounds in algae extract in conjunction with SMF, which can be responsible for these effects.

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