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Influence of Microalgae *Planktochlorella nurekis* Clones on Seed Germination

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Abstract: Microalgae are a rich source of plant hormones, vitamins, and other substances that can influence plant physiological metabolism, which in turn affects plant development, biotic and abiotic stress resistance, and yield. This study aimed at testing microalgae *Planktochlorella nurekis* clones obtained by co-treatment with colchicine and cytochalasin on four plant species to check their potential use as biostimulators in agriculture. The results are valuable for breeders, farmers, and microgreen producers. Eleven clone extracts in 1%, 5%, and 10% concentration were tested on four plant species: lettuce, wheat, broccoli, and radish. Germination and seedling characteristics (leaf and root length, fresh weight) were measured for each species. *P. nurekis* extracts show both a stimulating and inhibitory effect on tested plants, depending on the tested concentration, plant species, and algal clone tested. Co-treatment with colchicine and cytochalasin may be a good source of clones for potential use in agriculture as biostimulators and herbicides.

Keywords: microalgae; *Planktochlorella nurekis*; seed germination; sprouts

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1. Introduction

Continuous population growth leads to an increase in the demand for food [1]. In addition, modern agriculture is increasingly looking for products of natural origin in order to reduce the use of chemicals [2]. The biotechnology of microalgae, which is used for the production of natural fertilizers, is helpful in this respect. Microalgae are increasingly attracting the attention of scientists around the world [3,4]. In Roman times, plant seedlings were wrapped with algae to improve their growth. Nowadays, algal extracts are regaining popularity, and a few products based on algal extracts are already commercially available [5].

Microalgae are single-celled, autotrophic and photosynthetic organisms. Their natural habitats are freshwater and saltwater. Microalgae can be divided into four groups: cynaobacteria, rhodophytes, chlorophytes, and chromophytes [6]. Microalgae have many uses, ranging from use as food additives for humans and animals to applications in the cosmetics industry. They are also used for the production of such molecules as, for example, dyes or fatty acids as well as biofuels [7].

Plant germination is initiated by the activation of gibberellins (GA). The activation of GA inhibits abscisic acid (ABA) activity as it is active during seed dormancy and plays a role in germination inhibition [8]. Apart from germination, GA are involved in a few developmental pathways in the plant, namely stress regulation and cell division, which affect plant growth and development [9]. Auxin (IAA) is involved in the cell cycle, growth, and development of plant parts, including pollen, embryo, leaves, and roots [10]. It also plays a role in seed dormancy [11]. Cytokinins boost cell division in the plant and also during seed germination. Together with auxins, they play a role in shoot and root elongation, respectively [12]. It is worth mentioning that the individual application of the phytohormones BAP, NAA, or GA3 did not improve the germination rate compared to the water baseline control [13]. Microalgae are a great source of phytohormones, and in

inner, natural composition with other substances, they can boost germination, which is important not only in crop plant cultivation but also in sprout and microgreen production. Nowadays, consumers are seeking food that provides health benefits, hence the increasing popularity of sprouts [14]. The results suggest that extracts from algae are suitable as an alternative to synthetic biostimulants used in the production of sprouts [15,16].

Biostimulants are defined as materials other than fertilizers that promote plant growth when used in small amounts [17]. Plant growth regulators (PGRs) can promote germination and plant development, speed up the production cycle, and increase production efficiency, which is significant when it comes to high-value crops, not only in field environments but especially in controlled environments [18]. Phytohormones are produced by plants as well as other organisms, including microalgae [19]. In recent years, the use of algae extracts as fertilizers in organic farming has been increasing [20–22]. Algae extracts contain a rich set of phytohormones, amino acids, fatty acids, and microelements responsible for controlling the growth and development of plants and increasing resistance to pathogens. Data confirming the positive effect of algae and algal extracts on the growth of vegetables, fruits, and other crops are available in the literature. Algae extracts are used for seed conditioning as well as soil or foliar application during the growing and flowering period. They stimulate seed germination, growth, and yields of various crops. Algae components such as macro- and micronutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid-like hormones (ABA) influence metabolism in treated plants, leading to increased growth and yield [3,20]. They also improve soil fertility as well as pest and disease control, and they contribute to the removal of toxins. Despite the analysis of various chemical components of algae extracts, their mechanism of action on plants is not fully understood. We are probably dealing with a synergistic effect of many substances, both known and unknown [23].

Planktochlorella nurekis Skaloud & Nemcová is a new species of microalgae reported in 2014. This species has mononuclear, spherical cells surrounded by a two-layer cell wall. *Planktochlorella nurekis* is characterized by an asexual mode of reproduction using autospores [7]. Recently, this species was characterized in terms of biochemistry, and 11 clones were obtained by co-treatment with colchicine and cytochalasin. Clones showed increased antioxidant activity, increased content of B vitamins, higher lipid levels, and antimicrobial activity [24–26]. This study investigated the influence of the 11 clones on the germination capacity of selected crop plants and the morphological features of the tested plant sprouts.

2. Materials and Methods

2.1. Materials

Research material, from the company Bioorganic Technologies Sp. z o.o. (Sielec, Poland), was the dry mass of 11 selected algae clones and was compared to the wild type (WT). A detailed description of the research material procured and its characteristics is presented in the publication by E. Szpyrka et al., 2020 [25].

Studies were conducted on selected crops:

- *Brassica oleracea* L.—cultivar: Cezar (W. Legutko, Przedsiębiorstwo Handlowo-Nasienne Sp. z o.o.)
- *Lactuca sativa* L.—cultivar: May King—(W. Legutko, Przedsiębiorstwo Handlowo-Nasienne Sp. z o.o.)
- *Triticum aestivum* L.—spring wheat, cultivar: Serenada, (Hodowla Roślin Strzelce)
- *Raphanis sativus* L.—cultivar: Mino Early (W. Legutko, Przdsiębiorstwo Handlowo-Nasienne Sp. z o.o.)

2.2. Preparation of Seaweed Extract

The dry mass of 12 strains of algae was ground with laboratory grinder WŻ-1S (Sadkiewicz Instruments) for 5 s, then 15 g of each were selected. The prepared samples were flooded with 150 mL distilled water, incubated 12 h, and constantly shaken at room temper-

ature. The extracts obtained were diluted in distilled water to obtain test concentrations of 1%, 5%, and 10% (related to dry mass) (Figure 1) The control test was distilled water (0%).

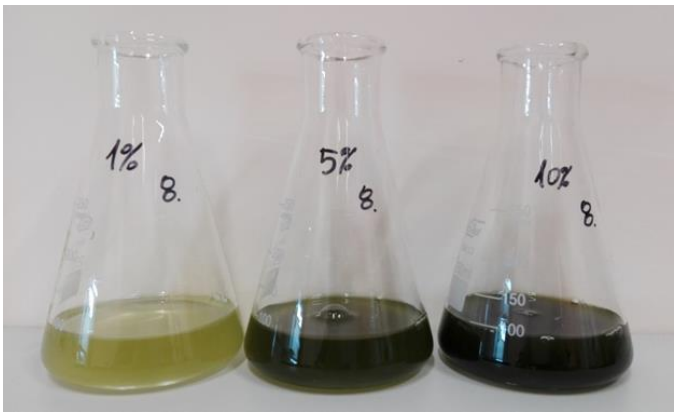

Figure 1. Extracts obtained from clone number 8 (1%, 5%, and 10%).

2.3. Germination Test

The seeds of the test plant were sterilized in 4% sodium hypochlorite solution for 10 min and then rinsed three times in sterile water. Plant cultivation was carried out in Petri dishes (diameter 9 cm) lined with two discs of filter paper (medium quality filters, weight 80, ash content 0.1). Each pan was lined with ten seeds of the test plant. The studies were conducted in three biological repetitions for each concentration of the extract and control. The seeds were exposed to different concentrations of the algae strains tested by watering them with 5 mL of the appropriate concentration extract and 5 mL of distilled water (control). The plants were grown in a breeding room at 24 ± 2 °C day/night (16/8) for 5 days. During this time, seed germination was observed, and the number of germinated plants was counted each day of the experiment. The length of the longest roots, the length of the leaves, and fresh mass were measured after 5 days (Table 1) [27]. The following parameters were determined from the results obtained:

- Germination energy (%GE) [28] after 3 days: %GE = (number of germinated seeds/total number of seeds) × 100
- Growth parameters—length of root and leaves (cm)
- Fresh plant weight (g).

Table 1. Plant seedlings measurements.

Observation	Method	Unit	Day of Measurement
Germination energy	%GE formula	%	at day 3
Length of root and leaves	Ruler	cm	at day 5
Fresh plant weight	Scale (Radwag PS 210.R2, precision 1 mg)	g	at day 5

2.4. Statistical Analysis

Mean values ± SD were calculated on the basis of at least three independent experiments. All data were analyzed for significant differences by analysis of variance—ANOVA. Statistical significance was evaluated using GraphPad Prism 8 using one-way ANOVA and Dunnett's test. A p-value < 0.05 was considered as a statistically significant.

3. Results

3.1. Germination Energy

Seed germination for all tested plants differed depending on the algae clone used and the concentration of extracts; there were also visible differences in seed germina-

tion in relation to the control sample. Differences in the germination energy of lettuce (*Lactuca sativa* L.), wheat (*Triticum aestivum* L.), and radish (*Raphanis sativus* L.) seeds treated with 1%, 5%, and 10% concentration of extracts reached statistical significance as compared to the control. In the case of broccoli (*Brassica oleracea* L.), statistically significant differences in germination energy appeared in relation to the control when the extract with 1% concentration was used.

The conducted broccoli seed germination test showed that treatment with 1% concentration of clone 5 and 11 extracts decreased the germination energy as compared to the control (Figure 2a, Table 2). The remaining clones did not significantly affect seed germination. The biggest differences between the action of the extracts are visible in the germination energy of lettuce seeds. Six algae clones decreased germination energy (Figure 2b, Table 2). Clones 1 and 9 had the greatest impact. For wheat, clone 8 decreased the seed germination rate (Figure 2c, Table 2). On the other hand, clone 7, used in radish seeds, increased the germination energy (Figure 2d, Table 2).

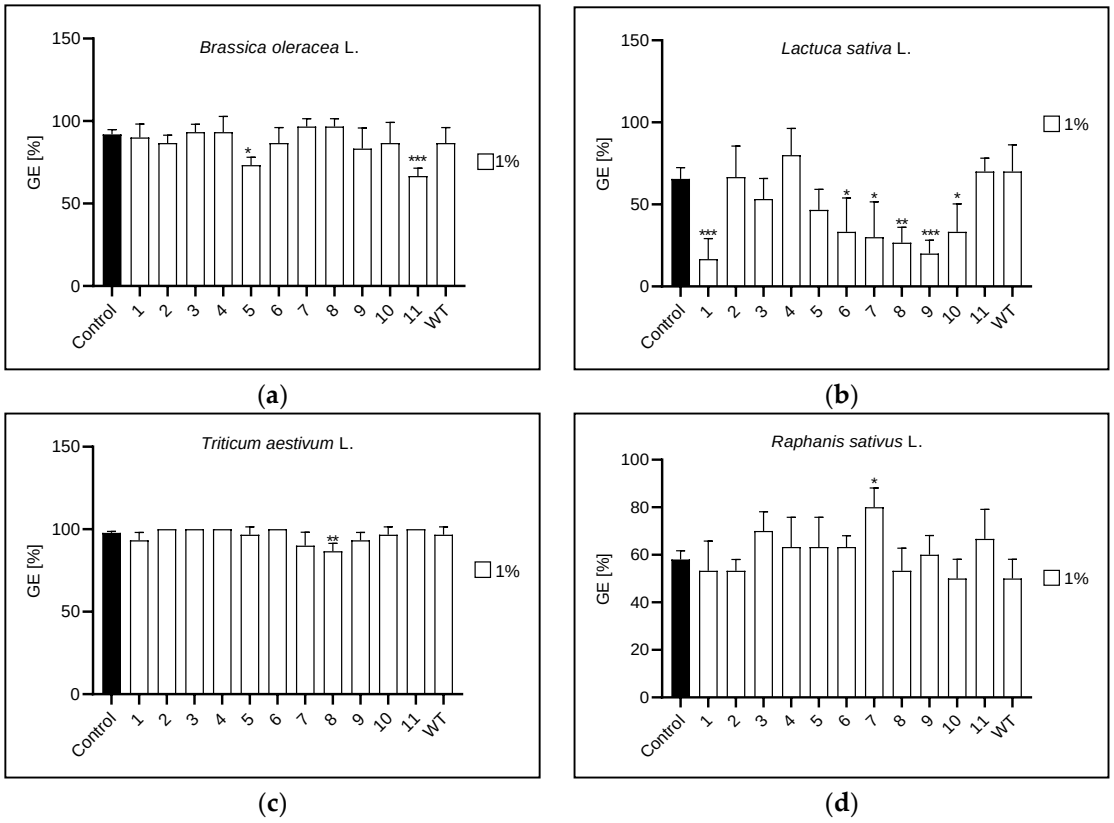

Figure 2. Percentage of germination of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) seeds treated with 1% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ compared to control (ANOVA and Dunnett's a posteriori test).

The changes in the germination energy of broccoli seeds resulting from the use of algae extracts at a concentration of 5% did not reach a statistically significant level (Figure 3a, Table 2). Therefore, it can be concluded that this concentration does not affect the germination energy of broccoli seeds. Germination of lettuce seeds differed depending on the algae clone used at 5% concentration (Figure 3b, Table 2). Clone 11 caused a slight increase in the germination energy, while clones 1, 2, 3, 6, 7, 8, 9, and WT caused a decrease in the germination energy of lettuce seeds. Clones 1, 3, 6, 7, 8, and 9 had the greatest impact on seed germination ($p < 0.0001$). In the case of wheat seeds, no significant changes in the germination energy were observed; only clone 10 caused a slight decrease in the germination energy (Figure 3c, Table 2). The application of 5% concentrations of algae

clones to radish seeds caused a decrease in the germination energy for clones 2 and 3 but at a small level ($p < 0.1$) (Figure 3d, Table 2).

Table 2. Influence of different algal clones concentrations (conc.) on four tested parameters in four plant species. Each number represents the clone. In green are positive effects, and in red are negative effects. Neutral effects are represented by n. Significant effects are displayed in bold for *** $p < 0.001$ or **** $p < 0.0001$ or without bold for * $p < 0.1$ or ** $p < 0.01$.

Parameter	Conc.	Broccoli	Lettuce	Wheat	Radish
Germination energy	**1%**	5, **11**	**1**,6,7,8,**9**,10	8	7
	5%	n	**1**,2,**3**,**6**,**7**,**8**,**9**,11,WT	10	2,3
	10%	n	**1**,**2**,**3**,**4**,**5**,**6**,**7**,**8**,**9**,**10**,**11**,**WT**	4	2,3,4,7,10,WT
Root length	**1%**	1,3	1,**3**,4,**5**,6,7,	1,5,6,7,8,**WT**	9,WT
	5%	3,9,10	7,10,11,WT	**1**,**3**,5,7,**8**,9,**10**,**WT**	3,**6**,**11**
	10%	n	10	**1**,2,3,5,**7**,**8**,9,**10**,11,WT	7,11
Leaf length	**1%**	n	10	2,8,9,11	2,4,**8**,9,11,**WT**
	5%	11	1,WT	1,**2**,**4**,**6**,7,9,11	1,3,**11**
	10%	n	9	4,6,8	**1**,**2**,**3**,**4**,**5**,**6**,**7**,**8**,**9**,**10**,**11**,**WT**
Fresh weight	**1%**	n	6,8	**WT**	7,**9**,10,**WT**
	5%	10	**1**,3,**5**,**6**,**7**,**8**,**9**,11,**WT**	3	1,3,**4**,**8**,11
	10%	n	2,3,4,5,6,7,9,WT	4	1,**4**,**5**,**7**,**9**,**10**,11

For broccoli seeds, the use of a 10% concentration of the studied algae clones did not cause statistically significant changes (Figure 4a, Table 2). This concentration had a great influence on the germination energy of lettuce seeds. Each of the used algae clones caused a significant decrease in the germination energy at the level of $p < 0.0001$ (Figure 4b, Table 2). Clone 1 completely inhibited the sprouting of lettuce seeds. Germination of wheat seeds after applying a 10% concentration of clone 4 slightly decreased the germination energy (Figure 4c, Table 2). The remaining clones did not affect seed germination. In the case of radish, clones 2, 3, 4, 7, 10, and WT slightly decreased germination energy ($p < 0.1$) (Figure 4d, Table 2).

Analysis of the influence of algae clones at different concentrations on the germination of selected seeds showed that lettuce seeds were the most susceptible to their effects. With an increase in the concentration of the extract used, the germination energy of seeds decreased. The exception was clone 11, which, when used at 5% concentration, caused a slight increase in the germination energy. For all seeds, the use of algae extracts was observed to cause a decrease in germination energy, with a few exceptions (clone 7–1%—radish, clone 11–5%—lettuce), but these changes were small.

3.2. Root and Leaf Length

The length of the roots and leaves of the sprouts of the tested plants also depended on the algae clone used and the concentration of its extract. Differences in root and leaf length with respect to control for each plant reached statistical significance under the influence for all test concentrations.

3.2.1. Root Length

The root length of broccoli sprouts and lettuce increased under the influence of some algae clones at a concentration of 1%. For broccoli, clones 1 and 3 caused increased root growth (Figure 5a, Table 2); in the case of lettuce, it was clones 1, 3, 4, 5, 6, and 7 (Figure 5b, Table 2). Algae clones at a concentration of 1% caused slight wheat root growth. The remaining algae clones (1, 6, 7, 8, and WT) reduced the root length of wheat germ (Figure 5c,

Table 2). In the case of radish sprouts, clone 9 and WT slightly decreased root length (Figure 5d, Table 2).

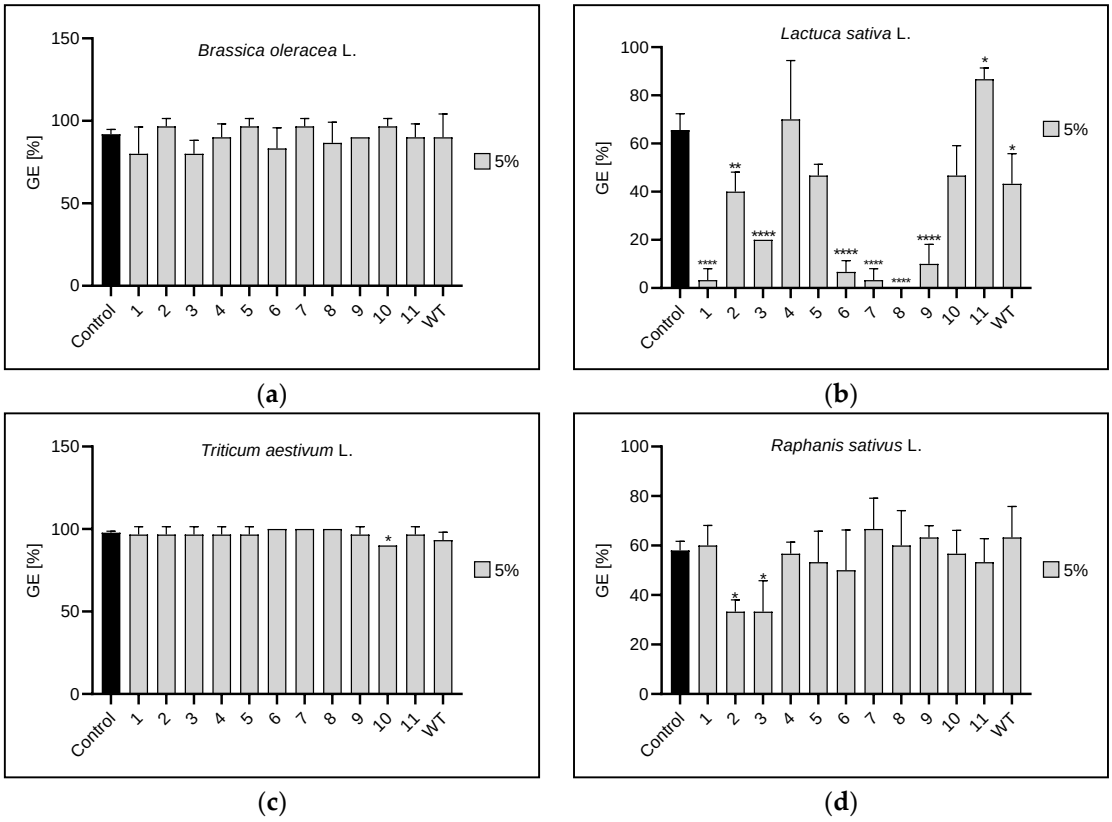

Figure 3. Percentage of germination of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) seeds treated with 5% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

The use of algae clones at a concentration of 5% had an effect on the root length for all the sprouts of the tested plants. In the case of broccoli sprouts, three algae clones (3, 9, and 10) slightly increased the root length (Figure 6a, Table 2). Similarly, in the case of lettuce sprouts, algae clone 10, 11, and WT increased the length of the roots, while clone 7 caused a decrease in the length of the roots of the lettuce sprouts (Figure 6b, Table 2). The greatest changes in root length were seen in wheat germ. Clones 3 and 5 caused an increase in root length, while clones 1, 7, 8, 9, 10, and WT caused a decrease in the root length of wheat germ (Figure 6c, Table 2). In the case of radish sprouts, three clones caused changes in root length (Figure 6d, Table 2). The third and eleventh clones reduced the length of the roots, while the sixth significantly contributed to the growth of the length of the radish sprouts.

The use of a 10% concentration of algae clones did not affect the root length of broccoli sprouts (Figure 7a, Table 2). Clone 10 caused an increase in the length of lettuce sprouts to a small extent (Figure 7b). In the case of wheat germ, clones 4 and 6 did not affect the size of the roots. Clone 3 and 5 caused a slight growth of sprout roots. The remaining algae clones reduced the root length of wheat germ (Figure 7c, Table 2). Two clones (7 and 11) influenced the length of radish sprouts, causing a reduction in the length of the sprouts' roots (Figure 7d, Table 2).

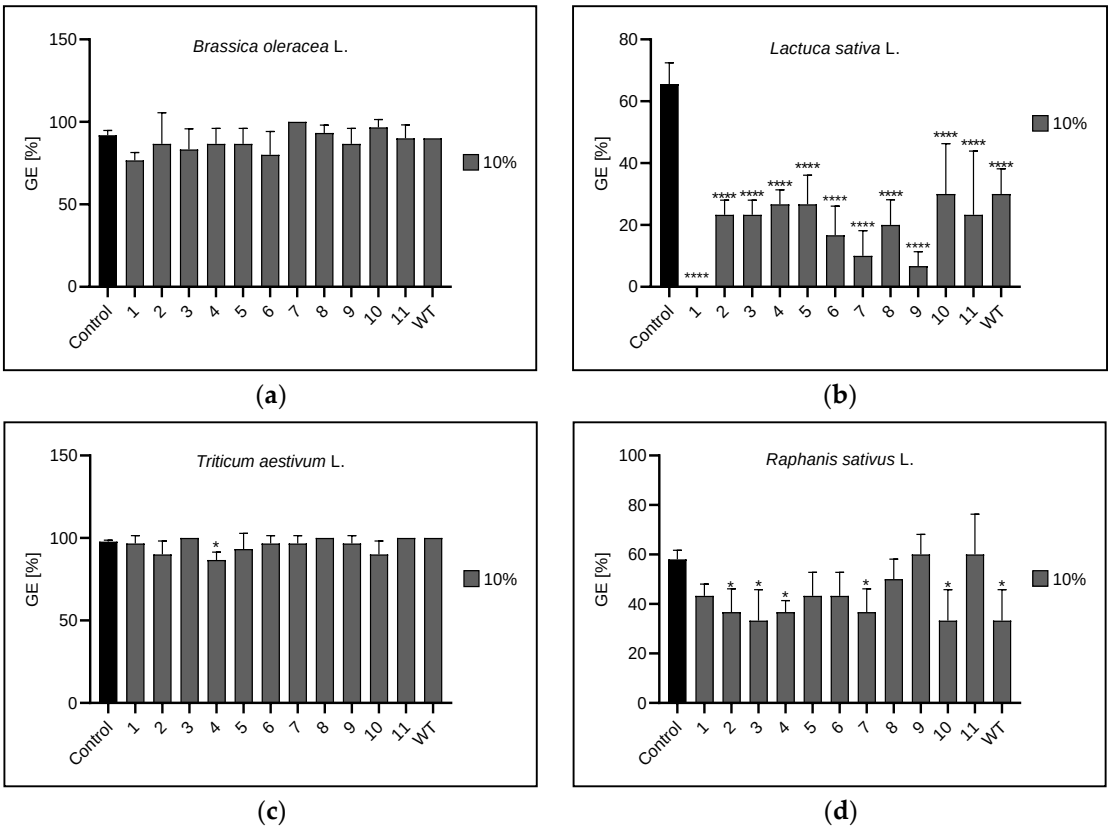

Figure 4. Percentage of germination of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) seeds treated with 10% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

3.2.2. Leaf Length

Algae clones applied at 1% concentration did not change the length of broccoli sprouts (Figure 8a, Table 2). Clone 10 caused a decrease in leaf length of the lettuce sprouts (Figure 8b, Table 2). In the case of wheat germ, the leaf length increased when four algae clones were applied (Figure 8c, Table 2). Clone 2 had the greatest impact at $p < 0.001$. The greatest changes were seen in the size of the leaves of the radish sprouts. Six algae clones reduced the leaf length of the sprouts (Figure 8d, Table 2).

The use of algae extract at a concentration of 5% caused a slight increase in the length of broccoli sprouts treated with clone 11 (Figure 9a, Table 2). The leaf length of lettuce sprouts decreased under the influence of the first clone and WT (Figure 9b, Table 2). Seven algae clones had an effect on the leaf length of wheat germ (Figure 9c, Table 2). Clone 4 caused a significant reduction in leaf length, while the remaining ones elongated the leaves. Radish sprout leaf length was reduced under the influence of clones 1, 3, and 11 (Figure 9d, Table 2).

The leaf length of broccoli sprouts did not change under the influence of a concentration of 10% (Figure 10a, Table 2). In the case of lettuce sprouts, clone 9 caused a reduction in the length of sprout leaves (Figure 10b, Table 2). The length of wheat leaves was reduced under the influence of two strains (clones 4 and 6); meanwhile, clones 2 and 8 caused an increase in the length of wheat germ leaves (Figure 10c, Table 2). The concentration of 10% for all algae clones caused a significant reduction in the length of the leaves of radish sprouts (at the level of $p < 0.0001$) (Figure 10d, Table 2).

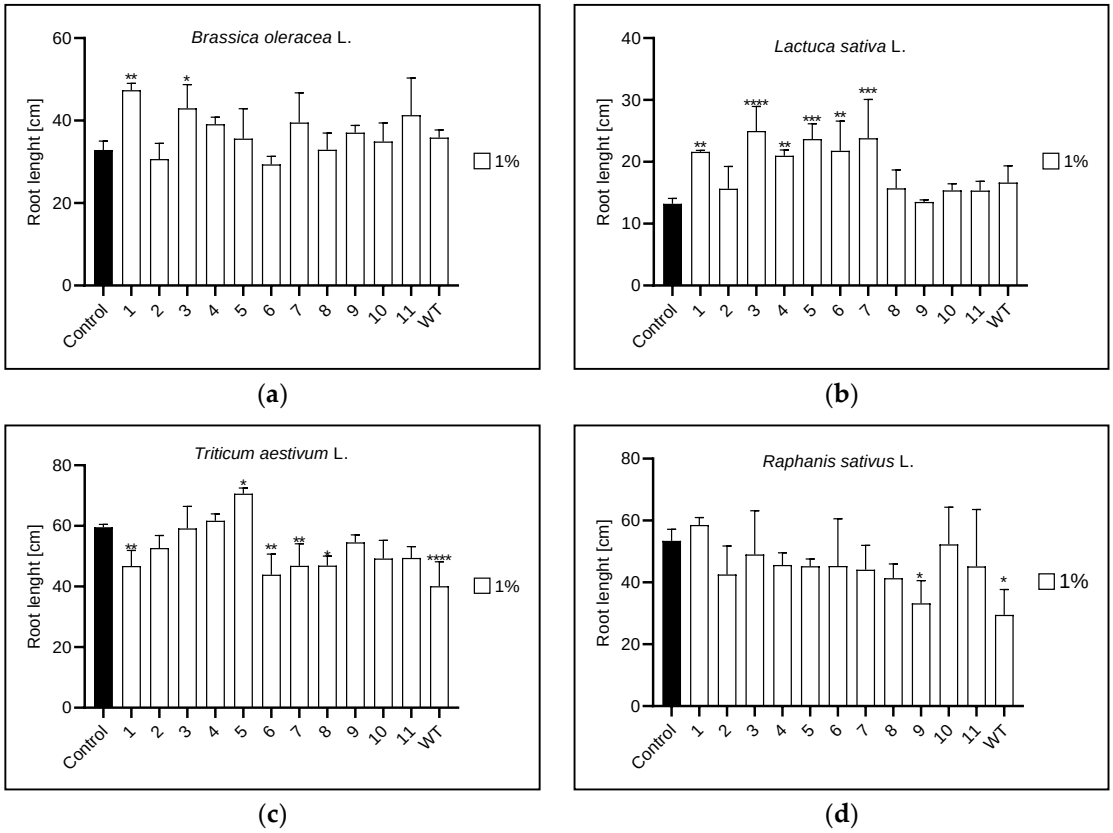

Figure 5. Root length of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 1% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

3.3. Fresh Plant Weight

The influence of selected algae clones on the plants' fresh mass was also investigated. Apart from the concentration of 1% in the case of broccoli sprouts, the remaining concentrations reached statistically significant differences in the weight of the plants compared to the control.

The changes that occurred in the weight of sprouts of the tested plants under the influence of algae extracts at a concentration of 1% are presented in the graphs (Figure 11a–d, Table 2). The changes in the weight of broccoli sprouts were not significant (Figure 11a, Table 2). In the case of lettuce sprouts, the sixth clone caused a significant decrease in weight, while clone 8 was marked by a significant increase in the weight of the lettuce sprouts (Figure 11b, Table 2). The WT clone caused a decrease in the weight of wheat germ (Figure 11c, Table 2). The weight of radish sprouts slightly increased under the influence of clone 10, and clone 7 caused a slight decrease in weight, while clone 9 and WT had the greatest effect on the weight loss of radish sprouts (Figure 11d).

Under the influence of algae extracts at a concentration of 5%, the weight of the tested plants' sprouts either decreased or increased. Clone 10 caused an increase in the weight of broccoli sprouts (Figure 12a, Table 2). Nine out of twelve analyzed algae clones caused a decrease in the weight of lettuce sprouts to a greater or lesser extent (Figure 12b, Table 2). In the case of wheat germ, the weight increased as a result of the action of the third algae clone (Figure 12c, Table 2). The weight of radish sprouts decreased under the influence of clones 1, 3, 4, 8, and 11 (Figure 12d, Table 2).

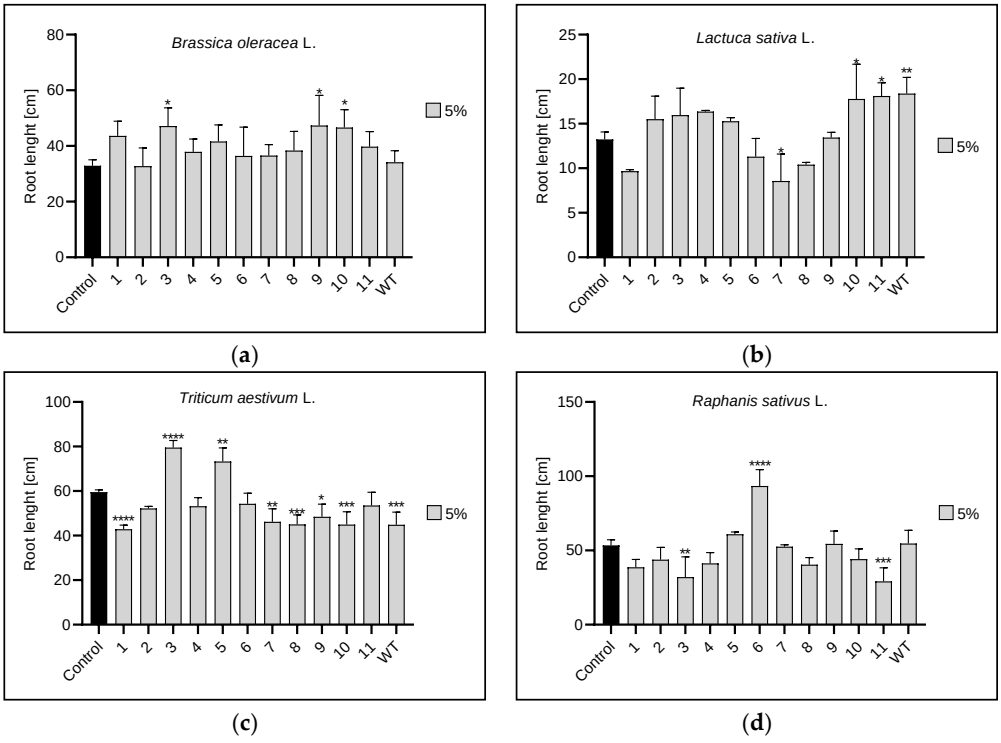

Figure 6. Root length of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 5% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

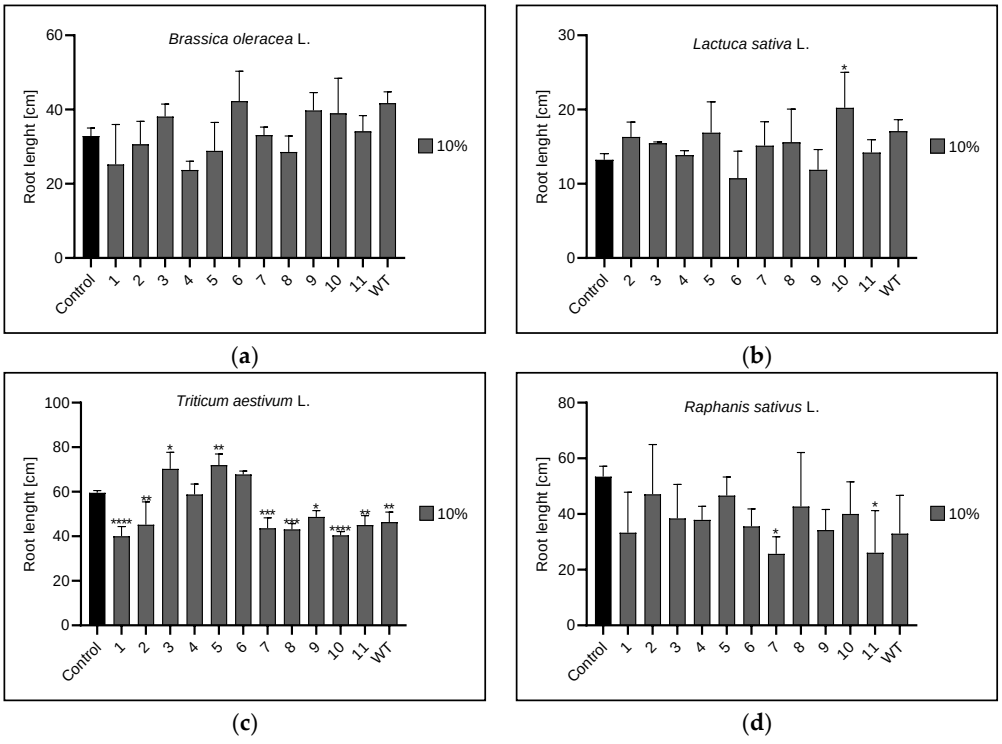

Figure 7. Root length of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 10% concentration for all tested algae clones (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

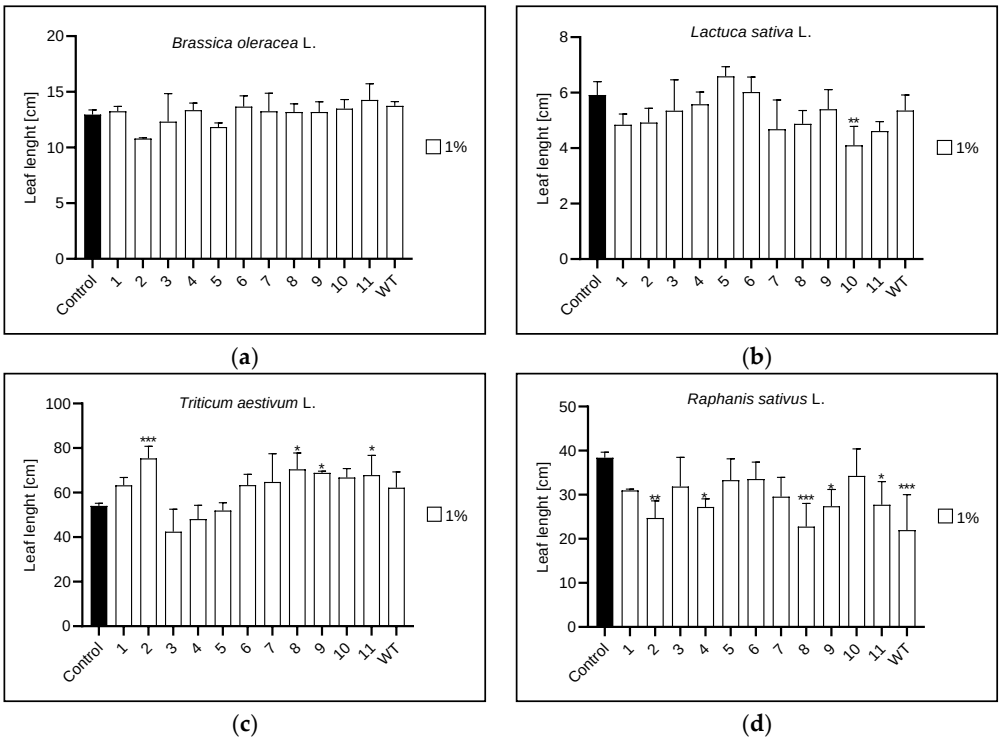

Figure 8. Leaf length of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 1% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ compared to control (ANOVA and Dunnett's a posteriori test).

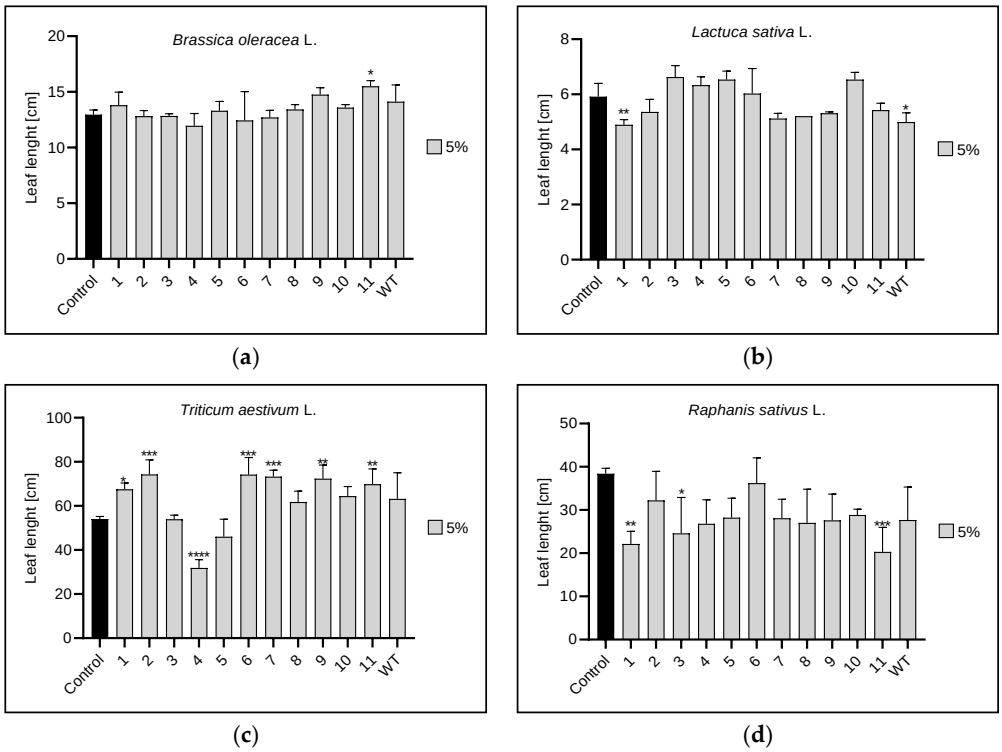

Figure 9. Leaf length of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 5% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

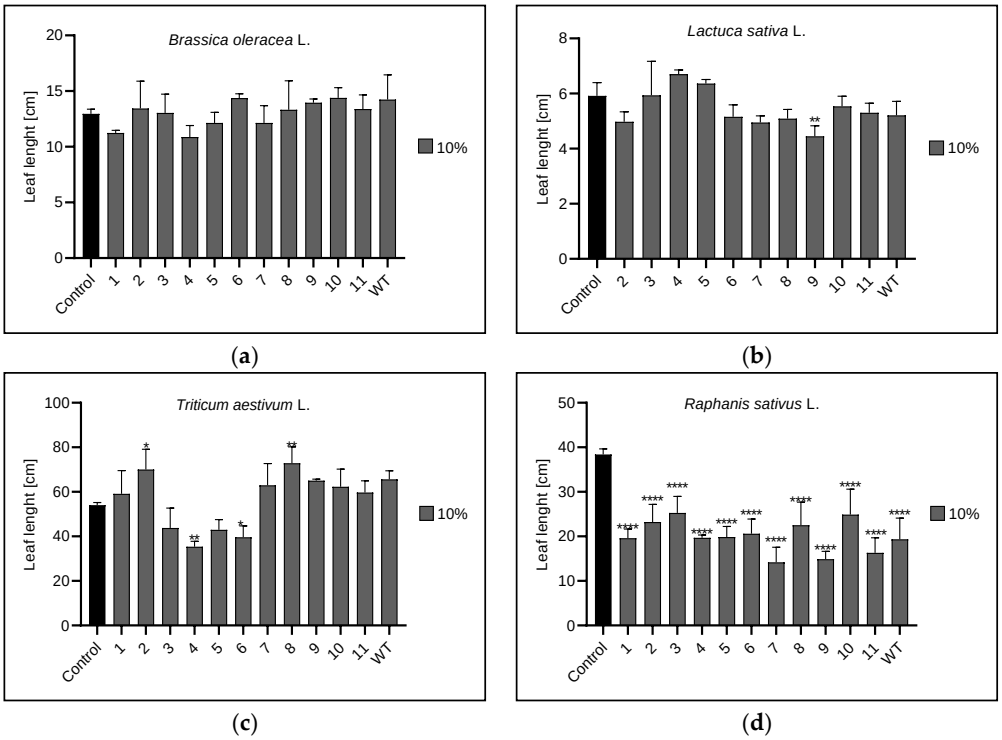

Figure 10. Leaf length of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 10% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

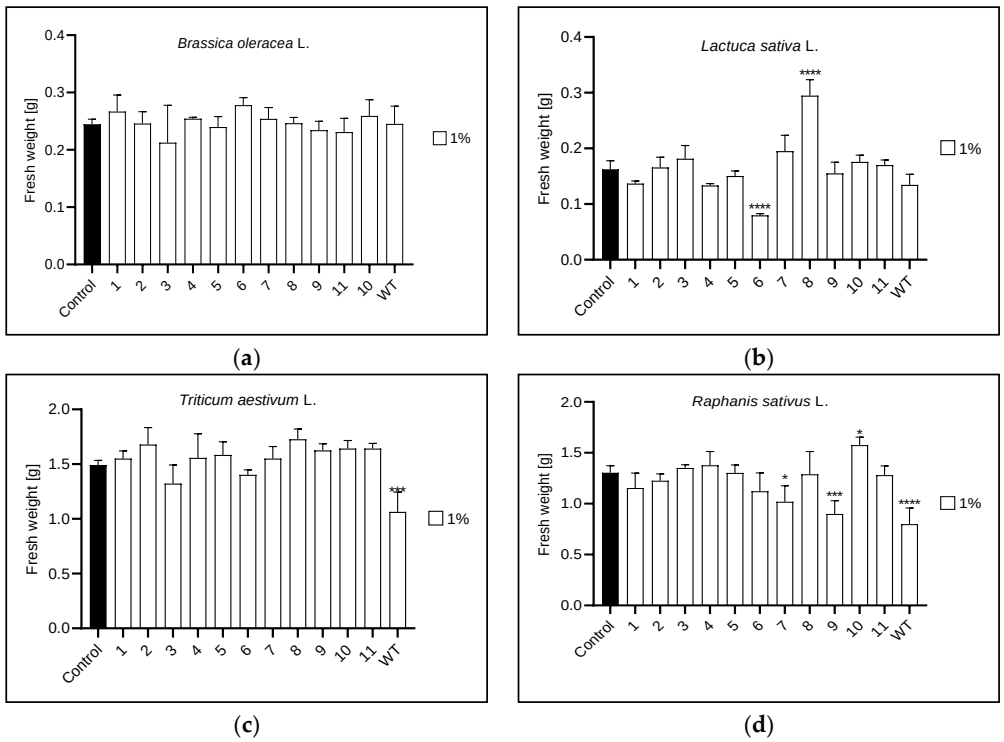

Figure 11. Fresh weight of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 1% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, *** $p < 0.001$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

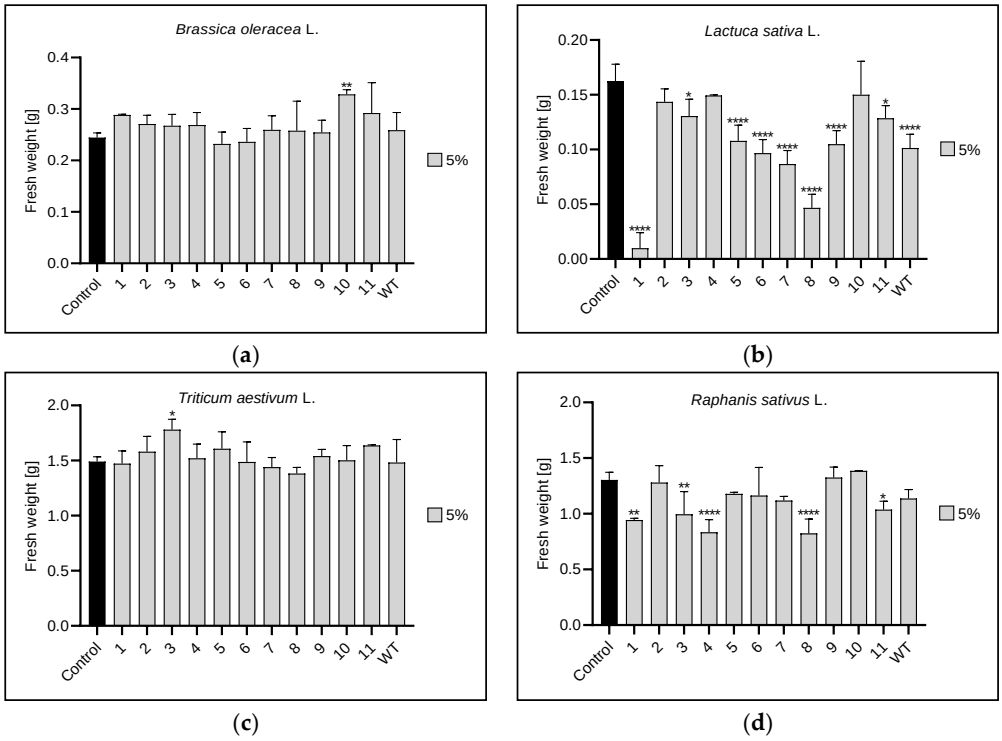

Figure 12. Fresh weight of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with 5% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, ** $p < 0.01$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

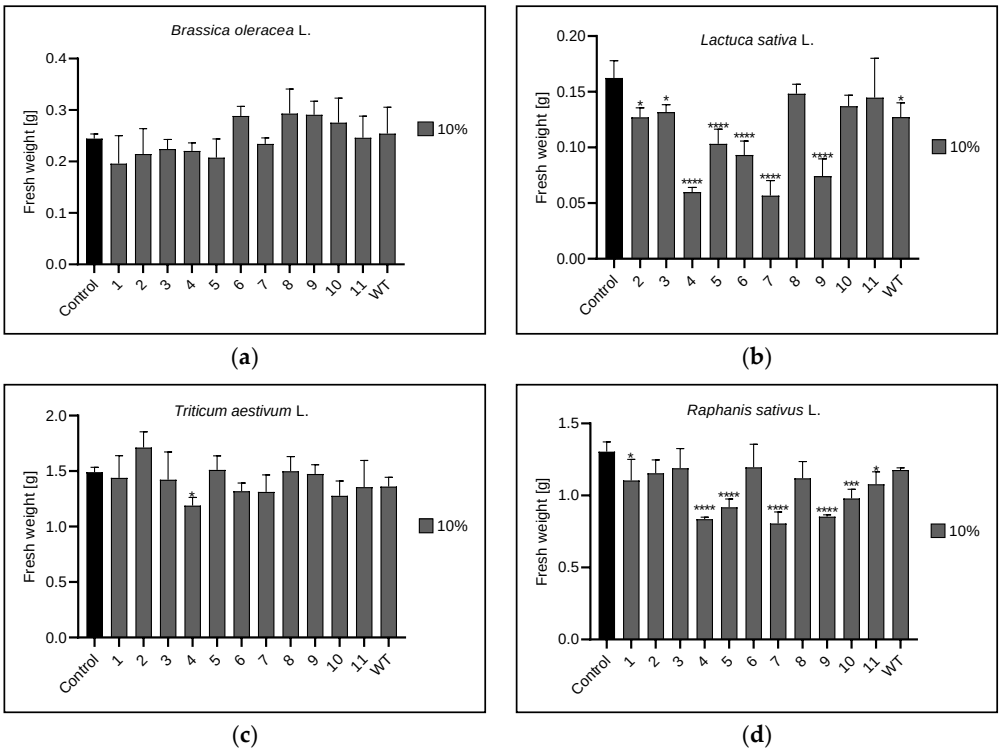

Figure 13. Fresh weight of broccoli (**a**), lettuce (**b**), wheat (**c**), and radish (**d**) sprouts treated with a 10% concentration for all algae clones tested (1-11, WT) and control (Control). Values represent mean ± SD, n = 10 plants, * $p < 0.1$, *** $p < 0.001$, **** $p < 0.0001$ compared to control (ANOVA and Dunnett's a posteriori test).

Algae extracts at a concentration of 10% did not cause changes in the weight of broccoli sprouts compared to the control (Figure 13a, Table 2). In the case of lettuce sprouts, the weight decreased under the influence of the action of eight algae clones. Clones 4, 5, 6, 7, and 9 had the greatest impact (Figure 13b, Table 2). The weight of wheat germ decreased slightly under the influence of clone 4 (Figure 13c, Table 2). The radish sprouts also showed a decrease in weight under the action of seven algae clones (Figure 13d, Table 2). Four clones (4, 5, 7 and 9) caused a significant decrease in the weight of radish sprouts.

4. Discussion

Algae extracts have been shown to contain substances that positively affect plant growth and development. Algae extracts are rich in vitamins, polysaccharides, amino acids, lipids, polyphenols, and plant hormones such as cytokinins, gibberellins, or auxins; they are also a source of macro- and microelements. The use of algae extracts results in improved seed germination, root and leaf development, as well as plant resistance to a variety of pathogens [3,28].

Many algal strains improve soil properties, crop growth, and fruit quality and have therefore been used as fertilizers [29]. However, no study has been performed to date on *Planktochlorella nurekis*. This is the first time that extracts obtained from the biomass of eleven *Planktochlorella nurekis* clones were tested on plants and resulted in improved biochemical characteristics compared to their unmodified counterpart. Environmental factors (light intensity, temperature) were constant during all treatments, so the differences between treatments are due to the different concentrations of extracts and different clones tested.

As a result of the analysis of the effect of algae extracts on the seedlings of four plant species (lettuce, wheat, radish and broccoli), it can be concluded that, in most cases, they have a negative or neutral effect on the species tested. In some cases, however, the effect was positive at low concentrations (1% and 5%). The overall germination efficiency was affected only in lettuce, for which a decrease was observed. Studies by Kumar et al. show that diluted algae extracts promote seed germination more than concentrated ones; growth of seedlings appears delayed at higher concentrations (15 and 20%) [30]. Our results show a similar trend in lettuce. In 1% concentration, three clones show an inhibitory effect on germination efficiency, in 5%—six, and in a concentration of 10%, it is exhibited by all clones and WT. Priming wheat seeds with low concentrations of seaweed extracts from *U. linza* and *C. officinalis* (20%) resulted in high percentages of germination [31]. We did not observe this effect in any of the tested clones or concentrations. Extracts seemed to be neutral to tested wheat seedlings, maybe because our concentrations were lower. Low concentrations (1%, 5%) of *P. nureckis* extracts stimulated root length positively in lettuce and negatively in wheat (1%, 5%, 10%). *U. linza* 20% extract effectively increased the total plant length of wheat. The highest shoot length, root length, and fresh weight were observed in 20% concentration of *C. tomentosum* liquid fertilizer in wheat plants [32]. Leaf length decreased in radish. For all of the clones, the strongest effect was observed with 10% extract. In the rest of the tested plant species, the effect was positive or negative only for single clones. According to Kasim et al. the imposition of salinity stress inhibits the shoot length of radish. However, presoaking of seeds in seaweed extracts significantly improved this parameter under salinity stress [33]. Of the different doses of microalgae, 5% resulted in the greatest improvement in growth components in broccoli (shoot FW and DW, length, and leaf area per plant) under drought stress [34]. In our study, 5% extract did not affect all tested parameters. The fresh weight of seedlings was negatively affected by extracts in concentrations of 5 and 10% in lettuce and radish. Data on the effect of *Chlorella vulgaris* extract on lettuce sprouting can be found in the literature [35]. The authors report an increase of about 60% in the fresh weight of lettuce seedlings (3- and 6-day-old seedlings). The cultivation conditions were very similar, but the extract was added to the soil, and the plants were not watered in Petri dishes, as in our case. We observed mostly a negative effect on the fresh weight of lettuce, but one clone caused fresh weight to increase at a similar level.

In low concentrations, algal extracts efficiently stimulate growth and plant yield, which can be explained by the corresponding low concentration of phytohormones. The concentration of phytohormones depends on botanical origin, time and place of collection, and method of biomass extraction [5]. Germination is mainly regulated by phytohormones: ABA, cytokinins, brassinosteroids, ethylene, and gibberellic acid (GA3) [36]. Phytohormones are believed to be very active components of algal extracts. They are derived from groups with different chemical structures and can influence plant metabolisms in many ways. Apart from phytohormones, algae contain a variety of other substances, such as polysaccharides, fatty acids, phenolic compounds, betaines, and a diverse assortment of other biologically active components. This great diversity of active compounds may affect plant growth synergistically or antagonistically. They can have a stimulatory or inhibitory effect on plants. Our results show both effects, depending on the tested extract, its concentration, and the tested plant. For example: root length was affected positively in lettuce and negatively in wheat. Fresh weight was negatively affected in lettuce and radish. Overall, the effect was usually neutral or negative, which may be explained by the excessive concentration of phytohormones. In higher doses, phytohormones can inhibit important processes occurring in plants [37]. Additionally, recent studies on *Lemna minor* have shown that algal methanol extract can act similarly to herbicide [38]. Rupawalla et al. quantified 15 known phytohormones in microalgae and did not obtain a clear correlation between phytohormone profile and seed germination [13]. They suggest that more different phytohormones and other substances are present in microalgae and that more complex interactions are taking place during plant germination, both among them as well as between them and the plant. In microalgae, a range of different substances, including auxins, cytokinins, giberellins, abscisic acid, ethyle, brassinosteroids, salicylates, signal peptiades, jasmonic acid, and polyamines, were found [39–41]. These can influence seed germination and seed development.

Microalgae extracts were tested on spinach seedlings, and Rupawalla et al. concluded that the most important biostimulators were located inside microalgae cells, and their availability for plant seedlings could be increased through cell disruption. [13] Their potential for use in drip irrigation was identified. These results also suggested that the insoluble fraction, which contains cell debris and precipitants, showed inhibitory effects on seedling development. In our case, all extract (not fractioned) was applied to seeds, and the neutral or inhibitory effects observed may be due to the presence of the insoluble fraction. It would be important to identify the soluble fractions of the 11 tested clones on plant seedlings.

Apart from their effect on growth and development parameters, algal extracts can also play a role in the stimulation of defense responses to stress factors such as high salinity, drought, low temperature, or pathogen attack [42], but sometimes the effect is not stimulating. Algal extracts enriched in amino acids demonstrated a biostimulating effect on plant physiology characterized by an increase of photosynthetic pigments and anthocyanin. No positive effect was observed in terms of plant growth or yield. Additionally, algal extracts enriched in amino acids increased the susceptibility of lettuce to powdery mildew [43].

Here we present the results of screening tests of 11 microalgae clones on four cultivated plant species. However, further studies need to be conducted to optimize the dose and type of fraction added to seedlings. Microalgae are a potential source of plant growth regulators, but whole extracts can have a neutral or inhibitory effect as other substances are also present in the extract. It would be interesting for future research to test different fractions of the clones on plants to choose the clone and fraction that would best improve germination, root and shoot development, and have the strongest potential application in agriculture. Our results are valuable not only for breeders and farmers but also for sprout and microgreen producers. Current studies show the positive effect of algal extracts on Amaranth sprouts. Aqueous seaweed extracts from *P. durvillei* and *U. lactuca* exhibited biostimulant activity when applied to amaranth seeds, improving the growth, yield, and functional quality of amaranth sprouts [16]. The plant species we tested are all mentioned

in a recent paper by Ebert [44]. Broccoli is one of six commonly grown and consumed microgreen species [45]. Together with radish and four other species, it is a member of the *Brassicaceae* family, which dominated the global sprout market in 2019 [46]. Lettuce and wheat sprouts are less popular but nevertheless of interest to consumers.

Apart from having an overall negative or neutral effect, some single clones seem to increase activity in both stimulating and inhibitory ways, which proves that co-treatment with colchicine and cytochalasin is a good tool to obtain *P. nurekis* clones with great value to agriculture and a potential use as both biostimulators and herbicides. Interest in bio-based chemicals is increasing. There is a demand for plant biostimulators that increase crop yield and quality and reduce usage of synthetic agrochemicals in plant production. Further tests need to be performed to carefully establish suitable concentrations of extracts for each plant species. Such tests will undoubtedly become important given the ongoing growth of the global market for plant biostimulators, predicted to be worth more than USD 4000 million by 2023 [47]. Biostimulators based on microalgae are a great potential source of active substances, both for seedlings and older plants. They would have a great impact not only on plant yield and environmental protection but also on the quality of the sprouts, vegetables, and grains we consume. Microalgae offer a promising platform for biostimulant production, and tests such as ours have an important role to play providing proof of function and enabling further screening to be planned.

5. Conclusions

Our results show the importance of testing algal extracts in different concentrations on different plant species. The study presented here is preliminary. The response of the clones tested is highly variable in some species and regular in others. It would be interesting for further studies to establish why. Additionally, in some cases, WT is very similar to control, which suggests that co-treatment with colchicine and cytochalasin may be a good source of clones with properties that are desired in agriculture and are different than those of WT. Some clones can have a negative effect, while others have a positive effect. Our conclusion is that both effects are desired in agriculture, as both natural herbicides and biostimulators can have a great impact on the safe production of healthy food.

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