agriculture

MDPI

Article

Evidence of Allelopathy among Selected Moss Species with Lettuce and Radish

Nikolina A. Matić [1], Marija V. Ćosić [1], Djordje P. Božović [1], Silvia Poponessi [2], Sara D. Pavkov [3], Michal Goga [4], Milorad M. Vujičić [1,5], Aneta D. Sabovljević [1,5] and Marko S. Sabovljević [1,4,5,*]

[1] Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Takovska 43, RS-11000 Belgrade, Serbia; nikolina.matic@bio.bg.ac.rs (N.A.M.); marijac@bio.bg.ac.rs (M.V.Ć.)
[2] Department of Life and Environmental Sciences, Botany Section, University of Cagliari, Viale S. Ignazio, IT-09123 Cagliari, Italy
[3] Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, RS-21000 Novi Sad, Serbia
[4] Department of Botany, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Mánesova 23, SK-040 01 Košice, Slovakia
[5] Center of Plant Biotechnology and Conservation (CPBC), Takovska 43, RS-11000 Belgrade, Serbia
* Correspondence: marko@bio.bg.ac.rs

Abstract: There is limited evidence on bryophyte-tracheophyte allelopathic interactions. Even less is known about such relationships among commercially important plants and mosses. With the aim of screening such interactions, various extract concentrations of nine different mosses were tested on the seed germination and seedlings, i.e., hypocotyl elongation and total chlorophyll content of lettuce and radish. The allelopathic effects are documented to be pairwise (moss-vegetable) and species-specific. Based on the results, the extracts of *Leucodon sciuroides* and *Dicranum polysetum* are not harmful to lettuce and radish. Lower extract concentrations of *Leucodon sciuroides* and *Ctenidium molluscum* have a positive effect on lettuce development, while those of *Thuidium delicatulum*, *Ctenidium molluscum*, and *Dicranum polysetum* showed to be effective on radish. Further, negative effects were noticed when applying higher extract concentration of *Abietinella abietina*, *Isothecium alopecuroides*, *Dicranum polysetum*, and *Racomitrium elongatum* to lettuce and *Isothecium alopecuroides* to radish. The dataset presented in this study offers numerous possibilities for further target pest/vegetable type applications since some of the moss extracts are shown to be positive, negative, or indifferent to the tested features in lettuce and radish.

Keywords: bryophytes; plant interactions; development; extracts

Citation: Matić, N.A.; Ćosić, M.V.; Božović, D.P.; Poponessi, S.; Pavkov, S.D.; Goga, M.; Vujičić, M.M.; Sabovljević, A.D.; Sabovljević, M.S. Evidence of Allelopathy among Selected Moss Species with Lettuce and Radish. *Agriculture* **2024**, *14*, 812. https://doi.org/10.3390/agriculture14060812

Academic Editor: Daniele Del Buono

Received: 8 May 2024
Revised: 18 May 2024
Accepted: 22 May 2024
Published: 23 May 2024

1. Introduction

Being sessile, plants interact with other organisms in their habitats in a specific way, primarily through secondary metabolites. Interactions through such chemical compounds between two organisms, including plants, algae, fungi, and bacteria, are defined as allelopathy [1] and may be stimulatory or inhibitory [2]. While an organism can stimulate the growth of many other organisms, it may also inhibit developmental and physiological processes in another. Thus, allelopathy is a very complex and often species-to-species-specific trait, especially in plants that are abundant in secondary metabolites. Chemical compounds that exhibit allelopathic effects are called allelopathic compounds, allelopathins, or allelochemicals [3].

A large number of plants possess secondary metabolites, which exhibit biological activity in the organisms in their environment. Bryophytes, as the second-largest group of plants (over 20,000 species worldwide), are particularly interesting due to their richness in secondary metabolites [4]. Compared to vascular plants, bryophytes are smaller in size, with reduced morphological complexity, and a lack of highly differentiated tissues

and organs. On the other hand, bryophytes synthesise a wide array of different chemical compounds, some of which are unique and not synthesised by vascular plants [5]. Representatives of all three main lineages of bryophytes (mosses, hornworts, and liverworts) produce secondary metabolites [6], which mainly belong to the group of terpenoid and aromatic compounds [5,7]. Additionally, the chemical profile of liverworts is particularly important due to the presence of oil bodies in which larger amounts of terpenoid and lipid compounds are stored, some of which are exclusive to liverworts [4,5]. On the other hand, many phenolic compounds are present in mosses [8], including benzoic, cinnamic, and phthalic acid derivatives and coumarins [4,5]. Such compounds, the products of complex secondary metabolism in bryophytes, facilitate the interaction of bryophytes with other organisms, i.e., microorganisms, pathogens, vascular plants, and, to a lesser extent, herbivores, evolved over a long coevolutionary history to increase bryophyte fitness and survival [9,10]. Moreover, increased synthesis and the accumulation of certain secondary products can be induced by the effects of some abiotic or biotic stresses or their combinations [8,11,12]. Despite the large amount of information on the biological activity of secondary metabolites, their properties, and their use in pharmacy, medicine, and agriculture (e.g., [6,13–15]), there is still little knowledge on how bryophytes interact with the other plants present in their environment. Certain bryophyte species have an impact on the spore germination and growth of young gametophytes of other species of bryophytes through secondary metabolism products [16]. Vicherova et al. [17] documented peat-moss *Sphagnum flexuosum* Dozy and Molk. positively affected the shoot elongation of moss *Hamatocaulis vernicosus* (Mitt.) Hedenäs via volatile organic compounds, which increased the production of β-cyclocitral. This compound is commonly found in vascular plants as a signalling molecule for plant-to-plant communication during stress responses. Conversely, interactions between bryophytes and vascular plants through various chemical compounds are less studied, despite their potential ecological and functional significance. Extracts of some bryophyte species can affect and modify the seed germination process of vascular plants [18,19]. For example, *Porella platyphylla* (L.) Pfeiff. extracts inhibited the germination of seeds from cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) [19]. In contrast, extracts derived from the moss *Abietinella abietina* (Hedw.) M. Fleisch. stimulated the seed germination of spruce species *Picea crassifolia* Kom. when applied in low and moderate concentrations [20]. Besides the influence on seed germination, specific metabolites of bryophytes may also affect the growth and development of vascular plants by promoting or inhibiting the growth of the shoots and roots of vascular plants [21]. Thus, it is important to study the chemistry of bryophytes to test the isolates on various growth and developmental phases and physiological features of vascular plants since those metabolites can be used as potential bioherbicides or plant stimulants [10].

According to Kato-Noguchi [22], a variety of secondary metabolites produced by bryophytes are also secreted into the rhizosphere substrate and act as allelopathic agents. However, very few of those compounds have been studied. Some of the better-known ones are momilactones from *Hypnum plumaeforme* Wilson [23] and 3-hydroxy-β-ionene from *Rhynchostegium pallidifolium* (Mitt.) A. Jaeger. [24,25], both exhibiting inhibitory and phytotoxic effects on surrounding vascular plants. Kato-Noguchi [22] further speculates that these compounds may be responsible for monospecific mat formation as well as the elimination of vascular plants from their micro- to meso-habitats.

As shown above, while there is some knowledge of bryophyte allelopathy, the subject of allelopathic interactions and effects remains obscure. Therefore, the main goals of this study were to investigate how different concentrations of selected bryophyte species extracts affect the seed germination and hypocotyl length of lettuce and radish and to explore whether these extracts influence their physiological traits. The aim is to screen for non-harmful crop natural agents that may serve as alternatives in weed suppression and treatments.

2. Materials and Methods

2.1. Plant Material

The allelopathic effect on the lettuce and radish seed germination of a total of nine randomly selected moss species was tested. Namely, eight moss species, *Abietinella abietina* (Thuidiaceae), *Ctenidium molluscum* Mitt. (Hynaceae), *Dicranum polysetum* Sw. (Dicranaceae), *Hylocomium splendens* Hedw. (Hylocomiaceae), *Isothecium alopecuroides* Isov. (Lembophyllaceae), *Leucodon sciuroides* (Hedw.) Schwaegr. (Leucodontaceae), *Racomitrium elongatum* Ehrh. ex Frisvoll. (Grimmiaceae), and *Thuidium tamariscinum* Schimp. (Thuidiaceae) were collected in a wide area of Tara Mountain, W. Serbia, and one moss species (*Thudium delicatulum* (Hedw.) Schimp.) was collected on Zlatar Mountain, W. Serbia, in October 2020. Lettuce (*Lactuca sativa* L. "May Queen") and radish seeds (*Raphanus sativus* L. "Saxa Treib") were commercially obtained from (Semenarnacoop d.o.o., Petrovaradin, Serbia) with the aim of investigating the allelopathic potential of extracts from the selected mosses.

The vouchers of the bryophyte specimens were confirmed by the M. Sabovljević and S. Poponessi, and they are deposited in the University of Belgrade Bryophyte Collection (BEOU-Bryo) collection: *A. abietina* (BEOU-Bryo 09230), *C. molluscum* (BEOU-Bryo 09226), *D. polysetum* (BEOU-Bryo 09234), *H. splendens* (BEOU-Bryo 09239), *I. alopecuroides* (BEOU-Bryo 09225), *L. sciuroides* (BEOU-Bryo 09228), *R. elongatum* (BEOU-Bryo 09238), *T. delicatulum* (BEOU-Bryo 09224) and *T. tamariscinum* (BEOU-Bryo 09232).

2.2. Experimental Design

2.2.1. Preparation of the Moss Extracts

The collected mosses were cleaned of old parts and impurities and air-dried at room temperature for 10 days. Initially, 5 g of dried plant material for each moss species was measured, ground in liquid nitrogen, and extracted with 50 mL of 96% ethanol. The mixture was then transferred to a glass bottle and incubated for 24 h on an orbital shaker (BioSan PSU-20i, Riga, Latvia) at room temperature. After the incubation, the mixtures of ethanol and plant material were filtrated through filter paper, and the clarified extracts were evaporated to dry using a rotary vacuum evaporation apparatus (IKA RV 3 V, IKa-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) set at 40 °C and 800–1000 mbar. The mass of the distillation balloons used for evaporation was measured before and after the evaporation of the ethanol from the filtrates in order to calculate the mass of the dry residue (extraction yield) for each of the tested moss species. The mass of the dry residue was used for the preparation of the primary ethanolic stock solution (10 mg/mL). The dilution series of different concentrations (10^{-4}, 10^{-3}, 2×10^{-3} mg/mL) was prepared by dissolving the primary stock in distilled water. These diluted extracts were used in the subsequent experiments.

2.2.2. Testing the Allelopathic Potential of the Moss Extracts

The allelopathic effects of the moss extracts were tested on lettuce and radish seed germination. Commercially obtained seeds were already washed and prepared for germination. Ten lettuce and ten radish seeds were placed in each Petri dish (Ø55 mm) and soaked in 3 mL of moss extract at different concentrations (10^{-4}, 10^{-3}, 2×10^{-3} mg/mL) or distilled water (as the control). Seven replications were set for each of the experimental groups, comprising a total of 70 seeds of lettuce and radish per experimental group. All the Petri dishes were placed under controlled long-day low light intensity conditions provided by fluorescent tubes (Tesla, Pančevo, Serbia, 65 W, 50 μmol m^{-2} s^{-1}) at a temperature of 18 ± 2 °C (16 h: 8 h light: dark) for five days.

The percentage of germinated seeds and the hypocotyl length of the seedlings were measured after three and five days. The length of the hypocotyl was measured using a millimetre ruler and all the changes were photographed and documented using a stereomicroscope (OPTIKA Microscopes, Ponteranica (BG), Italy).

2.2.3. Analysis of the Total Chlorophyll Concentration

The photosynthetic pigments were extracted in 96% (*v*:*v*) ethanol from 20 mg of frozen lettuce and radish seedling leaves. After incubation at 70 °C for 10 min and centrifugation (Tehtnica Centric 200R, Zelenzniki, Slovenia), the supernatant was transferred to a microtiter plate, and the absorbance was measured at 648 and 664 nm using the Multiscan Sky Thermo Scientific (Waltham, MA, USA) plate reader (method modified according to Lichtenhthaler [26]). The total chlorophyll concentration in the lettuce and radish seedlings was calculated according to the equation $C(a + b) = 5.24 \times A_{664} + 22.24 \times A_{648}$ and expressed as $\mu g\ g^{-1}$ fresh weight.

2.3. Statistical Analysis

Complete statistical analysis was carried out using R (v. 4.3.1) [27]. The data gathered in this research underwent two different types of statistical analysis. Considering that the seed germination data has two responses, the seeds either germinated or did not, a binomial logistic regression model [28] was used in order to predict the probability of germination using two categorical predictors and their interaction (extract concentration and treatment duration) in nine different moss species. Subsequently, the estimated marginal means (EMMs) were computed using the emmeans() function of the emmeans R package (v. 1.8.9; http://cran.r-project.org/package=emmeans, accessed on 7 January 2024), followed by contrast tests for pairwise comparisons with the Benjamini-Hochberg *p*-value adjustment, using the contrast() function of the same R package. The second type of analysis was done for the other two parameters (hypocotyl length and total chlorophyll content). Firstly, a preliminary exploration of the data was carried out using Lavene's test in order to test the homogeneity of variance and the Shapiro-Wilk normality test to check whether the data followed a normal distribution. It was found that not all the experimental groups followed a normal distribution and that the homogeneity of variance was violated across the groups, therefore a nonparametric factorial ANOVA was carried out using the Aligned Rank Transform (ART) procedure [29,30] employing the ARTool R package (v0.11.1) [31]. The factorial models were created for both parameters and nine bryophyte species using the art() function. Subsequently, the significance of these effects was assessed using the anova() function, and ultimately contrast tests were conducted using the art.con() function.

3. Results

3.1. The Effects of the Moss Extracts on the Lettuce Seed Germination

Different concentrations of the extracts of all the investigated moss species as well as the treatment duration did not affect the germination of the lettuce seeds (Table 1). No statistically significant differences were observed in the germination of the lettuce seeds between the third and fifth days within the same treatment or between different treatments (Figure 1A–I).

Table 1. A summary of the results of nine logistic regression models that predict the probability of germination of the lettuce seeds treated with the ethanolic extracts from nine bryophyte species. The predictors include the concentration of the ethanolic extracts (C), treatment duration (T), and their interaction (C × T).

Species	Parameter	(Intercept)	C [I]	C [II]	C [III]	T [5]	C [I] × T [5]	C [II] × T [5]	C [III] × T [5]	Observation	R^2 Tjur
Abietinella abietina	Odds ratios	6.00 ***	1.78	1.00	1.00	1.00	1.22	1.13	1.13	560	0.007
	p	<0.001	0.293	1.00	1.00	1.00	0.803	0.860	0.860		

Table 1. *Cont.*

Species	Parameter	(Intercept)	C [I]	C [II]	C [III]	T [5]	C [I] × T [5]	C [II] × T [5]	C [III] × T [5]	Observation	R^2 Tjur
Ctenidium molluscum	Odds ratios	6.78 ***	1.57	0.89	0.59	1.33	0.75	0.97	0.83	560	0.015
	p	<0.001	0.415	0.805	0.257	0.596	0.725	0.970	0.780		
Dicranum polysetum	Odds ratios	10.67 ***	0.56	0.38	0.56	1.55	0.84	0.71	0.65	560	0.017
	p	<0.001	0.293	0.060	0.293	0.514	0.830	0.665	0.597		
Hylocomnium splendens	Odds ratios	6.78 ***	0.59	0.79	0.71	1.33	0.75	0.75	0.75	560	0.007
	p	<0.001	0.257	0.630	0.479	0.596	0.677	0.689	0.685		
Isothecium alopecuroides	Odds ratios	4.38 ***	1.55	1.77	3.76 *	1.00	1.00	1.38	1.00	560	0.021
	p	<0.001	0.355	0.241	0.027	1.000	1.000	0.655	1.000		
Leucodon sciuroides	Odds ratios	10.67 ***	1.00	1.00	0.73	1.55	1.00	0.65	0.89	560	0.004
	p	<0.001	1.000	1.000	0.574	0.514	1.000	0.628	0.894		
Racomitrium elongatum	Odds ratios	9.00 ***	0.67	1.44	1.00	1.19	0.84	0.84	1.00	560	0.008
	p	<0.001	0.440	0.548	1.000	0.771	0.823	0.847	1.000		
Thuidium delicatulum	Odds ratios	7.75 ***	1.00	0.87	1.38	1.68	0.60	0.79	0.92	560	0.006
	p	<0.001	1.000	0.796	0.574	0.386	0.518	0.771	0.928		
Thuidium tamariscinum	Odds ratios	4.83 ***	1.00	1.40	1.40	1.24	0.89	0.92	0.81	560	0.003
	p	<0.001	1.000	0.479	0.479	0.643	0.864	0.906	0.753		

* $p < 0.05$, *** $p < 0.001$.

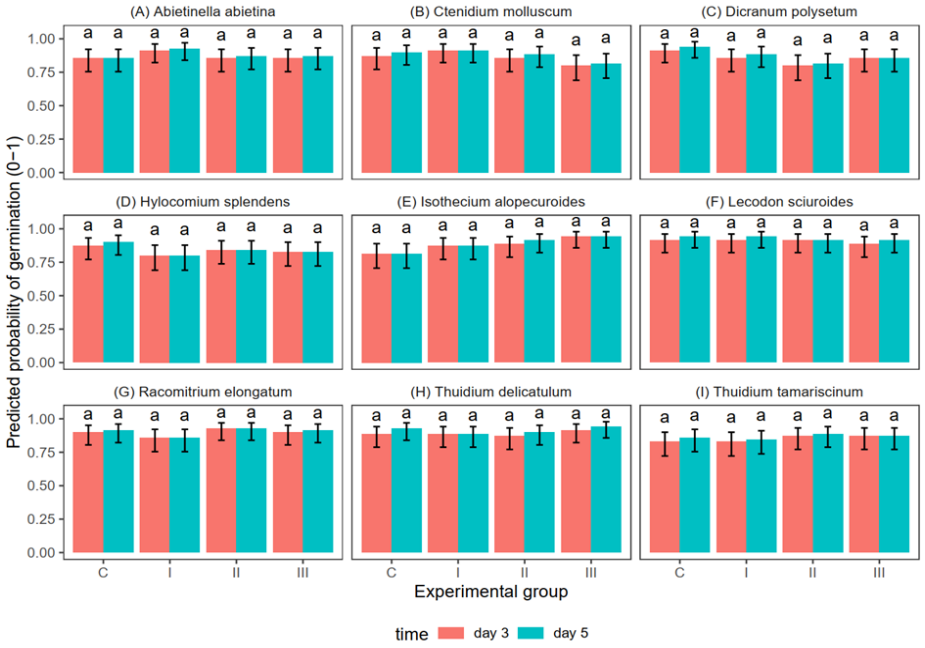

Figure 1. The predicted probability of the germination of the lettuce seeds. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively). The bars represent the predicted probability of germination (0–1) with the error bars denoting the 95% confidence intervals (CI). Statistically significant differences ($p < 0.05$) among the experimental groups are indicated by the letters above the bars.

3.2. The Effects of the Moss Extracts on the Radish Seed Germination

The extract concentration of 10^{-3} mg/mL of four moss species (*A. abietina*, *C. molluscum*, *H. splendens*, and *L. sciuroides*), affected the radish seed germination ($p < 0.05$, Table 2, predictor C [II]). Also, the highest extract concentrations (2×10^{-3} mg/mL) of four moss species (*C. molluscum*, *D. polysetum*, *H. splendens* and *I. alopecuroides*) inhibited the germination of the radish seeds ($p < 0.01$, Table 2, predictor C [III]). However, neither the treatment duration nor the interaction between the extract concentrations and the treatment duration impacted the germination of the radish seeds (Table 2).

Table 2. A summary of the results of nine logistic regression models that predict the probability of the germination of the radish seeds treated with the ethanolic extracts from nine bryophyte species. The predictors include the concentration of the ethanolic extracts (C), treatment duration (T), and their interaction (C × T).

Species	Parameter	(Intercept)	C [I]	C [II]	C [III]	T [5]	C [I] × T [5]	C [II] × T [5]	C [III] × T [5]	Observation	R^2 Tjur
Abietinella abietina	Odds ratios	1.26	0.94	0.39 **	0.60	1.43	0.88	1.35	0.83	560	0.035
	p	0.340	0.865	0.007	0.129	0.301	0.798	0.543	0.703		
Ctenidium molluscum	Odds ratios	1.19	1.43	0.44 *	0.36 **	1.43	1.04	1.42	1.16	560	0.076
	p	0.474	0.304	0.018	0.004	0.304	0.943	0.470	0.767		
Dicranum polysetum	Odds ratios	1.26	0.89	0.53	0.30 ***	1.12	1.19	1.12	1.41	560	0.048
	p	0.340	0.734	0.064	0.001	0.733	0.718	0.809	0.491		
Hylocomnium splendens	Odds ratios	1.80 *	0.74	0.22 ***	0.25 ***	1.30	0.92	1.01	0.77	560	0.107
	p	0.018	0.387	<0.001	<0.001	0.472	0.870	0.990	0.613		
Isothecium alopecuroides	Odds ratios	1.19	1.12	0.60	0.34 **	1.52	0.84	1.32	1.10	560	0.057
	p	0.474	0.734	0.129	0.002	0.229	0.717	0.570	0.849		
Leucodon sciuroides	Odds ratios	2.89 ***	0.71	0.35 **	0.66	1.52	0.70	1.05	0.99	560	0.036
	p	<0.001	0.354	0.003	0.270	0.311	0.521	0.928	0.990		
Racomitrium elongatum	Odds ratios	2.50 ***	0.68	0.82	0.82	1.25	1.03	1.22	1.05	560	0.009
	p	0.001	0.281	0.583	0.583	0.566	0.949	0.708	0.922		
Thuidium delicatulum	Odds ratios	1.80 *	1.39	1.21	1.73	1.21	1.11	1.01	0.89	560	0.009
	p	0.018	0.366	0.592	0.142	0.592	0.839	0.977	0.833		
Thuidium tamariscinum	Odds ratios	2.04 **	0.62	1.41	0.73	1.14	1.74	1.11	1.36	560	0.022
	p	0.005	0.166	0.354	0.380	0.716	0.278	0.845	0.545		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The extracts derived from four moss species (*A. abietina*, *R. elongatum*, *T. delicatulum*, and *T. tamariscinum*) did not affect the germination of the radish seeds (Figure 2A,G–I). No statistically significant differences were detected in germination, either between the different treatments or within the same experimental group, between the third and fifth days from the beginning of the experiments.

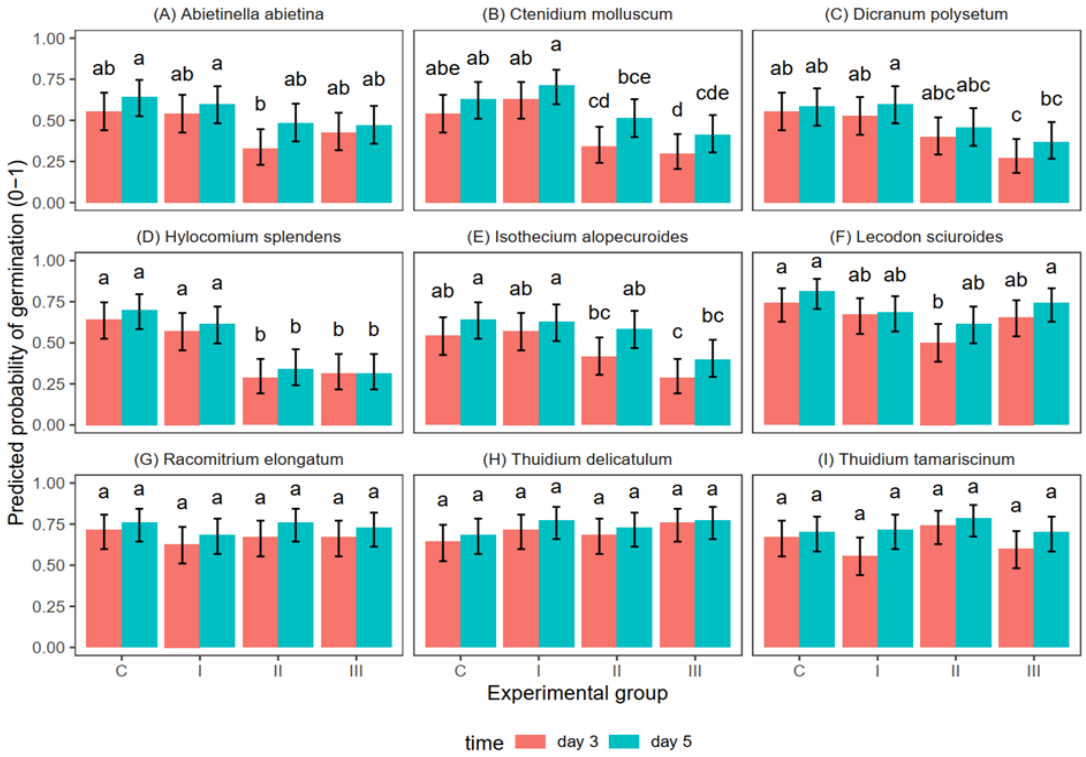

Figure 2. The predicted probability of the germination of the radish seeds. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively). The bars represent the predicted probability of germination (0–1) with the error bars denoting the 95% confidence intervals (CI). Statistically significant differences ($p < 0.05$) among the experimental groups are identified by the letters above the bars.

The treatment with the extract concentration of 10^{-3} mg/mL from moss *L. sciuroides* resulted in a decrease in the germination of the radish seeds compared to the control only after three days, i.e., short-term treatment had a slight inhibitory effect on the seed germination (Figure 2F). On the other hand, the highest applied concentration (2×10^{-3} mg/mL) of the two moss species extracts (namely *D. polysetum* and *I. alopecuroides*) had a significant negative effect on the germination of the radish seeds on the third and the fifth days (Figure 2B,D).

A significant decrease in the germination of the radish seeds occurred when the seeds were treated with the extract concentrations 10^{-3} and 2×10^{-3} mg/mL derived from *C. molluscum* and *H. splendens* after three and five days compared to the control group (Figure 2B,D).

No statistically significant differences were documented within the same experimental groups between the predicted germination percentage of the lettuce and radish seeds after three and five days (Figures 1 and 2). This contrasts with the findings for the other measured parameters in this study, where statistically significant differences were observed within the same treatments between the third and fifth days (Figures 3–6).

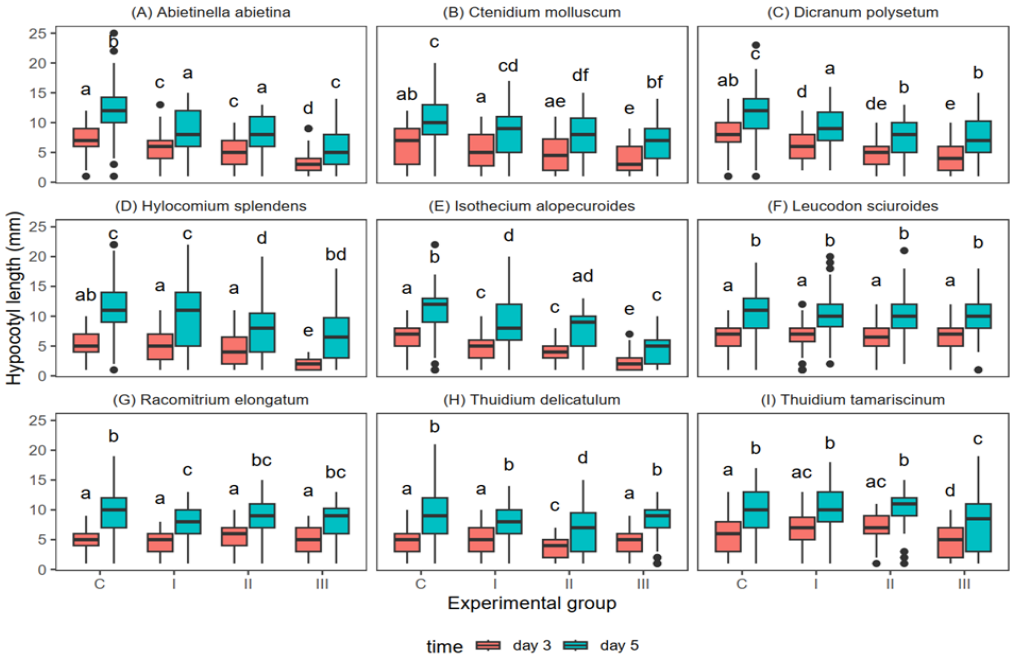

Figure 3. The effects of the ethanolic moss extracts on the lettuce seedling hypocotyl length. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively). Black horizontal lines represent the median, while the box ranges from the first (Q1) to the third (Q3) quartile, with the whiskers extending to the 1.5 × IQR (interquartile range) from the edge of the boxes. The black dots represent observations outside the 1.5 × IQR (interquartile range). Statistically significant differences ($p < 0.05$) among the experimental groups are identified by the letters above the boxplots.

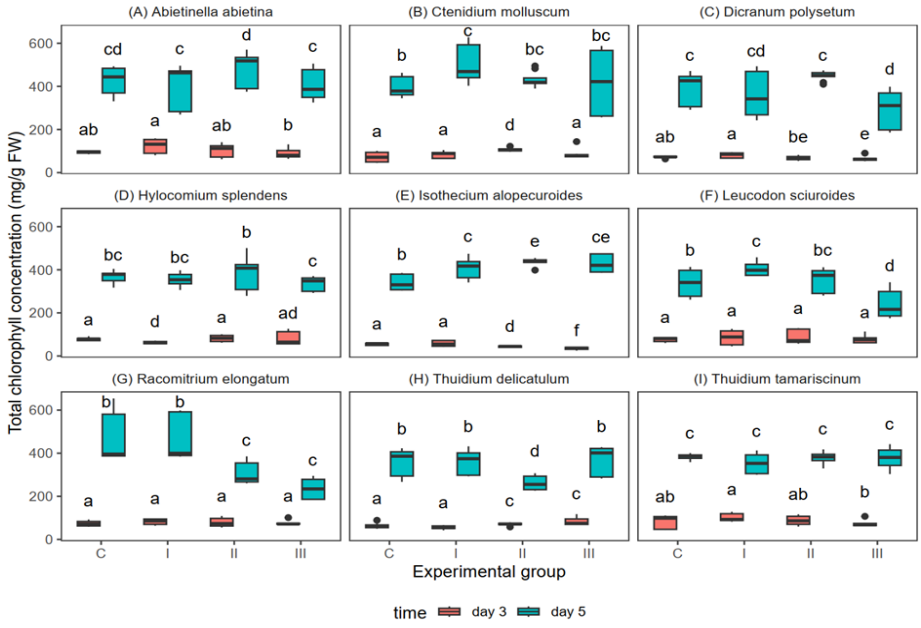

Figure 4. The total chlorophyll concentration of the lettuce seedlings. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively). Black horizontal lines represent the median, while the box ranges from the first (Q1) to the third (Q3) quartile, with the whiskers extending to the 1.5 × IQR (interquartile range) from the edge of the boxes. The black dots represent observations outside the 1.5 × IQR (interquartile range). Statistically significant differences ($p < 0.05$) among the experimental groups are indicated by the letters above the boxplots.

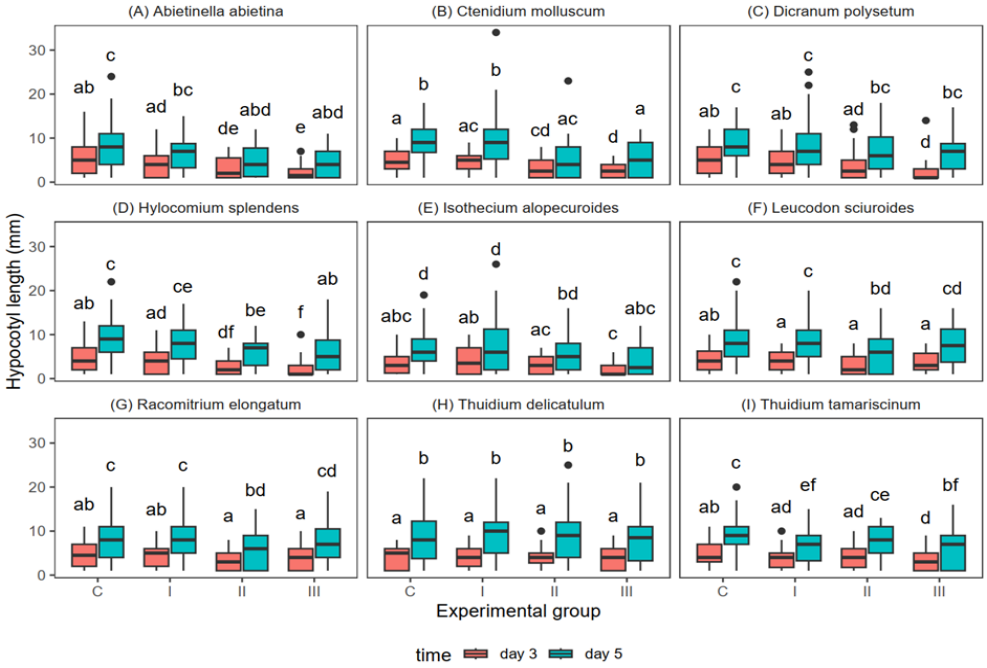

Figure 5. The effect of the ethanolic moss extracts on the radish seedling hypocotyl length. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively). Black horizontal lines represent the median, while the box ranges from the first (Q1) to the third (Q3) quartile, with the whiskers extending to the 1.5 × IQR (interquartile range) from the edge of the boxes. The black dots represent observations outside the 1.5 × IQR (interquartile range). Statistically significant differences ($p < 0.05$) among the experimental groups are indicated by the letters above the boxplots.

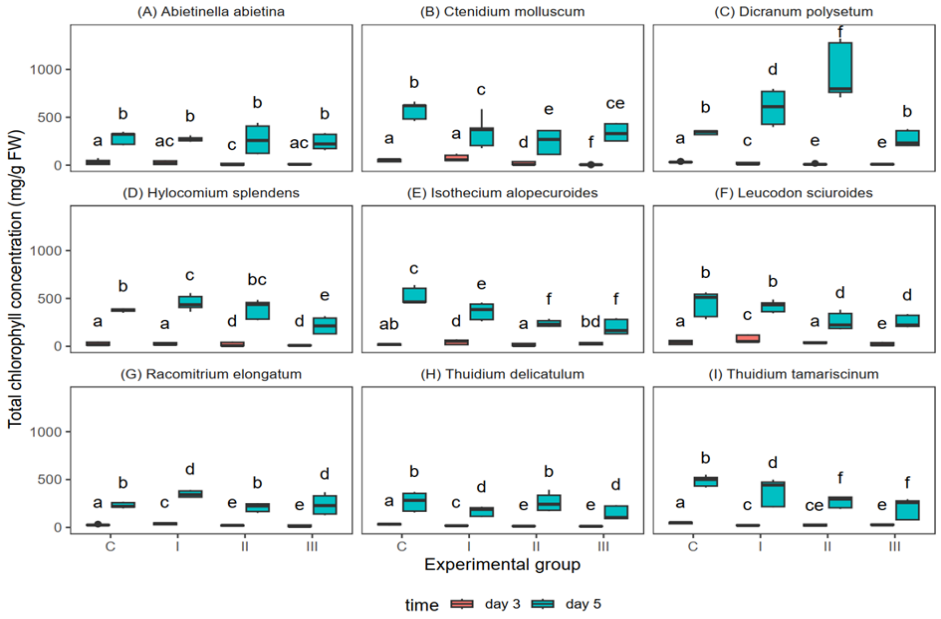

Figure 6. The total chlorophyll concentration of the radish seedlings. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively). Black horizontal lines represent the median, while the box ranges from the first (Q1) to the third (Q3) quartile, with the whiskers extending to the 1.5 × IQR (interquartile range) from the edge of the boxes. The black dots represent observations outside the 1.5 × IQR (interquartile range). Statistically significant differences ($p < 0.05$) among the experimental groups are indicated by the letters above the boxplots.

3.3. The Effect of the Moss Extracts on the Lettuce Seedling Hypocotyl Length

The extract concentrations of eight out of nine tested moss species (namely *A. abietina*, *C. molluscum*, *D. polysetum*, *H. splendens*, *I. alopecuroides*, *R. elongatum*, *T. delicatulum*, and *T. tamariscinum*) significantly affected the lettuce seedling hypocotyl length ($p < 0.01$, Table 3). The treatment duration of all the tested moss extracts affected the lettuce seedling hypocotyl length. ($p < 0.001$, Table 3). Additionally, the interaction between the extract concentrations and the treatment duration significantly impacted the lettuce seedling hypocotyl length in the treatments of three moss species (*A. abietina*, *H. splendens*, and *I. alopecuroides*) ($p < 0.05$, Table 3). Conversely, the extracts derived from *L. sciuroides* exhibited no effects on the lettuce hypocotyl length, irrespective of treatment duration (Figure 3F). In addition, no statistically significant differences were observed between the treatments of different extract concentrations of *L. sciuroides* (Figure 3F). Furthermore, different extract concentrations of two tested mosses (*R. elongatum* and *T. delicalutum*) did not show any clear pattern in the effects on the hypocotyl length (Figure 3H,G).

Table 3. The results of the factorial analysis which examined the influence of the ethanolic extracts from nine tested bryophyte species on the hypocotyl length and total chlorophyll content of the lettuce seedlings by assessing the main effects of concentration (C), treatment duration (T), and their interaction (C × T).

Species	Parameter	C	T	C × T
Abietinella abietina	Hypocotyl length	51.995 ***	131.77 ***	3.6807 *
	Total Chl	2.5974	194.98 ***	1.6675
Ctenidium molluscum	Hypocotyl length	10.428 ***	91.365 ***	0.3476
	Total Chl	5.9260 **	197.28 ***	4.6742 **
Dicranum polysetum	Hypocotyl length	31.86 ***	109.3 ***	0.152
	Total Chl	9.8232 ***	193.99 ***	10.399 ***
Hylocomium splendens	Hypocotyl length	34.266 ***	193.94 ***	3.5096 *
	Total Chl	3.594 *	193.1 ***	1.703
Isothecium alopecuroides	Hypocotyl length	66.155 ***	174.001 ***	4.3349 **
	Total Chl	9.7412 ***	193.69 ***	21.769 ***
Leucodon sciuroides	Hypocotyl length	0.5698	225.72 ***	0.9065
	Total Chl	11.596 ***	193.25 ***	9.8766 ***
Racomitrium elongatum	Hypocotyl length	4.2257 **	251.05 ***	1.1268
	Total Chl	29.512 ***	201.79 ***	30.297 ***
Thudium delicatulum	Hypocotyl length	5.7759 ***	203.78 ***	1.1136
	Total Chl	8.875 ***	193.8 ***	8.136 ***
Thudium tamariscinum	Hypocotyl length	15.065 ***	119.10 ***	0.3762
	Total Chl	0.41353	192.87 ***	2.3583

The values represent the F values with the asterisks denoting the corresponding level of statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Three tested species of mosses (namely, *C. molluscum*, *H. splendens* and *T. tamariscinum*) exhibited a mild inhibitory effect on the lettuce hypocotyl length (Figure 3B,D,I). The highest concentration extracts (2×10^{-3} mg/mL) derived from *C. molluscum*, *H. splendens* and *T. tamariscinum* resulted in a reduction of the lettuce seedling hypocotyl length both after three and five days, i.e., statistically significant differences were noted between the treatments with the highest extract concentrations of these mosses and the control group (Figure 3B,D,I). Also, a decrease in the lettuce seedling hypocotyl length was detected when the lettuce seedlings were treated with extracts of *C. molluscum* and *H. splendens* at a concentration of 10^{-3} mg/mL after five days. The long-term treatment with extracts of this concentration from two moss species (*C. molluscum* and *H. splendens*) negatively affected the lettuce seedling hypocotyl length (Figure 3B,D).

However, the extracts of three tested moss species (*A. abietina*, *D. polysetum*, and *I. alopecuroides*) exhibited a strong allelopathic effect on the length of the lettuce hypocotyl (Figure 3A,C,E). Each of the used concentrations of the *A. abietina* extract demonstrated an inhibitory effect on the lettuce hypocotyl length compared to the control group (Figure 3A). Additionally, statistically significant differences were detected between the treatments with lower concentrations of the extracts (10^{-4} and 10^{-3} mg/mL) as well as the highest concentration of the extract (2×10^{-3} mg/mL) (Figure 3A). Similar effects on the length of the lettuce hypocotyl were detected when the seeds were treated with the *I. alopecuroides* extracts (Figure 3E). All concentrations of the extract derived from *D. polysetum* resulted in a significant decrease in the lettuce hypocotyl length compared to the control group. Statistically significant differences were detected between treatments of lower extract concentrations (10^{-4} and 10^{-3} mg/mL) obtained from *D. polysetum* after five days (Figure 3C).

Among all the tested extracts on the lettuce hypocotyl length, the strongest allelopathic effects were documented for the *A. abietina*, *D. polysetum* and *I. alopecuroides* extracts, while the extracts of *L. sciuroides*, *R. elongatum*, and *T. delicatulum* exhibited no clear allelopathic patterns.

3.4. The Effect of the Moss Extracts on the Total Chlorophyll Concentration of the Lettuce Seedlings

Regarding the effect of extract concentration on the total chlorophyll concentration of the lettuce seedlings, all of the tested moss extracts affected the total chlorophyll concentration except that of *A. abietina* ($p < 0.05$, Table 3). However, the treatment duration of all the tested moss extracts did affect the total chlorophyll concentration of the lettuce seedlings ($p < 0.001$, Table 3). When considered multifactorially, the interaction, extract concentrations, and time affected the total chlorophyll concentration of the lettuce seedlings in treatments of six moss species (*C. molluscum*, *D. polysetum*, *I. alopecuroides*, *L. sciuroides*, *R. elongatum* and *T. delicatulum*) ($p < 0.01$, Table 3).

The total chlorophyll concentration of the lettuce seedlings differed significantly after three and five days within the same treatment. In the first three days, the young seedlings started the process of biosynthesis of photosynthetic pigments, so variations in the total chlorophyll concentration were observed between the third and fifth days. Extracts from two moss species (namely, *A. abietina* and *T. tamriscinum*) did not affect the total chlorophyll concentration of the lettuce seedlings either after three or five days i.e., this moss species did not have any significant effects on chlorophyll biosynthesis (Figure 4A,I). On the other hand, the extract concentrations of three moss species (*C. molluscum*, *H. splendens*, and *T. delicatulum*) did not show a clear effect on the total chlorophyll concentration of the lettuce seedlings (Figure 4B,D,H). However, extract concentrations of 10^{-3} and 2×10^{-3} mg/mL derived from *T. delicatulum* significantly decreased the total chlorophyll concentration of the lettuce seedlings, but only after three days (Figure 4H).

Nevertheless, the extracts obtained from *D. polysetum*, *I. alopecuroides*, *L. sciuroides*, as well as *R. elongatum* affected the total chlorophyll concentration of the lettuce seedlings (Figure 4C,E–G). The highest used concentration (2×10^{-3} mg/mL) of the *D. polysetum* moss extract had a significant effect on the reduction of photosynthetic pigment synthesis at both three and five days—long exposure (Figure 4C). Lower chlorophyll concentration was noticed in the lettuce seedlings treated with extract concentrations of 10^{-3} and 2×10^{-3} mg/mL derived from *I. alopecuroides* after three days. In contrast, on the fifth day, none of the used extract concentrations influenced the expected developmental increase in the total chlorophyll concentration of the lettuce seedlings. Statistically significant differences were observed in the total chlorophyll concentration of the lettuce seedlings for all the experimental groups after three and five days compared to the control (Figure 4E). Two moss species (*L. sciuroides* and *R. elongatum*) had a similar effect on the total chlorophyll concentration of the lettuce seedlings. No statistically significant differences regarding the total chlorophyll concentration were observed between the treatments and the control group or between different treatments after three days. However, those moss species affected a decrease in the total chlorophyll concentration of the lettuce seedlings when

treated with somewhat higher extract concentrations of 10^{-3} and 2×10^{-3} mg/mL after five days (Figure 4F,G).

3.5. The Effect of the Moss Extracts on the Radish Seedling Hypocotyl Length

The extract concentrations from eight out of nine moss species (*A. abietina*, *C. molluscum*, *D. polysetum*, *H. splendens*, *I. alopecuroides*, *L. sciuroides*, *R. elongatum*, and *T. tamariscinum*) also had a significant impact on the hypocotyl length of the radish seedlings ($p < 0.05$, Table 4). The treatment duration with the extracts of all the tested moss species affected the radish seedlings' hypocotyl length ($p < 0.001$, Table 4).

Table 4. The results of the factorial analysis examining the influence of the ethanolic extracts from nine tested bryophyte species on the hypocotyl length and total chlorophyll content of the radish seedlings by assessing the main effects of concentration (C), treatment duration (T), and their interaction (C × T).

Species	Parameter	C	T	C × T
Abietinella abietina	Hypocotyl length	11.729 ***	27.274 ***	0.547
	Total Chl	4.1820 ***	194.21 ***	1.3239
Ctenidium molluscum	Hypocotyl length	13.789 ***	58.425 ***	2.4708
	Total Chl	23.895 ***	198.63 ***	13.959 ***
Dicranum polysetum	Hypocotyl length	3.561 *	37.84 ***	0.232
	Total Chl	43.586 ***	200.45 ***	65.753 ***
Hylocomnium splendens	Hypocotyl length	9.8567 ***	60.798 ***	0.3352
	Total Chl	18.55 ***	200.0 ***	16.7 ***
Isothecium alopecuroides	Hypocotyl length	6.9103 ***	29.532 ***	0.8748
	Total Chl	37.950 ***	193.96 ***	51.563 ***
Leucodon sciuroides	Hypocotyl length	4.3754 **	90.711 ***	0.4317
	Total Chl	17.531 ***	197.61 ***	9.1641 ***
Racomitrium elongatum	Hypocotyl length	3.7890 *	60.649 ***	0.4399
	Total Chl	19.070 ***	192.75 ***	15.849 ***
Thuidium delicatulum	Hypocotyl length	0.5598	91.984 ***	0.3833
	Total Chl	9.615 ***	193.6 ***	7.518 ***
Thuidium tamariscinum	Hypocotyl length	7.2198 ***	87.760 ***	0.2007
	Total Chl	30.479 ***	198.20 ***	25.062 ***

The values represent the F values with the asterisks indicating the corresponding level of statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Again, only one species out of nine exhibited no effect. However, it was not the same one as in the case of the lettuce. The extract obtained from *T. delicatulum* did not affect the hypocotyl length of the radish seedlings, either after three or five days (Figure 5H), i.e., this extract did not affect the hypocotyl elongation. Moreover, no statistically significant differences regarding the radish hypocotyl length were observed between treatments and the control group or between different treatments (Figure 5H). However, the elongation of the radish seedlings' hypocotyl length did not correlate with increased extract concentrations in two of the used moss species (*L. sciuroides* and *R. elongatum*) (Figure 5F,G).

Three moss species (*D. polysetum*, *I. alopecuroides*, and *T. tamariscinum*) exhibited a mild inhibitory effect on the radish hypocotyl length when the seeds were treated with the highest extract concentration (2×10^{-3} mg/mL) (Figure 5C,E,I). Lower extract concentrations (10^{-4} and 10^{-3} mg/mL) did not affect the length of the radish hypocotyl at all when compared with the control group (Figure 5C,E,I). The highest extract concentration of *D. polysetum* (2×10^{-3} mg/mL) showed an inhibitory effect on the radish hypocotyl length only after three days (Figure 5C). The same effect on the elongation of the radish hypocotyl was observed in the treatment with the highest extract concentration of *I. alopuroides*, but only after five days (Figure 5E). However, the highest extract concentra-

tion of 2×10^{-3} mg/mL obtained from *T. tamariscinum* resulted in a decrease in the radish hypocotyl length both after three and five days, compared to the control group (Figure 5I).

On the other hand, the extracts obtained from *A. abietina*, *C. molluscum*, and *H. splendens* significantly affected the decrement of the radish hypocotyl length, especially after three days (Figure 5A,B,D). Increased concentrations of the *A. abietina* moss extracts gradually inhibited the formation of the radish hypocotyl, which was statistically significant (Figure 5A). Additionally, the extracts derived from *C. molluscum* and *H. splendens* exhibited a similar pattern regarding the inhibition of the radish hypocotyl growth (Figure 5B,D).

In general, a strong allelopathic effect on the radish hypocotyl formation was documented for the extracts derived from *A. abietina*, *C. molluscum*, and *H. splendens* while the extract of *T. delicatulum* had no effect.

3.6. The Effect of the Moss Extracts on the Total Chlorophyll Concentration of the Radish Seedlings

The extract concentrations of all the tested moss species significantly affected the total chlorophyll concentration of the radish seedlings ($p < 0.01$, Table 4). Also, the treatment duration with the extracts of all the tested moss species affected the total chlorophyll concentration of the radish seedlings ($p < 0.001$, Table 4). The interaction between the treatment duration and the extract concentration of all the tested moss species (with the exception of *A. abietina*) showed a significant effect on the total chlorophyll concentration of the radish seedlings ($p < 0.001$, Table 4).

Our results indicated that radish seedlings start the process of chlorophyll biosynthesis slightly later than lettuce under the laboratory conditions provided. Therefore, an extremely low total chlorophyll concentration was observed after the first three days. Thus, statistically significant differences in the total chlorophyll concentration were detected between the third and fifth days within the same experimental groups, which may be regarded as an artefact (Figure 6).

Abietinella abietina did not affect the synthesis of the photosynthetic pigments of the radish seedlings; moreover, no statistically significant differences were noticed between the control and all the experimental groups (Figure 6A). Increasing extract concentrations from two moss species (*R. elongatum* and *T. delicatulum)* did not show a clear trend regarding the total chlorophyll content of the radish seedlings after five days (Figure 6G,H). Nevertheless, increasing extract concentrations from *R. elongatum* exhibited a slight stimulatory effect on the chlorophyll biosynthesis of the radish seedlings after three days (Figure 6G). In contrast, with increasing concentrations of the *T. delicatulum* extracts, a gradual decrease in chlorophyll concentration was detected in the radish seedlings after three days (Figure 6H).

The extract concentration of 10^{-4} mg/mL derived from *H. splendens* influenced a slight increase in the chlorophyll concentration, while the highest extract concentration (2×10^{-3} mg/mL) of *H. splendens* resulted in a decrease in the total chlorophyll concentration after five days (Figure 6D).

On the other hand, gradually increased concentrations of the extract of five moss species (*C. molluscum*, *I. alopecuroides*, *L. sciuroides*, and *T. tamariscinum*) caused a gradual decrease in the total chlorophyll concentration of the radish seedlings after five days (Figure 6B,E,F,I). No statistically significant differences were noticed between the experimental groups of two extract concentrations 10^{-3} and 2×10^{-3} mg/mL, indicating that these extract concentrations had a similar effect on the chlorophyll synthesis of the radish seedlings (Figure 6B,E,F,I).

The total chlorophyll concentration increased when the radish seedlings were treated with lower extract concentrations (10^{-4} and 10^{-3} mg/mL) of the moss *D. polysetum* after five days (Figure 6C). Those two extract concentrations of *D. polysetum* had a significant stimulatory impact on the chlorophyll synthesis of the radish seedlings. However, the highest extract concentration (2×10^{-3} mg/mL) of *D. polysetum* did not affect the total chlorophyll concentration of the radish seedlings (Figure 6C).

A summary of the effects of various moss extracts for better visibility is presented as a heat map in Figure 7.

		Lettuce								Radish							
	Treatment duration	3 days				5 days				3 days				5 days			
	Experimental groups	C	I	II	III	C	I	II	III	C	I	II	III	C	I	II	III
Hypocotyl length	*Abietinella abietina*	7.15	5.42	4.97	3.37	11.85	8.48	8.00	5.61	5.54	4.13	3.26	2.37	8.62	6.36	4.82	4.58
	Ctenidium molluscum	5.97	5.28	4.78	3.88	9.87	8.38	7.56	6.77	4.87	4.34	3.04	2.60	8.82	9.28	5.03	5.28
	Dicranum polysetum	7.73	5.85	4.79	4.32	11.30	9.05	7.44	7.32	5.03	4.86	3.79	2.74	8.56	8.12	7.16	6.38
	Hylocomium splendens	5.56	4.89	4.58	1.97	11,27	10.46	7.61	6.59	4.87	3.98	2.90	2.36	9.18	7.72	6.13	5.77
	Isothecium alopecuroides	6.52	4.72	3.97	2.38	10.86	8.70	7.61	4.48	3.76	4.18	3.21	2.25	6.64	7.52	5.37	4.00
	Leucodon sciuroides	6.36	6.39	6.52	6.60	10.38	10.32	9.94	9.83	4.37	3.87	3.23	3.67	8.58	8.06	6.02	7.40
	Racomitrium elongatum	5.00	4.38	5.06	4.94	9.45	7.93	8.62	8.25	4.76	4.45	3.55	3.94	8.09	7.61	5.87	7.37
	Thuidium delicatulum	4.68	4.98	3.62	4.61	8.69	7.89	6.76	8.09	4.04	3.98	3.81	3.62	8.58	8.69	8.92	8.06
	Thuidium tamariscinum	6.02	6.72	7.16	4.77	9.76	10.12	10.47	7.52	4.81	3.70	4.02	3.17	8.78	6.62	7.50	6.29

	Experimental groups	C	I	II	III	C	I	II	III	C	I	II	III	C	I	II	III
Total chlorophyll content	*Abietinella abietina*	94.52	125.56	105.21	87.08	424.11	405.42	483.40	407.30	30.76	24.60	9.09	10.64	272.03	266.50	266.50	240.89
	Ctenidium molluscum	71.94	84.24	106.48	85.06	395.90	499.63	432.49	417.71	50.74	71.96	16.49	4.51	578.60	357.41	247.73	338.71
	Dicranum polysetum	72.40	81.35	67.19	64.00	392.73	357.57	448.07	295.69	32.52	16.79	9.27	8.63	340.63	600.51	941.80	265.37
	Hylocomium splendens	79.16	61.73	81.03	83.93	369.48	354.22	389.34	336.06	24.25	24.91	18.86	9.40	376.17	451.41	397.17	216.71
	Isothecium alopecuroides	55.72	57.97	43.59	34.84	337.93	408.02	437.71	426.75	17.48	46.58	14.40	28.69	514.55	368.35	238.00	194.19
	Leucodon sciuroides	77.08	86.32	86.68	76.27	336.16	404.68	353.19	240.44	41.34	72.96	37.60	21.56	457.42	418.76	254.09	250.98
	Racomitrium elongatum	73.58	82.58	79.01	74.52	470.09	460.94	305.79	236.46	25.03	38.49	20.01	14.52	233.81	347.01	210.60	238.29
	Thuidium delicatulum	63.13	54.81	70.44	83.80	360.53	362.33	260.15	371.44	32.11	18.93	13.51	10.96	269.25	170.66	262.63	140.75
	Thuidium tamariscinum	83.94	100.65	87.80	72.70	383.30	353.65	378.69	378.96	46.55	21.74	23.22	27.77	487.29	378.86	272.53	208.98

min max min max

Figure 7. The effects of various concentrations of the selected moss ethanolic extracts on the total chlorophyll content (mg/g FW) and hypocotyl length (mm) in radish and lettuce. C—control groups; I–III—applications of 10^{-4}, 10^{-3}, 2×10^{-3} mg/mL concentrations of the selected moss species extract (respectively).

4. Discussion

As allelopathy is a very complex phenomenon, it is not surprising to encounter contrasting results regarding the extract's effects on seed germination and seedling development. The results obtained in this study clearly show the presence of chemical communication among moss species with lettuce and radish. These interactions can be regarded as stimulatory, inhibitory, or indifferent, depending on the tested features in lettuce and radish. In similar studies conducted to date, both inhibitory and promoting effects have been documented [10,32]. These different allelopathic interactions are mostly driven by the chemical composition of the moss and the concentration of the extracts [32]. Moreover, seed germination is likely to be dosage-dependent, i.e., higher extract concentrations usually exhibit stronger effects than lower concentrations. Such results were also documented for the undiluted aqueous extracts of the liverwort *Bazzania trilobata* (L.) Gray which inhibited the seed germination of cress [10]. Moreover, the inhibition of seed germination is likely to occur when ethanolic, methanolic, and acetone extracts are applied rather than aqueous because of the chemical nature of allelochemicals [10,33,34].

4.1. The Effect of the Moss Extracts on Tested Vegetable Seed Germination

In our study, none of the tested bryophyte extracts affected the germination of the lettuce seeds (Figure 1). On the other hand, only four extracts, those from the mosses *C. molluscum*, *D. polysetum*, *H. splendens*, and *I. alopecuroides* inhibited the germination of the radish seeds (Figure 2). However, the effects on the seedling growth phase, i.e., the hypocotyl length were *species*-specific and somewhat dependent on the concentrations (Figures 3 and 5).

Although some extracts did not affect seed germination, their effects were noticeable in the later developmental stages. Those results are not surprising since allelopathic effects can act primarily at the seedling phase rather than on germination [32,35].

4.2. The Effect of the Moss Extracts on Tested Vegetable Hypocotyl Length

In this study, we found that the interaction is species-specific and depends on the tested counterparts (Figure 7). Despite being unable to infer very much from the hypocotyl length and total chlorophyll content after 3 day-long treatments with various concentrations of different moss extracts, it is noticeable that hypocotyl elongation was slightly suppressed in both lettuce and radish. Although there are certain dissimilarities between all the tested moss extracts concerning the lettuce and radish hypocotyl growth, the extracts of *L. sciuroides* and *R. elongatum* did not affect the elongation of the hypocotyl length either in lettuce or in radish. On the other hand, the extracts of *A. abietina* inhibited the elongation of the hypocotyl in both lettuce and radish, indicating a strong allelopathic effect. The other tested extracts manifested variable effects depending on concentration, the duration of the experiment, and the species tested. Therefore, the allelopathic effects can be described as species-to-species specific for most of the tested taxa.

Moreover, the results also show that the extracts of *L. sciuroides* do not affect the hypocotyl length in either lettuce or radish. These patterns are also confirmed after 5 days for the same moss species extracts, which makes this species a good candidate for testing among lettuce pests as a nature-friendly treatment, i.e., for potential biohericides. The extracts of the moss *T. tamariscinum* also showed promising potential for use in lettuce treatments. Previous studies have indicated that some isolated compounds from bryophytes such as those from the liverwort *Porella obtusata* (Tayl.) Trevis [36] and moss *Dicranum scoparium* Hedw Ref. [37] display repellent potential on slugs during the consumption of lettuce leaves. Regarding the radish, the pattern shown by the *T. delicatulum* extracts suggests that this species could be a potential source for testing as a radish pest-effective agent.

4.3. The Total Chlorophyll Content Is Rather Unstable under Moss Extract Treatments

On the other hand, the total chlorophyll content measured after three days of treatments may not serve as an adequate reference point since there were no significant differences between the development of the plantlets and the control group. Also, the short period of underdeveloped primarily leaves during which energy is derived from the cotyledons should be considered when interpreting these effects rather than after the fifth day of treatments. Indeed, non-harmful or even slightly stimulative effects of the extracts applied to lettuce as indicated by the total chlorophyll content compared to the control plant group under the same laboratory conditions were recorded in the extracts of most of the moss species applied. However, this was not the case in the radish tests, where the total chlorophyll content decreased compared to the control plant group, except in low extract concentrations of *D. polysetum* and *H. splendens.* The extract of *D. polysetum* at a concentration of 10^{-3} mg/mL even showed a stimulatory effect on the total chlorophyll content in radish.

5. Conclusions

Mosses communicate with vascular plants chemically, and the type of interaction and its effect are species-to-species-specific. Apart from species, the targeted effect depends on the type of extract, concentration, and duration of extract applications. Among the tested species, *L. sciuroides* exhibited the least harmful effect on lettuce overall, while for radish, that was *D. polysetum*. The most negative effect on both vegetable species was documented for *I. alopecuroides*, although higher concentrations of its extracts were stimulative for lettuce as indicated by the chlorophyll content. A stimulatory effect was also documented for lower concentrations of *D. polysetum* extracts in radish. Although the tested bryophyte extracts showed bryophyte-crop interaction, it is clear that more pairwise tests are needed along with additional approaches to clarify the mechanisms of these interactions.

Author Contributions: Conceptualization, M.S.S. and A.D.S.; methodology, M.S.S. and A.D.S.; software, D.P.B.; validation, M.G., M.M.V., A.D.S. and M.S.S.; formal analysis, N.A.M., M.V.Ć., D.P.B., S.D.P. and S.P.; investigation, N.A.M., M.V.Ć. and D.P.B.; resources, A.D.S. and M.S.S.; data curation, N.A.M. and D.P.B.; writing—original draft preparation, N.A.M., M.V.Ć., D.P.B., S.D.P. and S.P.; writing—review and editing, M.S.S. and A.D.S.; visualization, D.P.B.; supervision, M.S.S. and A.D.S.; project administration, A.D.S.; funding acquisition, A.D.S. and M.S.S. All authors have read and agreed to the published version of the manuscript.

Funding: The project is supported by the Serbian Ministry of Science, Technological Development and Innovations, contract no: 451-03-65/2024-03/200178 and 451-03-66/2024-03/200178.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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

References

1. Gniazdowska, A.; Bogatek, R. Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiol. Plant.* **2005**, *27*, 395–407. [CrossRef]
2. Rice, E.L. *Allelopathy*, 2nd ed.; Academic Press Inc.: Orlando, FL, USA, 1984; p. 424.
3. Soltys, D.; Krasuska, U.; Bogatek, R.; Gniazdowska, A. Allelochemicals as bioherbicides—Present and perspectives. In *Herbicides-Current Research and Case Studies in Use*; Price, A.J., Kelton, J.A., Eds.; IntechOpen: London, UK, 2013; pp. 517–542.
4. Asakawa, Y.; Ludwiczuk, A.; Nagashima, F. Phytochemical and biological studies of bryophytes. *Phytochemistry* **2013**, *91*, 52–80. [CrossRef] [PubMed]
5. Asakawa, Y.; Ludwiczuk, A.; Nagashima, F. *Chemical Constituents of Bryophyte*, 1st ed.; Springer: Vienna, Austria, 2013; pp. 563–605.
6. Sabovljević, M.S.; Sabovljević, A.D.; Ikram, N.K.K.; Peramuna, A.; Bae, H.; Simonsen, H.T. Bryophytes—An emerging source for herbal remedies and chemical production. *Plant Genet. Res.* **2016**, *14*, 314–327. [CrossRef]
7. Xie, C.; Lou, H. Secondary metabolites in bryophytes: An ecological aspect. *Chem. Biodivers.* **2009**, *6*, 303–312. [CrossRef] [PubMed]
8. Ćosić, M.V.; Mišić, D.M.; Jakovljević, K.M.; Giba, Z.S.; Sabovljević, A.D.; Sabovljević, M.S.; Vujičić, M.M. The selected moss analyses of quantitative and qualitative content of phenolic compounds under NaCl stress. *Molecules* **2023**, *28*, 1794. [CrossRef] [PubMed]
9. Willis, R.J. *The History of Allelopathy*; Springer Science & Business Media: Dordrecht, The Netherlands, 2007.
10. Whitehead, J.; Wittemann, M.; Cronberg, N. Allelopathy in bryophytes—A review. *Lindbergia* **2018**, *41*, 1–7. [CrossRef]
11. Chen, F.; Ludwiczuk, A.; Wei, G.; Chen, X.; Crandall-Stotler, B.; Bowman, J.L. Terpenoid secondary metabolites in bryophytes: Chemical diversity, biosynthesis and biological functions. *Crit. Rev. Plant Sci.* **2018**, *37*, 210–231. [CrossRef]
12. Kulshrestha, S.; Jibran, R.; Van Klink, J.W.; Zhou, Y.; Brummell, D.A.; Albert, N.W.; Schwinn, K.E.; Changé, D.; Landi, M.; Bowman, J.L.; et al. Stress, senescence, and specialized metabolites in bryophytes. *J. Exp. Bot.* **2022**, *73*, 4396–4411. [CrossRef]
13. Harris, E.S. Ethnobryology: Traditional uses and folk classification of bryophytes. *Bryologist* **2008**, *111*, 169–217. [CrossRef]
14. Mandić, M.R.; Oaldje, M.M.; Lunić, T.; Sabovljević, A.D.; Sabovljević, M.S.; Gašić, U.M.; Duletić-Laušević, S.N.; Božić, B.D.; Božić Nedeljković, B.D. Chemical characterization and in vitro immunomodulatory effects of different extracts of moss *Hedwigia ciliata* (Hedw.) P. Beauv. from the Vršačke Planine Mts., Serbia. *PLoS ONE* **2021**, *16*, e0246810. [CrossRef]
15. Drobnik, J.; Stebel, A. Four centuries of medicinal mosses and liverworts in European ethnopharmacy and scientific pharmacy: A review. *Plants* **2021**, *10*, 1296. [CrossRef] [PubMed]
16. Newton, A.E.; Mishler, B.D. The evolutionary significance of asexual reproduction in mosses. *J. Hattori Bot. Lab.* **1994**, *76*, 127–145.
17. Vicherová, E.; Glinwood, R.; Hájek, T.; Šmilauer, P.; Ninkovic, V. Bryophytes can recognize their neighbours through volatile organic compounds. *Sci. Rep.* **2020**, *10*, 7405. [CrossRef] [PubMed]
18. Löbel, S.; Dengler, J.; Hobohm, C. Species richness of vascular plants, bryophytes and lichens in dry grasslands: The effects of environment, landscape structure and competition. *Folia Geobot.* **2006**, *41*, 377–393. [CrossRef]
19. Frahm, J.P.; Risse, S.; Saan-Klein, B.V. Are bryophytes extracts inhibiting or promoting seed growth? *Arch. Bryol.* **2012**, *127*, 1–10.
20. Wang, Q.; Zhao, C.; Gao, Y.; Gao, C.; Qiao, Y.; Xie, H.; Wang, W.; Yuan, L.; Liu, J.; Ma, W.; et al. Effects of *Abietinella abietina* extracts on the germination and seedling emergence of *Picea crassifolia*: Results of greenhouse experiments. *Pol. J. Ecol.* **2016**, *64*, 357–368.
21. Huneck, S.; Meinunger, L. Plant growth regulatory activities of bryophytes: A contribution to the chemical ecology of mosses and liverworts. Bryophytes, their chemistry and chemical taxonomy. *Proc. Phytochem. Soc. Eur.* **1990**, *29*, 289–298.
22. Kato-Noguchi, H. Allelopathic chemical interaction of bryophytes with vascular plants. *Mini Rev. Org. Chem.* **2016**, *13*, 422–429. [CrossRef]
23. Kobayashi, K.; Shigemori, H. Allelopathy of the moss *Hypnum plumaeforme* by the production of momilactone A and B. *Weed Res.* **2009**, *49*, 621–627.

24. Kato-Noguchi, H.; Seki, T. Allelopathy of the moss *Rhynchstegium pallidifolium* and 3-hydroxy-β-ionene. *Plant Signal. Behav.* **2010**, *5*, 702–704. [CrossRef]
25. Kato-Noguchi, H.; Seki, T.; Shigemori, H. Allelopathy and allelopathic substance in the moss *Rhynchostegium pallidifolium*. *J. Plant Physiol.* **2010**, *167*, 468–471. [CrossRef] [PubMed]
26. Lichtenthaler, H.K. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382.
27. R Core Team, R. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022; Available online: https://www.R-project.org/ (accessed on 25 December 2023).
28. Agresti, A. *An Introduction to Categorical Data Analysis*, 2nd ed.; Wiley: Hoboken, NJ, USA, 2007.
29. Wobbrock, J.O.; Findlater, L.; Gergle, D.; Higgins, J.J. The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. In Proceedings of the ACM Conference on Human Factors in Computing Systems (CHI 2011), Vancouver, BC, Canada, 7–12 May 2011; ACM Press: New York, USA, 2011; pp. 143–146.
30. Elkin, L.A.; Kay, M.; Higgins, J.J.; Wobbrock, J.O. An aligned rank transform procedure for multifactor contrast tests. In Proceedings of the 34th Annual ACM Symposium on User Interface Software and Technology, Virtual, 10–14 October 2021; Nichols, J., Kumar, R., Bebeling, M., Eds.; Association for Computing Machinery: New York, NY, USA, 2021.
31. Kay, M.; Elkin, L.A.; Higgins, J.J.; Wobbrock, J.O. mjskay/ARTool: ARTool 0.11.0 (v0.11.0). Zenodo. 2021. Available online: https://zenodo.org/records/4721941 (accessed on 7 January 2024). [CrossRef]
32. Michel, P.; Burritt, D.J.; Lee, W.G. Bryophytes display allelopathic interactions with tree species in native forest ecosystems. *Oikos* **2011**, *120*, 1272–1280. [CrossRef]
33. Thakur, S.; Kapila, S. Allelopathic effect of some liverworts on seed germination of the weed *Bidens pilosa* L. *Int. J. Pharm. Sci. Rev. Res.* **2015**, *32*, 77–80.
34. Bhadauriya, G.; Singh, S.; Srivastava, K. Promoting and inhibiting effects of bryophyte extracts on the seed germination and seedling growth of *Vigna radiata* and *Cicer arietinum*. *Biol. Forum* **2016**, *8*, 410–416.
35. Tsubota, H.; Kuroda, A.; Masuzaki, H. Preliminary study on allelopathic activity of bryophytes under laboratory conditions using the sandwich method. *J. Hattori Bot. Lab.* **2006**, *100*, 395–409.
36. Frahm, J.P.; Kirchhoff, K. Antifeeding effects of bryophyte extracts from *Neckera crispa* and *Porella obtusata* against the slug *Arion lusitanicus*. *Cryptogam. Bryol.* **2002**, *23*, 271–275.
37. Rempt, M.; Pohnert, G. Novel acetylenic oxylipins from the moss *Dicranum scoparium* with antifeeding activity against herbivorous slugs. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 4755–4758. [CrossRef]