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Wider Use of Honey Plants in Farming: Allelopathic Potential of *Phacelia tanacetifolia* Benth.

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Abstract: *Phacelia tanacetifolia* Benth. is a melliferous, phytosanitary fodder plant. An important factor in understanding the biology of this plant is to investigate its allelopathic potential. In the experiment conducted here, ×*Triticosecale* Wittm. cv. Mamut was treated with water extracts from the roots, stems, leaves, and flowers of phacelia at concentrations of 10%, 12.5%, 15%, and control with 0% of extract. After 7 days of exposure, the germination of grains was assessed by analysing, seedling growth, mass parameters, water content, and electrolytes leakage. Aqueous extracts from the stalks, leaves, and flowers of phacelia significantly inhibited kernel germination at a 10% concentration, and from roots at a 12.5% concentration. The elongation growth of triticale seedlings was significantly inhibited by each of the extracts. Extracts from the leaves and flowers caused a significant reduction in fresh mass at a 10% concentration and extracts from the stalks at a 12.5% concentration. A significant reduction in water content was also found in seedlings watered with extracts of 10% from roots, stalks, and leaves and 12.5% from flowers. Extracts from phacelia roots at a 12.5% concentration and extracts from stalks, leaves, and flowers at a 10% concentration significantly increased the leakage of electrolytes. In general, phacelia exhibits allelopathic potential at higher concentrations of extracts.

Keywords: allelopathy; lacy phacelia; stress factors; stubble crop; sustainable agriculture

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1. Introduction

Allelopathy is considered one of the natural phenomena that affects the functioning of ecosystems at a significant level [1,2]. It includes different type of chemical interactions between organisms, realised by the synthesis and release of metabolites with inhibiting or facilitating properties for other organisms in the immediate vicinity. This term is mainly used in relation to plants. The knowledge of this phenomenon has a long history [3], however in recent decades studies on allelopathy have grown in respect to sustainable agriculture and forestry [4,5].

The knowledge of the positive or negative allelopathic effects of plants should be used in the selection of species for mixed crops and crop rotation [6–9]. Proper selection of plants can potentially reduce the negative allelopathic effects of crops (these effects in the long term can significantly reduce yields). The solution to these undesirable phenomena is the use of catch crops that interrupt unfavourable interactions between different or the same successive crop species and also contribute to the improvement of habitat conditions. Good agricultural practice proposes the use of catch crops to improve the physico-chemical and biological properties of the soil, thereby accelerating the decomposition of biomass by soil engineers, reducing weed growth, preventing soil erosion, and enriching the soil

with organic matter [10,11]. Proper crop selection should also consider plant interactions such as pest repelling and disease resistance; such considerations result in less chemical interference in crop protection, which is essential in sustainable agriculture [12,13]. Plant allelochemicals may be a substitute for synthetic pesticides, although their effectiveness and specificity are limited [14]. However, this mechanism may prove to be very important for management practices in organic farming.

One of the plants that is now increasingly used in stubble crops is *Phacelia tanacetifolia* Benth. Lacy phacelia (other common names: blue tansy, purple tansy) is a herbaceous annual plant that originates from the semi-deserts of California (North America), Once included in the borage family (Boraginaceae Juss.), it is now in a separate family waterleaf (Hydrophyllaceae R. Br. in Ker. Gawl.). It is one of the melliferous species popular among beekeepers [15]. It secretes nectar regardless of the weather and is eagerly visited by bees, even after dark [16]. It is also cultivated as a fodder plant in mixtures for direct feeding or silage [17,18]. For optimal development, it needs sufficiently moist, fertile, and warm soil [19]. As pointed out by Schappert et al. [20] phacelia more effectively covers the soil when grown in monoculture rather than in mixtures, which has a large impact on reducing surface erosion. In addition, it suppresses weeds [21] and improves the soil structure [16,22]. Tursun et al. [23] proved that phacelia as a cover crop in apricot orchards eliminates weeds by almost 75%. Live phacelia is less effective than, for example, glyphosate or mechanical weed control, but after mowing or ploughing it is more effective than these treatments. Some authors indicate [24] that the site after phacelia, compared to *Sinapis alba* L., is characterised by a greater number and biodiversity of accompanying plants, e.g., in organic oat cultivation. Based on the study of drought stress in plants in a greenhouse experiment, it was found that *P. tanacetifolia* has a much higher tolerance to water reduction compared to *Sinapis alba* and *Avena strigosa* Schreb. [19]. Furthmore, Handlířová et al. [25] found that, in an agroecosystem with a high average annual temperature and low rainfall totals, phacelia achieves higher and more stable yields compared to *Fagopyrum esculentum* Moench. In a Petri dish experiment designed to measure the palatability of organic matter of various stubble plants for earthworms *Lumbricus terrestris* L., Kliszcz, Puła [26] proved that during the first 24 h of breeding the soil-phacelia mixture the substrate uptake rate was the fastest by earthworms. In addition, on the phacelia plots, the population of endogenous earthworms increased compared to the plots with white mustard, indicating better trophic conditions for earthworms on the substrate with phacelia [27].

So far, few studies on the allelopathic potential of phacelia have been conducted to weeds [13,28], and in particular to crop plants with which this species occurs in crop rotation as a catch crop [24]. Therefore, the main aim of this laboratory experiment was to investigate the direct allelopathic effects (positive or negative) of organs of *Phacelia tanacetifolia*(flowers, stems, leaves, and roots) on the germination process, growth, and physiology (dry and fresh mass, water content, and electrolytes leakage) of cereals seedlings—×*Triticosecale* Wittm. cv. Mamut.

2. Short Characteristic of Study Plant

Phacelia tanacetifolia could grow up to 100 cm high with branches in the upper part. The whole plant is rough-hairy. It has single or double even-divided leaves, with strongly notched sections (Figure 1A). The flowers are quintuple, bell-shaped, and violet-blue in colour (e.g., caused by facelianin—[29]), with a discoid nectary located on the flower base that is protected by throat scales. They are clustered in inflorescences of the helicoid cyme and scorpioid types (Figure 1A,B). The fruit here is a capsule [30]. *P. tanacetifolia* is also considered an ornamental plant; it is a summer flower that can be sowed directly into the ground (it does not like replanting). In chemical terms, it is a relatively well-known species, and the main groups of compounds found in its organs are characterised in Table 1.

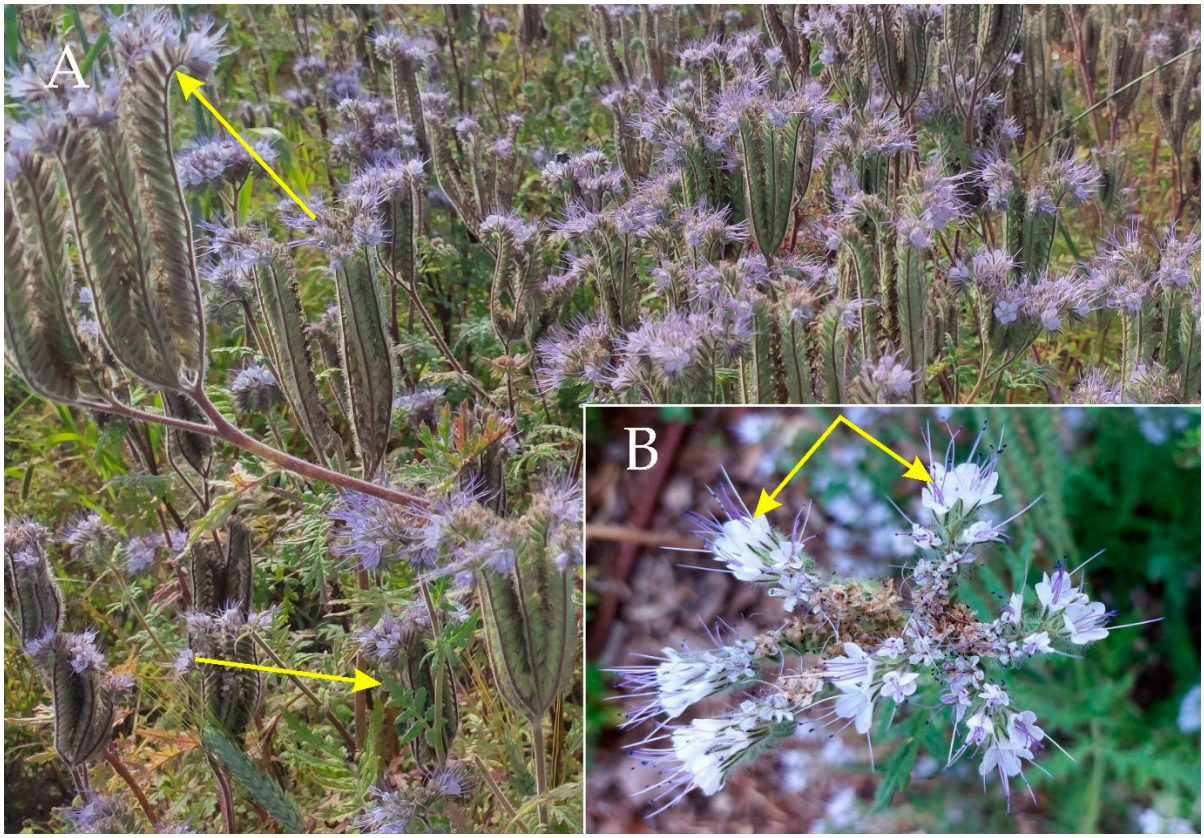

Figure 1. The cultivation of *Phacelia tanacetifolia* Benth.: (**A**)—specimens in blooming, (**B**)—flowers in detail; arrows indicate features of inflorescence and leaf (Photos. A. Stachurska-Swakoń and B. Barabasz-Krasny).

Table 1. The contents of chemicals in different parts of *Phacelia tanacetifolia* Benth., according to Bajkacz et al. [31]—(B), Kruk et al. [32]—(K), and Puig et al. [28]—(P).

Chemical Compound	Concentration [ng·g^{-1}]				[µL·ml^{-1}]
	Roots	Stalk	Leaf	Flower	Whole Plant
Aliphatic acid:					
3-HBA (3-Hydroxybutyric acid)	2.47 (B)	0.27 (B)	0.34 (B)	21.60 (B)	-
Aromatic acids:					
3,4-DHBA (3,4-Dihydroxybenzoic acid)	211.90 (B)	212.90 (B)	230.20 (B)	347.50 (B)	-
3-HPA (3-Hydroxypicolinic acid)	0.22 (B)	0.20 (B)	1.37 (B)	7.90 (B)	-
3,4-HPPA (4-Hydroxyphenylpyruvic acid)	237.20 (B)	141.40 (B)	161.50 (B)	77.90 (B)	-
BA (Benzoic acid)	197.30 (B)	170.90 (B)	360.90 (B)	648.50 (B)	-
CA (Caffeic acid)	1444.00 (B)	873.00 (B)	811.90 (B)	404.10 (B)	98.31 (P)
DOPAC (3,4-Dihydroxyphenylacetic acid)	380.60 (B)	1199.00 (B)	1956.00 (B)	868.90 (B)	-
FA (Ferulic acid)	823.80 (B)	608.10 (B)	610.10 (B)	385.60 (B)	-
HA (Hippurid acid)	0.73 (B)	0.76 (B)	27.10 (B)	0.80 (B)	-
HVA (4-hydroxy-3-methoxyphenylacetic acid)	43.50 (B)	25.00 (B)	13.00 (B)	24.10 (B)	-
p-CA (*para*-Caffeic acid)	324.80 (B)	11,778.00 (B)	297.90 (B)	173.40 (B)	-
p-HBA (p-Hydroxybenzoic acid)	-	-	-	-	28.29 (P)
Ester:					
4-HBA (4-Hydroxybutyl Acrylate)	4049.00 (B)	3809.00 (B)	3915.00 (B)	4784.00 (B)	-

Table 1. *Cont.*

Chemical Compound	Concentration [ng·g^{-1}]				[µL·ml^{-1}]
	Roots	**Stalk**	**Leaf**	**Flower**	**Whole Plant**
Polyphenols:					
Ellagitannin '1'	-	-	-	-	231.59 (P)
Ellagitannin '2'	-	-	-	-	7.49 (P)
ERC (Eriocitrin)			3.09 (B)	3.09 (B)	-
ERI (Eriodictyol)	0.19(a B)	2.50 (B)	25.80 (B)	21.30 (B)	12.51 (P)
FIS (Fisetin)	-	-	-	14.70 (B)	-
HSD (Hesperidin)	0.97 (B)	57.60 (B)	66.50 (B)	93.10 (B)	-
HST (Hesperetin)	0.42 (B)		0.67 (B)	0.16 (B)	-
Luteolin derivative '4'	-	-	-	-	1.82 (P)
NAR (Naringenin)	1.00 (B)	0.99 (B)	1.88 (B)	1.60 (B)	-
NARG (Naringin)	-	0.31 (B)	-	0.78 (B)	-
NHSD (Neohesperidin)	0.52 (B)	34.10 (B)	22.20 (B)	62.10 (B)	-
NRI (Narirutin)	4.75 (B)	7.60 (B)	3.60 (B)	7.80 (B)	-
PIN (Pinocembrin)	-	-	0.33 (B)	0.24 (B)	-
QUE (Quercetin)	0.27 (B)	74.30 (B)	58.10 (B)	20.60 (B)	-
R-ERI (R-enantiomer of Eriodictyol)	-	365.00 (K)	2574.00 (K)	1502.00 (K)	-
R-NAR (R-enantiomer of Naringenin)	-	141.00 (K)	230.00 (K)	311.00 (K)	-
RUT (Rutin)	2336.00 (B)	1129.00 (B)	10,296.00 (B)	13,922.00 (B)	-
S-ERI (S-enantiomer of Eriodictyol)	-	460.00 (K)	4752.00 (K)	4461.00 (K)	-
S-HST (S-enantiomer of Hesperetin)	-	210.00 (K)	380.00 (K)	244.00 (K)	-
S-NAR (S-enantiomer of Naringenin)	-	724.00 (K)	1298.00 (K)	1656.00 (K)	-
TAX (Taxifolin)	4.00 (B)	1.59 (B)	5.90 (B)	0.79 (B)	-

Its seeds are resistant to fire and do not contain the sucrose ester 6-O-linoleyl-α-D-glucopyranosyl-b-D-fructofuranoside that is present in the closely related fire-dependent *P. minor* (Harvey) Thell., *P. brachyloba* (Benth.) A. Gray and *P. grandiflora* (Benth.) A. Gray. The seeds of these species break the dormancy period after a fire. Egerton-Warburton and Ghisalberti [33] indicate that this compound has an inhibitory effect on fire-independent species (*P. tanacetifolia* and *P. campanularia* A. Gray.).

3. Materials and Methods

3.1. Plants Material

P. tanacetifolia specimens used in the experiment conducted here were collected from the cultivation field of the Experimental Station of the University of Agriculture in Krakow—Mydlniki (Southern Poland: 50°05′02.4″ N 19°51′13.8″ E) in favourable conditions for the plant's growth. Divided into parts, the plants (roots, stalks, leaves, and flowers) were dried in laboratory conditions: in the dark, at room temperature, with an average air humidity of 60–70%. The dried plant material was stored in paper bags in the dark at room temperature for the duration of the experiment.

Spring triticale grains (×*Triticosecale* cv. Mamut), as a testing plant in this experiment, were purchased from Małopolska Hodowla Plant Sp. z o.o., Poland.

3.2. Preparation of Extracts

Dried parts of *P. tanacetifolia* (roots, stalks, leaves, and flowers) were each separately ground into a mortar and water extracts were prepared with the following concentrations: 10%, 12.5%, 15%, and control (only distilled water). To extract chemical substances, the plant material was flooded with water and stored for 24 h in the dark and at room temperature 23 ± 2 °C (the sample was sealed with cellophane). After this time, the aqueous extracts were filtered through gauze and stored in a refrigerator at 8 ± 2 °C for the duration of the experiment.

3.3. Preparation of Cereal Grains and Conditions of Their Germination

Grains of ×*Triticosecale* cv. Mamut was sterilised in a 1% acetone solution for 1 min and then rinsed 3 times with distilled water. Twenty-five grains were placed in each sterile Petri dish (Ø 9 cm) with three layers of paper moistened with a suitable extract of *P. tanacetifolia*. The control group consisted of grains moistened only with distilled water. The Petri dishes were placed in the dark at room temperature (23 ± 2 °C) and 60–70% humidity.

The duration of the experiment was 7 days, as this was deemed sufficient time for the germination of triticale grains [34]. During this time, the number of germinated grains was checked every 24 h. Germinated seeds were considered those whose germinal root was equal to half the size of the grains. The experiment was carried out in 3 repetitions (each n = 25) for each concentration and type of phacelia extract and for the control group. On this basis, the germination parameters were determined as listed in Table 2.

Table 2. The germination coefficients formulas used in the statistical analyses.

No	Name of Parameter	Formula	According to Authors
1.	GP—Germination Percentage [%]	$GP = \frac{\sum_{i=1}^{k} n_i}{N} \times 100$ where: n_i: number of seeds newly germinating on day i; N: total number of seeds tested, and k: last day of germination	Khan et al. [35]
2.	MGT—Mean Germination Time [day]	$MGT\ (day) = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$ where: k—last day of germination, n_i—number of seeds newly germinating on day i, t_i—number of days from sowing	Możdżeń et al. [9]
3.	CVG—Coefficient of Velocity Germination [%]	$CVG\ (\%) = \frac{\sum_{i=1}^{k} n_i}{\sum_{i=1}^{k} n_i t_i} \times 100$ where: k—last day of germination, n_i—number of seeds newly germinating on day i, t_i—number of days from sowing	Bewley, Black [36], Jones, Sanders [37], Chiapusio et al. [38]
4.	GI—Germination Index [unit less]	$GI = \sum_{i=1}^{k} \frac{n_i}{t_i}$ where: k—last day of germination, n_i—number of seeds newly germinating on day i, t_i—number of days from sowing	AOSA [39]
5.	T_{50}—Time required for 50% germination [day]	$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)\left(t_j - t_i\right)}{\left(n_j - n_i\right)}$ where $n_i < \frac{N}{2} < n_j$ where : N—final number of germination, n_i, n_j—cumulative numbers of seeds germinated by adjacent counts at times t_i and t_j where $n_i < \frac{N}{2} < n_j$	Coolbear et al. [40], Farooq et al. [41]
6.	U—Uncertainty of germination process [bit]	$U\ (\text{bit}) = -\sum_{i=1}^{k} f_i \log_2 f_i$ where : U—Uncertainty of germination process, f_i is the relative frequency of germination (estimated as $f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$), k—last day of germination, n_i—number of seeds newly germinating on day i, t_i—number of days from sowing	Ranal, Santana [42]

3.4. Seedling Elongation Growth

Based on the measurement of seedling length ×*Triticosecale* cv. Mamut, watered with phacelia extracts (after 7 days of experiment), the growth inhibition index [%] of this species was determined according to the formula:

$$IP = [1 - (L_E/L_C)] \times 100, \quad (1)$$

where LE is the length of plants (mm) watered with a given emitter and LC is the length of plants (mm) from the control [43]. Seedling length measurements were made in 10 repetitions using the caliper (Topex 31C615, Warszawa, Poland) with an accuracy of 1 mm.

3.5. Plant Biomass and Water Content

Fresh mass (FM) of seedlings ×*Triticosecale* cv. Mamut was weighed on a laboratory balance (Radwag, WPS-120, Radom, Poland). They were then dried at 105 °C (Wamed SUP-100, Zabrze, Poland) to obtain dry matter (DM). The calculations of the percentage water content were made according to the following formula:

$$H_2O\ (\%) = 100 - [(DM \times 100)/FM] \quad (2)$$

H_2O—water, DM—dry mass, FM—fresh mass [44].

3.6. Electrolytes Leakage

Seedlings of ×*Triticosecale* cv. Mamut were placed in polypropylene falcons tubes with 30 mL of distilled water, with a conductivity of 0.05 µS. Each falcon tube was shaken for 3 h on a shaker (Labnet, Rocker, NJ, USA) to determine electrolytes leakage from live leaves (L1). Then, the seedlings in distilled water were frozen at −75 °C for 24 h to macerate the material. The next day, the samples were thawed and subjected to the same procedures as described above, and the amount of electrolytes leakage from the dead seedlings (L2) was determined. Analyses of the degree of cell membrane destabilisation were measured using a conductivity meter (CX-701, Elmetron, Zabrze, Poland), with an electrode with constant K = 1.02 (Elmetron, Zabrze, Poland). Calculations were made according to the following formula:

$$EL = (L1/L2) \times 100 \quad (3)$$

EL—a percentage of electrolytes leakage, L1—electrolytes leakage in living cells, L2—a percentage of electrolytes leakage from dead cells [45]. An electrolytes leakage was measured on the seventh day of grains germination on 10 specimens for every experiment probe.

3.7. Statistical Analyses

The data sets were tested for normality using the W. Shapiro-Wilk normality test. Levene's test was used to test the homogeneity of variances. To test for differences in the experimental objects, one-way analyses of variance (ANOVA) was used as well as the post-hoc Duncan test for independent samples (Statistica 13.0 software).

4. Results

4.1. GP Germination Percentage [%]

The process of germination of grains ×*Triticosecale* cv. Mamut in the presence of extracts from the roots and stem of *Phacelia tanacetifolia* was quite homogeneous (Figure 2). Compared to the control, all types of phacelia extracts had an inhibitory effect on the germination of triticale; in the case of the root, the greatest inhibition was observed at 12.5%, and in the case of the stalk, the greatest inhibition was observed at 15% of the extract concentration (Figure 2). When using leaf extracts, strong inhibition of grain germination was observed at 12.5% and even 10% concentrations, and in the case of flowers at 12.5% and 15% concentrations of the extracts.

4.2. Other Germination Coefficients

The highest values of the MGT coefficient were recorded for grains of ×*Triticosecale* cv. Mamut that were watered with 15% extract of flowers and roots of *P. tanacetifolia*. However, they did not differ statistically significantly compared to the control. The lowest values of this parameter were found for grains watered with 10% and 12.5% phacelia leaf extract.

Only these extracts statistically significantly reduced the mean germination time of grains concerning the control (Table 3).

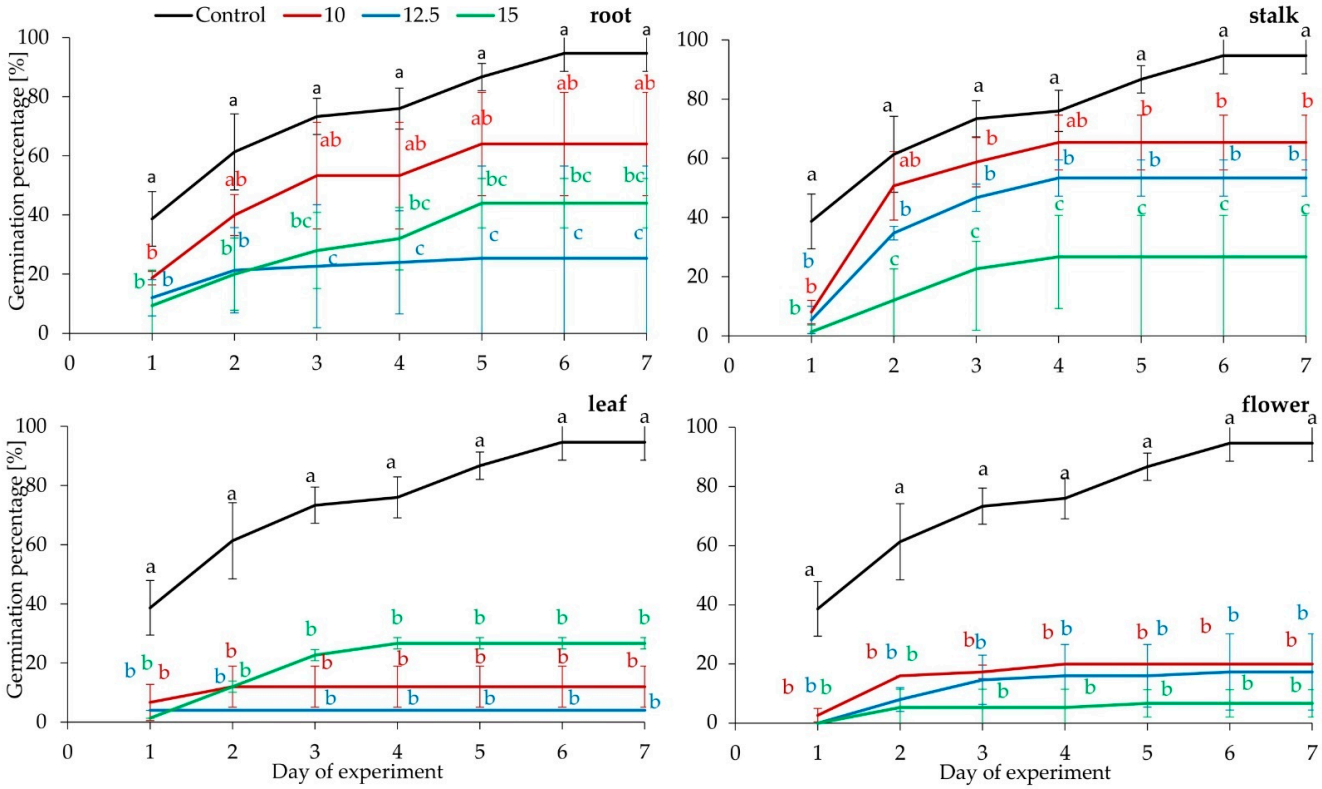

Figure 2. The cumulative germination percentage of ×*Triticosecale* cv. Mamut grains watered with *Phacelia tanacetifolia* Benth. organs extracts (root, stem, leaf, flower), with concentrations of 10%, 12.5%, 15%, and control (distilled water); in each sampling, means (±SD) marked with the same letters do not differ significantly according to Duncan's test, at $p \leq 0.05$.

The highest coefficient CVG value concerned triticale grains watered with 12.5% phacelia leaf extract, and the lowest concerned grains watered with 15% root extract (Table 3). The CVG coefficient was statistically significantly different from the control only for grains treated with 10 and 12.5% leaf extracts. This means that with a 12.5% concentration of phacelia leaf extract, triticale grains germinated quickly, but their further development was inhibited, while in the presence of 15% phacelia root extract, the germination process itself was already inhibited.

All noticed GI values were statistically significantly lower compared to controls. The highest GI values were found for triticale grains watered with 10% phacelia root extract, and the lowest with 12.5% leaf extract. In the case of the T_{50} coefficient, the highest values of this parameter concerned grains watered with 15% phacelia flower extract, and the lowest with 12.5% phacelia leaf extract. The U coefficient values turned out to be lower than the control in all extracts used here. The highest values of this coefficient were recorded for triticale grains watered with 10% root extract, and the lowest with 15% phacelia flower extract (Table 3).

4.3. Seedling Organ Length [cm]

The tested extracts inhibited the growth and development of individual organs ×*Triticosecale* cv. Mamut, but the effect was not uniform (Figures 3 and 4A,B). The stimulating effect of extracts was observed only in the case of the leaf sheath among seedlings watered with all concentrations of roots extracts (av. 5.18 cm compared to the control equal to 4.4 cm), 10% and 12.5% extracts of stalks (av. 5.05 cm), and 10% extracts of phacelia flowers (Figure 4B). In the case of root extracts, the stimulating effect on the leaf sheath

intensified with the increase in the concentration of the extract, and the differences were statistically significant. However, 15% phacelia stalks extract strongly inhibited the growth of this organ in the analysed seedlings (av. 1.86 cm).

Table 3. The comparison of the MGT, CVG, GI, T50, and U coefficients was determined for grains of ×*Triticosecale* Wittm cv. Mamut [%], which germinated on extracts from various parts of *Phacelia tanacetifolia* Benth. (root, stem, leaf, flower), with concentrations of 10%, 12.5%, 15%, and control (distilled water); means (±SD) marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$.

Extract Type [%]	MGT [Day]	CVG [%]	GI [Unit Less]	T_{50} [Day]	U [Bit] *
			Root		
Control	2.45a ± 0.34	41.41a ± 6.01	14.53a ± 1.85	1.48a ± 0.32	2.07a ± 0.16
10	2.41a ± 0.17	41.65a ± 3.08	8.98b ± 1.84	1.59a ± 0.21	1.78a ± 0.19
12.5	2.07a ± 1.05	56.37a ±24.67	4.43b ± 2.80	1.61a ± 1.23	1.25b ± 0.38
15	2.90a ± 0.35	34.87a ±4.47	5.18b ± 3.51	2.22a ± 0.54	1.79a ± 0.29
			Stalk		
Control	2.45ab ± 0.34	41.41ab ± 6.01	14.53a ± 1.85	1.48b ± 0.32	2.07a ± 0.16
10	2.20b ± 0.07	45.41a ± 1.39	8.42b ± 0.85	1.58b ± 0.03	1.45ab ± 0.34
12.5	2.37ab ± 0.06	42.13ab ± 1.06	6.42b ± 1.06	1.73ab ± 0.07	1.57ab ± 0.33
15	2.66a ± 0.06	37.60b ± 0.81	2.81c ± 1.71	2.12a ± 0.28	1.16b ± 0.46
			Leaf		
Control	2.45a ± 0.34	41.41c ± 6.01	14.53a ± 1.85	1.48ab ± 0.32	2.07a ± 0.16
10	1.47b ± 0.50	73.81b ± 25.08	2.33b ± 1.53	0.94bc ± 0.51	0.32b ± 0.56
12.5	1.00b ± 0.00	100.00a ± 0.0	1.00b ± 0.00	0.50c ± 0.00	0.00b ± 0.00
15	2.11a ± 0.19	47.62c ± 4.12	1.28b ± 0.25	1.58a ± 0.12	0.31b ± 0.53
			Flower		
Control	2.45a ± 0.34	41.41a ± 6.01	14.53a ± 1.85	1.48a ± 0.32	2.07a ± 0.16
10	2.20a ± 0.00	45.45a ± 0.00	2.61b ± 0.24	1.54a ± 0.07	1.15b ± 0.37
12.5	2.65a ± 0.42	38.29a ± 5.63	1.69b ± 1.09	2.03a ± 0.29	1.24b ± 0.49
15	3.00a ± 1.73	40.00a ± 17.32	0.73b ± 0.68	2.50a ± 1.73	0.00c ± 0.00

* MGT—Mean germination time; CVG—Coefficient of velocity germination; GI—Germination index; T_{50}—Time required for 50% germination, U—Uncertainty of germination process.

The highest inhibitory effect, after using extracts from phacelia leaves, was visible in the case of triticale roots, which showed nearly 30 times lower values for the average length of roots of seedlings watered with extracts compared to the control (Figure 4A). Length parameters closest to the control values were found in the aboveground parts of triticale seedlings watered with 10% phacelia root extracts; lower values were recorded only at 12.5% and 15% concentrations of extracts, but the 15% extracts inhibited the growth of this part of seedlings to a lesser extent than the 12.5% (av. 7.8 cm and 13.0, respectively). Extracts from phacelia leaves and flowers in almost every percentage contributed to a strong inhibition of leaf sheath growth, but the effect of leaf extracts most strongly inhibited the growth of this organ (Figure 4B).

4.4. Fresh and Dry Mass [g] and Water Content in Seedlings [%]

The 12.5% phacelia root extract caused a significant decrease in the fresh mass of triticale seedlings compared to the control (Table 4). At other concentrations of extracts, the fresh weight was higher, but these differences are statistically insignificant. All extracts from the stalks, leaves, and flowers of phacelia significantly decreased the fresh mass of triticale seedlings compared to the control.

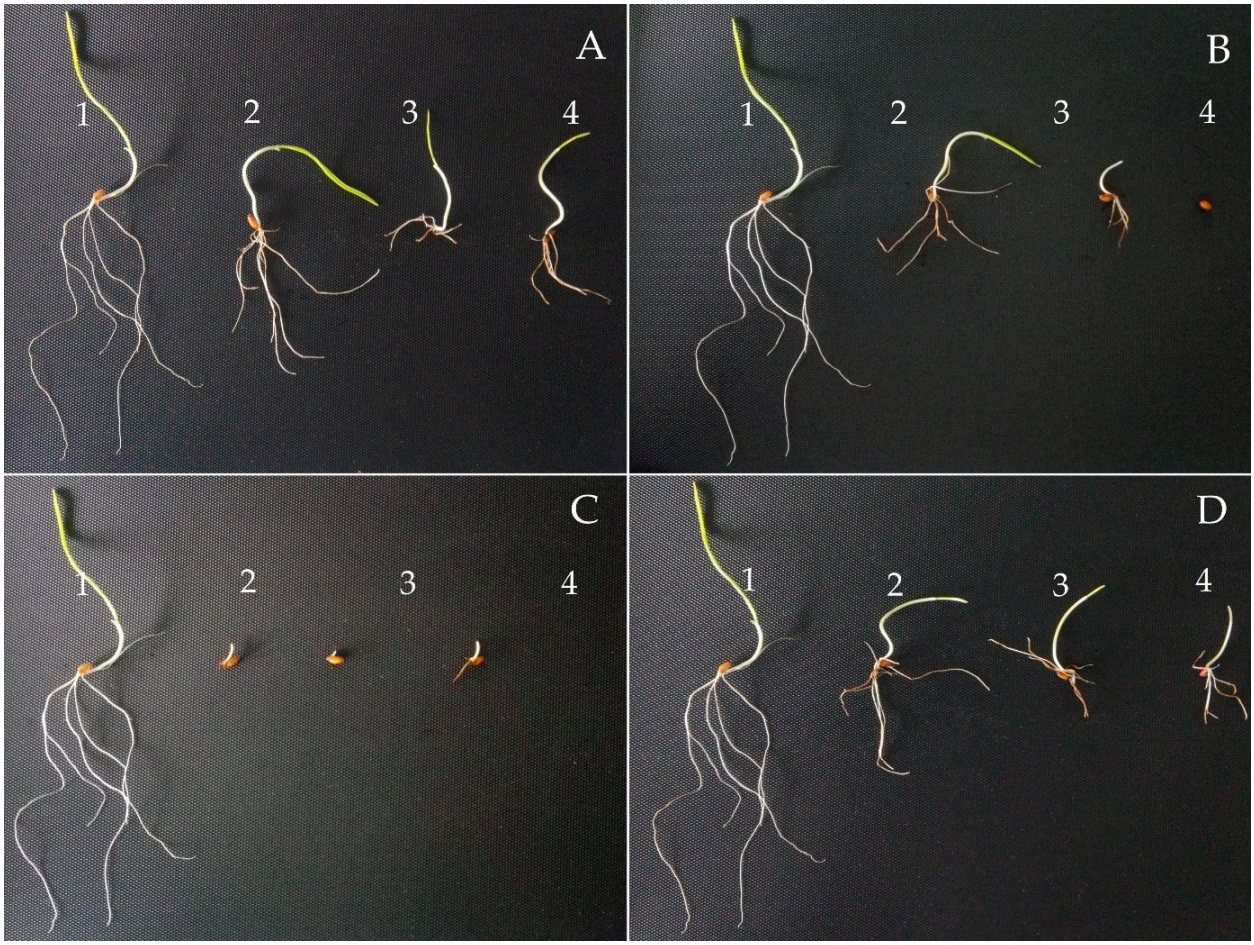

Figure 3. The seedlings of ×*Triticosecale* Wittm cv. Mamut after watering with extracts from *Phacelia tanacetifolia* Benth. organs (extracts from (**A**)—roots, (**B**)—flowers, (**C**)—leaves, (**D**)—stems), with different concentrations (1—control: distilled water, 2—10%, 3—12.5%, 4—15%), on the 7th day of experiment (Photo: A. Kliszcz).

Higher dry mass values of spring triticale seedlings were noted after the use of all types of extracts and their concentrations, with statistically significant relationships noted only for the comparison of the control with the concentrations used. Between concentrations, the results were statistically insignificant. For seedlings watered with extracts from phacelia flowers in all concentrations used, the dry mass values obtained did not differ statistically significantly from the control (Table 4).

The percentage of water content was lower in seedlings watered with extracts from phacelia organs in different concentrations, compared to the control (Table 4). The lowest values of this parameter were recorded for triticale seedlings watered with 12.5% phacelia leaf extract; the results for seedlings watered with 10% flower extract were statistically insignificant.

4.5. Electrolytes Leakage from Seedlings

All concentrations of extracts from phacelia organs used here caused a statistically significant, concerning the control, leakage of electrolytes from the cells of triticale seedlings (Figure 5). The highest electrolytes leakage was observed for all concentrations of leaf extracts (especially 10%), and these leakages were comparable regardless of the concentration. The smallest values of leakages were recorded for extracts from phacelia roots (also 10%).

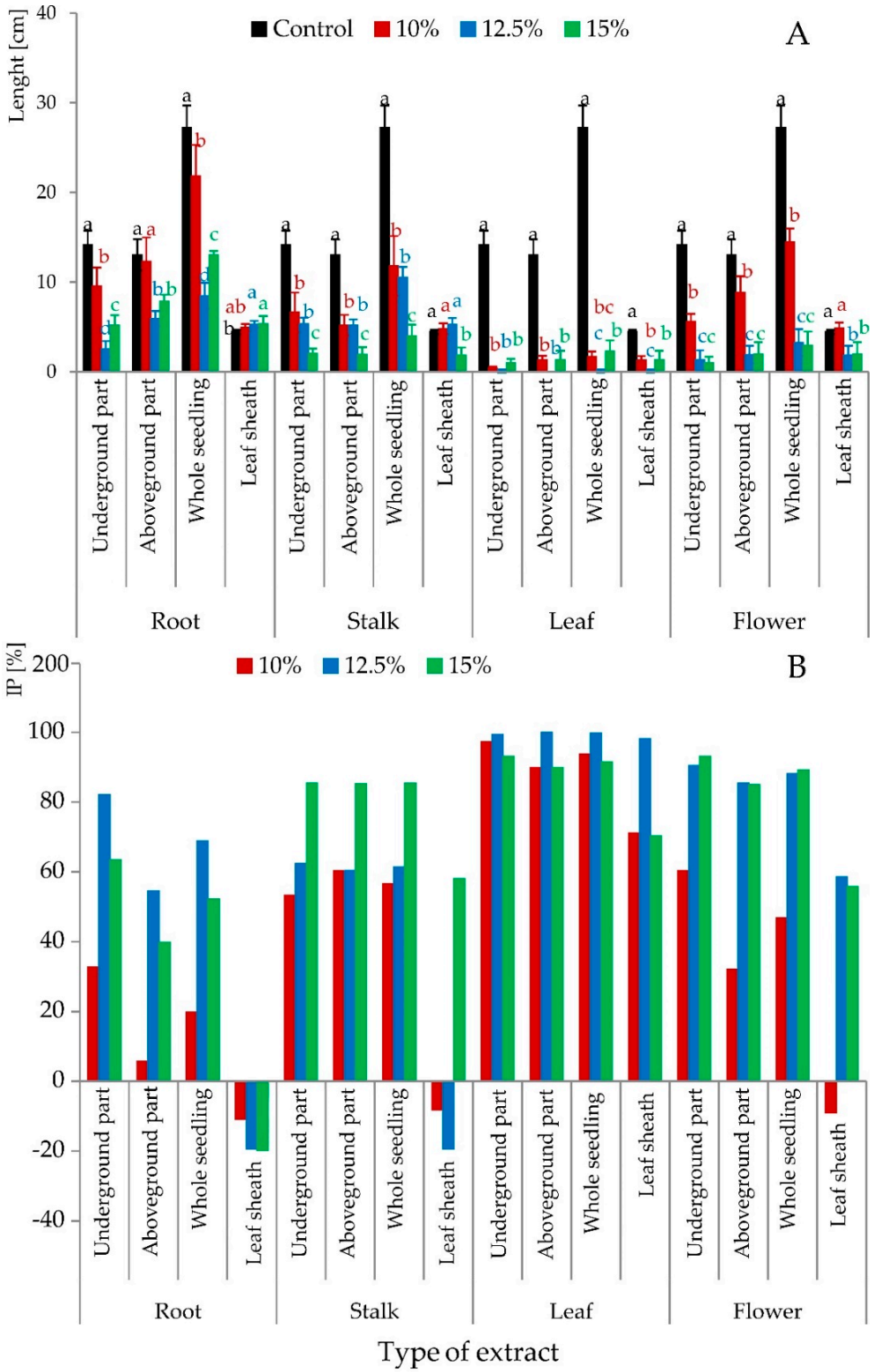

Figure 4. The comparison of organ length [cm]—(**A**) and growth inhibition index IP [%]—(**B**) of ×*Triticosecale* Wittm. cv. Mamut seedlings, watered with extracts from the roots, stalks, leaves, and flowers of *Phacelia tanacetifolia* Benth., in various concentrations; means (±SD) marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$.

Table 4. The comparison of fresh and dry mass [g] and water content [%] in ×*Triticosecale* Wittm cv. Mamut seedlings, which have grown in the presence of extracts from various parts of *Phacelia tanacetifolia* Benth. with concentrations of 10%, 12.5%, 15%, and control (distilled water); means (±SD) marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$.

Extract Type [%]	Fresh Mass [g]	Dry Mass [g]	Dry Mass/Fresh Mass	Total Water Content [%]
		Root		
Control	0.2703a ± 0.0632	0.0205b ± 0.0052	0.0754d ± 0.0038	92.46a ± 0.38
10	0.3005a ± 0.0317	0.0266ab ± 0.0017	0.0890c ± 0.0070	91.10b ± 0.70
12.5	0.2048b ± 0.0477	0.0316a ±0.0086	0.1541a ± 0.0124	84.59d ± 0.94
15	0.2726a ± 0.0220	0.0329a ± 0.0050	0.1204b ± 0.0123	87.96c ± 1.23
		Stalk		
Control	0.2703a ± 0.0632	0.0205b ± 0.0052	0.0754c ± 0.0038	92.46a ± 0.38
10	0.2224ab ± 0.0294	0.0348a ± 0.0032	0.1598b ± 0.0368	84.02b ± 3.68
12.5	0.1925b ± 0.0170	0.0338a ± 0.0024	0.1761b ± 0.0108	82.39b ± 1.08
15	0.1200c ± 0.0206	0.0334a ± 0.0059	0.2795a ± 0.0346	72.05c ± 3.46
		Leaf		
Control	0.2703a ± 0.0632	0.0205b ± 0.0052	0.0754c ± 0.0038	92.46a ± 0.38
10	0.0958b ± 0.0234	0.0322a ± 0.0098	0.3332b ± 0.0472	66.68b ± 4.72
12.5	0.0751b ± 0.0079	0.0321a ± 0.0024	0.4312a ± 0.0610	56.88c ± 6.10
15	0.0915b ± 0.0216	0.0301a ± 0.0051	0.3431b ± 0.0832	65.69b ± 8.32
		Flower		
Control	0.2703a ± 0.0632	0.0205a ± 0.0052	0.0754b ± 0.0038	92.46a ± 0.38
10	0.1910b ± 0.0221	0.0250a ± 0.0044	0.1329b ± 0.0342	86.71a ± 3.42
12.5	0.0924c ± 0.0287	0.0268a ± 0.0079	0.2934a ± 0.0321	70.66b ± 3.21
15	0.0923c ± 0.0274	0.0262a ± 0.0085	0.2896a ± 0.0929	71.04b ± 9.29

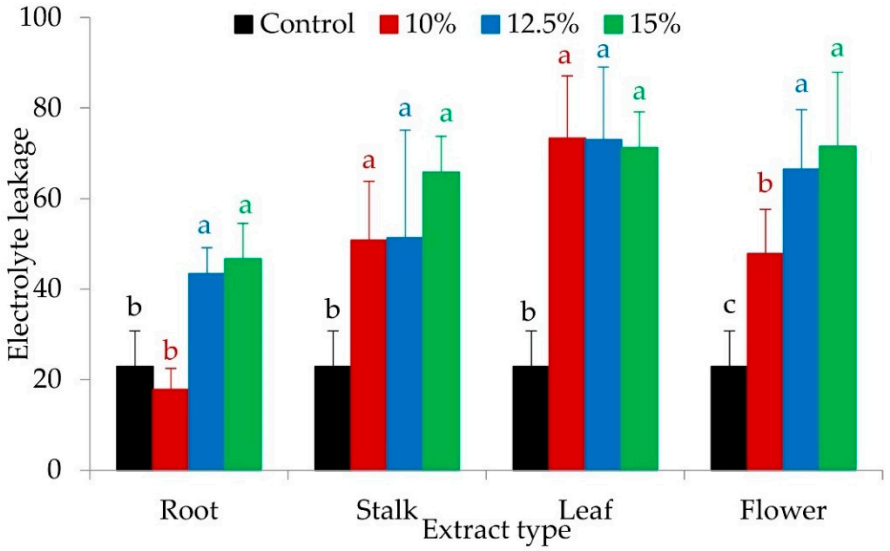

Figure 5. The electrolytes leakage [%] in seedling cells of ×*Triticosecale* Wittm. cv. Mamut, watered with extracts from root, stalk, leaf, and flower of *Phacelia tanacetifolia* Benth. at concentrations of 10%, 12.5%, 15%, and control (distilled water); means (±SD) marked with the same letters do not differ significantly according to Duncan's test at $p \leq 0.05$.

5. Discussion

In laboratory conditions, the interactions between plants are direct and primary, while in field conditions, the direction and strength of these interactions are modified by the components of the soil agroecosystem and climatic conditions. As previous studies have shown, crop plants can also have high allelopathic potential [46–50]. Higher concentrations of allelopathic substances can have a negative effect on seed swelling, inhibit

hormones and enzymes, damage seed coats and the aleurone layer, and inhibit cell elongation. As a result, these anatomical and morphological distortions significantly delay seed germination [51,52]. However, at lower concentrations, allelopathic compounds may exhibit stimulating properties [8,53]. In an experiment carried out here with *Phacelia tanacetifolia* extracts, all types of extracts inhibited the germination of ×*Triticosecale* cv. Mamut, compared to the control. This is clearly illustrated by the lower values of the GP and GI coefficients obtained here (Figure 2; Table 3). The extracts from the leaves and flowers of phacelia were probably characterised by a higher content of allelochemicals because they inhibited the germination of triticale more significantly than extracts from the roots or stalks of this plant. Bajkacz et al. [31], Kruk et al. [32], and Puig et al. [28], analysed aqueous extracts from various parts of *P. tanacetifolia* (flowers, stalks, leaves, and roots) and deciphered a cocktail of secondary metabolites within these parts (Table 1). However, it is not known exactly which of these compounds plays the most important role in the allelopathic potential of the analysed species. There are probably numerous phenolic compounds and flavonoids.

Allelopathic compounds inhibit the elongation of the germinal root, leading to its death, which was most evident in the case of phacelia leaf extracts (Figure 4A). During the contact of the root with allelochemicals, cell and tissue dysfunction occurred, which resulted in, among other things, the lack of root hairs formation, lateral roots (horizontal growth of the roots), and an increase in the chromosome aberration index of the root cells [54]. In many previous experiments, the negative effect of allelochemicals from crop plants on the elongation growth of other crop plants has beenm documented, e.g., rye for cucumber roots [55], sesame and rapeseed [56], mung bean for tobacco [48], cotton and sesame [57], or peppermint for radish [7]. Inhibition of root growth by allelopathic compounds may be a consequence of mitotic disturbances due to damage to the chromatin and the karyokinetic spindle [58]. With the increase of the concentration of phacelia extracts, their negative effect on grain germination increased (Figure 3), which is probably related to the increase in the concentration of allelopathic compounds in the tested extracts. The reactions of germinating triticale grains to the applied extracts, such as inhibition of the growth of aboveground parts, lower increase in seedling biomass, and inhibition of root growth, may therefore be the effect of the allelopathic compound's action mechanisms that are contained in different parts of the phacelia

However, not all allelochemicals have an inhibitory effect, as already mentioned above. The experiment carried out here pointed that allelopathic substances from phacelia root extracts have a stimulating effect on the initial growth of the triticale leaf sheath (Table 3); they at least inhibit the growth and development of the aboveground and underground parts of its seedlings. The leaf sheath is the organ responsible for the physical protection of the germinating seed and young plant, and for protecting the hypocotyl developing inside it (until it is ready to start photosynthesis). The analysed data show that this structure is not indifferent to substances contained in its environment (probably also soil) and grows in length, and its growth is stimulated by allelochemicals.

All extracts from the stalks, leaves, and flowers of phacelia significantly decreased the fresh mass of triticale seedlings, compared to the control. A similar trend was related to the percentage of water content in triticale seedlings. In the case of a dry mass of seedlings, the opposite tendency was observed (Table 4). Allelochemicals can change the content of plant growth regulators or cause imbalances in various phytohormones, which inhibits plant development and consequently affects seedling mass parameters, e.g., [59,60]. Most phenolic allelochemicals can, for example, stimulate IAA oxidase activity and inhibit the reaction of POD (peroxidase) with IAA (indoleacetic acid), bound GA (gibberellins), or IAA, to affect endogenous hormone levels [61]. Allelopathic substances also cause enzyme dysfunction and mineral and water absorption disorders, resulting in changes in plant growth [62]. They have a clear influence on the water relations in the plant [63,64]. For example, the presence of phenolic acids plays an important role in the regulation of diffusion, as well as in maintaining the correct water potential in cells [65,66].

The published studies show that changes in the permeability of cell membranes under the influence of stress are accompanied by the generation and accumulation of reactive oxygen species (ROS) [67–69]. ROS cause peroxidation of membrane lipids, thus affecting changes in membrane permeability, composition, and structure. Many studies have shown that allelochemicals significantly inhibit the activity of antioxidant enzymes and increase the level of free radicals. This, in turn, reduces the scavenging effect of activated oxygen and damages the entire membrane system of the plants. In extreme cases, this leads to programmed cell death [70–73]. The ability of many natural plant products to bind to membranes is manifested not only by conformational changes in ion channels and proteins but also by increasing or decreasing the flow of ions [68]. This generally indicates the presence of environmental stress caused by these extracts. Probably, such mechanisms acted also on the cells of the triticale seedlings studied here. It was found in our experiment with phacelia that all types of extracts increased the electrolytes leakage from triticale seedling cells, except for 10% phacelia root extract (Figure 5). However, regardless of the concentration, the leaf extracts caused the leakage of electrolytes at the same—highest level (av. 72.48%).

Most of the tested parameters of germination and early growth of triticale indicate the negative impact of aqueous extracts of *P. tanacetifolia* (Table 5). At the same time, it is worth emphasising that the allelopathic potential of phacelia compared to other species, for example, *Juglans regia* L. [4], or *Mentha* ×*piperita* L. [8,45,74], is relatively small, and is noticeable only at concentrations of extracts of 10% and above. The use of stubble crops with weak allelopathic potential is one of the sustainable development strategies in agriculture and is currently implemented within the scope of "*Agri-environment-climate measures in the European Union*" [75]. It is beneficial especially in cereal monocultures, e.g., [21,59,76–80]. It has already been proven that the cultivation of phacelia as a catch crop in apple and peach orchards alleviates soil sickness, significantly lowering the concentration of phytotoxic phenols in the soil solution and improving soil biological activity. Patkowska, Konopiński [81] showed that soil mulching with *P. tanacetifolia* reduces fungal infections of seedling roots in *Tragopogon porrifolius* L. var. *sativus* (Gaterau) Br., while according to Konopiński [82], mulching the soil with phacelia has no significant effect on the emergence of *Scorzonera hispanica* L. 'Lange Jan'.

Table 5. The comparison of the studied parameters concerning the allelopathic effect of *Phacelia tanacetifolia* Benth on ×*Triticosecale* Wittm. cv. Mamut; colour indicates the type of impact: favourable (green), unfavourable (red), or neutral (grey).

Parameter	Extract Concentrate [%]											
	10				12.5				15			
	R	S	L	F	R	S	L	F	R	S	L	F *
GP(%)												
MGT												
CVG												
GI												
T_{50}												
U												
Seedling length												
Fresh mass												
Dry mass												
Total water content												
EL (%)												

* R—root; S—stalk; L—leaf; F—flower.

In the experiment carried out, the aboveground parts of phacelia (especially leaves and flowers) showed a strong inhibitory effect on the growth of the entire triticale seedling (Table 3). Concerning field conditions, this type of action may contribute to delaying the germination process of triticale or other cereal plants. However, this effect is probably

significantly weakened by the deposition of plant material from phacelia in the soil and is shaped by the biotope. Leaves and flowers are the organs that, due to the structure of the tissues, decompose the fastest after the termination of the stubble crop (they contain less difficult-to-decompose lignin and cellulose), hence the quick release of their allelopathic substances. The main factors levelling the adverse effects of allelopathic compounds are the mesofauna and soil microflora. These organisms can decompose polyphenolic compounds and flavonoids from plant biomass. For example, Liebeke et al. [83] indicated that drillodefensins (surface active lipophilic ions 259.1013 Da, which m/z are consistent with a molecular formula of $C_{12}H_{19}O_4S^-$) are produced in the foregut section of earthworms' intestine system and help them digest phenolic-rich residues of plant materials. Earthworms belonging to the *anecic* ecological group feed on litter (mulch), while the *endogeic* group processes the ploughed remains of the stubble crop in the soil. The affinity of some fungal decomposers, such as *Mucor* sp., concerning the fresh mass of plant residues introduced into the soil has also been proven [84]. In this context, it seems important to maintain an appropriate time regime between the termination of the stubble crop and the sowing of the cereal plant, so that the decomposition of allelochemicals by living organisms can take place and be accelerated by physical processes (freezing, thawing, drying, moistening, solar radiation, including UV). It can be assumed that the efficiency of decomposition of this type of compound will be a derivative of the network of connections and the efficiency of individual components of the agroecosystem, their abundance, and biodiversity [27,85].

In the term of the laboratory tests carried out here and the primary directions of interactions between phacelia and triticale recorded, it would be worth undertaking further experiments to determine the effect of phacelia on the growth and development of cereal plants, in conditions of field. Another interesting direction of research would be to reveal the impact of phacelia on other crop species to indicate those that are most resistant to its allelochemicals, which is important from the point of view of implementing research into agricultural practice.

6. Conclusions

Our experiment proves that *Phacelia tanacetifolia* Benth. has an allelopathic potential. All concentrations of extracts from roots, stems, leaves, and flowers of *P. tanacetifolia* inhibited the germination of ×*Triticosecale* Wittm. cv. Mamut, compared to the control. Extracts from the roots of phacelia had the smallest effect on the length of the underground and aboveground parts, and leaf extracts showed the strongest inhibitory effect on individual parts of triticale seedlings. Leaf sheath length was significantly stimulated by all concentrations of phacelia root extract. All concentrations of stalks, leaves, and flowers extracts as well as 12.5% roots extract resulted in a statistically significant reduction in the fresh mass of triticale seedlings, compared to the control. All types of extracts (in all concentrations) caused an increase in the dry mass of triticale seedlings regarding the control, and a statistically significant leakage of electrolytes from the cells of triticale seedlings.

Negative allelopathic effects of *P. tanacetifolia* on germinating triticale grains were found in these laboratory tests, but only in higher concentrations (the positive effect concerned the stimulation of the growth of the leaf sheath). Given the possibility of using phacelia as a stubble crop in the cultivation of cereal plants, its biomass may have a positive effect on cereals through the accumulation of organic matter in the soil, with a relatively weak allelopathic potential.

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