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ORIGINAL ARTICLE

Allelopathic interaction between two common meadow plants: *Dactylis glomerata* L. and *Trifolium pratense* L.

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Abstract

Under natural conditions, plants compete for environmental resources, including by the release of allelopathic compounds with a various spectrum of activity. Therefore, the effect of aqueous extracts of cock's-foot *Dactylis glomerata* L. on germination and early growth phases and electrolyte leakages of a red clover *Trifolium pratense* L. was investigated. The 5, 10, and 15% of the aqueous extracts of cock's-foot separately from shoots and inflorescences were used in two type of treatments tested in parallel. In first treatment the red clover seeds were watered directly with the aqueous extracts by eight days of experiment time, in second the seeds were pretreated with extracts for 24, 48 and 72 h and next they were watered with distilled water during experiment time. The results showed that the germination capacity of red clover seeds decreased with increasing concentrations of cock's-foot aqueous extracts. Regardless of the duration of seed treatment with the extracts, the highest inhibition of germination was found when the 15% cock's-foot shoot extracts was used. For red clover seedlings pretreated with extracts for 72 h, the highest and statistically significant differences in the growth were observed. With the increasing of concentration of cock's-foot extracts significant inhibition of the underground and aboveground organs growth were observed. The increase of fresh and dry masses of red clover seedlings varied depending on the duration of contact with the extracts and their concentrations. The electrolyte leakage, as compared to the control, increased with the concentration of extracts, regardless of types and duration of extracts. The obtained results clearly confirm that leaving biomass of cock's-foot on the field can lead to the release of phytotoxins that may inhibit germination and growth of red clover.

Keywords Allelopathy · cock's-foot · Electrolyte leakage · Germination · Red clover · Seeds

Introduction

Plants during development are subjected to the influence of various stress factors, and one of them is the positive or negative impact of other plants (Możdżeń et al. 2018, 2019; Szafraniec et al. 2019). Substances that arise during the decomposition of plants or are part of their natural metabolic pathways, eluate with atmospheric precipitation and can cause stress in other species that grow in the immediate environment (e.g. Einhellig 1995; DA Inderjit et al. 2011; Cheng and Cheng 2015; Latif et al. 2017). The intensity of allelopathic compounds depends on their concentration in the plant and environmental conditions of growth. There is a close correlation between the occurrence of environmental stresses that directly effect on plant growth and stimulation of phytotoxic production (Saxena et al. 1996; San Emeterio et al. 2007; Gioria and Osborne 2014). In natural ecosystems, allelopathic interactions

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begin already during seeds germination and have influence on the subsequent growth and development of plants (Narwal 1994; Możdżeń et al. 2019). Many allelopathic substances that inhibit the growth of certain plants at higher concentrations can stimulate the growth of the same or different species at lower concentrations. However, these phenomena still require detailed research that will allow to draw general conclusions about their mechanisms (Narwal 1994; Możdżeń et al. 2018). From the point of the agricultural economy, this is particularly interesting for fodder species, such as grasses or legumes, which are very desirable in compound feeds and can have a positive or negative impact (DA Inderjit et al. 2011; Leather and Einhellig 1988; Puła et al. 2018).

Cock's-foot, orchard grass, or cat grass (*Dactylis glomerata* L.), belongs to grasses family (*Poaceae* (R. Br.) Barnh. = *Gramineae* Juss.). It occurs around the world, dominating in meadow communities and rarely in ruderal (Harris et al. 2008). In the classification of plant communities, it is a species characteristic of the *Arrhenatheretalia elatioris* Pawł. 1928 order (Matuszkiewicz 2019). It belongs to high, durable and loose-water grasses. It produces a well-developed root system (up to 1 m deep) and generative shoots (up to a height of 60–90 cm). The stalks are stiff, with a green-blue color, and the leaves are rough with hooks on the edges. The inflorescence forms a one-sided and straightened panicle, which is initially focused, and in the flowering phase (from May to August) becomes scattered. The fruit is husked, bony seeds that germinate at 17–19 °C. Cock's-foot is a photophilous, nitrous and drought-resistant species. High yields are achieved on medium-fertile soils and rich in absorbable forms of elements with regulated water ratios. It is resistant to harsh winters, but sensitive to late spring frosts and excess moisture. When considering its nutrients, it contains very large amounts of protein and nitrogen (accumulated in aboveground parts), and has low content of sugars (cellulose, hemicellulose and lignin). However, as the plants age, the content of structural carbohydrates and lignins increases (Volaire et al. 2005; Turner et al. 2012).

Red clover (*Trifolium pratense* L.) belongs to legume family (*Fabaceae* Lindl. = *Papilionaceae* Giseke). It occurs throughout Europe, Asia and Africa, mainly in non-forest communities (in meadows and pastures) and it is used as a crop plant. In the classification of plant communities, it is a species characteristic of the *Molinio-Arrhenatheretea* R.Tx. 1937 class (Matuszkiewicz 2019). It is a biennial or perennial plant, reaching a height from 15 to 50 cm. It has a pile, branched and spindle root system. Its stem is also branchy, rising or raised, slightly hairy and richly foliage. The inflorescence is formed in the form of a large, spherical head with numerous dark purple or pink flowers. Cultivated since the eleventh century, and now is one of the basic butterfly plants intended for animal feed, thanks to the high content of protein and mineral salts (Narwal 1994; Dear and Cocks 1997).

The study was conducted to determine the allelopathic effect of aqueous extracts from cock's-foot (*Dactylis glomerata* L.) shoots and inflorescences, at different concentrations, on germination and early development of red clover seeds (*Trifolium pratense* L.). The following hypotheses were tested: (1) the aqueous extract of *D. glomerata* inhibit the germination process of *T. pratense*, (2) the elongation of seedlings of *T. pratense* watered with the extract from *D. glomerata* is smaller when the extract is stronger, (3) the fresh mass of red clover seedlings negatively depends on extract concentration, (4) electrolyte leakage depends negatively on extract concentration, (5) the influence of aqueous extract differs in the case of plants part used.

Material and methods

Plant material and growth conditions

The experiment was performed under strictly controlled conditions, established by the method of complete randomization. Biotests were carried out in the dark, at a constant temperature of 20 °C ± 1 °C and relative humidity of 90–95%. Plant material with *Dactylis glomerata* L. was collected in the nature (in May and June 2018), in the southern part of Poland (Suchoraba 49°58′37″N 20°11′49″E). The dry plant material was stored in the laboratory in the dark to avoid microbiological destruction of allelopathic compounds present in it. The seeds of *Trifolium pratense* L. were purchased in the ordinary market from POLAN Sp.z.o.o. Breeding and Seed Horticulture in Kraków (Poland).

Preparation of aqueous extracts solutions

The aqueous extracts at concentrations of 5, 10 and 15% (5% = 5 g plant material +95 ml distilled water, 10% = 10 g plant material +90 ml distilled water, 15% = 15 g plant material +85 ml distilled water) were prepared separately from dried shoots and dried inflorescences cock's-foot. Each extract, depending on the concentration, was flooded with a suitable amount of distilled water and left for 24 h, at room temperature to extract the compounds. The extracts obtained were filtered through a filter paper on a Büchner funnel by Whatman filter paper, using a vacuum pump, and then stored in a refrigerator for the duration of the experiment.

Seed germination

Red clover seeds were sterilized in 1% sodium hypochlorite for 10 min, then rinsed with distilled water and dried at room temperature. Two treatments of experiment with seed germination were made in parallel. First treatment: the red clover seeds put on the sterile Petri dishes (on filter paper) were

watered with aqueous extract of cock's-foot: separately with different concentrations from shoots and inflorescences. Second treatment: the red clover seeds were soaked in different extract concentrations separately from shoots and inflorescences for 24 h, 48 h and 72 h. After that the seeds were put on Petri dishes and watered with distilled water. The experiment with germination lasted for 8 days. In summary, there were 28 combinations using 100 seeds of red clover for every type with control group watered with distilled water. The experiment was five time repeated.

During the experiment, the number of germinated seeds was checked every 24 h. Those that had a hypocotyl at least 2 mm in length were considered germinated. After 8 days, the effect of *D. glomerata* aqueous extracts on germination indexes of *T. pratense* seeds was calculated. To check the influence of aqueous extract on germination process the following indexes were used: germination percentage (GP), germination index (GI), speed of emergence (SE), coefficient of the rate of germination (CRG), seedling vigour index (SVI) and required for 50% germination (T_{50}). The calculation methods of these indexes are described in the Table 1. In the case of germination percentage the daily differences between extract concentrations were compared.

Length of seedlings, fresh and dry mass

The length of underground and aboveground parts as well as whole seedlings was determined using a caliper (Topex 31C615, Poland), with an accuracy of 1 mm. The inhibition percentage (IP) of growth was calculated according to Islam and Kato-Noguchi (2012) (Table 1). Fresh mass [g] of 8 day seedlings was marked on the weight (1600 C Medicat). To check the influence of the extract on fresh mass the generalized linear method (GLM) was used with the inhibition percentage (IP). In order to obtain a dry mass [g], the plant material was dried for 48 h at 105 °C in a dryer (Termaks 8430) and then weighed. On the basis of the mass values obtained, the percentage water content was determined.

Electrolyte leakage

Electrolyte leakage measurements were made on 8-day-old seedlings according to method used by Skrzypek et al. (2015). Seedlings were placed in polypropylene falcons with 15 ml distilled water. Then it was shaken for 3 h on a shaker and for 10 min. on the vortex. Electrolyte leakage was measured by conductometer with electrode (K = 1.02). The measured material was frozen at −75 °C to macerate the tissues. After 24 h the falcons were defrosted and subjected to the same procedure as samples with live material. On the basis of the obtained results, the percentage electrolyte leakage was determined according to the formula EL = (EL live/ EL dead) × 100.

Statistical analysis

The experiment was carried out in 5 independent replicates, on 100 seeds in each experimental groups. STATISTICA (data analysis software system), version 13.1 www.statsoft.com, and the differences among the treatments were compared using Duncan test at 5% level of probability ($p < 0.05$).

Results

Germination indexes

Germination percent (GP) was the smallest in all cases of using 15% of the cock's-foot shoots extracts (Fig. 1). Regardless of the type of extract, the smallest number of

Table 1 List of germination indexes used to examine the influence of the extracts from *Dactylis glomerata* L. on germination process of *Trifolium pratense* L.

Index	Formula	Source
GP (Germination percentage)	GP = [Number of germinated seeds at everyday/Total number of seeds sets for bioassay] × 100	Global method
GI (Germination index)	[Number of germinated seeds/Days of first count] + ... + [Number of germinated seeds/Days of last or final count]	AOSA (1983)
SE (Speed of emergence)	SE = (Number of germinated seeds at the starting day of germination/Number of germinated seeds at the final days of measurement) × 100	Islam et al. (2009)
CRG (Coefficient of the rate of germination)	$CRG = [(n_1 + n_2 + nn)/((n_1 \times T_1) + (n_2 \times T_2) + (n3 \times T3) + \ldots)] \times 100$ n_1 = number of germinated seeds on time T_1 n_2 = number of germinated seeds on time T_2 n_3 = number of germinated seeds on timeT_3	Bewley and Black (1985), Chiapusio et al. (1997)
SVI (Seedling vigour index)	SVI = (Seedling length(mm) × Germination percent)/100	Islam et al. (2009)
T_{50} (Time required for 50%germination)	$T_{50} = t_i + [(N/2) - n_j) \times (t_i\text{-}t_j)]/(n_i\text{-}n_j)$ N is the final number of germination and n_i, n_j cumulative numbers of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$	Coolbear et al. (1984), Farooq et al. (2006)
IP (Inhibitory percentage)	$IP = [1 - (L_E/L_C)] \times 100$ L_E = length of seedlings in aqueous plant extract L_C = length of seedlings in control (without extract)	Islam and Kato-Noguchi (2012)

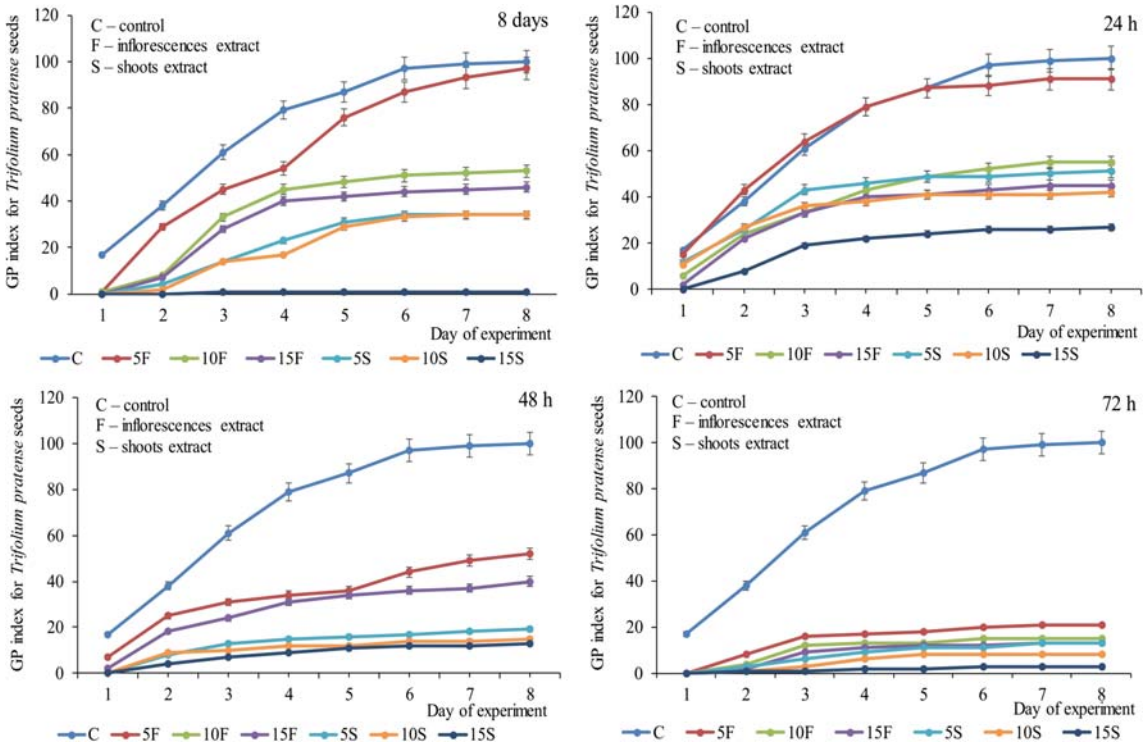

Fig. 1 The comparison of germination percentage (GP) indexes of *Trifolium pratense* L. seeds treated of *Dactylis glomerata* L. aqueous extracts at concentrations 5, 10, 15% in different time

germinated seeds was found in the group of seeds treated with extracts for 72 h. Seeds watered with extracts for 8 days of experiment demonstrated slightly higher germination capacity. In the group of seeds treated with extracts for 48 h intermediate germination values were found. In comparison to the control, in the group of seeds watered with extracts for 24 h the highest germination capacity was observed.

The smallest changes in germination index (GI) values were demonstrated for seeds treated with 5% inflorescence extracts for 24 h (Fig. 2). In other cases, regardless of the concentration and type of the extract, the GI values were clearly lower as compared to the control.

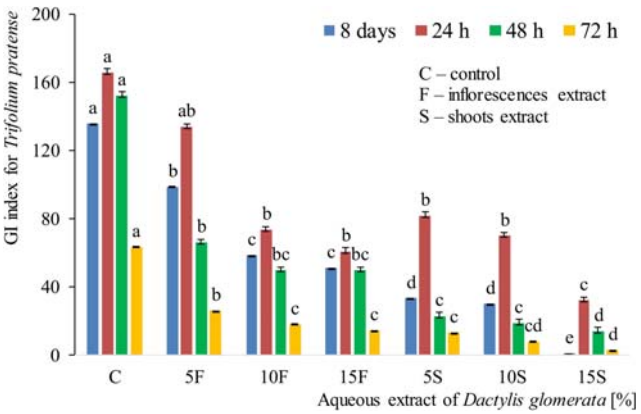

Fig. 2 The comparison of germination index (GI) of *Trifolium pratense* L. seeds treated of *Dactylis glomerata* L. aqueous extracts at concentrations 5, 10, 15% in different time

Speed of emergence (SE) values decreased with increasing concentration of extracts in relation to the control (Tables 2 and 3). The smallest SE values were observed for seeds treated with extracts for 72 h, and the highest for seeds treated with cock's-foot extracts by 24 h at concentrations of 5 and 10%.

Coefficient of the rate of germination (CRG) and seedling vigour index (SVI) significantly decreased with increasing concentrations of extracts, in relation to control values, irrespective of the time of treatment of seeds and type of extract. The only exception was the seeds watered with 5% inflorescence extracts for 8 days, in which the seedlings SVI values slightly increased, in comparison to the control. Whereas T_{50} was increased, except for seeds treated for 8 days with 15% shoot extracts (Table 2 and 3).

Length of seedlings

With the increasing of cock's-foot extracts concentration, significant inhibition of roots and hypocotyls clover growth were observed (Fig. 3). Regardless of the time of seeds contact with the extracts, inhibition percentage (IP) reached positive values that indicate a significant inhibition of clover seedlings growth. Only for seeds watered with 5% extracts for 24 h and 8 days of experiment, and 5 and 10% extracts for 72 h, stimulation of clover seedlings growth, expressed as negative IP values was noted.

Table 2 Indexes values of SE, CRG, T_{50}, SVI of *Trifolium pratense* L. seedlings watered with *Dactylis glomerata* L. aqueous extracts (5, 10, and 15%) from shoots (S) or inflorescences (F) by 8 days and pre-treated with extracts for 24 h before germination process. Values (±SD) marked (a, b, c) differ significantly according to Duncan test at $p < 0.05$

Treatment	Indexes					
	SE	CRG	T_{50}	SVI seedlings parts		
				underground	aboveground	whole
Time 8 days						
Control	16.60 a ± 2.41	18.61 a ± 0.25	3.80 b ± 0.12	12.80 a ± 0.58	33.00 a ± 1.48	45.80 a ± 1.69
5F	1.00 b ± 0.71	17.59 a ± 0.19	4.46 a ± 0.03	13.16 a ± 0.85	35.68 a ± 2.41	48.84 a ± 2.02
10F	1.14 b ± 1.74	17.80 a ± 0.15	4.46 a ± 0.06	1.49 b ± 0.20	7.32 b ± 1.24	8.80 b ± 1.36
15F	0.44 b ± 0.99	17.83 a ± 0.07	4.48 a ± 0.04	0.73 c ± 0.12	2.72 d ± 0.57	3.45 d ± 0.68
5S	0.00 b ± 0.00	17.18 a ± 0.18	4.50 a ± 0.00	1.09 b ± 0.12	4.53 bc ±0.41	5.62 cd ±0.48
10S	0.00 b ± 0.00	16.88 a ± 0.07	4.50 a ± 0.04	0.47 c ± 0.09	1.37 e ± 0.61	1.84 ced ±0.70
15S	0.00 b ± 0.00	7.25 b ± 9.93	0.00 c ± 0.00	0.00 d ± 0.58	0.00 f ± 0.00	0.00 d ± 0.00
Time 24 h						
Control	16.60 c ± 2.41	18.61 d ± 0.25	3.80 c ± 0.12	12.80 a ± 0.58	33.00 a ± 1.48	45.80 a ± 1.69
5F	16.64 c ± 0.92	19.00 c ± 0.09	3.80 c ± 0.05	1.44 b ± 0.08	3.25 b ± 0.36	4.69 a ± 0.40
10F	11.67 d ± 2.25	18.61 c ± 0.29	4.04 b ± 0.10	0.34 c ± 0.04	1.66 c ± 0.23	2.00 c ± 0.20
15F	3.97 e ± 3.95	18.76 d ± 0.18	4.35 a ± 0.15	0.25 c ± 0.04	0.85 cd ±0.17	1.10 cd ±0.19
5S	22.75 b ± 2.26	19.44 b ± 0.10	3.47 d ± 0.13	0.23 c ± 0.03	1.38 c ± 0.18	1.61 cd ±0.20
10S	26.55 a ± 3.21	19.83 a ± 0.18	3.23 e ± 0.20	0.23 c ± 0.03	1.38 cd ±0.11	1.61 cd ±0.10
15S	0.00 f ± 0.00	18.26 e ± 0.26	4.50 a ± 0.00	0.04 c ± 0.58	0.09 d ± 0.02	0.13 d ± 0.03

SE, speed of emergence; *CRG*, coefficient of the rate of germination; T_{50}, time required for 50% germination; *SVI*, seedling vigour index

Table 3 Indexes values of SE, CRG, T_{50}, SVI of *Trifolium pratense* L. seedlings pre-treated with *Dactylis glomerata* L. aqueous extracts (5, 10, and 15%) from shoots (S) or inflorescences (F) before germination process for 48 and 72 h; values (±SD) marked (a, b, c) differ significantly according to Duncan test at $p < 0.05$

Treatment	Indexes					
	SE	CRG	T_{50}	SVI seedlings parts		
				underground	aboveground	whole
Time 48 h						
Control	16.60 a ± 2.41	18.61 a ± 0.25	3.80 d ± 0.12	12.80 a ± 0.58	33.00 a ± 1.48	45.80 ± 1.68
5F	12.69 b ± 2.48	18.67 a ± 0.26	3.99 c ± 0.11	0.37 b ± 0.04	1.38 b ± 0.36	1.74 ± 0.39
10F	4.49 c ± 4.22	18.50 a ± 0.28	4.33 b ± 0.17	0.32 b ± 0.04	0.94 b ± 0.06	1.26 ± 0.06
15F	4.49 c ± 2.04	18.50 a ± 0.44	4.33 b ± 0.08	0.11 bc ±0.02	0.87 b ± 0.04	0.98 ± 0.05
5S	0.00 d ± 0.00	18.37 a ± 0.25	4.50 a ± 0.00	0.08 bc ±0.01	0.41 b ± 0.04	0.49 ± 0.04
10S	0.00 d ± 0.00	18.69 a ± 0.19	4.50 a ± 0.00	0.05 bc ±0.01	0.12 b ± 0.03	0.17 ± 0.04
15S	0.00 d ± 0.00	17.90 b ± 0.34	4.50 a ± 0.00	0.02 bc ±0.01	0.03 b ± 0.01	0.05 ± 0.01
Time 72 h						
Control	16.60 a ± 2.41	18.61 a ± 0.25	3.80 b ± 0.12	12.80 a ± 0.58	33.00 ± 1.48	45.80 ± 1.68
5F	0.00 b ± 0.00	18.42 a ± 0.21	4.50 a ± 0.00	0.06 b ± 0.02	0.43 ± 0.12	0.50 ± 0.12
10F	0.00 b ± 0.00	18.22 a ± 0.35	4.50 a ± 0.00	0.04 b ± 0.01	0.06 ± 0.01	0.10 ± 0.02
15F	0.00 b ± 0.00	17.88 a ± 0.30	4.50 a ± 0.00	0.02 b ± 0.01	0.14 ± 0.02	0.16 ± 0.03
5S	0.00 b ± 0.00	17.54 a ± 0.73	4.50 a ± 0.00	0.42 b ± 0.07	0.12 ± 0.06	0.53 ± 0.08
10S	0.00 b ± 0.00	17.18 b ± 0.70	4.50 a ± 0.00	0.15 b ± 0.06	0.04 ± 0.03	0.18 ± 0.09
15S	0.00 b ± 0.00	17.88 a ± 1.68	4.50 a ± 0.00	0.01 b ± 0.01	0.001 ± 0.0008	0.01 ± 0.01

SE, speed of emergence; *CRG*, coefficient of the rate of germination; T_{50}, time required for 50% germination; *SVI*, seedling vigour index

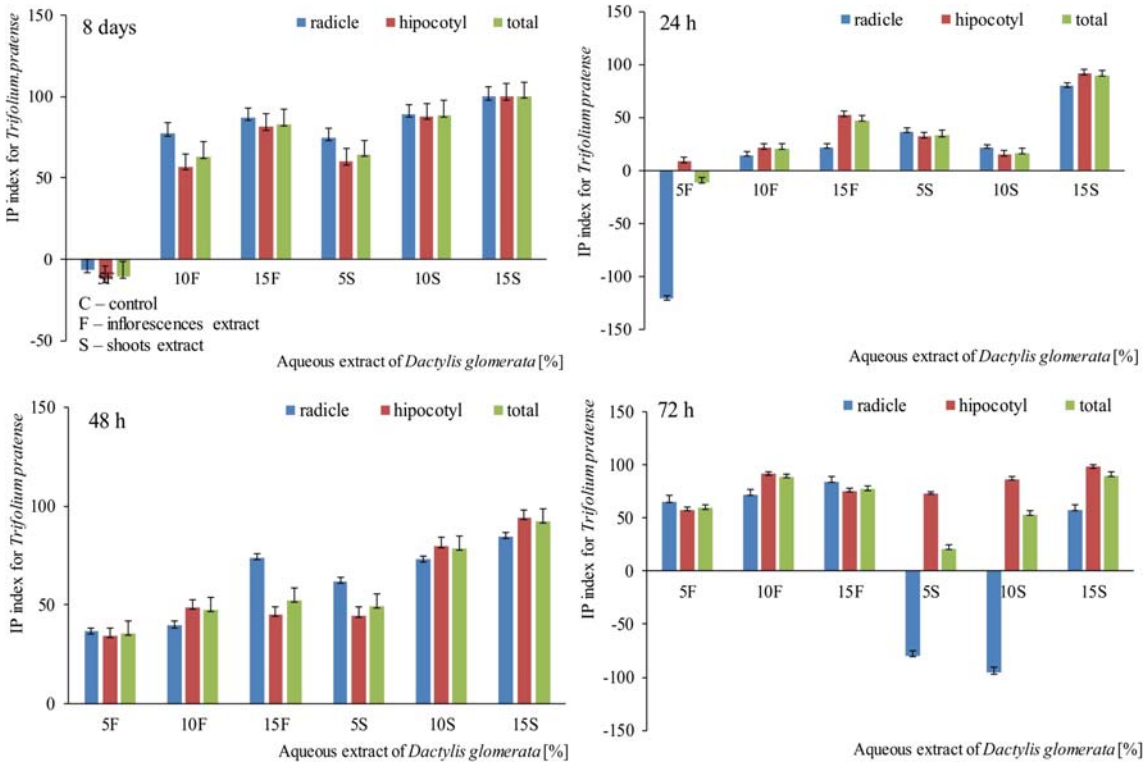

Fig. 3 The comparison of inhibition percentage (IP) indicator of growth of *Trifolium pretense* L. seedlings by *Dactylis glomerata* L. aqueous extracts at concentrations 5, 10, 15% used in different time; a negative value (−) on the Y axis indicates growth stimulation, and a positive (+) value indicates the inhibition of growth

Fresh and dry mass

The fresh and dry masses of clover seedlings varied depending on the time of contact with the extracts and their concentrations (Table 4). The fresh mass values increased in seedlings watered with 5% *D. glomerata* inflorescences extracts, compared to the control. In other cases, aqueous extracts from the cock's-foot clearly inhibited the growth of clover seedlings fresh mass. The dry mass significantly increased in each of the inflorescence extracts and 5% shoots extracts, in comparison to the control. However, with increasing concentration of allelopathins in shoots extracts, this parameter value increased in seedlings treated with extracts for 8 days, 24 and 48 h. However, the seeds reaction after 72 h of treated with the extracts was reversed.

We observed negative correlation between fresh mass of red clover and IP index (Fig. 4). This correlation was stronger for seeds watered with extracts than for seeds pretreated before they were exposed to germination. However, the treatment of seeds with extracts by 72 h caused inhibition of growth.

The water content of red clover seedlings germinated on cock's-foot extracts was not significantly different from the control (Table 4). In seedlings germinated on the 5% inflorescence extracts for 8 days and 24 h and 10% inflorescence extracts in each of the applied times, a small but statistically significant reduction in water content was demonstrated.

Electrolyte leakage

The percentage of electrolyte leakage increased with the concentration of extracts, regardless of their types and times of use, in comparison to the control (Table 5). The destabilization of cell membranes increased to 83% in seeds treated with 5% inflorescence extracts for 3 days. The smallest disturbances in the electrolyte leakage were demonstrated in seedlings watered with extracts only one day (24 h). The other two inflorescences extracts caused the highest changes in the electrolyte leakage in red clover seedlings treated by them in times: 72 h and 8 days.

In the case of shoots extracts, with increasing their concentration, an increase in the destabilization of cell membranes was observed, regardless of the time of contact with the extracts. The largest, over 90%, electrolyte leakage was recorded in groups watered with 10 and 15% shoot extracts for 48 h. The seedlings treated by 24 h of the 5% shoot extracts and inflorescence extracts in all concentrations did not cause changes in the electrolyte leakage. The only exception, were seedlings germinated on 10 and 15% shoot extracts in which the electrolyte leakage increased by half.

Table 4 Fresh, dry masses and water content values of *Trifolium pratense* L. seedlings under stress of *Dactylis glomerata* L. aqueous extracts (5, 10, and 15%) from shoots (S) or inflorescences (F) in two treatments: watered with extracts for 8 days and pre-treated with extracts before germination process for 24, 48, and 72 h. Values (±SD) marked (a, b, c) differ significantly according to Duncan test at $p < 0.05$

Time	Aqueous extracts from *Dactylis glomerata* [%]						
	Control	5F	10F	15F	5S	10S	15S
	Fresh mass [g]						
8 days	0.0222 a ± 0.002	0.0210 a ± 0.002	0.0070 b ± 0.001	0.0080 b ± 0.001	0.0100 b ± 0.001	0.0090 b ± 0.001	0.0050 bc ±0.001
24 h	0.0264 b ± 0.002	0.0280 a ± 0.0009	0.0248 a ± 0.0013	0.0212 b ± 0.0013	0.0137 b ± 0.00077	0.0146 bc ±0.0003	0.0173 bc ±0.00168
48 h	0.0200 b ± 0.002	0.0280 a ± 0.0001	0.0210 ab ±0.0001	0.0140 c ± 0.0001	0.0110 c ± 0.0002	0.0080 cd ±0.0001	0.0040 cd ±0.0001
72 h	0.0195 b ± 0.002	0.0400 a ± 0.0001	0.0100 bc ±0.0016	0.0100 bc ±0.0001	0.0030 b ± 0.0001	0.0050 b ± 0.0001	0.0080 b ± 0.0001
	Dry mass [g]						
8 days	0.0005 b ± 0.0002	0.0010 a ± 0.0001	0.0010 a ± 0.00006	0.0003 a ± 0.0001	0.0010 a ± 0.0002	0.0001 b ± 0.0000	0.0001 b ± 0.0000
24 h	0.0004 b ± 0.0002	0.0010 a ± 0.0001	0.0010 a ± 0.000060	0.0004 bc ±0.0001	0.0010 a ± 0.0002	0.0010 a ± 0.0001	0.0003 bc ±0.0001
48 h	0.0003 b ± 0.0002	0.0010 a ± 0.0001	0.0010 a ± 0.0016	0.0034 a ± 0.0001	0.0006 b ± 0.0002	0.0001 b ± 0.0001	0.0001 b ± 0.0001
72 h	0.0002 b ± 0.0002	0.0004 a ± 0.0001	0.0002 b ± 0.0001	0.0001 b ± 0.0001	0.0001 b ± 0.0001	0.0001 b ± 0.0001	0.0001 b ± 0.0001
	Water content [%]						
8 days	98.11 a ± 0.85	94.99 b ± 0.34	83.85 b ± 2.93	95.43 a ± 1.50	93.95 a ± 2.164	98.76 a ± 0.183	97.32 a ± 0.70
24 h	99.31 a ± 0.85	96.96 b ± 0.0001	96.74 b ± 0.0001	98.02 a ± 0.0001	96.17 ab ±0.00038	95.25 ab ±0.0001	98.65 a ± 0.0001
48 h	97.01 a ± 0.85	96.07 a ± 0.0001	86.70 b ± 0.0001	97.28 a ± 0.0001	96.94 a ± 0.0001	98.22 a ± 0.0001	96.77 a ± 0.0001
72 h	98.00 a ± 0.85	97.98 a ± 0.0001	97.45 b ± 0.0010	98.96 a ± 0.0001	92.98 a ± 0.0001	97.15 a ± 0.0001	98.14 a ± 0.0001

Discussion

In the natural environment, plants compete not only for light, water and mineral salts, but also for the vital space they need (Müller-Schärer et al. 2000; Martiniello and Teixeira da Silva 2011; Barabasz-Krasny et al. 2017, 2018a, b). An important role, in this case is played by allelopathy, associated with the secretion of chemicals by plants with various phytotoxic potential for the environment. These substances are, for example, phenolic acids, alcaloids, coumarins, tannins, flavonoids, steroids, quinines etc., occurring in and underground and above-ground plant organs (Chung and Miller 1995; Khanh et al. 2005; Andrew et al. 2015; Cheng and Cheng 2015). Even in small amounts, they can effect on the structure and functioning of plant populations, and sometimes even entire plant communities. (Wardle et al. 1998; Możdżeń et al. 2018). For example, many studies have

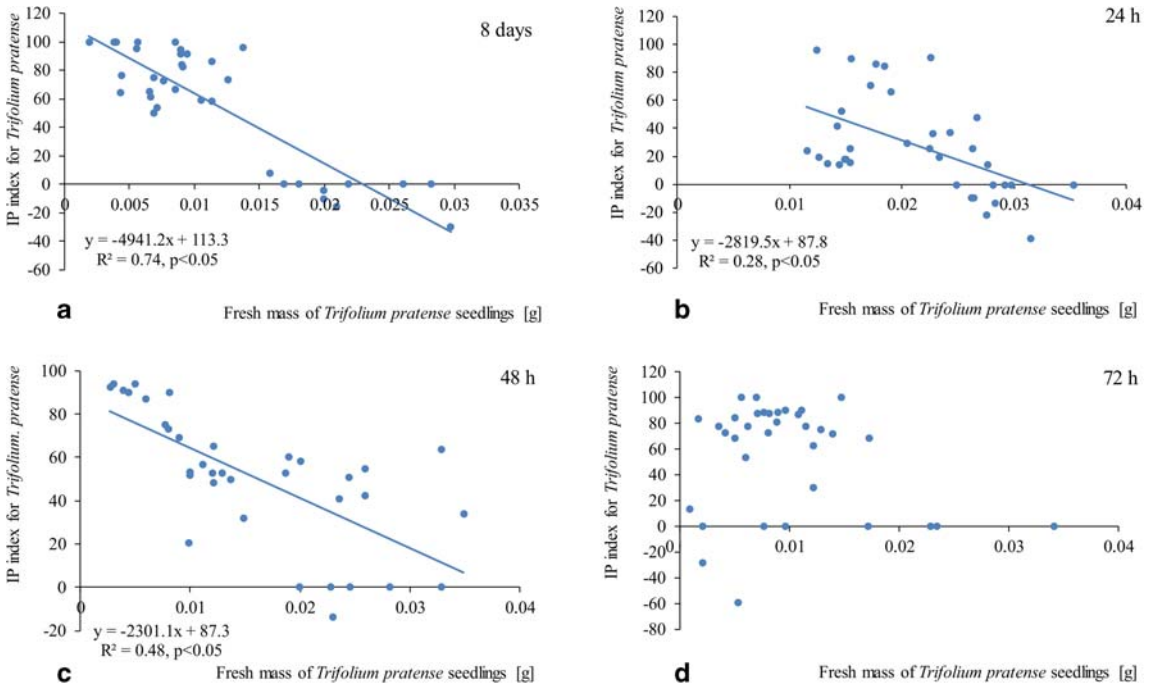

Fig. 4 Correlation between inhibition index (IP) and fresh mass of *Trifolium pratense* L. seedlings treated with *Dactylis glomerata* L. aqueous extracts at concentrations 5, 10, and 15% from shoots and inflorescences in two types of experiment: watered with extracts for 8 days and pretreated with extracts for 24, 48 and 72 h. *p* value shows Pearson significant correlation

Table 5 Electrolyte leakage from *Trifolium pratense* L. seedlings treated with *Dactylis glomerata* L. aqueous extracts at concentrations 5, 10, and 15% from shoots (S) and inflorescences (F) in different time: 24, 48, and 72 h, and watered with extract for 8 days; values (±SD) marked (a, b, c) differ significantly according to Duncan test at $p < 0.05$

Treatment	Time			
	8 days	24 h	48 h	72 h
Control	44.00 b ± 5.12	26.09 c ± 4.00	38.78 c ± 2.10	32.27 c ± 5.00
5F	36.47 b ± 9.75	29.93 b ± 12.20	49.94 ab ±10.64	82.75 a ± 24.48
10F	72.47 a ± 16.11	27.81 b ± 11.63	57.67 ab ±11.12	74.34 a ± 12.60
15F	82.44 a ± 2.04	27.70 b ± 19.17	53.54 ab ±3.79	63.40 ab ±25.75
5S	75.17 a ± 3.04	32.32 b ± 11.90	65.69 ab ±15.80	61.80 ab ±11.98
10S	78.46 a ± 3.88	63.57 a ± 29.25	94.24 a ± 2.03	68.98 a ± 19.97
15S	76.70 a ± 6.78	60.71 a ± 7.02	93.70 a ± 4.54	80.33 a ± 13.77

found that cock's-foot had a suppressive effect on volunteer species (Nie et al. 2004; Tozer et al. 2009; Clark et al. 2016; Li et al. 2016).

The experiment carried out examined the allelopathic influence of aqueous extracts from *Dactylis glomerata*, including the *Trifolium pratense* seeds germination (Figs. 1 and 2; Tables 2 and 3). The degree of inhibition of germination of red clover seeds and their growth depended both on the concentration of extracts and the time of contact of seeds with the cock's-foot extracts. The degree of inhibition increased with increasing extract concentration and time treatment. The highest changes in the values of germination indexes were demonstrated at the highest concentrations of the cock's-foot extracts and together with the increase in contact time of clover seeds with extracts (Tables 2 and 3). Stimulatory effects at low concentrations of allelochemicals may become inhibitory at higher concentrations (Kushima et al. 1998; Mandal 2001). An important role in the germination process of all seeds is played by enzymes, such as proteases, lipases and α-amylases, which action in the presence of allelopathic compounds is destabilized (Nie et al. 2005). Reducing the activity of hydrolytic enzymes means that the seeds are not able to provide enough energy and germination materials, which reduces the germination rate and causes the formation of weakened seedlings. For example, the germination of lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds treated by 6-methoxy-2-benzoxazolinone (MBOA) was positively correlated with the activity of α-amylase (Kato-Noguchi and Macías 2005). In soybean (*Glycine max* (L.) Merr.) seeds, lipases act to mobilize storage triglycerides by hydrolysis to fatty acids during the early germination phases. Fatty acids released by lipases are used to generate energy and supply it to growing embryos and seedlings (Staubmann et al. 1999). Probably the enzymatic mechanisms are also responsible for the weakening of the clover germination parameters in higher concentrations, with longer contact with the cock's-foot extracts.

In the present study, almost in each of the studied cases, there was a higher inhibition of root growth than seedling shoots (Fig. 3). This can be explained by the fact that the roots of seedlings tend to be more sensitive to the presence of allelochemical substances than the aboveground parts. In a specific range, their increase is proportional to the concentration of allelopathic substances. After exceeding the threshold concentration, there may be distortions and even dying of meristematic areas and, as a result, complete death of the root system (Levizou et al. 2002). Poor roots development can limit plant resistance to other environmental factors and weaken their competitive opportunities.

The results obtained are consistent with previous researches on other allelopathic interactions (Zandi et al. 2018; Możdżeń et al. 2019). For example, Li et al. (2016) stated that the cock's-foot roots can produce metabolites that inhibit seed germination and the growth of accompanying species. This can contribute to a longer sustainability of this species and have important practical implications for weed control. When sown in mixture with clovers and grasses, cock's-foot tended to become more dominant with and depress the regeneration of companion sub clover (*T. subterraneum* L.) (Dear and Cocks 1997). High allelopathic inhibition of a related neighbour in the early phases of development reduces the risk that later it will become a competitor for environmental resources (San Emeterio et al. 2004).

Aqueous extracts from shoots and inflorescences of *D. glomerata* showed variable allelopathic activity on the *T. pratense* mass growth. Allelopathic interactions depended on both the concentrations of extracts, the time of their application, and the organ from which they were prepared (Table 4). Changes in the fresh mass of clover seedlings were correlated with the stimulation of their growth (Fig. 4). The maximum reduction both in the fresh and dry masses, occurred at extracts with the highest concentrations, regardless of the time of watering with them. The decrease in the dry mass of grass growing in the presence of the cock's-foot was demonstrated earlier, e.g. Tozer et al. (2009). Differences in mass values can in this case be attributed to a different allelopathic force, resulting from the constitutional variability of allelopathic compounds (Kwiecińska-Poppe et al. 2011). However, the percentage of water content in *T. pratense* seedlings was not significantly inhibited by the aqueous extracts of

D. glomerata (Table 4). This type of reaction may be related to the construction of the seeds and their physical properties.

Like other stress factors, the allelopathic compounds also effect on the electrolyte leakage of cell membranes and ions transport. They can damage the cell membranes by direct interaction with the membrane component or cause impairment of some of the metabolic processes necessary for the proper functioning of the membranes (Galindo et al. 1999; Lehman and Blum 1999). During stress, in the cells occur, among others lipid peroxidation by disorders in the formation of polyunsaturated fatty acids (Foyer et al. 1994). Most likely the chemical compounds present in the extracts cause oxidative stress by generating reactive oxygen forms. Singlet oxygen, superoxide radicles and hydroxyl radicle (OH), hydrogen peroxides are highly reactive and toxic. These molecules can cause oxidative damage to membranes, DNA, proteins, photosynthetic pigments and lipids (Apel and Hirt 2004). The study showed an excessive leaking of ions (electrolytes) in each of the applied extracts, in relation to the control (Table 5). This phenomenon may indicate the inability to maintain consistent membranes in *T. pratense* cells, in the presence of *D. glomerata* extracts, resulting in disturbances in seeds germination (Nie et al. 2005).

The laboratory tests carried out clearly indicate the presence of water-soluble phytotoxins in the cock's-foot extracts, which unfavorably effect on clover seeds. Higher toxicity of shoots extracts than inflorescences is probably related to the occurrence of allelopathic compounds of different concentration in various parts of the plant. Their toxicity may be caused by a synergistic effect, not only by the action of individual substances.

Conclusion

In the laboratory conditions, detailed responses were received regarding the allelopathic effect of *Dactylis glomerata* L. on the *Trifolium pratense* L. seeds and seedlings:

[1] the aqueous extracts from *D. glomerata* shoots and inflorescences inhibited the germination *T. pratense* seeds; with the increase of contact time with extracts, this was most evident at the highest 15% extracts concentrations;
[2] the elongation growth of clover seedlings was clearly weaker with the increase in the concentration of cock's-foot extracts and the longer contact time with these extracts;
[3] the *D. glomerata* shoots and inflorescences extracts showed variable allelopathic activity on the *T. pratense* masses growth; maximum reduction in the growth, both fresh and dry masses, occurred at extracts with the highest concentrations, regardless of the time of watering with extracts;
[4] with the time of contact seeds with extracts, an increase in the destabilization of cell membranes in red clover cells was observed;
[5] the most negative effect on red clover seeds had aqueous extracts from cock's-foot shoots, compared to the control and tests carried out with inflorescence extracts.

The obtained results clearly confirm the importance of allelopathic substances in the potential shaping of the species composition of meadow phytocenoses. They also indicate the importance of proper management in hay meadows, since leaving biomass can lead to the release of phytotoxins that may inhibit germination and growth of other plants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of Interest.

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