

Allelopathic influence of medicinal plant *Filipendula vulgaris* Moench on germination process

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Abstract

The allelopathic potential of medicinal plant *Filipendula vulgaris* Moench, originated from two natural habitats: calcareous grasslands and humid meadows, was investigated using radish (*Raphanus sativus* L. var. *radicula* Pers.) as a model plant. Suppression influence of aqueous extracts at concentrations: 1%, 5%, 10%, obtained separately from under and aboveground parts of the species, was studied testing germination capacity, biomass and biometry of seedling, and electrolyte leakage. Additionally, two cultivars of the radish were used to test the sensitivity of frequently used plant in the laboratory tests. This study revealed the inhibiting effect of high concentration aqueous extracts from *F. vulgaris* organs on the germination of radish. It was significant for seed vigour index, speed emergence, germination stress tolerance index, and mean germination time. However, the stimulating effect of 1% of aqueous extracts from dropwort roots and shoots on the germination indexes was documented. Similarly, low concentration extracts had a positive effect on seedling elongation which was visible in biometric analyses and inhibition percentage index but it was not so obvious in the biomass analyses. In general, the aboveground extracts from dropwort grown on calcareous grassland demonstrated higher allelopathic potential than extracts from underground parts and meadow habitat.

Keywords: dropwort; electrolyte leakage; germination; medicinal plant; seeds

Introduction

Medicinal plants have a long history as a source of potential therapeutic agents, that have been incorporated into modern medicine (e.g. Kamboj, 2000; Tapsell *et al.*, 2006; Verma and Singh, 2008; Atanasov *et al.*, 2015). It is estimated that almost 53,000 flowering plant species are considered medicinal plants (Wakdidar, 2004). They are used mainly due to their natural compounds with biological activity that have a positive effect on maintaining human health. Additionally, secondary metabolites in medicinal plants have been reported as potent growth inhibitory agents indicating that such plants serve as a storehouse for allelopathic compounds (Batish *et al.*, 2006). In the last decade, there is a growing interest to estimate the allelopathic potential of medicinal plants (e.g. Fujii *et al.*, 2003; Amini *et al.*, 2014; Skrzypek *et al.*, 2016; Puła

et al., 2020). Behind the researches of allelopathic activity is the purpose of better practice in the cultivation of medicinal plants as well as a future prospect in controlling major crop weeds for sustainable agricultural production (Islam *et al.*, 2018). Increasing consumer awareness of herbicide residues in production practices leads to increased demands for organic products or safer foods (McErlich and Boydston, 2013; Tal, 2018).

Possibility of using plants compounds as natural pesticide promote sustainable agriculture and address audience concerns on the effects of pesticides on the environment and human health (Kropff and Walter, 2000; Dayan *et al.*, 2009). There are suggestions that most allelopathic compounds will be biodegradable and also less polluting than traditional pesticides owing to their shorter half-lives (Daizy *et al.*, 1995).

Filipendula vulgaris Moench (syn. *F. hexapetala* Gilib., *Rosaceae*), commonly known as dropwort (or fern-leaf dropwort), is one of the plant species used in traditional medicine. The use of the species in folk medicine was reported from Europe in countries such as Poland (Mowszowicz, 1985; Oszmiański *et al.*, 2007), Romania (Imbrea *et al.*, 2010), Russia (Olennikov and Kruglova, 2013) or Serbia (Tucakov, 1973). The entire plant or their parts (flowers, leaves, tubers) were used in case of breathlessness, wheezing, sore throats and congestion, stomachache and diarrhea, rheumatism (Mowszowicz, 1985). Published studies reveal antibacterial, anti-inflammatory, antipyretic, antihyperalgesic, and antioxidant properties, as well as nootropic activity (Maksimović *et al.*, 2007; Pavlovic *et al.*, 2007; Katanić *et al.*, 2015; 2018; Shilova and Suslov, 2015; Shilova *et al.*, 2015; Samardžić *et al.*, 2016).

The plant contains active substances such as spiraeoside, phenolic acids (ellagic, gallic, caffeic, salicylic, syringic, chlorogenic, rosmarinic), flavonoids (quercetin, hyperoside, kaempferol, apigenin, luteolin, dihydroquercetin, isoquercitrin, avicularin, spiraeoside, rutin), tannins (rugosins, tellimagrandins), saponins, amino acids and other (Oszmianski *et al.*, 2007; Bączek *et al.*, 2011, 2012; Vengerovsky *et al.*, 2011; Capecka *et al.*, 2012; Olennikov and Kruglova, 2013; Movsumov *et al.*, 2017, Shilova and Korotkova, 2017; Katanić *et al.*, 2018).

Filipendula vulgaris is a rosette-forming perennial and clonal herb bearing short rhizomes and tubers (Klimešová and Klimeš, 2006). The flowering stalks are up to 80 cm high, consisting of numerous cream-white flowers, mainly pollinated by insects or wind (Clapham *et al.*, 1987; Weidema *et al.*, 2000). *Filipendula vulgaris* is classified as euro-siberian species (Zajac and Zajac, 2009) and occurs in north-western Africa through Europe and middle Asia range. Dropwort is included as diagnostic species for plant communities of *Festuco-Brometea* class, where calcareous grasslands belongs (Medwecka-Kornaś *et al.*, 1966; Towpasz and Stachurska-Swakoń, 2011, 2012). Moreover, the species may also grow in moist meadows from the *Caricetalia davalianae* order and *Molinietalia* order (Dubiel *et al.*, 1999; Towpasz and Stachurska-Swakoń, 2009). The studies focusing on biology of this species are scarce (Kostrakiewicz-Gieralt and Stachurska-Swakoń, 2017).

The aim of the study was to determine the allelopathic influence of aqueous extracts from *Filipendula vulgaris* on germination process and early growth on model plant *Raphanus sativus* L. var. *radicula* Pers. We used separately underground and aboveground parts of dropwort originated from two natural habitats: humid meadow and calcareous grassland to examine the potential of allelopathy and the influence of habitat on production of allelopathic compounds. The germination capacity of seeds was tested by determining the germination indexes, the biometric analysis of seedlings was performed, the value of fresh and dry mass was determined and the percentage of destabilisation of cell membranes was measured. In the experiment two cultivars of radish were used to test the sensitivity of the model plant.

Materials and Methods

Plant material

Filipendula vulgaris specimens were collected from two natural habitats in southern Poland: humid meadows and calcareous grasslands in July 2019 (Figure 1). The humid meadows from the alliance *Molinion caeruleae*, *Molinio-Arrhenatheretea* class, were situated on the low flood terrace of the Vistula river where the

limestone monadnocks (Jurassic-Cretaceous) and tectonic depressions of the Brama Krakowska gate occur (50°01'48.8"N 19°52'26.7"E, Silesian-Krakow Upland). Histic Aerosol is reported as the soil unit for the place where the meadows occurred (Skiba *et al.*, 2010). The calcareous grasslands, represented *Thalictro-Salvietum* association from *Festuco-Brometea* class, were located in the Miechów Upland (50°18'59.1"N 20°04'14.3"E, Małopolska Upland). Rendzic and calcareous leptosols are the soil units that prevailed in the area.

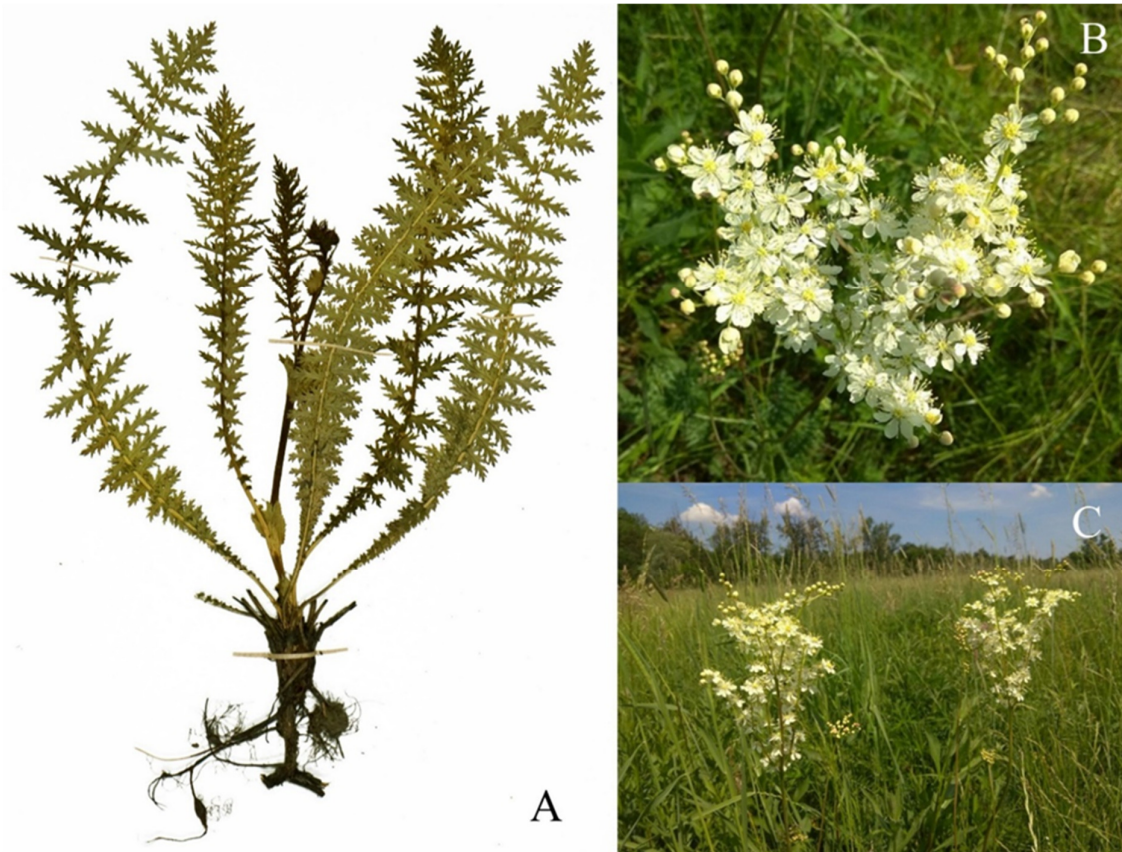


Figure 1. *Filipendula vulgaris* Moench (dropwort) collected from southern Poland
A – before flowering with a rosette of leaves, rhizomes, and tubers, B – inflorescence, C – specimens in meadow habitat
(Photo M. Fecowicz)

Fresh material of the species was selected in the laboratory to eliminate defective parts (e.g. damaged, infected leaves, roots). Then the plants were separated into underground parts (roots, tubers, rhizomes) and aboveground parts (shoots, leaves). In the text, the term “roots” is used for underground parts and “shoots” for aboveground parts of the species. The plant material was dried at the temperature of 25 °C, in the dark for 3 weeks in a dryer (Wamed SUP-100, Poland). The dry plant material of *F. vulgaris* was stored in the paper bags, in the dark, in a desiccator to avoid microbiological destruction of allelopathic compounds present in it.

Radish *Raphanus sativus* L. var. *radicula* Pers was selected for the study as a model organism due to the short life cycle and low environmental requirements for germination. Two commonly cultivars of radish: ‘Rowa’ and ‘Krakowianka’ were used in the experiment. Both cultivars require sunny areas, rich and permeable soil with low nitrogen content. They differ in the length of the growing season: ‘Rowa’: 28-32 days, ‘Krakowianka’: 33-37 days (Kupczak, 2011). The seeds of radish cultivars were purchased from POLAN Sp.z.o.o. Breeding and Seed Horticulture in Kraków (Poland).

Preparation of aqueous extract solutions

Dry plant material was ground separately for under and aboveground parts of dropwort in a mill (Braun, 4 045 type, Germany) and used to prepare the aqueous extracts of various concentrations: 1%, 5%, and 10%. Each extract, depending on the concentration, was flooded with a suitable amount of distilled water (e.g. 5% = 5 g plant material + 95 ml distilled water, 10% = 10 g plant material + 90 ml distilled water) and left for 24 hours, at 25°C temperature, in the dark to extract the compounds. The extracts obtained were filtered through a filter paper on a Büchner funnel by Whatman (Grade 1: 11 µm – medium flow filter paper) filter paper, using a vacuum pump (PL2/3, Al-Conbest, USA), and then stored in a refrigerator for the duration of the experiment.

Seed germination conditions

Radish seeds were sterilised in 1% sodium hypochlorite for 10 min, then rinsed with distilled water (3x). In the experiment, seeds were put on the sterile Petri dishes (25 seeds per dish) with 3 layers of filter paper (Whatman, Grade 1: 11 µm - medium flow filter paper) soaked with 5 ml the aqueous extract of dropwort: separately with different concentrations from under and aboveground parts. Then seeds on dishes were placed in a growth chamber (Angelantoni Industrie, Italy) at 25 °C / 20°C, photoperiod 200 µmol·m⁻²·s⁻¹ (12h day / 12h night), and relative air humidity (RH) 80-90%. The control group was radish seeds treated with distilled water in the same volumes as extract watered seeds. 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 7 days, the effect of *F. vulgaris* aqueous extracts on germination and early growth of radish was measured.

Germination indexes

To check the influence of aqueous extract on the germination process the following indexes were used: seedling vigour index (SVI), speed of emergence (SE) (Mazur, 2020), rate of germination (RG). Mean germination time (MGT) and germination stress tolerance index (GSTI) were calculated according to the formulas used by Możdżeń *et al.* (2020).

$$SVI = [\text{seedling length (cm)} \times \text{percentage of germinated seeds}] / 100$$

$$SE = [\text{number of germinated seeds on the first day of germination} / \text{number of germinated seeds on the last day of germination}] \times 100$$

$$RG = [(n1 + n2 + nn) / (n1 \times T1) + (n2 \times T2) + (n3 \times T3) + \dots] \times 100$$

where: n1 = number of germinated seeds on time T1; n2 = number of germinated seeds on time T2; n3 = number of germinated seeds on time T3

$$MGT = \sum fx / \sum f$$

where: x is the number of new seeds germinating on day f and f is the number of days from the beginning

$$GSTI (\%) = [\text{PI of stressed seeds} / \text{PI of control seeds}] \times 100$$

where: PI is a speed of germination index calculated according to the formula: $PI = nd2 \times (1.00) + nd4 \times (0.75) + nd6 \times (0.50) + nd8 \times (0.25)$; nd2, nd4, nd6 and nd8 are the seed numbers, germinating on days 2, 4, 6 and 8, respectively.

Seedlings elongation

The length of roots and hypocotyls, as well as whole seedlings, was measured using a caliper (Topex 31C615, Poland), with an accuracy of 1 mm. Besides, the seedling length was expressed as a percentage of control by using an inhibition percentage (IP) index:

$$IP = [1 - (LE/LC)] \times 100$$

where: IP – growth inhibition index (%), LE – plant length (cm) treated with aqueous extract, LC – plant length (cm) from the control.

Inhibition percentage (IP) of growth (as control %) – a minus (-) value indicates growth stimulation, and a plus (+) value indicates growth inhibition.

Fresh and dry masses, water content

Fresh mass (g) of single seedlings was weighted (Ohaus Adventurer Pro, USA). Dry mass of seedlings was determined after 48 h of drying the fresh material in a dryer (Wamed SUP-100, Poland) at temperature 105 °C. Additionally, the percentage of dry mass and total water content were determined.

$$\text{Dry mass (\%)} = (\text{dry mass} / \text{fresh mass}) \times 100$$

$$\text{H}_2\text{O (\%)} = [(\text{fresh mass} - \text{dry mass}) / \text{fresh mass}] \times 100$$

Electrolyte leakage

The percentage of destabilisation of cell membranes of radish seedlings was determined by slightly modifying the method described in the study of Możdżeń *et al.* (2020). A single radish seedling was placed in polypropylene falcons with 30 ml distilled water. Each of the samples was shaken for 3 h on a shaker (Labnet, Rocker, USA) to measure the electrolytes leakage from living seedlings (EL). Electrolyte leakage was measured by conductometer (CX-701, Elmetron, Poland) with electrode (K = 1.02) (Elmetron, Poland). The seedlings in distilled water were frozen at -75 °C for 24 h. The next day, samples were defrosted and shaken for 3 h to determine the total electrolyte leakage from dead seedlings (ED). The total percentage of electrolyte leakage was determined according to the formula:

$$\text{EL} = (\text{EL} / \text{ED}) \times 100$$

Statistical analysis

The experiment was performed in three repetitions. The obtained results were statistically tested between repetitions of the experiment ($n = 3$, \pm SE) as well as between cultivars, habitats extracts, and organ extracts using the Duncan test at $p < 0.05$ with Statistica software.

Results

Germination indexes

The percentage of seeds germinated was the highest in the control. A decrease in seed germination capacity was observed with the increase in the concentration of aqueous extracts. The smallest differences were found between the control and 1% of *Filipendula vulgaris* root and shoot extracts regardless of the habitats. The lowest number of germinated radish seeds was found on the 10% dropwort extracts. More germinated seeds were observed on root extracts than on shoots (Figure 2).

The seed vigour index (SVI) reached the significantly highest values for the investigated radish cultivars watered with the 1% aqueous extracts of *F. vulgaris* roots, compared to the control. In the case of extracts from shoots, the highest values of this parameter were recorded for the 'Krakowianka' cultivar treated with 1% and 5% of the extracts of plants growing on calcareous grassland. Regardless of the radish cultivar and the extract type, the lowest SVI values were found for seeds germinated on the 10% extracts (Table 1).

Speed emergence (SE) values for radish seeds watered with root extracts, both from meadows and calcareous grasslands, were similar to the control at each concentration. The exception was 10% calcareous grassland root extracts, which significantly limited the SE. Aqueous extracts from shoots with a concentration of 1% did not affect the values of this parameter. Compared to the control samples, 5% and 10% extracts, especially from shoots obtained from calcareous grasslands, inhibited SE.

In comparison with the control, the only significant differences in the values of the rate of germination (RG) index were demonstrated for the 'Krakowianka' cultivar watered during germination with 10% shoot

extracts from calcareous grassland. In other cases, in the values of this index, no statistically significant differences were observed.

Mean germination time (MGT) reached similar values between the control and 1% *F. vulgaris* root and shoot extracts from both meadow and calcareous grassland. In the 'Rowa' cultivar, a lower value of this index was revealed for seeds treated, regardless of the concentration of the extract, with dropwort plant root extracts from the meadow. For the 'Krakowianka', significant differences were found only for seeds watered with 10% extracts.

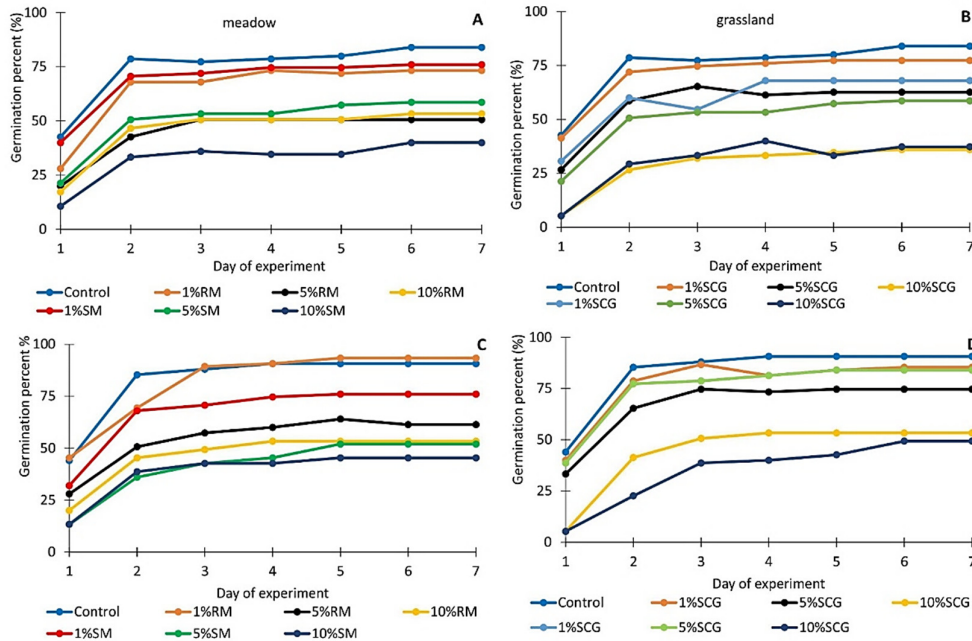


Figure 2. Germination capacity of *Raphanus sativus* L. var. *radicula* Pers. cv under influence of *Filipendula vulgaris* extracts

(A, B) – 'Rowa', (C, D) – 'Krakowianka' in seedlings 'watered with aqueous extracts from root and shoot *Filipendula vulgaris* Moench in different concentrations; Control – distilled water, 1, 5, 10RM – 1%, 5%, 10% extract from root plant grown on meadow, 1, 5, 10RCG – 1%, 5%, 10% extract from root plant grown on calcareous grasslands, 1, 5, 10SM – 1%, 5%, 10% extract from shoot plant grown on meadow, 1, 5, 10SCG – 1%, 5%, 10% extract from shoot plant grown on calcareous grasslands

In the remaining cases, irrespective of the cultivar, significant differences were observed between the control and extracts of 10% root extracts from calcareous grasslands and 5% and 10% shoot extracts from plants grown on meadows and calcareous grasslands.

Germination stress tolerance index (GST) achieved significantly the highest values for two radish cultivars watered with 1% extracts of plant shoots harvested from calcareous grasslands. Additionally, for the 'Rowa', a significant increase in the GST value was demonstrated for seeds watered with 1% extracts from the roots of plants growing on calcareous grasslands and shoots collected from meadows. The lowest values of this index were observed for 'Rowa' seeds watered with 10% root extract and for 'Krakowianka' treated with 10% shoot extracts obtained from calcareous grasslands (Table 1).

Table 1. Germination parameters of *Raphanus sativus* L. var. *radicula* Pers. cv (A) – ‘Rowa’, (B) – ‘Krakowianka’ in seedlings ‘watered with aqueous extracts from root and shoot *Filipendula vulgaris* Moench in different concentrations

Aqueous extracts (%)	SVI		SE		RG		MGT		GST	
	A	B	A	B	A	B	A	B	A	B
Control	6.52 b ±0.02	6.94 b ±0.02	50.88 a ±2.12	49.92 a ±2.00	23.47 a ±1.12	23.49 a ±1.02	0.51 a ±0.02	0.49 a ±0.01	1.00 b ±0.01	1.00 b ±0.01
1RM	9.55 a ±0.05	10.78 a ±0.05	37.38 ab ±1.56	48.43 a ±1.82	23.10 a ±1.40	23.04 a ±1.00	0.38 b ±0.01	0.49 a ±0.01	0.89 b ±0.02	0.94 b ±0.01
5RM	4.19 d ±0.01	7.15 b ±0.04	37.25 ab ±1.55	45.37 a ±1.79	22.98 a ±0.99	23.03 a ±1.11	0.39 b ±0.01	0.46 a ±0.01	0.67 cd ±0.01	0.68 cd ±0.01
10RM	4.99 c ±0.01	5.25 c ±0.01	32.14 ab ±1.46	37.78 ab ±1.52	22.81 a ±1.12	22.92 a ±1.14	0.33 b ±0.01	0.38 b ±0.01	1.05 b ±0.03	0.88 bc ±0.01
1RCG	10.52 a ±0.05	11.14 a ±0.05	53.88 a ±2.15	46.42 a ±1.92	23.51 a ±1.10	23.41 a ±1.10	0.53 a ±0.02	0.47 a ±0.01	1.50 a ±0.04	1.63 b ±0.05
5RCG	6.79 b ±0.02	7.55 b ±0.04	42.86 a ±1.92	44.22 a ±1.56	22.89 a ±1.10	23.22 a ±1.12	0.43 a ±0.01	0.45 a ±0.01	0.81 b ±0.02	0.87 bc ±0.02
10RCG	2.84 ef ±0.01	4.23 d ±0.01	14.07 c ±1.02	9.21 cd ±1.00	22.36 a ±1.15	21.93 ab ±1.52	0.15 cd ±0.01	0.10 d ±0.05	0.52 d ±0.01	0.69 cd ±0.01
1SM	7.46 b ±0.04	5.03 c ±0.01	52.63 a ±2.00	42.33 a ±1.62	23.56 a ±1.10	23.14 a ±1.00	0.53 a ±0.01	0.42 ab ±0.01	2.33 a ±0.06	1.49 b ±0.05
5SM	7.76 b ±0.04	5.31 c ±0.01	37.25 ab ±1.44	26.03 b ±1.02	23.35 a ±1.12	22.10 a ±0.99	0.36 b ±0.01	0.26 c ±0.01	0.73 c ±0.01	0.60 d ±0.01
10SM	3.06 e ±0.01	4.06 d ±0.01	22.29 b ±1.38	32.96 ab ±1.52	21.80 ab ±1.01	22.83 a ±1.00	0.27 c ±0.01	0.29 c ±0.01	0.66 cd ±0.01	0.96 b ±0.01
1SCG	7.84 b ±0.04	10.06 a ±0.05	44.13 a ±1.73	45.58 a ±2.10	23.08 a ±0.89	23.27 a ±1.22	0.45 a ±0.01	0.46 a ±0.01	1.81 a ±0.07	1.92 a ±0.08
5SCG	6.49 b ±0.02	9.98 a ±0.05	22.46 b ±1.11	16.68 b ±1.12	22.48 a ±1.00	21.80 ab ±1.02	0.36 b ±0.01	0.46 a ±0.01	0.83 b ±0.01	1.00 b ±0.01
10SCG	4.08 d ±0.01	5.15 c ±0.01	14.39 b ±1.04	11.61 bc ±1.01	22.26 a ±1.12	21.02 b ±1.00	0.14 cd ±0.01	0.11 d ±0.01	0.65 cd ±0.01	0.45 e ±0.01

Control – distilled water, 1, 5, 10RM – 1%, 5%, 10% extract from root plant grown on meadow, 1, 5, 10RCG – 1%, 5%, 10% extract from root plant grown on calcareous grasslands, 1, 5, 10SM – 1%, 5%, 10% extract from shoot plant grown on meadow, 1, 5, 10SCG – 1%, 5%, 10% extract from shoot plant grown on calcareous grasslands; mean values (n = 5, ±SE) marked different letter differ statistically; SVI – seedling vigour index, SE – speed of emergence, RG – rate of germination, MGT – mean germination time, GSTI – germination stress tolerance index; mean values (n = 5, ±SE) marked different letter differ statistically (in column) according to Duncan test at p < 0.05

Seedling elongation

Regardless of the cultivar, 1% aqueous extracts from the *F. vulgaris* roots stimulated the length of seedlings' roots.

In the case of plant shoots collected from meadows, the elongation of this radish organ was decreased. The 10% extracts from roots obtained from calcareous grasslands and meadows also had a negative impact on root length. For seedlings watered with 5% extracts from shoots collected on calcareous grasslands, significant root elongation was observed (Figure 3A).

The aqueous extracts from the roots and shoots of dropwort had a positive effect on the elongation of the hypocotyl of radish seedlings, ‘Rowa’ and ‘Krakowianka’ cultivars, concerning the control. Generally, hypocotyl growth stimulation was observed with increasing concentrations (Figure 4A).

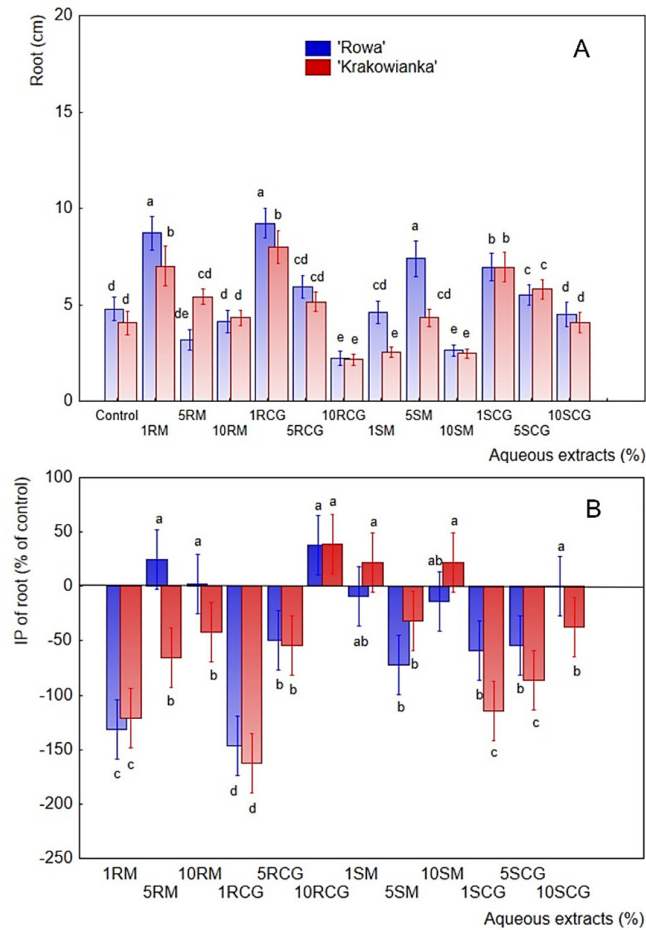


Figure 3. A – Length of roots of *Raphanus sativus* L. var. *radicula* Pers. 'Rowa' and 'Krakowianka' after 7 days of watering with from roots and shoots *Filipendula vulgaris* Moench with different concentrations (1, 5, 10%) and under control conditions; B – Inhibition percentage (IP) of growth (as control %) The horizontal line is the border with negative values that indicate elongation stimulation and positive values that indicate the percentage of inhibition of seedling growth; RM – extract from root plant grown on the meadow, SCG – extract from shoot plant grown on calcareous grasslands; mean values (n = 3, ±SE) marked different letter differ statistically according to Duncan test at p < 0.05

Biometric analysis of whole seedlings revealed a stimulating effect for both radish cultivars, extracts from 1% of roots and 5% of shoots. Besides, a positive effect of shoot extracts at concentrations of 1% and 5% from calcareous grasslands was observed. Compared to the control, 5% root extracts of calcareous grassland plants also showed stimulation of seedling elongation (Figure 5A).

Morphometric analysis of the under- and aboveground organs of radish seedlings by determining the IP index as a percentage of control confirmed the positive and negative effect of extracts from *F. vulgaris* on the roots, hypocotyls, and whole radish seedlings in two cultivars. For this index, positive values indicate a higher percent growth inhibition. On the other hand, negative values show the positive influence of the extracts on organ elongation. The root length was inhibited by extracts with 10% concentrations, regardless of the dropwort organs. Contrary to the 1% extracts, which stimulated the growth of radish seedlings (Figure 3B). Elongation of the hypocotyls was stimulated relative to the control (Figure 4B). The length of the whole seedlings was generally stimulated, but to the highest extent by 1% dropwort extracts (Figure 5B). The smallest effects were found for extracts from shoots from calcareous grasslands (Figures 3-5).

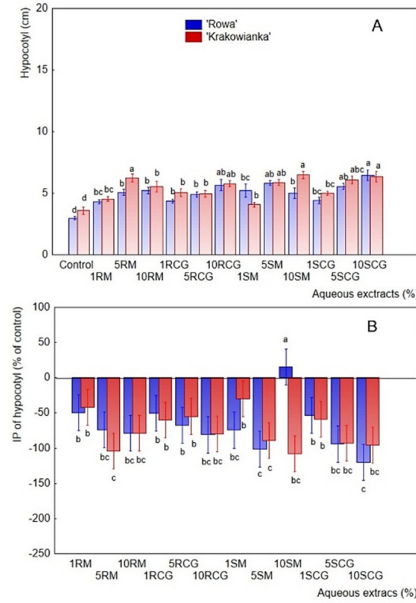


Figure 4. A – Length of hypocotyls of *Raphanus sativus* L. var. *radicula* Pers. 'Rowa' and 'Krakowianka' after 7 days of watering with aqueous extracts from roots and shoots *Filipendula vulgaris* Moench with different concentrations (1, 5, 10%) and under control conditions; B – Inhibition percentage (IP) of growth (as control %)

The horizontal line is the border with negative values that indicate elongation stimulation and positive values that indicate the percentage of inhibition of seedling growth; RM – extract from root plant grown on the meadow, SCG – extract from shoot plant grown on calcareous grasslands; mean values (n = 3, ±SE) marked different letter differ statistically according to Duncan test at p < 0.05

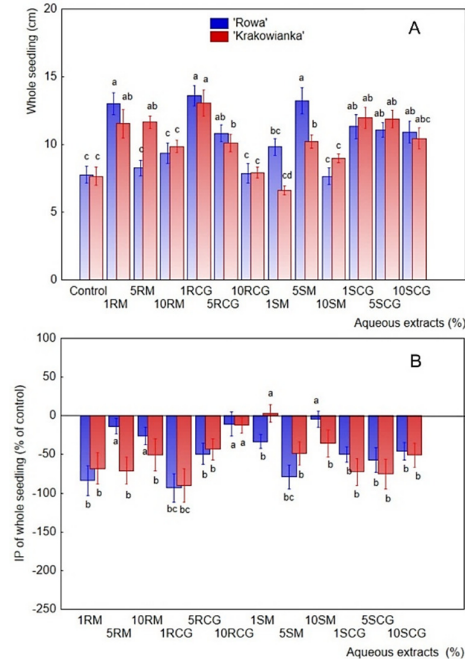


Figure 5. A – Length of whole seedlings of *Raphanus sativus* L. var. *radicula* Pers. 'Rowa' and 'Krakowianka' after 7 days of watering with aqueous extracts from roots and shoots *Filipendula vulgaris* Moench with different concentrations (1, 5, 10%) and under control conditions; B – Inhibition percentage (IP) of growth (as control %)

The horizontal line is the border with negative values that indicate elongation stimulation and positive values that indicate the percentage of inhibition of seedling growth; RM – extract from root plant grown on the meadow, SCG – extract from shoot plant grown on calcareous grasslands; mean values ($n = 3, \pm SE$) marked different letter differ statistically according to Duncan test at $p < 0.05$

Table 2. Fresh and dry mass and water content in seedlings *Raphanus sativus* L. var. *radicula* Pers. cv 'Rowa', 'Krakowianka' treatment the aqueous extracts from root and shoot *Filipendula vulgaris* Moench in different concentrations

Radish cultivar	Control	1RM	5RM	10RM	1RCG	5RCG	10RCG	1SM	5SM	10SM	1SCG	5SCG	10SCG
	Fresh mass (g)												
'Rowa'	0.1633 bc ± 0.03	0.1611 bc ± 0.02	0.1837 ab ± 0.04	0.1960 ab ± 0.05	0.1685 b ± 0.01	0.1721 abc ± 0.02	0.1734 abc ± 0.04	0.1868 ab ± 0.06	0.1962 ab ± 0.02	0.2042 a ± 0.07	0.1411 bc ± 0.01	0.1240 c ± 0.01	0.1865 ab ± 0.04
'Krakowianka'	0.2015 a ± 0.01	0.2038 a ± 0.01	0.1837 ab ± 0.03	0.2238 a ± 0.06	0.1842 ab ± 0.03	0.1824 ab ± 0.04	0.1832 ab ± 0.04	0.1587 bc ± 0.03	0.2091 a ± 0.03	0.2121 a ± 0.02	0.1924 ab ± 0.03	0.2019 a ± 0.02	0.2053 a ± 0.04
	Dry mass (g)												
'Rowa'	0.0068 b ± 0.001	0.0067 b ± 0.002	0.0100 a ± 0.001	0.0091 ab ± 0.002	0.0081 ab 0.001	0.0087 ab ± 0.001	0.0097 ab ± 0.005	0.0081 ab ± 0.002	0.0082 ab ± 0.001	0.0094 ab ± 0.002	0.0065 b ± 0.001	0.0066 b ± 0.002	0.0083 ab ± 0.001
'Krakowianka'	0.0097 ab ± 0.002	0.0094 ab ± 0.002	0.0089 ab ± 0.001	0.0106 a ± 0.001	0.0093 ab ± 0.001	0.0091 ab ± 0.001	0.0101 a ± 0.002	0.0088 ab ± 0.001	0.0095 ab ± 0.001	0.0109 a ± 0.001	0.0086 ab ± 0.002	0.0090 ab ± 0.001	0.0093 ab ± 0.002
	Dry mass (%)												
'Rowa'	4.15 b ± 0.97	4.17 b ± 0.96	5.42 a ± 0.93	4.65 ab ± 0.54	4.78 ab ± 0.30	5.03 a ± 0.61	5.62 a ± 1.50	4.33 b ± 0.63	4.17 b ± 1.23	4.60 ab ± 0.35	4.61 ab ± 1.23	5.29 a ± 0.49	4.43 b ± 0.55
'Krakowianka'	4.80 ab ± 0.74	4.60 ab ± 0.86	4.84 ab ± 1.91	4.71 ab ± 1.57	5.06 a ± 0.37	5.01 a ± 0.77	5.52 a ± 1.59	5.54 a ± 0.22	4.53 ab ± 0.39	5.15 a ± 0.29	4.47 ab ± 0.78	4.44 ab ± 0.78	4.55 ab ± 0.53
	Water content (%)												
'Rowa'	95.85 a ± 0.97	95.83 a ± 0.96	94.58 b ± 0.93	95.35 a ± 0.54	95.22 a ± 0.30	94.98 ab ± 0.61	94.38 ab ± 1.50	95.67 a ± 0.63	95.83 a ± 0.44	95.40 a ± 1.23	95.39 a ± 0.35	94.71 ab ± 1.23	95.47 a ± 0.49
'Krakowianka'	95.20 a ± 0.55	95.40 a ± 0.74	95.16 a ± 0.86	95.29 a ± 1.95	94.94 ab ± 0.16	94.99 ab ± 0.37	94.48 ab ± 0.77	94.46 ab ± 1.59	95.47 a ± 0.22	94.85 ab ± 0.39	95.53 a ± 0.29	95.56 a ± 0.78	95.45 a ± 0.53

Control – distilled water, 1, 5, 10RM – 1%, 5%, 10% extract from root plant grown on meadow, 1, 5, 10RCG – 1%, 5%, 10% extract from root plant grown on calcareous grasslands, 1, 5, 10SM – 1%, 5%, 10% extract from shoot plant grown on meadow, 1, 5, 10SCG – 1%, 5%, 10% extract from shoot plant grown on calcareous grasslands; mean values ($n = 5, \pm SE$) marked different letter differ statistically (in row) according to Duncan test at $p < 0.05$

Fresh, dry mass, and water content

The fresh mass of 'Rowa' radish seedlings was significantly highest for the seeds watered with 10% of the shoots from the meadows, compared to the control. Significantly the lowest fresh mass value of seedlings was found for seeds watered with 5% of *F. vulgaris* shoot extracts from calcareous grasslands. For the 'Krakowianka', no statistically significant differences in the fresh mass values were observed between the control and the extracts from the dropwort organs used (Table 2).

In the case of dry mass, its significant increase was demonstrated for seedlings of 'Rowa' cultivar grown from seeds watered with 5% root extract from meadow areas, compared to the control. In other cases, no significant differences in the values of this parameter were observed.

In general, for the 'Rowa' cultivar a percentage of the dry mass index was significantly higher for seedlings grown on root extracts than shoot extracts. For the 'Krakowianka', no differences in the values of this index were found. The total water content was the lowest in 'Rowa' seedlings grown from seeds watered with 5% root extracts from the meadow, compared to the control and all tested extracts. For the 'Krakowianka' cultivar, no changes in the percentage of water content were observed (Table 2).

Electrolyte leakage

The percentage of electrolyte leakage was the highest in the control for both radish cultivars. For radish seedlings watered with 1% root extracts from meadows, similar values of this parameter were observed. The lowest degree of cell membrane destabilisation, for seedlings watered with 10% of the root extracts from meadows and 5% of the shoot extracts from calcareous grasslands, was measured. In the remaining extracts, the electrolyte leakage values were significantly lower than in the control group (Figure 6).

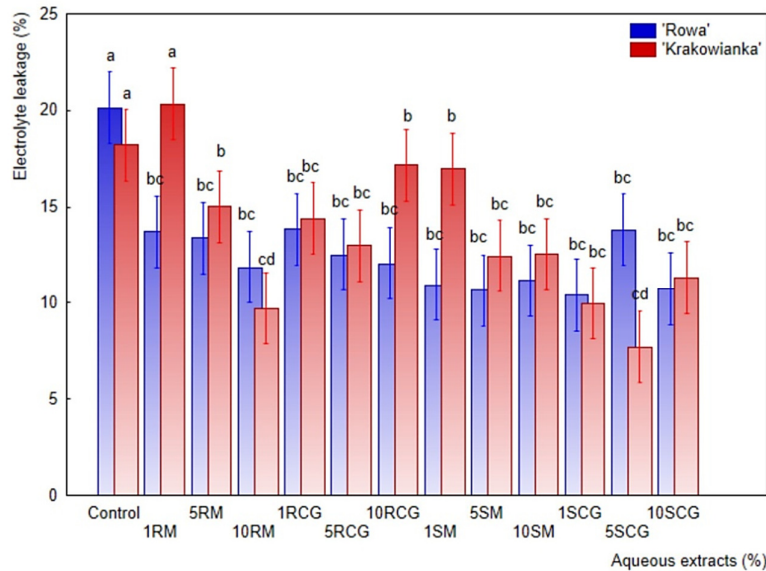


Figure 6. Electrolyte leakage of *Raphanus sativus* L. var. *radicula* Pers. 'Rowa' and 'Krakowianka' seedlings after 7 days of watering with aqueous extracts from roots and shoots *Filipendula vulgaris* Moench with different concentrations (1, 5, 10%) and under control conditions
RM –extract from root plant grown on the meadow, SCG – extract from shoot plant grown on calcareous grasslands; mean values (n = 3, \pm SE) marked different letter differ statistically according to Duncan test at $p < 0.05$

Discussion

A growing interest in the use of allelopathic plants is observed, both in the direct use as plants controlling weeds as well as attempts to isolate specific compounds of high biological activity as natural herbicides (Duke *et al.*, 2002; Singh *et al.*, 2003; Golisz *et al.*, 2004). The medicinal plants are considered an important source of secondary metabolites and recently they are studied in case of herbicidal potential in controlling major crop weeds for sustainable agricultural production. The possibility to have more bioactive compounds in medicinal plants than in other plants stand behind the special interest in this group of species. As a result of the researches, a considerable number of examined medicinal plants exhibited allelopathic properties (Islam *et al.*, 2018). The studies investigated the suppression of extracts from medicinal plants on model plants like lettuce (e.g. Amini *et al.*, 2016), radish (e.g. Haugland and Brandsaeter, 1996), chosen weeds (e.g. Boonmee and Kato-Noguchi, 2019), or other cultivated species (e.g. Puła *et al.*, 2020). This study showed the allelopathic effect of aqueous extracts from *Filipendula vulgaris* roots and shoots on germination and early stages of growth of model radish seeds in two cultivars. The percentage of seed germination was decreased with the increase in the concentration of aqueous extracts. The smallest differences were found between the control and 1% of *Filipendula vulgaris* root and shoot extracts regardless of the habitats. The highest inhibition of germination capacity seeds was observed on the 10% dropwort extracts. On the root extracts, more percentage of germinated seeds were observed than shoots extracts (Figure 2). In general, the performed determinations of germination indexes

showed that extracts with 1% concentration significantly stimulated seed germination. On the other hand, dropwort extracts with a concentration of 10% had an inhibitory effect on the germination capacity, regardless of the radish cultivars (Table 1). Germination depends on many factors biochemical and molecular, from the activation of the metabolism of the embryo embedded in the seed, the quality of the RNA and the integrity of the DNA, plant hormones (Koorneef *et al.*, 2002; Rajjou *et al.*, 2012). In the germination process many enzymes participate, which activity in the presence of allelopathic compounds can be destabilized. Reducing the activity of hydrolytic enzymes means that the seeds are not able to provide enough energy for germinating seeds, which reduces the germination capacity and causes the formation of weakened seedlings (Zandi *et al.*, 2018; Możdżeń *et al.*, 2020). Allelochemical substances increase the vacuolization of cells, which is accompanied by a reduction in the number of mitochondria, the density of ribosomes, and dictyosomes (Burgos *et al.*, 2004). The seed coat can also play an important role, as it protects the seed against harmful external factors and mechanical damage (Rzepka and Możdżeń, 2016; Stachurska-Swakoń *et al.*, 2018; Mazur, 2019). It is easily permeable to oxygen and water. The lower concentration of allelopathic compounds in 1% extracts means that more water is absorbed by the seeds, hence perhaps a positive influence of extracts of low concentrations on the values of selected germination indexes, and no differences between the control samples and dropwort extracts was observed. Root or leaf extracts may also contain diverse phytotoxins with different modes of action (Inderjit *et al.*, 2007). Therefore, it can be assumed that the different reactions of radish seeds result from a different concentration of allelochemical compounds in aqueous extracts from roots and shoots.

Understanding the mechanisms of action of allelochemical compounds on plants is difficult, if only because they belong to different chemical classes and it is difficult to expect them to act in the same way. It is assumed that allelochemical compounds affect comprehensively. Moreover, allelochemicals present in the plant after reaching the rhizosphere can be quickly converted into compounds with greater or lesser activity. Therefore, it is important to assess the concentration and form in which the allelochemical is available for the species. It should also be considered how the habitat, physical, chemical, and biological soil factors, climatic conditions influence the qualitative effects and quantitative concentration of a given compound (Kaur *et al.*, 2005). There is a close correlation between the occurrence of environmental stresses that directly shape plant growth and stimulation of phytotoxic production (Saxena *et al.*, 1996; San Emeterio *et al.*, 2007; Gioria and Osborne, 2014). As a result, it leads to morphogenetic changes and affects the size and quality of the crop (Rice, 1984; Einheling, 1995). The growth of plants is a very sensitive process to the presence of allelochemical compounds. Allelochemical compounds reveal their effect already during seed swelling, affecting the cells of the seed coat and aleurone layer, mitotic cell division, shaping cell organelles, and also elongation. They usually distort the cell walls in the cells of the apex of the root growth and contribute to the accumulation of lipids in the cytoplasm of the cells (Wójcik-Wojtkowiak *et al.*, 1998; Kaur *et al.*, 2005). According to Burgos *et al.* (2004), inhibition of seed cell growth is caused by disturbances in lipid metabolism and the reduction of protein synthesis. In our study, when considering biometry, a stimulating effect of 1% of *F. vulgaris* root and shoot extracts obtained from the meadow and calcareous grassland on hypocotyle length was observed. As the concentrations increased, the elongation of seedling organs was inhibited (especially on 10% extracts) (Figures 3-5).

The aqueous extracts of the dropwort roots and shoots did not significantly influence the fresh and dry mass (Table 2). The allelopathic effect of *F. vulgaris* depended on the concentration of the extracts. Only the 'Rowa' presented a slight increase in the fresh mass of seedlings on 5% of *F. vulgaris* shoot extracts from calcareous grasslands and dry mass on 5% root extract from meadow areas. Similarly, no differences in water content in seedlings were found. These results suggest the presence of different concentrations of soluble allelochemical substances in the aqueous extracts of dropwort. The higher toxicity of meadow extracts than calcareous grassland is probably related to the presence of more amounts of allelopathic compounds in plants. Their toxicity may be due to also a synergistic action, and not only to the action of individual substances. The concentration of a chemical in solution may be significantly less than the concentration of a single allelopathic substance required to produce a plant growth inhibitory effect (Xuan *et al.*, 2004; Inderjit *et al.*, 2007).

Allelopathic compounds have a primary effect on cytoplasmic membranes. Their damage affects the entire metabolism and all physiological processes (Wójcik-Wojtkowiak *et al.*, 1998). In this research, a significant reduction in the degree of destabilization of cell membranes, regardless of the extract used, was found. However, it was lower than in the control, which is an interesting result (Figure 6). This radish seedling response may be due to the lower content of phenolic compounds in leaves than in flowers (Bączek *et al.*, 2012). Recent studies on the extracts of *F. vulgaris* revealed that they contain several classes of phenolic compounds (Katanić *et al.*, 2018). Flavonoid glycosides have been described as a major constituent of the upper parts of dropwort, and catechin and procyanids were identified as a major constituent of roots (Oszmiański *et al.*, 2007). Such a reaction of seedlings also depends on the harvest period of the plants from which the extracts were prepared. Bączek *et al.* (2012) have found seasonal variation in the content of phenolic compounds in the upper parts of the species, they were higher at the beginning of flowering as compared with the full flowering stage. Additionally, the content of catechins and polyphenolic acids found in the roots did not change during the season. In the present study, *F. vulgaris* plants were harvested in the late generative stage, which probably reduced the electrolyte leakage. On the other hand, we cannot exclude the probable presence of calcium in *F. vulgaris* extracts, the role of which in membrane stabilization is known (Hepler, 2005).

It is commonly believed that plants have a negative allelopathic effect on other plants, but some of them also play a positive role. Some plants contribute to the protection of soils against erosion, improve their fertility, and have a positive effect on the condition of the soil microflora. Allelochemical compounds secreted by plants that stimulate the growth of other plants are also known. However, they constitute a small group compared to allelochemicals compounds inhibitory (Haupt *et al.*, 2001; Goufo *et al.*, 2014; Kopczewski, 2014). Our study pointed at the inhibiting effect of all extracts on germination capacity. At low concentrations of aqueous extracts from the roots and shoots of dropwort the germination indexes had higher values than control. The biometric and biomass parameters of seedlings were generally stimulated by these extracts, regardless of the plant harvesting habitats, organs, and concentrations, as compared to the control seedlings. Therefore, it is necessary to conduct further research on the allelopathic interactions of plants to understand their effects not only on seed germination but also their physiology and plants grown from them. Additionally, it seems necessary to study the chemical composition to identify chemical compounds responsible for this type of reaction and to strive for their practical application in various fields. Multidisciplinary studies involving plant ecology, genetics, physiology, biochemistry, soil science, and microbiology can answer the key questions of if and how allelopathy influences plant interactions.

Conclusions

Plants, in order to survive in the natural environment, developed many defence mechanisms, including the ability to synthesize and secrete compounds with allelopathic potential. In the literature, there are various groups of allelochemical compounds as well as the sources of their release into the environment. The release of substances with the allelopathic potential to the environment may inhibit or stimulate seed germination and plant growth. Their impact on other organisms depends on their chemical nature, the species they affect, and the prevailing environmental conditions.

This study revealed the inhibiting effect of aqueous extracts from *Filipendula vulgaris* organs on the germination of two cultivars of radish seeds. However, the stimulating effect of 1% of aqueous extracts from dropwort roots and shoots on the germination indexes was revealed. As the concentration of allelopathic compounds in the extracts increased, a decrease in the values of germination indexes was observed, compared to the control. Similarly, the biometry of radish seedlings revealed a positive effect of extracts with low concentrations, as opposed to extracts with a higher content of allelopathic substances (especially, 10% extracts). Observations of changes in the length of the root organs, hypocotyls, and whole seedlings were similar, regardless of the type and concentration of the extract, concerning seedlings grown on distilled water.

For the 'Rowa' cultivar, an increase in the fresh mass of seedlings with an increase in the concentration of extracts was demonstrated, in contrast to the 'Krakowianka'. The dry mass values generally no significant differences were changed. The exception was 'Rowa' cultivar grown from seeds watered with 5% root extract from meadow areas, where the dry mass was lower. The percentage of dry mass depended on the concentration and type of extract used for watering the radish seeds. A positive effect of 5% and 10% extracts was observed, regardless of the organ used in preparing the extract. A positive effect of all dropwort extracts on the electrolyte leakage was demonstrated.

Authors' Contributions

ASS planned and designed the research; MF, KM performed the experiment; ASS, KM, BBK, MF analysed the data and prepared figures and tables, ASS, KM, BBK wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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