

Effect of *Rosa gorenkensis* Besser aqueous extracts on germination and early growth of native plant species

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Abstract

In Europe, *Rosa gorenkensis* Besser is considered an invasive species. However, its negative impact on native flora components or other habitat components has not been described so far. In the experiment, the germination reactions of mono- and dicotyledonous plant seeds to the aqueous extracts of *R. gorenkensis* were investigated to determine the allelopathic potential of this plant. Seeds of common plants – wild-growing *Festuca rubra* L. and cultivated *Raphanus sativus* L. var. *radicula* Pers. cv. 'Rowa' were treated with aqueous extracts from the roots, stalks, leaves, and flowers of rosa at concentrations of 1%, 2.5%, and 5%. Along with the increase in the concentration of allelochemical compounds in the extracts, the negative influence of the extracts on the germination capacity of the tested seeds species was found. Regardless of the type of extract, inhibition of the growth of the underground and aboveground parts of seedlings was also observed. Changes in biomass and water content, depending on the concentration and type of the extract, were found. The greatest differences in the electrolytes leakage in seedlings watered with 5% extracts were revealed. The study showed that the aqueous extracts of leaves and flowers of this species had the greatest allelopathic potential.

Keywords: alien species; allelopathy; biomass; electrolyte leakage; invasion; seedlings

Introduction

Initially, the effects of alien species on native flora are not noticeable. In many cases, the so-called lag phase was observed, i.e., the period between the appearance of a new species and the disclosure of its invasive features (Hobbs and Humphries, 1995; Richardson and Pyšek, 2006). Therefore, it is important to undertake research to determine how a given species may interact with native components of the flora. The secretion of allelopathic compounds is often an important mechanism of expansion. These substances can directly affect

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the species diversity of native flora and vegetation by spreading over greater distances with seeping water or melting snow (Nilsson and Wardle, 2005).

Among the alien species in Western and Central Europe, there is, e.g., *Rosa gorenkensis* Besser (syn. *R. glabrifolia* auct. non C.A. Mey, *R. gorinkensis* Besser, *R. turbinata* Schmalh.), from the Cinnamomeae DC. ex Ser. section (Figure 1).



Figure 1. *Rosa gorenkensis* Besser – morphological features: A – part of fruiting short shoot, B – part of long shoot, C – stipule, D – petiole ± glandular, E – sepal, F – fruit with glandular sepals, G – fruit, H – leaflet, I – margin of leaflet
Solid bar = 1 cm, double bar – 0.5 cm; (Photo. 2021, A. Soltys-Lelek, specimen from Poland, Kłęka village: 52°04'31"N 17°25'10"E, leg. 2020, W. Gruszka)

The natural range of this taxon is limited to the southern regions of the European part of Russia, the northern part of Kazakhstan, Western Siberia, and Ukraine (Tzvelev, 2001; Popek, 2007; Shaulo *et al.*, 2010). This rose has long been cultivated or grows wild in Lithuania, Latvia, Estonia, and Poland (Zieliński, 1987; Popek, 2007; Kurtto, 2009; *Rosa gorenkensis* Besser in GBIF Secretariat, 2021). Its introduction to Poland is estimated in the 19th century. Now, it is considered in this country as a domesticated kenophyte (Tokarska-Guzik *et al.*, 2012). Like many similar species, it was brought from the east for decorative purposes. Spontaneous localities of this species occur mainly in anthropogenic, partially transformed habitats, e.g., in roadside ditches (Marciniuk *et al.*, 2011), in old cemeteries (Czarna, 2016), etc. In Poland, it has a few, scattered localities, most of which are grouped in the eastern part of the country, which confirms its Eastern European character (Kucharczyk, 2001; Adamowski *et al.*, 2002; Nobis, 2007; Czarna, 2009; Marciniuk *et al.*, 2011; Soltys-Lelek, 2012; Piwowarski, 2013).

R. gorenkensis was included in the “List of Species Alien in Europe and to Europe” from category E – European species which became alien outside its native range (Pyšek *et al.*, 2009). However, its negative impact on native flora species or other elements of the habitat has not been described so far. But this kind an impact cannot be ruled out. This species is resistant to frosts and low temperatures (even down to $-45\text{ }^{\circ}\text{C}$). It is characterized by a high reproductive potential, which is manifested by abundant flowering and fruiting, and also quickly takes over the habitat, producing stolons. It grows in the form of dense thickets, difficult to mow. Due to habitat preferences, species from meadows and thermophilic grasslands are the most endangered by its invasion (Marciniuk, 2011; Gruszka and Sołtys-Lelek, own observations).

The ecological effects of biological invasions appear at all levels of the organization of life, ranging from changes in population genetics to changes in the functioning of ecosystems. At the individual level, invasive species can reduce the growth rates and sizes of native species (Parker *et al.*, 1999; Hulme, 2007). For example, it has been observed that *Solidago canadensis* L. – an extremely invasive species in Europe – is a source of allelopathic substances released during the decomposition of its remains, which further promotes its expansion (Możdżeń *et al.*, 2020). In the case of *R. gorenkensis*, several disturbing symptoms have already been found, indicating its high invasive potential. For example, in Poland, vegetative growth of this species was observed under cultivation conditions: in 2009, one poor-rooted shoot, 20 cm long, was planted in the garden. In September 2011, the monitored specimen grew to 18 shoots, covering an area of 2 m^2 (Marciniuk *et al.*, 2011). This simple experiment illustrates the enormous possibilities of the vegetative spread of this taxon under favourable climatic and edaphic conditions. That is why it is so important to undertake research showing various aspects of the influence (including its allelopathy) of this species on native components of the flora.

Seeds of two species were selected for the experiment on the allelopathic potential of *R. gorenkensis*: red fescue *Festuca rubra* L., representing monocots, occurring on thermophilic grasslands, and red radish *Raphanus sativus* L. var. *radicula* Pers. cv. ‘Rowa’, a dicotyledonous taxon, widely cultivated all over the world.

The aim of the study was to check the effect of water extracts of various concentrations prepared from the organs of *R. gorenkensis* on the seed’s germination and early growth of mono- and dicotyledonous plants. The following parameters of the analysed plants were assumed as determinants of the impact of this alien species: seed germination capacity – through various germination indexes (1), morphometry (2) and seedling biomass (3), and the degree of destabilization of cell membranes in seedlings (4).

Materials and Methods

Plant material

Rosa gorenkensis specimens were acquired in June 2020 from the site by A. Czarna (2009). The rose appears here in the form of a compact cluster at the southern border of allotments in Klęka $52^{\circ}4'36.99''\text{N}$; $17^{\circ}25'18.87''\text{E}$ (central Poland). It is a site that has arisen spontaneously from specimens that have spread out of the cultivation.

Morphologically similar plants, not infected with viruses and fungi and undamaged, were selected for the experiments. The plant material was divided into underground parts (roots) and above-ground parts (stalks, leaves, and flowers), and then dried in the dark at a room temperature of $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, with an average air humidity of 60-70%. The flowers and leaves were dried for 7 days, and the roots and stalks for 10 to 12 days, which was related to their anatomical structure. The dried plant organs were stored in paper bags in the darkness, at room temperature for the duration of the experiment. Red radish (*Raphanus sativus* var. *radicula* cv. ‘Rowa’) seeds were purchased at the seed shop of Polan sp. z o.o. Breeding and Seed Horticulture in Krakow (Poland), while the seeds of red fescue (*Festuca rubra* L.) were obtained from DLF Seeds (Hladké Životice, Czech Republic).

Extract preparation

The dried organs of *R. gorenkensis* (each separately) were ground in an electric grinder and a mortar. Then, aqueous extracts were prepared from them with the following percentages: 1%, 2.5%, and 5%. For example, 1% flower extract was prepared by weighing out 1 g of dried rose petals and pouring 99 ml of distilled water over them; suitably – extract 2.5% – 2.5 g dry mass + 97.5 ml distilled water; 5% extract – 5 g dry mass + 95 ml distilled water. In the same way, extracts from the roots, stalks, and leaves of the rose were prepared. To extract the chemicals contained in the extracts, the beakers with the liquids were covered with cellophane and left in the dark at room temperature ($23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) for 24 h. After this time, the aqueous extracts were filtered through gauze and stored in a refrigerator at $8\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

pH of extracts

For each of the extracts, pH determinations were made from individual parts of *R. gorenkensis* using an electrode (Elmetron, Zabrze, Poland). Average pH values were determined from five replicates.

Seeds germination conditions

The seeds of *F. rubra* and *R. sativus* var. *radicula* cv. Rowa (each separately) were sterilized in 1% acetone solution for 1 min and rinsed 3 times with distilled water. 25 seeds were placed in sterile glass Petri dishes, 9 cm in diameter, with three layers of filter paper, moistened with an appropriate rose extract (5 ml every other day). The control group consisted of seeds watered only with distilled water. The Petri dishes with seeds were placed in the dark, at a room temperature of $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, with a relative humidity of about 60-70%. The number of germinating seeds was analyzed every 24 hours for 7 days. The experiment was performed in 3 replications for each concentration and type of rose extract and control group.

Germination parameters

The germination capacity of red fescue and radish seeds was performed by the germination parameters. Germination percentage – G (%) (global method), coefficient of velocity of germination – CVG (%) (Jones and Sanders, 1987), germination index – GI (unit less) (AOSA, 1983), uncertainty of germination process – U and synchrony of germination process – Z (Ranal and Santana, 2006), time to 50% germination – T_{50} (Coolbear *et al.*, 1984), \bar{t} – mean germination time (Orchard, 1977).

$$G\%age = \frac{\sum_{i=1}^k n_i}{N} \times 100 \quad (1)$$

where: n_i – number of seeds newly germinating on a day i ; N – total number of seeds tested and k – last day of germination

$$CVG (\%) = \frac{\sum_{i=1}^k n_i}{\sum_{i=1}^k n_i t_i} \times 100 \quad (2)$$

where: CVG – coefficient of velocity of germination, where k – last day of germination, n_i – number of seeds newly germinating on a day i , t_i – number of days from sowing

$$GI (\text{unit less}) = \sum_{i=1}^k \frac{n_i}{t_i} \quad (3)$$

where: GI – Germination Index, where k – last day of germination, n_i – number of seeds newly germinating on day i , t_i – number of days from sowing

$$U (\text{bit}) = - \sum_{i=1}^k f_i \log_2 f_i \quad (4)$$

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 a day i , t_i – number of days from sowing

$$Z \text{ (unit less)} = \frac{\sum_{i=1}^k C_{n_i,2}}{C_{\sum_{i=1}^k n_i,2}} \quad (5)$$

where: Z – Synchrony of germination process, $C_{n_i,2}$ is the partial combination of the two germinated seeds from among n_i , the number of seeds newly germinating on day 'i' (estimated as $C_{n_i,2} = \frac{n_i(n_i-1)}{2}$), and $C_{\sum_{i=1}^k n_i,2}$ is the partial combination of the two germinated seeds from among the total number of seeds germinated at the final count, assuming that all seeds that germinated did so simultaneously

$$\text{MGT (day)} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i} \quad (6)$$

where: MGT – mean germination time, k – last day of germination, n_i – number of seeds newly germinating on a day i , t_i – number of days from sowing

$$T_{50}(\text{day}) = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)} \quad (7)$$

where: T_{50} – time to 50% germination, N – final number of germinations, n_i, n_j – cumulative numbers of seeds germinated by adjacent counts at times t_i and t_j when $n_i < \frac{N}{2} < n_j$

Seedling's biometry

The seedling biometry of *F. rubra* and *R. sativus* var. *radicula* cv. 'Rowa' grown on various extracts from *R. gorenkensis* organs was based on the measurement of the length of the underground and aboveground parts using a caliper (Topex 31C615, Poland), with an accuracy of 1 mm. The percentage of seedling growth inhibition (IP) was determined according to the formula of Mominul Islam and Kato-Noguchi (2012):

$$\text{IP (\%)} = (1 - (\text{LE} / \text{LC})) \times 100 \quad (8)$$

where: LE – seedling length (cm) treated with the aqueous extract, LC – seedling length (cm) treated with the distilled water (control group)

Seedling's biomass

After 7 days of germination, the fresh mass of individual seedlings of the analysed species was determined on a laboratory balance with an accuracy of 0.0001 g (Ohaus Adventurer Pro, NY, USA). Dry mass of seedlings was determined after 48 h of drying, at the temperature of 105 °C obtained in a dryer (WAMED SUP 100, Zabrze, Poland). Based on the obtained results, the total water content was calculated according to the following formula:

$$\text{WC (\%)} = 100 - [(DM \times 100) / FM] \quad (9)$$

Where: WC – water content, DM – dry mass, FM – fresh mass

Electrolyte leakage

Single seedlings *F. rubra* or *R. sativus* var. *radicula* cv. 'Rowa' was placed in 30 ml of distilled water in polypropylene vials and shaken for 3 hours on a shaker (Labnet, Rocker, USA). After this time, each of the samples was additionally vortexed for 10 seconds. The leakage of electrolytes from living cells (E1) was measured using a CX-701 conductometer with an electrode ($K = 1.02$) (Elmetron, Zabrze, Poland). Then, the seedlings in vials with water were frozen for 24 h, at -25 °C temperature to macerate the cells. After this time, the samples were thawed and subjected to the same shaking and measurement procedure as the live seedling samples to determine total electrolyte leakage (E2). From the obtained results, the percentage of electrolyte leakage was calculated according to the formula (Pandey *et al.*, 2008):

$$\text{EL (\%)} = (E1 / E2) \times 100 \quad (10)$$

where: EL – electrolyte leakage, E1 – EL from live seedlings, E2 – EL from dead seedlings

Statistical analysis

The experiment was carried out in three replicates. One repetition consisted of 25 *F. rubra* seeds and 25 *R. sativus* var. *radicula* cv. 'Rowa' seeds, separately for each type and concentration of aqueous extracts from *R. gorenkensis* organs and a control sample (distilled water). The obtained mean results ($n = 10, \pm SD$) were subjected to one-way ANOVA statistical analysis. Differences between extracts for each type of extract and seed species are marked with different letters in the tables, both in columns and in rows, using Tukey's test at $p \leq 0.05$ in the program StatSoft Inc. (2018).

Results*pH values of water organ extract of Rosa gorenkensis*

Statistical analysis of the pH value of aqueous extracts from *R. gorenkensis* organs showed an increase in the value of this parameter in practically all types of extracts, along with an increase in their concentration. The exception was flower extracts, in which a significant decrease in the pH value was observed along with an increase in the concentration of allelochemical compounds (Table 1).

Table 1. pH values of water extracts of *Rosa gorenkensis* Besser organs

Part of rosa Extract (%)	Root	Stalk	Leaf	Flower
1	5.06 b	3.95 b	4.73 b	4.98 a
2.5	5.22 b	3.86 c	4.71 b	3.92 c
5	5.91 a	4.59 a	5.05 a	4.46 b

Values marked with different letters differ significantly according to Tukey's test for different N, $p \leq 0.05$

Germination parameters

As the concentration of extracts increased, the germination index (GI) of seeds of *Festuca rubra* reached significantly lower values (Table 2).

Regarding the control, all aqueous extracts from *Rosa gorenkensis* organs inhibited the germination of red fescue seeds. Only between the control and the seeds watered with 1% extracts no differences were found. Additionally, a clear inhibition of germination capacity was found for seeds watered with 2.5% flower extracts (Figure 2). For the coefficient of velocity of germination (CVG), there was a significant effect of almost all types of aqueous rose extracts, relative to the control. Root and leaf extracts, at each concentration, inhibited seed germination. Only stalk extracts did not affect the values of this index. In the case of *R. gorenkensis* flower extracts, significant differences in CVG values were demonstrated for *Festuca rubra* seeds watered with 2.5% and 5% extracts (Table 2).

As the concentrations of rose organ extracts increased, the Germination index (GI) was significantly lower than the control. In the case of root and leaf extracts, all extracts inhibited the GI values. For stalk and flower extracts, no significant difference was found between the control and the 1% extracts. The values of the U – synchrony of germination process index was similar between the control and *R. gorenkensis* organ extracts. Significant differences were shown only for seeds watered with 5% extracts from all organs. The Z index (uncertainty of germination process) was the highest, compared to the control, for seeds treated with 5% root extracts, and the lowest for those treated with 5% stalk extracts. In other cases, no differences in the values of this parameter were observed (Table 2).

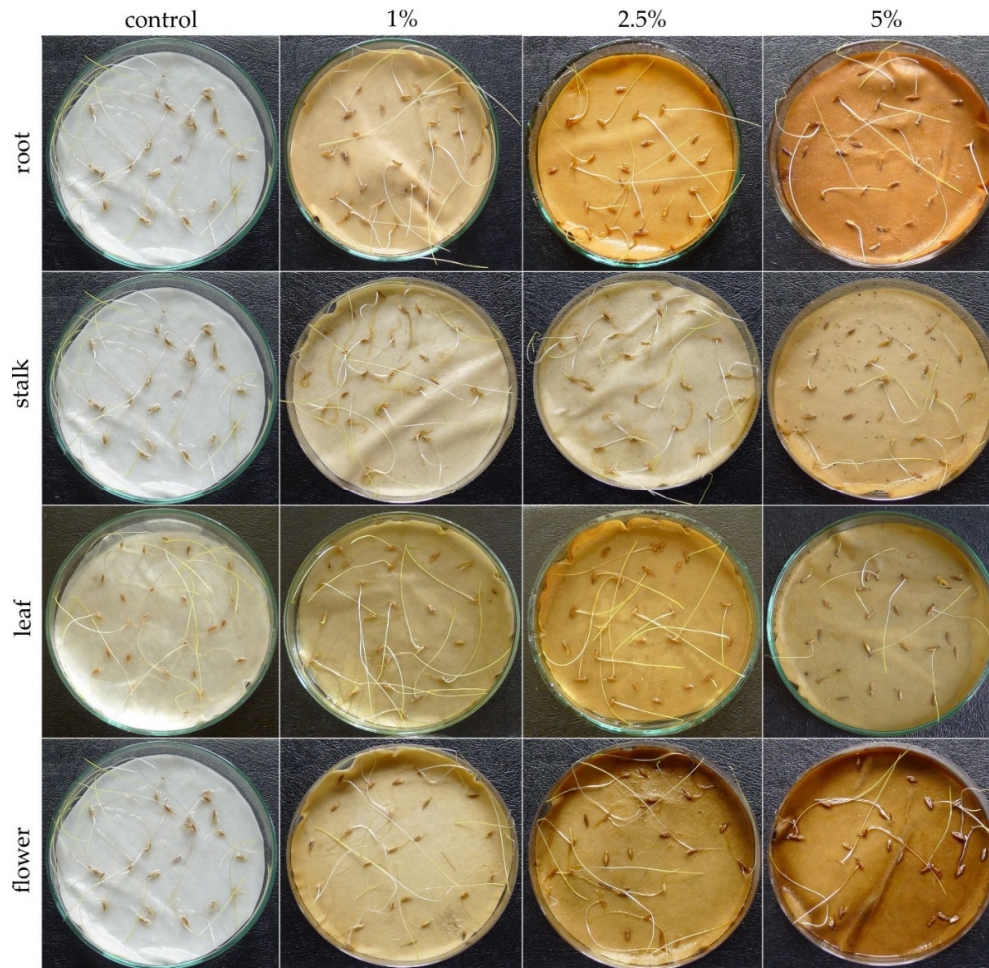


Figure 2. Comparison of the effect of aqueous extracts from organs of *Rosa gorenkensis* Besser, at different concentrations, on the germination of *Festuca rubra* L. seeds
The control was carried out on distilled water

For *Raphanus sativus* var. *radicula* cv. Rowa the GI values were similar between the control and the root and leaf extracts (Table 2). For red radish watered with 5% stalk extracts and 2.5% and 5% flower extracts of *R. gorenkensis* a significant reduction in the percentage of germinated seeds was demonstrated (Figure 3).

In the case of CVG, the values of this index did not differ between the control and the types of extracts used, in each concentration (Table 2). Similarly, the GI for red radish seeds did not differ between the control and rose root and leaf extracts. Stalk extracts at a concentration of 1% and 2.5% increased the GI value as compared to the control. On the other hand, extracts from 2.5% and 5% of flowers reduced the germination value. The germination indexes U and Z did not change significantly between the control and the used extracts from all *R. gorenkensis* organs.

Table 2. Selected seed germination indexes of *Festuca rubra* L. – FR and *Raphanus sativus* L. var. *radicula* Pers. cv. Rowa – RS, watered with *Rosa gorenkensis* Besser organ extracts of various concentrations (1%, 2.5%, 5%); mean values (\pm SD) marked with different letters in the column differ significantly according to Tukey's test for different N, $p \leq 0.05$.

Extracts type (%)	G (%)		CVG (%)		GI (unit less)		U (bit)		Z (unit less)	
	Root									
	FR	RS	FR	RS	FR	RS	FR	RS	FR	RS
Control	80 \pm 4.38 a	91 \pm 6.86 a	37.82 \pm 2.50 a	48.27 \pm 6.92 a	9.91 \pm 0.71 a	12.55 \pm 2.30 a	1.99 \pm 0.15 a	1.27 \pm 0.55 a	0.27 \pm 0.07 b	0.52 \pm 0.20 a
1R	71 \pm 9.24 a	96 \pm 4.00 a	27.12 \pm 1.68 b	47.08 \pm 0.88 a	5.81 \pm 1.30 b	12.03 \pm 0.63 a	1.97 \pm 0.13 a	0.98 \pm 0.19 a	0.23 \pm 0.03 b	0.65 \pm 0.09 a
2.5R	40 \pm 4.00 b	92 \pm 4.00 a	25.94 \pm 3.11 b	48.60 \pm 2.21 a	3.39 \pm 0.82 c	11.64 \pm 0.80 a	1.81 \pm 0.17 a	0.67 \pm 0.37 a	0.24 \pm 0.08 b	0.76 \pm 0.14 a
5R	24 \pm 6.93 c	96 \pm 4.00 a	25.60 \pm 1.56 b	48.02 \pm 0.91 a	1.59 \pm 0.50 c	12.00 \pm 0.44 a	0.83 \pm 0.13 b	0.78 \pm 0.11 a	0.52 \pm 0.11 a	0.70 \pm 0.06 a
Stalk										
Control	80 \pm 4.38 a	91 \pm 6.86 a	37.82 \pm 2.50 a	48.27 \pm 6.92 ab	9.91 \pm 0.71 a	12.55 \pm 2.30 bc	1.99 \pm 0.15 a	1.27 \pm 0.55 a	0.27 \pm 0.07 a	0.52 \pm 0.20 a
1S	76 \pm 4.00 a	97 \pm 4.62 a	41.84 \pm 2.93 a	62.06 \pm 5.75 a	9.94 \pm 0.10 a	17.92 \pm 2.45 a	1.77 \pm 0.29 ab	1.34 \pm 0.13 a	0.28 \pm 0.08 a	0.043 \pm 0.02 a
2.5S	64 \pm 4.00 b	95 \pm 2.31 a	40.34 \pm 1.23 a	52.03 \pm 3.56 ab	8.19 \pm 0.77 b	15.64 \pm 0.53 ab	1.88 \pm 0.10 ab	1.60 \pm 0.22 a	0.24 \pm 0.03 a	0.36 \pm 0.05 a
5S	9.33 \pm 4.62 c	76 \pm 4.00 b	55.56 \pm 3.49 a	47.17 \pm 1.29 b	1.06 \pm 0.05 c	9.87 \pm 0.91 c	1.41 \pm 0.30 b	0.91 \pm 0.13 a	0.00 \pm 0.00 b	0.66 \pm 0.07 a
Leaf										
Control	80 \pm 4.38 a	91 \pm 6.86 a	37.82 \pm 2.50 a	48.27 \pm 6.92 a	9.91 \pm 0.71 a	12.55 \pm 2.30 a	1.99 \pm 0.15 ab	1.27 \pm 0.55 a	0.27 \pm 0.07 a	0.52 \pm 0.20 a
1L	80 \pm 10.58 a	96 \pm 4.00 a	29.58 \pm 1.87 b	51.86 \pm 2.38 a	7.51 \pm 0.79 b	13.64 \pm 0.76 a	2.33 \pm 0.10 a	1.01 \pm 0.26 a	0.20 \pm 0.02 a	0.59 \pm 0.12 a
2.5L	60 \pm 10.58 b	92 \pm 4.00 a	24.20 \pm 1.80 c	49.58 \pm 3.79 a	4.23 \pm 1.07 c	12.96 \pm 0.26 a	2.02 \pm 0.15 ab	1.34 \pm 0.16 a	0.23 \pm 0.01 a	0.46 \pm 0.04 a
5L	21 \pm 2.31 c	83 \pm 4.62 a	20.79 \pm 0.08 c	49.36 \pm 7.82 a	1.22 \pm 0.20 d	11.36 \pm 2.30 a	1.58 \pm 0.30 b	1.20 \pm 0.38 a	0.22 \pm 0.11 a	0.49 \pm 0.10 a
Flower										
Control	80 \pm 4.38 a	91 \pm 6.86 a	37.82 \pm 2.50 ab	48.27 \pm 6.92 a	9.91 \pm 0.71 a	12.55 \pm 2.30 a	1.99 \pm 0.15 a	1.27 \pm 0.55 a	0.27 \pm 0.07 a	0.52 \pm 0.20 a
1F	59 \pm 15.14 b	100 \pm 0.00 a	45.63 \pm 5.54 a	48.24 \pm 3.44 a	8.28 \pm 2.53 ab	13.51 \pm 0.65 a	1.60 \pm 0.31 ab	1.09 \pm 0.26 a	0.33 \pm 0.09 a	0.59 \pm 0.07 a
2.5F	52 \pm 10.58 b	60 \pm 13.86 b	33.97 \pm 5.36 b	40.26 \pm 7.07 a	5.31 \pm 1.15 b	6.87 \pm 2.64 b	1.62 \pm 0.40 ab	1.39 \pm 0.20 a	0.32 \pm 0.01 a	0.42 \pm 0.12 a
5F	20 \pm 4.00 c	15 \pm 2.31 c	36.05 \pm 2.58 b	52.38 \pm 4.12 a	1.94 \pm 0.34 c	2.11 \pm 0.54 b	1.16 \pm 0.30 b	1.16 \pm 0.49 a	0.32 \pm 0.13 a	0.56 \pm 0.42 a

Control – distilled water, 1R – 1% aqueous extracts from roots, 2.5R – 2.5% aqueous extracts from roots, 5R – 5% aqueous extracts from roots, 1S – 1% aqueous extracts from stalks, 2.5S – 2.5% aqueous extracts from stalks, 5S – 5% aqueous extracts from stalks, 1L – 1% aqueous extracts from leaves, 2.5L – 2.5% aqueous extracts from leaves, 5L – 5% aqueous extracts from leaves, 1F – 1% aqueous extracts from flowers, 2.5F – 2.5% aqueous extracts from flowers, 5F – 5% aqueous extracts from flowers; G – germination percentage index, CVG – coefficient of velocity of germination, GI – germination index, U – synchrony of germination process, Z – uncertainty of germination process.



Figure 3. Comparison of the effect of aqueous extracts from the organs of *Rosa gorenkensis* Besser, at different concentrations, on the germination of seeds of *Raphanus sativus* L. var. *radicula* Pers. cv. 'Rowa' The control was carried out on distilled water.

Mean germination time (MGT) and time to 50% germination (T50) increased significantly for *F. rubra* seeds watered with 2.5% and 5% root and leaf extracts, compared to the control. In the remaining cases, between the seeds watered with distilled water and rose organ extracts of various concentrations no differences were observed (Figure 4A–B). For red radish, MGT values did not differ significantly between the control and the extracts used (Figure 4C). In the case of T50, a significant reduction in germination time was revealed only for red radish seeds watered with 2.5% of *R. gorenkensis* stalk extracts, and elongation in those treated with 2.5% flower extracts, relative to control (Figure 4D).

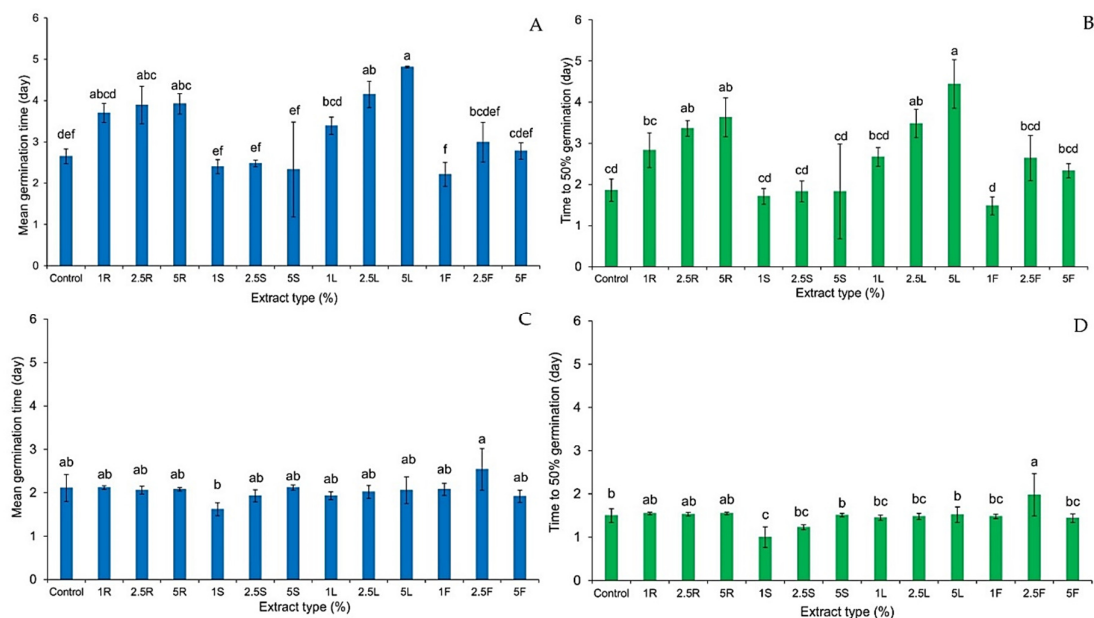


Figure 4. Mean germination time (MGT) and time to 50% germination (T50) of *Festuca rubra* L. seeds (FR) – A and C, and *Raphanus sativus* L. var. *radicola* Pers. cv. 'Rowa' (RS) – B and D, watered with *Rosa gorenkensis* Besser organ extracts of various concentrations (1%, 2.5%, 5%)

mean values (\pm SD) marked with different letters differ significantly according to Tukey's test for different N, $p \leq 0.05$; Control – distilled water, 1R – 1% aqueous extracts from roots, 2.5R – 2.5% aqueous extracts from roots, 5R – 5% aqueous extracts from roots, 1S – 1% aqueous extracts from stalks, 2.5S – 2.5% aqueous extracts from stalks, 5S – 5% aqueous extracts from stalks, 1L – 1% aqueous extracts from leaves, 2.5L – 2.5% aqueous extracts from leaves, 5L – 5% aqueous extracts from leaves, 1F – 1% aqueous extracts from flowers, 2.5F – 2.5% aqueous extracts from flowers, 5L – 5% aqueous extracts from flowers.

Seedling biometry

Biometric analysis of the roots of *F. rubra* seedlings showed a negative effect of *R. gorenkensis* aqueous extracts, irrespective of the organ and concentration that germinated seeds were watered with (Figure 5, Table 2). The shortest roots, compared to the control values, were recorded in seedlings watered with 5% of extracts. In the case of the aboveground part of the seedlings, significant differences were observed only between the control and the seedlings grown on the media with 5% rose extracts. At the remaining concentrations, the elongation growth of these organs was inhibited, but the differences were not statistically significant. Compared to the control, the length of whole seedlings was clearly inhibited with an increase in rose extract concentrations. Significant differences were already shown at concentrations of 2.5% and 5%. The stem extracts were an exception – they limited the elongation growth of whole seedlings only at a concentration of 5%.

The root length of red radish seedlings was significantly inhibited by 5% of extracts prepared from roots and stalks of rose (Figure 5, Table 3). In the case of leaf and flower extracts, at 2.5% concentration of allelochemical compounds, the inhibition of root growth was found. On the other hand, extracts from the roots of *R. gorenkensis* did not affect the growth of the aboveground part of *R. sativus* var. *radicola* cv. 'Rowa'. Significant inhibition of the growth of these organs was found with 5% stalk extracts, relative to the control data. Only flower extracts with concentrations of 2.5% and 5% caused a clear inhibition of the growth of red radish aboveground organs. Whole seedlings biometry showed the negative influence of all 5% of extracts, regardless of the rose organ from which they were prepared. In addition, it was found that the remaining two leaf extracts at concentrations of 1% and 2.5% and 2.5% flower extract inhibited the elongation growth of whole red radish seedlings.

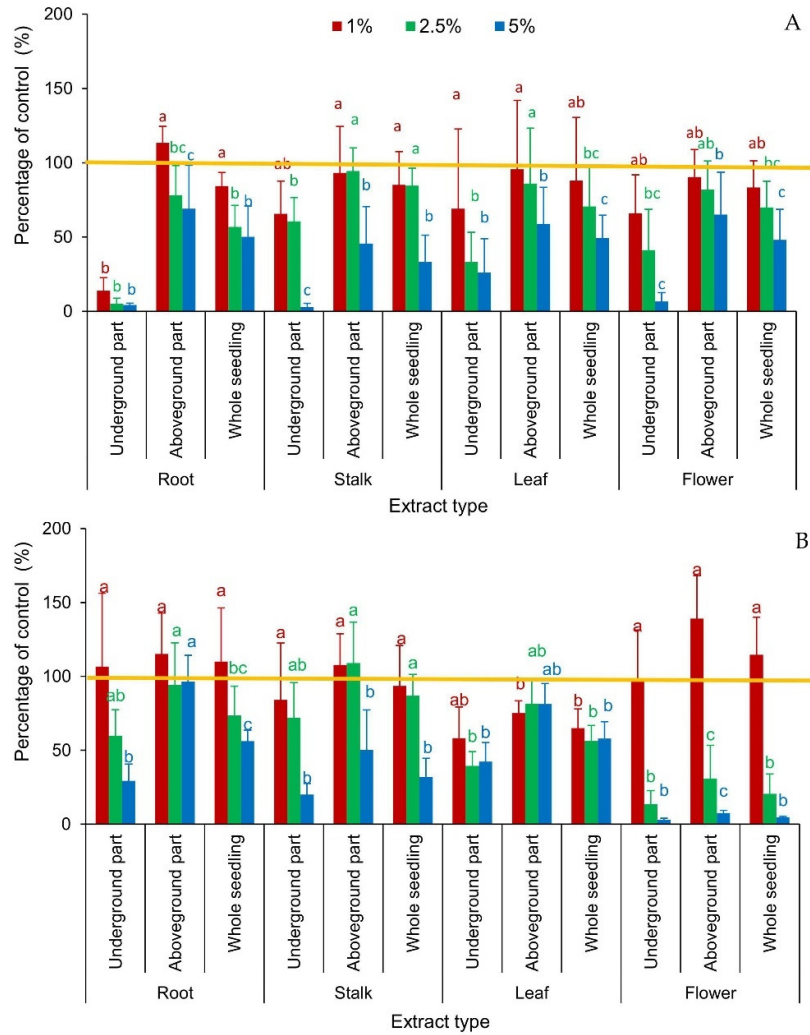


Figure 5. Seedling biometry of *Festuca rubra* L. (A) and *Raphanus sativus* L. var. *radicle* Pers. cv. 'Rowa' (B) watered with *Rosa gorenkensis* Besser organ extracts of various concentrations (1%, 2.5%, 5%) Mean values (\pm SD) marked with different letters differ significantly according to Tukey's test for different N, $p \leq 0.05$; orange line means the control (100%).

Table 3. Biometry of seedling organs of *Festuca rubra* L. (FR) and *Raphanus sativus* L. var. *radicula* Pers. cv. 'Rowa' (RS) watered with *Rosa gorenkensis* Besser organ extracts of various concentrations (1%, 2.5%, 5%)

Extracts type (%)	Underground part (cm)		Aboveground part (cm)		Whole seedling (cm)	
	Root					
	FR	RS	FR	RS	FR	RS
Control	2.53 ± 1.21 a	6.63 ± 4.22 a	6.13 ± 1.55 ab	4.43 ± 1.13 a	8.66 ± 2.36 a	11.06 ± 4.03 ab
1R	0.35 ± 0.22 b	7.06 ± 3.29 a	6.95 ± 0.69 a	5.10 ± 1.22 a	7.30 ± 0.79 a	12.16 ± 4.01 a
2.5R	0.13 ± 0.09 b	3.96 ± 1.17 ab	4.79 ± 1.22 bc	4.17 ± 1.26 a	4.92 ± 1.26 b	8.13 ± 2.18 bc
5R	0.11 ± 0.03 b	1.94 ± 0.75 b	4.24 ± 0.03 c	4.27 ± 0.79 a	4.35 ± 1.79 b	6.21 ± 0.82 c
	Stalk					
Control	2.53 ± 1.21 a	6.63 ± 4.22 a	6.13 ± 1.55 a	4.43 ± 1.13 a	8.66 ± 2.36 a	11.06 ± 4.03 a
1S	1.66 ± 0.55 ab	5.57 ± 2.55 a	5.71 ± 1.91 a	4.77 ± 0.94 a	7.37 ± 1.94 a	10.34 ± 3.02 a
2.5S	1.53 ± 0.41 b	4.77 ± 1.57 ab	5.79 ± 0.96 a	4.83 ± 1.22 a	7.32 ± 1.02 a	9.60 ± 1.60 a
5S	0.07 ± 0.07 c	1.32 ± 0.50 b	2.80 ± 1.53 b	2.22 ± 1.20 b	2.87 ± 1.57 b	3.54 ± 1.39 b
	Leaf					
Control	2.53 ± 1.21 a	6.63 ± 4.22 a	6.13 ± 1.55 a	4.43 ± 1.13 a	8.66 ± 2.36 a	11.06 ± 4.03 a
1L	1.74 ± 0.89 ab	3.85 ± 1.40 ab	5.87 ± 0.92 a	3.33 ± 0.37 b	7.61 ± 1.49 ab	7.18 ± 1.44 b
2.5L	0.84 ± 0.32 b	2.61 ± 0.64 b	5.27 ± 1.31 a	3.61 ± 0.74 ab	6.11 ± 1.35 bc	6.22 ± 1.17 b
5L	0.66 ± 0.37 b	2.80 ± 0.86 b	3.61 ± 0.86 b	3.60 ± 0.62 ab	4.27 ± 0.78 c	6.40 ± 1.26 b
	Flower					
Control	2.53 ± 1.21 a	6.63 ± 4.22 a	6.13 ± 1.55 a	4.43 ± 1.13 b	8.66 ± 2.36 a	11.06 ± 4.03 a
1F	1.67 ± 0.65 ab	6.5 ± 12.20 a	5.54 ± 1.14 ab	6.16 ± 1.29 a	7.21 ± 1.56 ab	12.67 ± 2.81 a
2.5F	1.04 ± 0.70 bc	0.90 ± 0.59 b	5.02 ± 1.18 ab	1.37 ± 0.99 c	6.06 ± 1.51 bc	2.27 ± 1.47 b
5F	0.17 ± 0.15 c	0.19 ± 0.07 b	3.99 ± 1.74 b	0.33 ± 0.08 c	4.16 ± 1.79 c	0.52 ± 0.06 b

Mean values (\pm SD) marked with different letters in the column differ significantly according to Tukey's test for different N, $p \leq 0.05$.

Seedling biomass

Fresh mass values of *F. rubra* seedlings watered with *R. gorenkensis* root extracts significantly differed between the control and the 2.5% extract (Table 4). In the case of leaf extracts, significant inhibition of the weight gain of red fescue seedlings was found at each concentration, compared to the value from the control (Table 4). For *F. rubra* seedlings watered with extracts of rose stalks and flowers, the fresh mass was similar to the control, regardless of the concentration of the extract. The dry mass of red fescue seedlings did not differ between the values from the control and those obtained for individual concentrations and types of rose extracts. Similarly, no statistically significant differences were observed for the ratio of dry mass to fresh mass and total water content in *F. rubra* seedlings.

Fresh mass of seedlings *R. sativus* var. *radicula* cv. 'Rowa' was significantly lower on 5% rose extracts, compared to the control (Table 4). It was also observed that 1% of the stalk extracts increased the fresh mass of red radish seedlings. In contrast to leaf extracts, which, regardless of the concentration, inhibited the growth of fresh mass of red radish, compared to the control. Extracts from flowers with a concentration of 2.5% also had a negative effect on the fresh mass values of the studied dicotyledonous seedlings. Red radish dry mass values did not differ between the control and the extracts used. In the case of the ratio of dry mass to fresh mass, each extract with a concentration of 5% increased the value of this parameter. The exception turned out to be leaf extracts, which at a concentration of 2.5% initiated higher values of this parameter, compared to the control. The percentage of water content was lower in the case of radish seedlings irrigated with 5% extracts in most of the extracts used. The leaf extract was the exception; a concentration of 2.5% had a negative impact on the values of this parameter, compared to the other data and the control.

Table 4. Fresh mass, dry mass and water content of seedlings of seeds of *Festuca rubra* L. (FR) and *Raphanus sativus* L. var. *radicula* Pers. cv. Rowa (RS) watered with *Rosa gorenkensis* Besser organ extracts of various concentrations (1%, 2.5%, 5%)

Extract type (%)	Fresh mass (g)		Dry mass (g)		Dry mass/Fresh mass (a.u.)		Total water content (%)	
	FR	RS	FR	RS	FR	RS	FR	RS
	Root							
Control	0.0079 ± 0.002 a	0.1047 ± 0.021 a	0.0007 ± 0.0004 a	0.0062 ± 0.002 ab	0.0855 ± 0.046 a	0.0600 ± 0.017 b	91.45 ± 4.60 a	94.00 ± 1.75 a
1	0.0069 ± 0.001 ab	0.0880 ± 0.027 ab	0.0005 ± 0.0003 a	0.0051 ± 0.002 b	0.0733 ± 0.052 a	0.0605 ± 0.020 b	92.67 ± 5.24 a	93.95 ± 2.00 a
2.5	0.0060 ± 0.001 b	0.0999 ± 0.021 a	0.0005 ± 0.0004 a	0.0067 ± 0.002 ab	0.0945 ± 0.072 a	0.0664 ± 0.014 b	90.55 ± 7.15 a	93.36 ± 1.40 a
5	0.0075 ± 0.002 a	0.0760 ± 0.019 b	0.0004 ± 0.0003 a	0.0072 ± 0.003 a	0.0556 ± 0.047 a	0.0921 ± 0.019 a	94.44 ± 4.67 a	90.79 ± 1.90 b
	Stalk							
Control	0.0079 ± 0.002 ab	0.1047 ± 0.021 b	0.0007 ± 0.0004 a	0.0062 ± 0.002 ab	0.0855 ± 0.046 a	0.0600 ± 0.017 b	91.45 ± 4.60 a	94.00 ± 1.75 a
1	0.0076 ± 0.002 ab	0.1350 ± 0.028 a	0.0005 ± 0.0003 a	0.0064 ± 0.002 ab	0.0696 ± 0.036 a	0.0481 ± 0.013 b	93.04 ± 3.57 a	95.19 ± 1.31 a
2.5	0.0085 ± 0.001 a	0.0993 ± 0.018 b	0.0004 ± 0.0003 a	0.0051 ± 0.001 b	0.0434 ± 0.032 a	0.0526 ± 0.014 b	95.66 ± 3.23 a	94.74 ± 1.42 a
5	0.0065 ± 0.001 b	0.0623 ± 0.012 c	0.0005 ± 0.0003 a	0.0076 ± 0.002 a	0.0835 ± 0.055 a	0.1264 ± 0.047 a	91.65 ± 5.45 a	87.36 ± 4.71 b
	Leaf							
Control	0.0079 ± 0.002 a	0.1047 ± 0.021 a	0.0007 ± 0.0004 a	0.0062 ± 0.002 a	0.0855 ± 0.046 a	0.0600 ± 0.017 b	91.45 ± 4.60 a	94.00 ± 1.75 a
1	0.0062 ± 0.001 b	0.0770 ± 0.018 b	0.0007 ± 0.0003 a	0.0058 ± 0.003 a	0.1039 ± 0.030 a	0.0785 ± 0.039 ab	89.61 ± 3.04 a	92.15 ± 3.93 ab
2.5	0.0052 ± 0.001 bc	0.0575 ± 0.020 b	0.0004 ± 0.0003 a	0.0063 ± 0.002 a	0.0764 ± 0.053 a	0.1135 ± 0.038 a	92.36 ± 5.29 a	88.65 ± 3.76 b
5	0.0045 ± 0.001 c	0.0621 ± 0.024 b	0.0004 ± 0.0003 a	0.0057 ± 0.004 a	0.0939 ± 0.069 a	0.0884 ± 0.048a b	90.61 ± 6.93 a	91.16 ± 4.85 ab
	Flower							
Control	0.0079 ± 0.002 a	0.1047 ± 0.021 a	0.0007 ± 0.0004 ab	0.0062 ± 0.002 a	0.0855 ± 0.046 ab	0.0600 ± 0.017 c	91.45 ± 4.60 ab	94.00 ± 1.75 a
1	0.0078 ± 0.001 a	0.1151 ± 0.019 a	0.0009 ± 0.0004 a	0.0061 ± 0.002 a	0.1089 ± 0.041 a	0.0528 ± 0.009 c	89.11 ± 4.12 b	94.72 ± 0.94 a
2.5	0.0088 ± 0.002 a	0.0578 ± 0.021 b	0.0004 ± 0.0003 b	0.0081 ± 0.002 a	0.0466 ± 0.027 b	0.1591 ± 0.066 b	95.34 ± 2.66 a	84.09 ± 6.62 b
5	0.0076 ± 0.002 a	0.0224 ± 0.009 c	0.0005 ± 0.0002 ab	0.0073 ± 0.002 a	0.0714 ± 0.014 a	0.3451 ± 0.079 a	92.86 ± 1.43 ab	65.49 ± 7.88 c

Mean values (± SD) marked with different letters in the column differ significantly according to Tukey's test for different N, $p \leq 0.05$.

Electrolyte leakage

Aqueous extracts of roots, stalks, and leaves did not affect the destabilization of cell membranes of *F. rubra* seedlings. Only in the case of *R. gorenkensis* flower extracts, an increase in electrolytes leakage was observed relative to the control value (Figure 6A). In the case of *R. sativus* var. *radicula* cv. 'Rowa' seedlings, the different effect of extracts on electrolytes leakage was observed (Figure 6B). Root extracts did not disturb the water balance of seedlings. Stalk extracts, at each concentration, reduced electrolytes leakage, relative to the control. Leaf extracts in all the concentrations used caused a significant increase in membrane destabilization. For flower extracts, a reduction in electrolyte leakage was observed only for seedlings watered with 1% rose extracts.

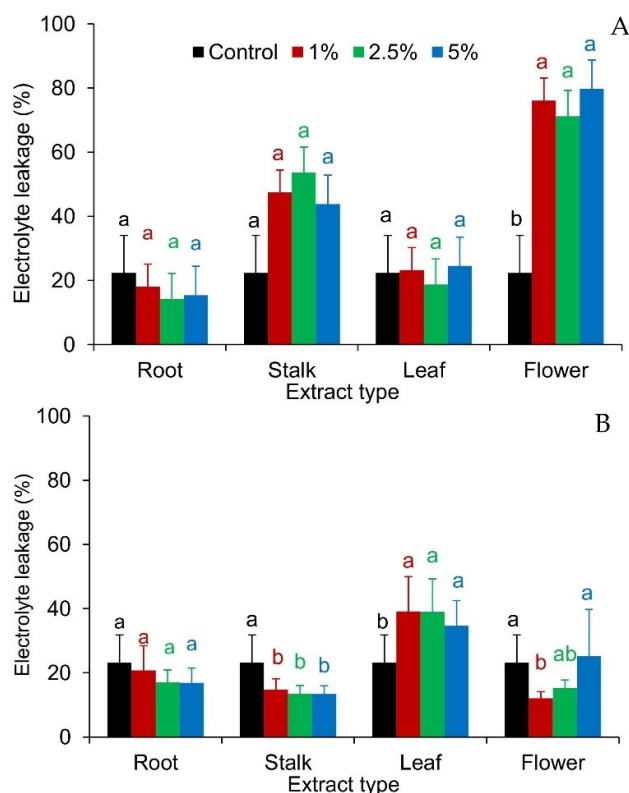


Figure 6. Electrolyte leakage from seedlings of *Festuca rubra* L. (FR) and *Raphanus sativus* L. var. *radicula* Pers. cv. Rowa (RS) watered with *Rosa gorenkensis* Besser organ extracts of various concentrations (1%, 2.5%, 5%)
Mean values (\pm SD) marked with different letters differ significantly according to Tukey's test for different N, $p \leq 0.05$.

Discussion

Invasive plants in agricultural areas occupy large areas, making it difficult to use the land, which causes economic losses. However, most of all, they threaten entire ecosystems, not only transformed but also natural ones. Their expansion often leads to the extinction of local species, which is a particular problem, especially in protected areas (Rhymer and Simberloff, 1996; Buhler *et al.*, 2001). In all ecosystems, plant interactions begin with the germination of seeds and continue throughout their growth and development period. Allelopathic interactions can be one of the factors changing the species composition of plant communities, especially when their source is foreign chemicals from new arrivals taxa (Nilsson and Wardle, 2005).

The experimental analyzes undertaken in this study showed a negative effect of water extracts from *Rosa gorenkensis* on the germination capacity of red fescue and radish seeds (Figure 4, Table 5, 6). Based on the germination indicators, it was shown that the extracts from the organs of the analyzed rose had a negative effect on seed germination with increasing concentrations (Table 2, Figure 4). When assessing the allelopathic properties of individual extracts (regardless of their type), it should be stated that, to the control objects, the greatest inhibitions were caused by extracts with 5% concentrations, prepared from leaves and flowers. *Festuca rubra* seeds were more sensitive to the extracts than those of *Raphanus sativus* var. *radicula* cv. 'Rowa'. These differences were most likely related to seed size and plant life cycle. Red radish has a very short life cycle. The time until the radish is ripe for consumption is 30–50 days, depending on the growing conditions, the variety, and the method of sowing the seeds (Krawiec *et al.*, 2012). *F. rubra* seeds germinate for 10 to 20 days, and their further development takes much longer. Thus, in this case, variation in seed germination rate may have positive ecological significance. Smaller seeds may favour the rapid recolonization of initial microhabitats that appear randomly and temporarily. On the other hand, seedlings developing from large seeds, thanks to the collected material, are better adapted to competing with other plant species in dense plant patches (Souza and Fagundes, 2004). All these factors may partially explain the differences in resistance of species to the prepared extracts noted here.

A more sensitive biotest than seed germination is the assessment of plant growth and development by determining: the length of the roots and the aboveground part of seedlings, biomass, or the degree of destabilization of cell membranes. In the analysed experiment, taking the initial growth of the species in the control as a reference point, the chemicals released from *R. gorenkensis* extracts had a negative effect on the growth of seedlings of both tested species (Table 3, 5; Figure 5).

Table 5. Comparison of selected parameters from germination and growth versus control to illustrate the response of *Festuca rubra* L. seeds to aqueous organ extracts of *Rosa gorenkensis* Besser

Parameter	Extract concentrate (%)											
	1				2.5				5			
	R	S	L	F	R	S	L	F	R	S	L	F
G (%)	Green	Green	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red
MGT	Green	Green	Green	Red	Green	Red	Red	Green	Red	Green	Red	Green
Seedling length	Green	Green	Green	Red	Green	Red	Red	Red	Red	Red	Red	Red
Biomass (fresh mass)	Green	Green	Red	Green	Red	Green	Red	Green	Green	Green	Red	Green
EL (%)	Green	Green	Green	Red	Green	Green	Green	Red	Green	Green	Green	Red

Red colour means inhibition, green colour means stimulation.

R – root, S – stalk, L – leaf, F – flower; G – germination percentage index, MGT – mean germination time, EL – electrolyte leakage

In most of the studied cases, both the length of the underground and above-ground organs was shorter than in the control objects. In the presence of rose root and stem extracts at low concentrations, the stimulating effect was observed. However, at a concentration of 5%, biotests showed a negative effect of the extracts on the elongation growth of seedlings of both tested species. Allelopathic compounds released in the aqueous extracts of *R. gorenkensis* leaves and flowers limited the growth of seedlings to the greatest extent. It is commonly believed in the subject literature that allelochemical substances released from various organs may inhibit or stimulate plant growth and development. A greater allelopathic potential is attributed to dicotyledonous species, however, there are also reports of an inhibitory effect of monocotyledonous species (Khanh *et al.*, 2008). The leaves are the organs in which the greatest amount of allelochemical compounds with a broad qualitative spectrum are accumulated. On the other hand, the weakest allelopathic properties are shown by compounds extracted from the roots (Gniazdowska *et al.*, 2004). In the case of *R. gorenkensis*, it is difficult to

determine which chemical compounds had inhibitory effects, as there are no detailed data available on this subject. Roses are primarily a source of vitamin C, mineral salts, polyphenolic compounds with strong antioxidant properties (Kaszuba *et al.*, 2019). Probably polyphenols may be responsible for these kinds of properties of this plant.

The speed of the initial growth of the roots and above-ground parts is specific for each species and is genetically determined. However, it shows great variability under the influence of environmental factors (Patrzalek, 2000). The roots of germinating seeds are more sensitive to allelochemical compounds than shoots, due to the first contact with the external environment after the seed coat has burst (Mazur, 2019). In the conducted research, it was observed that the allelochemical compounds produced by *R. gorenkensis* not only limited germination and growth but also negatively influenced the production of biomass. The differences in the value of the fresh and dry mass of seedlings depended primarily on the type of extract and its concentration (Table 3). Similar to *R. blanda* Ait. (Możdżeń *et al.*, 2021), the greatest negative impact was found here in the case of leaf and flower extracts.

The response of seedlings to water extracts from *R. gorenkensis* organs was also related to their influence on cell membranes. It was shown here that the greatest disturbances in their stabilization occurred in seedlings watered with leaf extracts (Figure 6; Table 4, 5).

Table 6. Comparison of selected parameters from germination and growth versus control to illustrate the response of *Raphanus sativus* L. var. *radicula* Pers. cv. Rowa seeds to aqueous organ extracts of *Rosa gorenkensis* Besser

Parameter	Extract concentrate (%)											
	1				2.5				5			
	R	S	L	F	R	S	L	F	R	S	L	F
G (%)	Green	Green	Green	Red	Green	Green	Red	Red	Green	Red	Red	Red
MGT	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Seedling length	Green	Green	Red	Green	Green	Green	Red	Red	Red	Red	Red	Red
Biomass (fresh mass)	Green	Green	Red	Green	Green	Green	Red	Red	Red	Red	Red	Red
EL (%)	Green	Green	Red	Green	Green	Green	Red	Green	Green	Green	Red	Green

Red colour means inhibition, green colour means stimulation.

R – root, S – stalk, L – leaf, F – flower; G – germination percentage index, MGT – mean germination time, EL – electrolyte leakage

The mechanisms and physiological role of electrolyte efflux are not fully understood, but it is known that maintaining the structural integrity and stability of cell membrane functions is a measure of stress tolerance. Most likely, this phenomenon is related to the disruption of the conductivity of K^+ , Cl^- , HPO_4^{2-} , NO_3^- ions (Demidchik *et al.*, 2014; Kocheva *et al.*, 2014). In the experiment, rose leaf extracts caused the greatest changes in the structure of proteins and lipids and contributed to a high concentration of ions in the vacuole and the cytoplasm, resulting in the denaturation of proteins located in the membranes. The effect of this was the observed increase in membrane permeability, causing an uncontrolled outflow of electrolytes.

The results of the research on the allelopathic properties of *R. gorenkensis* described above additionally confirm the fact that this rose poses a potential threat to native floristic components. In the long term and on a global scale, according to the most pessimistic forecasts related to the disappearance of geographic barriers, invasions may lead to a reduction in species diversity by over 50% (Mack *et al.*, 2000). Therefore, you should pay special attention to new arrivals species that pose a potential threat. Based on the obtained results, it can be concluded that this species shows significant allelopathic potential. Further research into the chemistry of this rose will identify and isolate allelochemicals responsible for this type of interaction.

Conclusions

(1) Among the extracts used in the experiment, allelochemical substances released from leaves and flowers of *Rosa gorenkensis* Besser to the greatest extent inhibited the germination of seeds of the test species. (2) As the concentration of the extracts increased, their inhibitory effect on the growth of seedlings was observed. (3) Depending on the extract and concentration, differences in the values of mass gain were observed. (4) The degree of destabilization of cell membranes was the highest in seedlings germinated on leaves extracts of *R. gorenkensis*. At this stage of the research, the seeds and seedlings of *F. rubra* (monocotyledonous) showed much greater sensitivity than that of *R. sativus* var. *radicula* cv. 'Rowa' (dicotyledonous). All of the *R. gorenkensis* extracts revealed allelopathic properties to the studied species. Therefore, it can be concluded that allelopathy may be one of the mechanisms of expansion of this species in new habitats.

Authors' Contributions

Conceptualization, W.G., A.S-L., and K.M.; methodology, K.M., A.T., P.Z.; software, A.T., K.M., B.B-K., and P.Z.; validation, K.M., A.T., W.G., and B.B-K.; formal analysis, K.M., A.T., P.Z., and B.B-K.; investigation, K.M., B.B-K., A.T., W.G., A. S-L., and P.Z.; resources, K.M., A.T., and W.G.; data curation, K.M., A.T., B.B-K., and W.G.; writing–original draft preparation, K.M., A.S-L., and B.B-K.; writing-review and editing, B.B-K., K.M., A.T., P.Z., A.S-L., and W.G.; visualization, K.M., A.T., and B.B-K.; supervision, K.M. and B.B-K.; project administration, K.M., A.T., and B.B-K.; funding acquisition, B.B-K. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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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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