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Allelopathic effect of preparations of *Betula pendula* Roth., *Chamomilla recutita* L. and *Urtica dioica* L. on the initial growth of *Hordeum vulgare* L.

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Abstract

The experiment investigated the effect of preparations of *Betula pendula*, *Chamomilla recutita*, and *Urtica dioica* on the initial growth of *Hordeum vulgare* under laboratory conditions. The experiment involved a water solution of sap from the trunk of *B. pendula*, an infusion from *Ch. recutita*, extracts from leaves and rhizomes as well as macerated herbage of *U. dioica*, at the concentrations of 2.5%; 5%; 10% and 20%. During the first four days of the growth of the test plants, the plant preparations tested had a stimulatory effect on the length of leaves and roots in all the cases. The leaf increases were greater than those of the roots and became smaller with an increase in the concentration of the preparations. Continued watering of *H. vulgare* seedlings resulted in a further increase in leaf length after the application of *U. dioica* macerate and *Ch. recutita* infusion, with a simultaneous increase in root length with the macerate of *U. dioica* and *B. pendula* sap used at the concentrations of 2.5–10%. The other extracts made from *U. dioica* leaves and roots, irrespective of the concentration applied, strongly reduced the increase in leaves and roots from 11 to 27%. The weight of roots and leaves in *H. vulgare*, over the entire range of the concentrations applied and irrespective of the duration of the experiment, was stimulated only by *B. pendula* sap. The extracts from *Ch. recutita* and *U. dioica*, especially the leaf and root extracts, significantly reduced the root weight of the test plant.

Keywords: herbal plants; extract application; *Hordeum vulgare*; interactions; seedlings; length; weight

Introduction

The interaction of plants is a multidimensional phenomenon which commonly occurs in nature, e.g. by the release of bioactive substances referred to as allelopathic compounds to the environment. Plants excreting allelopathins are described as donors, while the organisms in their neighborhood, exposed to those excretions, are called acceptors. The effects of these interactions can be of stimulatory or inhibitory nature, which, however, is not strictly determined, as stimulatory compounds, when in high concentrations, can exhibit an inhibitory effect. Similarly, inhibitory compounds in low concentrations can stimulate some physiological processes of the acceptor. Allelopathic compounds are present mostly in leaves and, at smaller amounts, in roots or seeds. The possibility of using these compounds in herbalism depends on their origin (roots, stems, leaves, fruits), by separating pure sap or making extracts, infusions, macerated oils, on concentrations or even on the plant development stage [1–2].

One of the conditions for the effect of some bioactive substances on other plants is their penetration into the soil in specific environmental conditions [3]. Allelopathic compounds, present in plant tissues, can not only stimulate or inhibit the growth and development of the neighboring species but also, indirectly, affect the structure of natural systems in biocenosis [4]. Most frequently, the allelopathic influence of plants is successfully made use of under the conditions of organic and bio-dynamic farming.

Over the recent years, research has also been performed on the potential of bioactive compounds to combat weeds and pests of crops [5–8]. An interesting issue is presently the potential of the application of bioactive substances derived from various plant organs which are commonly considered to be herbal material. During the growing season, *Betula pendula* sap, *Chamomilla recutita* inflorescences as well as *Urtica dioica* rhizomes and herbage (leaves with stems) are commonly available. The vast pharmacology literature claims that plant substances contained in these materials are rich in mineral compounds, saponins, tannins, essential oils, sterols, flavonoids, resins, and antiseptics [1–2,9–10]. In herbalism, plants have various applications; however, the effect on other plants is poorly identified.

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The aim of the research was to determine the effect of plant preparations of various origin (*Betula pendula*, *Urtica dioica*, and *Chamomilla recutita*) and obtained by different methods (sap, infusion, extract, macerate) on the growth of Hordeum vulgare seedlings.

The working hypothesis of the experiment was that active substances contained in the preparations of various origin and obtained by different methods had a stimulatory or inhibitory effect on the weight and length of leaves and roots of H. vulgare in the period of its initial growth.

Material and methods

The study on the effects of preparations obtained from medicinal plants on the initial growth of *Hordeum vulgare*, cultivar 'Justina', was performed under laboratory conditions. In the experiment, water solutions from the following plants were applied in the form of: (***i***) sap from the trunk of *B. pendula* – sap (Betula); (***ii***) *C. recutita* inflorescence – infusion (Chamomilla); (***iii***) *U. dioica* leaves – extract (Urtica 1); (***iv***) rhizomes *U. dioica* – extract (Urtica 2); (***v***) herbage (leaves and stems) *U. dioica* – macerate (Urtica 3).

The water solution of *B. pendula* sap was made from diluted natural sap collected directly from the tree trunks in spring and available throughout the entire year as a commercial product distributed by the company Oskoła. The extract from the inflorescence of *C. recutita* was obtained by pouring one liter of boiling water over 20 grams of dried material and leaving it to cool down. In herbalism, such a kind of preparation is referred to as "infusion". The leaf extract and the extract from rhizomes together with *U. dioica* roots were obtained by pouring one liter of cold water over 100 g of fresh plant material and leaving it for a period of 24 hours. The third kind of extract from *U. dioica* was prepared by pouring one liter of cold water onto 100 g of fresh leaves and stems and exposing it to slow maceration at a temperature of about 20°C for two weeks. In the experiment, an informed selection was made of herbal materials commonly available throughout the growing period and the effect of their separation method on their allelopathic influence was evaluated.

Betula pendula sap was diluted to obtain the solution concentrations of 2.5%, 5%, 10% and 20%, while the infusions, extracts and macerated preparations were screened using a 0.1 mm mesh sieve and diluted by applying identical percentage proportions. The control was poured only with distilled water.

The experiment with barley seedlings was carried out in Szmal germinators at a temperature of 20–22°C provided with access of daylight. These germinators (patent PL 105443 B1) are built from adequately profiled battens with hollows for germinating seeds which are separated with filter paper. The whole is placed on trays to be poured with the solutions. Each tray was initially poured with 20 ml of the plant solutions tested, and on successive days of the experiment, to maintain constant moisture content of the filter paper, the containers were poured with 10 ml of the liquid evaluated. The experiment was performed in three replicates for all the treatments in a cycle of four and seven days and later from each tray 50 germinating *H. vulgare* seedlings were collected for each treatment, including the control. After each series of the experiment, the length of roots and leaves (cm) as well as their dry weight (mg) was determined for the produced seedlings of the test plant. Using the measurements taken, the massiveness coefficient for the roots and leaves was calculated as the ratio of the weight of the roots and leaves (mg) to their length (cm) [11].

The results were subjected to statistical analysis using analysis of variance. The significance of the differences between treatments was evaluated with Tukey's test at a level of significance of $P \leq 0.05$, separately for the respective preparations and the concentrations applied as well as the means for the preparations.

Results

The results of the research on the morphological measurements of *H. vulgare* seedlings after four and seven days of the experiment are included in Tab. 1 and Tab. 2. The measurements of seedling leaf and root length after the first four days of the experiment showed that the plant preparations applied in indivdiual cases acted as stimulants. The greatest leaf elongation, as compared with the control, was found for the extracts obtained from macerated *U. dioica* herbage and the infusion of *Ch. recutita* inflorescence. The lowest leaf increases were induced by the extract from nettle leaves and rhizomes (Urtica 1 and Urtica 2). The beneficial effect of the preparations on the increase in seedling leaf length ranged from 9.4% (Urtica 2) to 82.5% (the macerate made from stinging nettle) (Urtica 3). The greatest increases in *H. vulgare* leaf were recorded for the following preparations at the concentrations given: Betula (5%), Chamomilla (20%), Urtica 1 (10%), Urtica 2 (20%) and Urtica 3 (2.5%).

On the other hand, the favorable effect of the preparations on root increases was lower than in the case of leaves and these increases ranged from 2.8% (Betula) to 27.6% (Urtica 3). Of all the preparations applied, the most significant increase in root length occurred in the presence of the 2.5% Urtica 3 preparation as well as 5% and 20% Chamomilla. Despite the lack of significant differences, after the application of the Urtica 1 preparation, a slight increase in root length was found at the concentrations of 5 and 10%.

After seven days of the experiment, a favorable effect of the preparations on leaf length was found after the application of the Chamomilla and Urtica 3 preparations, particularly at the higher concentrations (10% and 20%). The use of these preparations resulted in a greater increase in leaves, by about 10%, compared to the control. The other water solutions studied inhibited the length of *H. vulgare* leaves from 17.7% for the Betula preparation to 30% for Urtica 2.

At the second stage of the experiment, a significant increase in root length was found only after the application of macerated Urtica 3 at 20%. The root growth was definitely inhibited by the extracts from the leaves (Urtica 1) and rhizomes (Urtica 2). The other preparations at various concentrations caused negligible root growth or it was inhibited statistically insignificantly.

Tab. 1 Length of embryonic roots and leaf length (cm) of *Hordeum vulgare* seedlings in the presence of plant preparations after four and seven days of the experiment.

	Betula	Chamomilla	Urtica 1	Urtica 2	Urtica 3	Betula	Chamomilla	Urtica 1	Urtica 2	Urtica 3
	After 4 days					After 7 days				
Leaf length (cm)										
control	2.87 c	2.87 b	2.87 b	2.87 bc	2.87 c	14.48 a	14.48 bc	14.48 a	14.48 a	14.48 c
2.50%	3.37 bc	4.27 a	2.94 b	2.55 c	5.90 a	13.64 ab	14.47 c	10.77 d	9.45 c	15.06 bc
5%	4.14 a	4.38 a	3.39 ab	3.08abc	5.27 ab	12.44 b	15.84 ab	11.91 bc	10.89 b	15.45 bc
10%	3.57 b	4.17 a	3.76 a	3.29 ab	4.93 b	12.54 b	16.93 a	12.61 b	10.51 bc	16.07 ab
20%	3.39 b	4.45 a	2.92 b	3.63 a	4.87 b	10.74 c	16.26 a	11.25 cd	10.27 bc	16.79 a
Mean 2.5–20%	3.62 BC	4.32 B	3.25 C	3.14 C	5.24 A	11.91 B	15.87 A	11.63 B	10.28 B	15.84 A
*	+26.1	+50.5	+13.2	+9.4	+82.5	−17.7	+9.6	−19.7	−30.0	+9.4
Root length (cm)										
control	4.34 b	4.34 b	4.34 a	4.34 bc	4.34 b	9.00 a	9.00 a	9.00 a	9.00 a	9.00 b
2.5%	4.71 ab	5.13 a	4.09 a	4.00 c	5.89 a	9.10 a	7.00 b	7.02 b	6.33 c	9.84 b
5%	5.03 a	5.21 a	4.52 a	4.59 ab	5.71 a	9.24 a	8.77 a	6.79 b	6.86 c	9.40 b
10%	4.40 b	5.06 a	4.50 a	4.61 ab	5.29 a	9.26 a	9.05 a	7.52 b	7.98 ab	9.28 b
20%	3.70 c	5.32 a	4.26 a	4.97a	5.26 a	6.58 b	7.11 b	6.51 b	6.91 bc	10.90 a
Mean 2.5–20%	4.46 BC	5.18 AB	4.34 C	4.54 BC	5.54 A	8.55 B	7.98 AB	6.96 B	7.02 B	9.86 A
*	+2.8	+19.3	0.0	+4.6	+27.6	−5.0	−11.3	−22.7	−22.0	+12.2

Betula – sap made up from *B. pendula*; Chamomilla – infusion from the inflorescences of *C. recutita*; Urtica 1 – extract from the leaves of *U. dioica*; Urtica 2 – extract from the rhizomes of *U. dioica*; Urtica 3 – macerate from the herbage *U. dioica*. Values followed by the same letters (separated for each date and morphometric features) do not differ significantly at $P \leq 0.05$. * share in +/− mean value of the preparations evaluated against the control (%).

Tab. 2 Weight of embryonic roots and the weight of leaves (mg) of spring barley seedlings incubated in the solutions of the plant preparations after four and seven days of the experiment.

	Betula	Chamomilla	Urtica 1	Urtica 2	Urtica 3	Betula	Chamomilla	Urtica 1	Urtica 2	Urtica 3
	After 4 days					After 7 days				
Leaf length (cm)										
control	5.83 c	5.83 a	5.83 a	5.83 a	5.83 a	12.12 b	12.12 a	12.12 a	12.12 a	12.12 a
2.50%	7.15 b	4.53 c	4.62 b	4.92 cd	5.75 a	16.13 a	11.63 ab	9.21 c	8.44 c	10.04 b
5%	7.81 a	3.91 d	4.71 b	5.14 bc	4.74 b	14.91 a	10.52 c	10.82 b	9.52 b	10.22 b
10%	6.63 b	4.75 c	5.35 a	5.63 ab	4.74 b	16.73 a	11.31 b	10.50 b	8.63 c	10.81 b
20%	6.90 b	5.26 b	4.33 b	4.61 d	5.33 a	15.39 a	11.65 ab	9.53 c	8.61 c	12.14 a
Mean 2.5–20%	7.12 A	4.61 B	4.75 B	5.10 B	5.14 B	15.79 A	11.28 B	10.02 BC	8.80 C	10.80 B
*	+22.4	−20.7	−18.9	−12.1	−12.1	+29.7	−7.4	−17.3	−27.3	−10.7
Root length (cm)										
control	6.82 c	6.82 a	6.82 a	6.82 a	6.82 a	9.72 c	9.72 a	9.72 a	9.72 a	9.72 a
2.5%	8.62 a	4.63 b	3.33 b	4.55 d	6.13 b	10.43 b	7.23 d	8.53 bc	8.07 b	8.04 c
5%	8.32 ab	4.82 b	3.72 b	5.32 c	4.91 c	11.42 a	7.91 c	9.65 a	8.54 b	8.32 bc
10%	9.12 a	4.53 b	3.06 b	5.46 c	5.14 c	11.43 a	8.63 b	9.15 ab	7.73 b	7.83 c
20%	8.15 b	4.90 b	3.51 b	5.93 b	4.81 c	9.94 bc	7.61 cd	7.85 c	8.05 b	8.81 b
Mean 2.5–20%	8.55 A	4.72 B	3.41 C	5.32 B	5.25 B	10.81 A	7.85 B	8.80 B	8.10 B	8.25 B
*	+25	−30.9	−50.0	−22.1	−23.5	+11.3	−19.6	−9.3	−16.5	−15.5

Symbols, see Tab. 1.

The measurements of the weight of the leaves and roots of the test plant seedlings during the experiment show that only the solutions from Betula sap resulted in their increase in each application range (Tab. 2).

In the four-day treatment, the significant increase in leaf weight was on average 22.4% and for roots – 25%, while in the seven-day treatment, on the other hand, the leaf weight, compared to the control, was on average 29.7% higher and the root weight by 11.3%. The application of the other preparations, irrespective of the concentrations applied, resulted in the seedling weights being significantly lower than in the control, especially at the first stage of the experiment. In the four-day treatment, the lowest leaf weight, as compared with the control, was found after the use of the Chamomilla and Urtica 1 extracts, on average by 20.7% and 18.9%, whereas for the roots – 30.9% and 50%. In the seven-day experiment, the lowest leaf weights were noted after the application of the Urtica 2 (on average by 27.3%) and Urtica 1 extracts (on average by 17.3%).

To present the relationship between the effect of the various preparations and their concentrations on the increase in barley seedlings, the massiveness coefficients were calculated for the roots and leaves, as shown in Fig. 1–Fig. 4. At the first stage of the experiment, of all the solutions applied, a higher unit root weight, as compared with the control, was recorded only for the seedlings treated with Betula sap, especially at the concentrations of 10 and 20%. On the other hand, in the case of the other preparations the massiveness coefficient, both for leaves and roots, shows that their lengths increase more significantly than their weight. In the seven-day experiment with Betula as well as with the leaf (Urtica 1) and rhizome (Urtica 2) preparations, it was found that the higher the increase in leaves, the proportionally higher the *H. vulgare* root weight. For the Betula preparation, the effect is clearly visible for the higher concentrations, while in the case of the U. dioica preparations for the lower ones. The other preparations made the unit weight of seedlings of the test plant, both leaves and roots, lower than in the control, especially after the application of the Chamomilla and Urtica 3 preparations.

The values of the massiveness coefficients show that a further development of roots and leaves is mostly connected with an intensive growth of their length, as compared with the seedling weight. It was also found that despite the inhibiting effect of the preparations from nettle leaves and rhizomes (Urtica 1 and Urtica 2) on the seedling growth, their unit weights were similar to the values recorded for the plants treated with Betula sap.

Discussion

The results of the present research show that the effects of solutions of Betula sap, camomile (Chamomilla) infusion as well as of various forms of Urtica leaf, rhizome and herbage preparations (1–3), applied at different concentrations, on *H. vulgare* seedlings growing under laboratory conditions significantly differed and that they affected both the length and weight of the leaves and roots of the test plants. Their stimulating or inhibiting effect depended on the kind of the

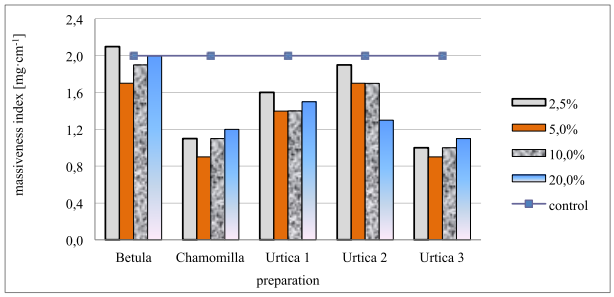

Fig. 1 Values of the massiveness coefficients of the leaves after 4 days of the experiment. Betula – sap made from *B. pendula*; Chamomilla – infusion from the inflorescence of *C. recutita*; Urtica 1 – leaf extract made from *U. dioica*; Urtica 2 – rhizome extract made from *U. dioica*; Urtica 3 – macerate from *U. dioica* herbage.

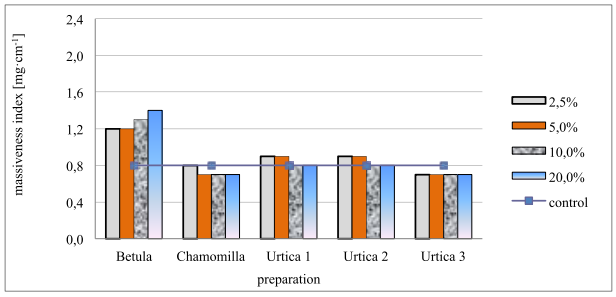

Fig. 2 Values of the massiveness coefficients of the leaves after 7 days of the experiment. See Fig. 1 for additional explanations.

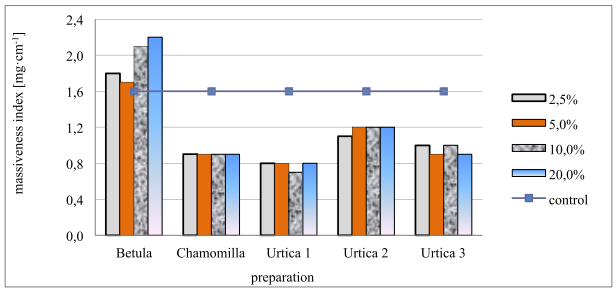

Fig. 3 Values of the massiveness coefficients of the roots after 4 days of the experiment. See Fig. 1 for additional explanations.

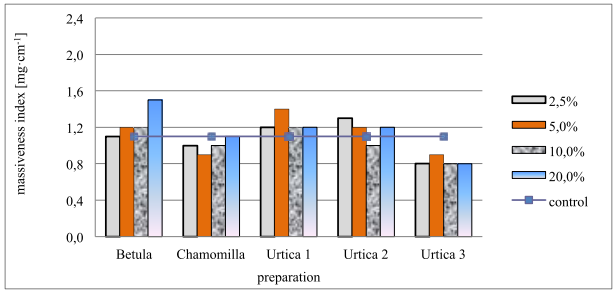

Fig. 4 Values of the massiveness coefficients of the roots after 7 days of the experiment. See Fig. 1 for additional explanations.

preparations and their concentrations. The research shows that in the case of *U. dioica* its negative allelopathic impact is comparable with the results of the study performed by Komorowska et al. [12] which concerned the effects of leaf extracts on rye and wheat seedlings. Other studies on various plants with herbal properties or treated as weeds and on their allelopathic effect demonstrate that the sensitivity of cereals to those substances varies and depends mostly on the test plant species, the concentration of bioactive substances in the preparations applied as well as the duration of their effect [3,13–16].

The present research confirms the observations made for barley by Islam and Begun [17] which indicate a greater sensitivity of the roots, compared to the aboveground parts, to the allelopathic factor, which at the same time increases proportionally together with the concentration of the substance.

The study reveals that plant roots affected by allelopathic substances respond stronger than the aboveground parts. What also changes is the proportion of the weight increases relative to the length, which results from the massiveness coefficient value calculated.

As far as the allelopathic effect is concerned, as confirmed by the present research, not only the plant species and herbal material obtained (sap, stem, leaves, roots) are essential, but also the method and technique of preparation of bioactive substances in the form of natural secretion (sap), infusion, extract, or macerate. Similarly, the duration of the effect of bioactive substances on the test plant is important.

The effect of active substances found in medicinal plants and tested under laboratory conditions may not quite reflect their effect on plants grown in the field. The reports by Gniazdowska et al. [3] show that the interaction of allelopathic compounds between species depends significantly on environmental conditions. With that in mind, a field experiment with the previously studied preparations should be established.

Conclusions

The water solutions from the plant preparations tested in the experiment showed an allelopathic potential and a stimulatory or inhibitory effect on the growth of seedlings of the test plant depending on their origin, concentration as well as the experiment duration.

The results of the experiment show that, irrespective of the impact, whether positive or negative, the applied preparations affect more considerably the elongation of the leaves than the roots and show a stronger effect on the root weight.

At the initial stage (four days) of the experiment, all the preparations stimulated the elongation of leaves and roots. At the further growth stage (seven days), the elongation of seedlings was limited, except for the application of the Urtica 3 macerate.

Betula sap, irrespective of the experiment duration and at all the concentrations applied, increased the weight of leaves and roots. The other preparations decreased the weight of leaves and roots, as compared with the test plant, especially Chamomilla, Urtica 1, and Urtica 2.

The calculated values of the massiveness coefficients for the leaves and roots show that the Urtica 3 macerate, especially the solution at the concentration of 20%, had the most beneficial effect on the growth of seedlings of the test plant after the completion of the experiment, but also the preparation with Chamomilla and Betula sap.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: concept: AD; laboratory work: ZS; data analysis and manuscript development: AD, ZS.

Competing interests

No competing interests have been declared.

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Allelopatyczne oddziaływanie preparatów z *Betula pendula* Roth., *Chamomilla recutita* L. i *Urtica dioica* L. na początkowy wzrost *Hordeum vulgare* L.

Streszczenie

W doświadczeniu badano wpływ preparatów roślinnych otrzymanych z *Betula pendula*, *Chamomilla recutita* oraz *Urtica dioica* a wykorzystywanych w ziołolecznictwie na początkowy wzrost *Hordeum vulgare* w warunkach laboratoryjnych. W doświadczeniu zastosowano sok z pnia *B. pendula*, napar z *Ch. recutita*, oraz ekstrakty z liści, kłączy oraz macerat ziela *U. dioica*, w stężeniach 2.5%, 5%, 10% i 20%. Testowane preparaty roślinne w ciągu pierwszych czterech dni wzrostu rośliny testowej wpływały stymulująco na długość liści i korzeni we wszystkich przypadkach. Intensywne przyrosty liści były większe niż korzeni i ulegały zmniejszeniu wraz ze wzrostem stężenia preparatów. Kontynuowanie podlewania siewek *H. vulgare* skutkowało dalszym wzrostem długości liści po aplikacji maceratem *U. dioica* oraz naparem *Ch. recutita* przy jednoczesnym wzroście długości korzeni wobec maceratu *U. dioica* i soku *B. pendula* w stężeniach 2.5–10%. Pozostałe ekstrakty z liści i korzeni *U. dioica*, niezależnie od aplikowanego stężenia silnie ograniczały wzrost liści i korzeni w zakresie 11–27%. Masę korzeni i liści *H. vulgare* w pełnym zakresie aplikowanych stężeń i niezależnie od długości trwania eksperymentu, stymulował jedynie sok z *B. pendula*. Ekstrakty z *Ch. recutita* i *U. dioica*, a zwłaszcza z jej liści i korzeni silnie ograniczały masę korzeni rośliny testowej.