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Allelopathic effect of couch grass (*Elymus repens* L.) on germination of common wheat seeds

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Abstract

The resistance of common wheat (*Triticum aestivum* L.), a traditional Ukrainian crop, to allelopathic weeds in the early stages of cultivation has not been sufficiently analysed up to now. Contradictory information on the chemical interaction between wheat and couch grass (*Elymus repens* L.) has led to a detailed investigation of the effect of couch grass. The aim of the experiment was to investigate the effect of the aqueous extracts of couch grass rhizomes collected in Vinnytsia region, western and south-western Ukraine on wheat seed germination and further seedling development. It was found that the presence of couch grass extracts inhibited the energy of seed germination in all experimental treatments. The couch grass extract with a concentration of 1:1000 had a stimulating effect on the germination and subsequent growth of wheat seedlings compared to the control treatment, in which the wheat seeds were germinated in the distilled water. On the 4th day of the experiment, the couch grass extracts at higher concentrations (1:100 and 1:10) significantly reduced the length of the germ root of wheat by 0.25 and 0.30 cm. On the 7th day of wheat seed germination, measurements of the stem length showed a decrease by 56.2% and 51.4% compared to the control treatment. Thus, the inhibitory effect was maybe decreasing within 5–11 days of the germination of wheat seeds depending on the concentration of allelopathically active substances of couch grass in an aqueous solution.

Keywords: Poaceae, rhizomes, weeds, *Elytrigia repens*, *Agropyron repens*, allelopathy.

Introduction

It is well known that the yield of crops is reduced in the weedy farmlands (Araniti et al., 2017; Sawicka et al., 2020; Woźniak, 2020). The compatible growth of unwanted plants with crops and their interactions have been sufficiently investigated. However, the chemical effects of weeds on crops need to be considered in more detail in the light of high allelopathic pressure (Jabran et al., 2015).

Allelopathy is one of the most important and influential forms of chemical relationships and interactions between plants in the phytocenosis (Grodzinskii, 2016; Кучерявий, 2020). It forms the species and numerical composition, structure, and productivity of phytocenoses. Each plant has two allelopathic characteristics, i.e., activity as the ability to form and release biologically active substances into the environment (phytoncides, allomone, and phytolins) and tolerance as the ability to withstand the accumulation of its own (autotolerance) or other species secretions.

Interaction of plant species is positive if one species secretes a substance that stimulates the growth and development of another, or negative if the interaction is inhibitory. Cases of negative chemical effects of weeds on crops are the most common (Akgün et al., 2018). The degree of the inhibitory effect of certain weed species depends on their toxicity (Yang et al., 2016; Wang et al., 2021).

Couch grass, or quackgrass (*Elymus repens*, syn. *Elytrigia repens* and *Agropyron repens*), and wheat are the representatives of Poaceae family. Both species have the ability for tillering. However, couch grass is a perennial plant. It stores a significant amount of nutrients in underground stems. Rhizomes of wheatgrass contain carbohydrates such as triticin, mannitol, agropyrene, glucunanil, malic acid salts, more than 9% protein, mucous substances, mineral salts, saponin, fatty and essential oils, carotene, and 150 mg 100 g^{-1} of ascorbic acid. Couch grass excretes various substances into the environment, and during the growing season it has a significant negative effect on neighbouring plants. Couch grass is considered a weed because of its toxic effect on crops, and it is very difficult to get rid of it in agrophytocenoses.

Couch grass is the most common weed in the artificial phytocenoses of the Forest-Steppe zone. It is a perennial weed with the ability to quickly displace other species from an area because its excretions are harmful to crops and other weeds (Ringselle et al., 2020). In the

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rhizomes of couch grass, essential oils, fructose, starch, organic acids, glycosides, inulin, inositol, mineral salts of calcium and iron, vitamins C, E and B, carotene, pectin, and mucus were found. The yield and quality of all crops is reduced in the couch grass farmlands (Brandsæter et al., 2017). However, the issue of couch grass effect at the stage of crop seed germination has not been sufficiently analysed (Tørresen et al., 2010). Problems of phytocoenotic interactions between plants have become especially relevant recently due to the constant growth of human needs for food and raw materials. Therefore, it is necessary to help crops to resist the negative effects of weeds by developing rules for the rational use of phytocenoses.

The inhibitory effect on seed oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) germination was 1.7–2.6% higher for thistle (*Cirsium arvense* L., *Sonchus arvensis* L.) and couch grass than for other perennial species (Tsytsiura, Tsaruk, 2021). During the experiment, stimulation of winter wheat seedlings growth was observed under the action of white mustard, oil radish, spring vetch, and buckwheat aqueous extracts. The seedling length increased significantly by 13.1 mm compared to the control. Sweet clover extracts greatly inhibited the growth and development of winter wheat seedlings. Extracts of oilseed radish and white mustard increased germination to 95.0%, while sweet clover and spring vetch reduced this number to 81.7% and 85.0%, respectively (Лисянський, 2017).

It was found that species of one genus are very different in their allelopathic activity. Physiologically active substances of the aboveground and underground organs of plants are not always toxic and not for all plant species. It has been found that the effect (negative or positive) of water-soluble allelochemicals depends on their concentration and the species they affect (Håkansson, 2003; Wei et al., 2010; Кобів, 2015).

It was found that the extract effect on growth was species-specific with different effects on the root system and stem (aboveground) part. Reducing the concentration of aqueous weed extract from 4.0% to 1.0% reduces the mismatch of stem and root ratios. The indicator meets the requirements; it is proved by the ratio dynamics. The influence of the extracts on the root system formation and intensive influence on both the root and the stem part of the species with a high allelopathic potential are dominant. It should be noted that the allelopathic potential is a measure of the allelopathic effect in the weed testing system. It shows the level of competitiveness of a particular weed species without taking into account the growth rate of vegetation, its vital activity and other factors. However, it divides the species according to the threshold value important at the beginning of the competition, which significantly determines the further success of any crop agrocenosis formation (Tsytsiura, 2021).

Mineralisation of water extracts from donors, i.e., leaves, stems, rhizomes, and rhizosphere soil of couch grass, was one of the reasons for their allelopathic activity, which reduced germination of tester seeds (radishes, black radish, and watercress) to 5–30% in the period of germination energy and up to 15–35% in the period of laboratory germination at 80–100% in the control treatment (Гринченко, Дріль, 2017). These results suggested that plant residues of local landraces could be left on the soil surface or soil-incorporated after wheat harvest for weed control. Furthermore, durum wheat landraces can be considered potential plants for the possible future production of bioherbicides (Scavo et al., 2022). All measures in agriculture should be aimed at eliminating allelopathic stress by inactivating toxic substances by chemical means selecting tolerant plants that can metabolise, bind, or neutralise their excretion products (Гангур, 2021).

Thus, today the most pressing methodological issues are the sufficient concentration for the effects of phytotoxicity in natural ecosystems, the dependence of allelochemicals on soil properties, and the distinction between the direct and indirect action of allelochemicals on plants (Grakhov et al., 2014; Gruznova et al., 2018). Any plant of the phytocenosis can be a producer, that is, a donor of physiologically active substances, and their consumer, i.e., an acceptor or recipient. Their chemical nature and influence in modern agrophytocenoses have not been investigated yet.

Considering the above-mentioned material, the aim of the experiment was to study the effect of different concentrations of water-soluble allelopathic active substances from the couch grass rhizomes on the germination of common wheat seeds.

Material and methods

The experiment was conducted in 2021–2022 in the Laboratory of Plant Physiology of Vinnytsia National Agrarian University, Ukraine. Common wheat (*Triticum aestivum* L.) seeds were germinated in Petri dishes 8 times in a thermostat under +22°C temperature. In the control treatment, the seeds were germinated by adding distilled water to Petri dishes. In the experiment, aqueous extracts of different concentrations (1:1000, 1:100, and 1:10) of couch grass (*Elymus repens* L.) rhizomes were the media for the germination of wheat seeds.

Rhizomes of couch grass were collected at the experimental field of the Vinnytsia National Agrarian University located in the central part of Vinnytsia region (49°11′31″ NW, 28°22′16″ E, 49.19194° N, 28.37111° E), western and south-western Ukraine in autumn, after the end of the growing season, i.e., when they have accumulated the greatest number of the allelopathy related substances. Wheatgrass rhizomes were collected outside the field where the peas were grown. In this area, no tillage system was carried out and no herbicides were used. The area of the experimental field has a flat topography. The soil of the study area is represented as gray medium-loam forest soil. According to the indicators of morphological, physical, and physico-chemical properties, they are typical for both the Vinnytsia region and Right-Bank Forest-Steppe of Ukraine.

Then the material was dried to an air-dry state and ground into particles of 3–4 mm. A weighed portion (0.5, 1, and 10 g) of couch grass rhizomes was transferred to a glass container, and 100 ml of distilled water at room temperature was added. The amount of water used to make infusions should ensure the complete removal of active substances. The rhizomes powder of couch grass was permanently stirred so that it was completely immersed in water.

The extraction process lasted one day at +20°C temperature. After 24 hours, the extracted solution was filtered from the plant mass using paper filters. Germination was performed using 10 seeds of experimental plants belonging to a single sowing fraction under the thermostatic regime with a temperature of +22°C in 8 replications. The indicators of germination of seeds in all experimental treatments were recorded on the 4th day after laying Petri dishes in a thermostat (according to DSTU 4138-2002). The seedling length was measured with a ruler at 24-hour intervals for six days since the first estimation. The obtained results were compared with the control ones, where the seeds were germinated in distilled water. Germination energy is the number expressing the percentage of fast-germinating seeds; it was planned for the 4th day.

Statistical analysis. All data represented are the mean ± standard deviation (SD) of eight replicates (n = 8) of each treatment. The experimental data were evaluated by the one-way analysis of variance (ANOVA).

Results and discussion

Common wheat crops are more competitive with weeds than row crops. They are bushy, which also helps to suppress unwanted vegetation. Herbological protection during the critical period allows a high level of potential yield. For germination of wheat seeds, the sum of daily temperatures 90°C in the soil should be reached during the first 15–20 days. Factors influencing the emergence of plant seedlings are divided into endogenous and exogenous. Endogenous factors include the level of nutrient supply to seeds, their own phytohormonal regulation, and genotype. Exogenous factors include the influence of temperature and water regimes, the exposure of light, and the duration of daylight. Its embryo consumes reserve substances from the grain during seed germination. This process requires that organic matter be converted into soluble forms. This transformation occurs due to the activity of enzymes activated by phytohormones of the gibberellin group. In the presence of sufficient moisture, gibberellins from the embryo pass into the aleurone layer of the epidermis, where they begin to stimulate the synthesis of enzymes, namely α-amylase for starch cleavage, proteases for proteins, lipases for fats, and nucleases for isocitrates and nucleic acids. Nucleic acids activate the synthesis of cytokinin, responsible for cell division and auxin, which ensures the growth of cells in length. As soon as the synthesis of hormones begins, the seeds begin to germinate. During this period, more moisture is used – wheat seeds use about 60% of their weight of moisture (Макрушин и др., 2006).

Allelochemicals can affect plant development by acting on metabolic pathways such as photosynthesis, respiration, and ion uptake mechanisms (Zhu et al., 2021).

The embryonic root first appears, then it begins to supply water and nutrients to the embryo. All sees expend a lot of energy during germination. The beginning of competitive relations within a species (in conditions of thickening) and interspecific relations (in conditions of co-germination with other species, weeds are especially dangerous) depends on the germination conditions provided by humans to crops.

Physiologists identify a number of successive stages of seed germination: (i) on the 1st day the enzymatic activity is activated, the intensity of respiration increases, and the hydrolysis of spare substances used by the embryo for germination and further development takes place; (ii) at the 2nd–3rd stage embryonic roots appear due to the development of a sprout (this stage ends for wheat when coleoptile appears); (iii) at the 4th stage the seedlings reach a length of 0.5–0.7 cm (https://growex.ua/ua/blog/prorostannya-ta-otrimannya-shodiv-pshenitsi). If the seedlings of crops are in an environment saturated with allelopathic active substances, their growth may be accelerated or inhibited. Accordingly, the seedlings of these crops may sooner or later appear on the soil surface. There is a fierce competition between plants in the horizontal plane at the beginning of the growing season.

Therefore, a rapid germination of cultivated plants has a better chance of dominance and the formation of a favourable phytoenvironment. The ecological and microclimatic conditions of such a cenosis will be favourable for the growth and development of crops, so they will be able to control the level of weeds and reduce their harmful effects (Yang, Kong, 2017). Thus, the studies of the conditions of seeds germination and the allelopathic effect of weed species on this process are important for modern agricultural science (Silva, Vieira, 2019).

In vegetative conditions, aqueous extracts of biomass of white mustard, oil radish, spring vetch, and buckwheat reduce the energy of seed germination and have a phytoprotective (stimulating) effect on the length and weight of winter wheat seedlings, and aqueous extracts of biomass of sweet clover have allelopathic pressure. Toxic or stimulating effects of green manure crops are not observed at the time of winter wheat sowing in the field (Лисянський, 2017).

Phytocompetition in the cenosis depends on the allelopathic activity of the species participating in it (Coelho et al., 2017). Therefore, it is difficult to assess the allelopathic pressure of each weed species on the yield in the field – usually the effects of weed species on crops are studied in the laboratory under controlled conditions.

The chemical composition of aqueous extracts from the couch grass rhizomes had a significant effect on the rate of the germinal root emergence compared to the control treatment. Measurements were performed on the 4th day of the experiment. According to the Table 1 data, the higher concentration (1:10) of couch grass rhizome extract caused a decrease in the length of the wheat germinal root. The data represents the average values for the treatments of the experiment. The analysis of the obtained data showed the inhibition of seed germination. All the seeds began to germinate in the control media, then in the other treatments part of the seeds was in the swelling phase.

The couch grass rhizome extract at a concentration of 1:1000 was the environment for the germination of wheat seeds, and 87% of the seeds formed the primary root. An increase in the concentration to 1:100 caused the germination of 74% of wheat seeds. In the conditions of the highest concentration (1:10) of the couch grass extract, only 62% of the seeds germinated. On the 4th day of the experiment, the average root length

Table 1. Dynamics of the wheat germination and 4th day seedling morphometric parameters depending on the germination media with different concentrations of couch grass rhizome extract

Treatment	Distilled water (control)	Extract concentration		
		1:1000	1:100	1:10
Root length cm	0.48 ± 0.03	0.32 ± 0.02	0.23 ± 0.02	0.18** ± 0.02

Note. Results were represented as mean ± SD; significant at ** – $P < 0.01$.

was 0.18 cm, which is significantly different from that of the control treatment. Thus, the germination energy of wheat seeds depended on the allelopathic effect of substances extracted into an aqueous solution from couch grass rhizomes. It decreased in proportion to the extract concentration increase.

The inhibitory effect on the initial growth of all analysed species was proportional to the increase in concentration. The effect on initial growth was stronger than the effect on the germination of the analysed species. Lower concentrations stimulated germination (Novak et al., 2021).

The calculations performed on the 7th day of the experiment confirmed the inhibitory effect of higher concentrations (1:100 and 1:10) of couch grass rhizome extract on the height of the wheat seedling and the length of the root system (Table 2).

Table 2. Morphometric parameters of the 7-day-old wheat seedlings depending on the germination media with different concentrations of couch grass rhizome extract

Analysed parameters	Distilled water (control)	Extract concentration			LSD
		1:1000	1:100	1:10	
Stem height cm	2.49 ± 0.09	2.55 ± 0.09	1.08* ± 0.06	1.21* ± 0.07	0.05
Embryo root length cm	3.80 ± 0.17	4.91 ± 0.18	1.22* ± 0.07	1.37* ± 0.08	0.07
Total root system length cm	10.16 ± 0.38	11.64 ± 0.42	3.46* ± 0.13	3.66* ± 0.13	0.20

significant at * – $P < 0.05$

However, the data on the germination of wheat seeds in the treatment with the lowest concentration (1:1000) of couch grass rhizome extract are of interest. All three experimental indicators were significantly higher than in the control treatment. This concentration had a stimulating effect on the further growth of both the root system and stem. This is consistent with the literature suggesting that the allelopathic effect of allelochemicals depends on the concentration level and other factors (Novak et al., 2021; Tsytsiura, Tsaruk, 2021; Scavo et al., 2022). This also explains the contradictions in the information concerning the phytoncide effect between plants (Кобів, 2015; Лисянський, 2017; Кучерявий, 2020). It is necessary to take into account changes in the chemical composition of plants during the growing season, the presence or absence of certain substances, their percentage in the plant, the activity of metabolic processes, and the environment characteristics.

The lowest values of seedling height and root system length were recorded in the treatment with the extract concentration of 1:100. The length of the stem was by 56.6% shorter, the length of the germinal root was by 67.9% lower, and the length of the root system was by 65.9% lower than in the control treatment.

It is also important to study the obtained data on the ratio of aboveground and underground parts of wheat depending on the effect of couch grass extract. It was determined that the length of the seedling was a different proportion according to the treatments of the experiment.

According to Table 3 data, higher concentrations (1:100 and 1:10) of the couch grass rhizome extracts inhibit the growth of the root system, which should provide the plant with nutrients from the environment. Accordingly, such weakened plants will not be able to provide high yield, weed competition, and good immunity to disease.

Table 3. The ratio (%) of stem height to the root system length of wheat seedlings depending on the germination media with different concentrations of couch grass rhizome extract

Treatment	Distilled water (control)	Extract concentration		
		1:1000	1:100	1:10
Ratio	19.7:80.3	18.0:82.0	23.8:76.2	24.8:75.2***

Significant at *** – $P < 0.001$

It should be taken into account that wheat seeds also emit certain substances into the environment during germination. These compounds can chemically react with those in the aqueous environment. Their effect on further growth processes also needs to be studied. Further experiment was conducted with two treatments: the control and with a concentration of 1:10. The above-mentioned indicators were measured four days after the previous measurements. The difference in stem height between the control and the treatment with a concentration of 1:10 was 1.28 cm; it increased to 1.63 cm on the 11th day after the germination. It can be concluded that the allelopathic pressure of couch grass on wheat does not lose its inhibitory effect on the stem growth over time (Figures 1 and 2).

Within four days, the stem height increased by 81.2% in the control treatment and by 58.6% in the treatment with a 1:10 concentration of the couch grass rhizome extract. The length of the root system also reacted negatively to the presence of allelopathically active substances in the aqueous solution. The inhibitory effect on the length of the root system was even stronger, i.e., it increased by 66.4% in the control treatment and by 34.4% in the treatment with a concentration of 1:10 (Figures 1 and 3).

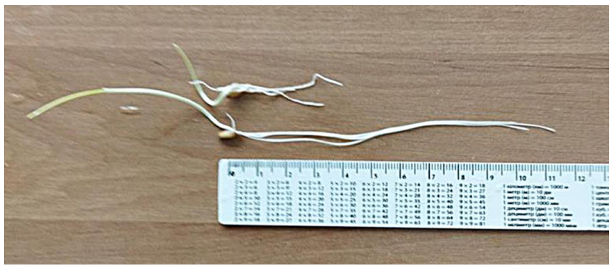
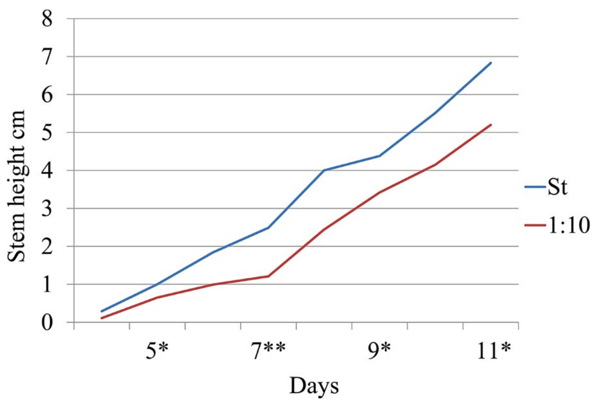


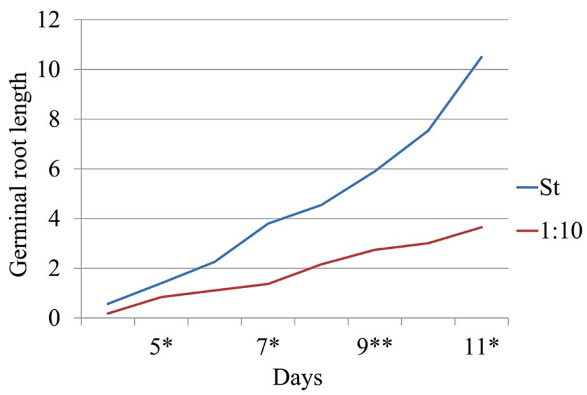
Figure 1. Typical samples of wheat seedlings of the control treatment (below) and of the treatment with a 1:10 concentration of couch grass rhizome extract – 11th day after the beginning of germination (on top)

In all experimental treatments, the indicators of germination of seeds were recorded on the 4th day.



Significant at * – $P < 0.05$ and ** – $P < 0.01$

Figure 2. Dynamics of the stem height (cm) of wheat seeds after 4–11 days germination in the water containing medium (blue line) and in the couch grass rhizome extract with a concentration of 1:10 (red line)



Significant at * – $P < 0.05$, ** – $P < 0.01$ compared to the 4th day of germination

Figure 3. Dynamics of the germinal root length (cm) of wheat after 4–11 days of germination in the water containing medium (blue line) and in the couch grass rhizome extract with a concentration of 1:10 (red line)

The seedling length was measured with a ruler at 24-hour intervals. Allelochemicals significantly inhibited the growth of both aboveground and underground wheat organs throughout the experiment.

The growth retardation of seedlings in a Petri dish was clearly visible in the treatment with a 1:10 concentration of the couch grass rhizome extract (Figure 4). It is the result of the inhibition of germination energy and growth processes. Samples in the Petri dish

Figure 4. Appearance of wheat seedlings after 11 days of germination in the water containing medium (left) and in the couch grass rhizome extract with a concentration of 1:10 (right)

(pictured right) varied in size. It means that uneven seedlings will be formed in the field. As a result, higher plants will suppress lower ones, which also suffer from the negative effects of weeds. Thus, there will be an inhibitory effect of intraspecific competition for such wheat plants.

The growth retardation of samples exposed to the extracted substances of couch grass will inevitably affect the height of plants and the formation of grain yield in the future. As a result, wheat plants will lag in growth and development at the beginning of growing in weedy areas of the field.

The allelopathic pressure created by couch grass rhizomes has a negative effect on the formation of the root system of wheat. It can cause a weak competitiveness of crops and their low productivity in the future.

Conclusions

1. The aqueous extract from the couch grass (*Elymus repens* L.) rhizomes significantly inhibits the germination energy of common wheat (*Triticum aestivum* L.) seeds. An increase in the extract concentration leads to a decrease of the germination energy to 84% and 74%.

2. The extracts of the couch grass rhizomes at concentrations of 1:100 and 1:10 had a negative effect on the initial stages of germination of wheat seeds. Such conditions also inhibit the emergence and growth of the germinal root by 52.1% and 62.5%, respectively, and the stems by 56.2% and 51.4%.

3. The treatment with the couch grass rhizome extract at a concentration of 1:1000 had a stimulating effect on the further growth of the root system and stems of wheat.

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Paprastojo varpučio (*Elymus repens* L.) alelopatinė įtaka kviečių sėklų dygimui

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Santrauka

Paprastųjų kviečių, tradicinių Ukrainos javų, atsparumas piktžolių alelopatinei įtakai ankstyvaisiais augimo tarpsniais dar nėra pakankamai ištyrinėtas. Prieštaringi duomenys apie kviečių ir varpučių cheminę sąveiką paskatino išsamiau ištirti varpučių įtaką. Tyrimo tikslas – nustatyti Vinicos srityje, Vakarų ir Pietvakarių Ukrainoje surinktų paprastojo varpučio (*Elymus repens* L.) šakniastiebių vandeninio ekstrakto poveikį paprastojo kviečio (*Triticum aestivum* L.) sėklų dygimui ir daigų augimui. Nustatyta, kad varpučių ekstraktas slopino kviečių sėklų dygimo energiją visuose eksperimento variantuose. Varpučių 1:1000 koncentracijos ekstraktas skatino kviečių sėklų dygimą ir vėlesnį augimą, palyginti su kontrolinio varianto sėklomis, daigintomis distiliuotame vandenyje. Didelės koncentracijos (1:100 ir 1:10) varpučių šakniastiebių ekstraktai kviečių daigų šaknų ilgį reikšmingai sumažino 4-tą dygimo dieną – atitinkamai 0,25 ir 0,30 cm. Septynių dienų amžiaus daigų stiebo ilgis sumažėjo 56,2 % ir 51,4 %, palyginti su kontroliniu variantu. Taigi, slopinamasis poveikis mažėjo sėklų dygimo 5–11 dienomis ir priklausė nuo varpučių šakniastiebių ekstrakto koncentracijos.

Reikšminiai žodžiai: Poaceae, šakniastiebiai, piktžolės, *Elytrigia repens*, *Agropyron repens*, alelopatija.

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