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Allelopathic effect of buckwheat extract on seedlings of selected weed species

Allelopatyczny wpływ ekstraktu z gryki na siewki wybranych gatunków chwastów

Summary. The influence of 1% aqueous extract obtained from a 14-day plants of common buckwheat (*Fagopyrum esculentum* Moench) on weed species was evaluated. Seedling roots of wild oat (*Avena fatua* L.), yellow foxtail (*Setaria glauca* L.), barnyardgrass (*Echinochloa crus galli* (L.) P. Beauv.), common windgrass (*Apera spica-venti* (L.) P. Beauv.), catchweed bedstraw (*Galium aparine* L.), scentless mayweed (*Matricaria inodora* L), gallant soldier (*Galinsoga parviflora* Cav.) and tiny vetch (*Vicia hirsuta* L.) were exposed to buckwheat extract and compared with control plants grown in water. The obtained results show that the buckwheat extract had lower influence on the growth of shoot than on roots of the evaluated weed species. The use of buckwheat extract in the medium caused the inhibition of root growth in all species except from tiny vetch. In the case of shoot of weeds, inhibition of growth by buckwheat extract occurred only in wild oat. Whereas in yellow foxtail, scentless mayweed and tiny vetch, the stimulation of shoot growth was demonstrated. These results may indicate that the buckwheat extracts act on directly exposed tissues. A different metabolic reaction of wild oat to buckwheat extract after 5 days of exposure than after 2 days may indicate a quick adaptation of wild oat seedlings to stressful conditions.

Key words: common buckwheat, aqueous extract, weeds, seedling, growth

INTRODUCTION

Worldwide intensive herbicide use has led to the evolution of herbicide-resistant weed populations which causes crop yield losses and increases production costs. Barn-

yardgrass (*Echinochloa crus galli* (L.) P. Beauv.) is one of the most economically important weeds in agriculture. Different biotypes of barnyardgrass have reported resistant to a variety of herbicides, including glyphosate [Bajwa et al. 2015]. Wild oat (*Avena fatua* L.) belongs to weeds that are the most susceptible to the development of resistance to herbicides [Beckie et al. 2012, Adamczewski et al. 2013].

The resistance of weeds to herbicides causes the search for other methods of limiting their occurrence. Allelopathic weed control methods can be a solution to this problem**.** The allelopathic influence of common buckwheat (Fagopyrum esculentum Moench) on other plants has been known for a long time [Xuan and Tsuzuki 2004, Falquet et al. 2015]. Buckwheat tissues contain many phenolic compounds having weed suppressive activity [Golisz et al. 2007, Gfeller et al. 2018]. The allelopathic influence of the common buckwheat on dicotyledonous plants was higher than on monocots [Kato-Noguchi et al. 2007]. Buckwheat is also useful for soil improvement and reduction of pests and weeds there [Xuan and Tsuzuki 2004]. According to Tominaga and Uezu [1995] decaying buckwheat residues in soil drastically reduced the biomass of *Digitaria ciliaris* and *Galinsoga ciliata*, and biomass of *Echinochloa crus-galli*, *Portulaca oleracea*, *Chenopodium album* and *Amaranthus lividus*.

Water extracts of various plants widely used as natural herbicides [Tanveer et al. 2012]. On the other hand, allelopathic water extracts application at lower concentrations stimulates germination and growth of some crops [Anwar et al. 2003]. Tissues of common buckwheat are rich in phenolic acids and flavonoids, including flavone C-glycosides [Wiczkowski et al. 2014, Wiczkowski et al. 2016]. de Bertoldi et al. [2009] have found that C-glycosides of iso-vitexine and iso-orientine completely inhibited germination of lettuce (*Lactuca sativa* L.). Also, Mioduszewska et al. [2013] have noted that aqueous extract from tissues of buckwheat inhibited the seed germination and seedling growth of winter wheat and lettuce.

The resistance of wild oat and barnyardgrass to numerous herbicides causes the search for alternative methods of limiting the population of this weeds. According to Szwed et al. [2019] the buckwheat residues in the soil inhibits the growth of several weed species, including wild oat and barnyardgrass. The purpose of this study was whether the aqueous extract obtained from buckwheat tissues has a similar allelopathic effect on seedlings of weed species grown *in vitro*. In this study, growth of weed seedling and content of some metabolites in the roots of wild oat were measured.

MATERIAL AND METHODS

Plant material

Common buckwheat (*Fagopyrum esculentum* Moench ‘Hruszowska’) and four monocots weed species: wild oat (*Avena fatua* L.), yellow foxtail (*Setaria glauca* L.), barnyardgrass (*Echinochloa crus galli* (L.) P. Beauv., and common windgrass (*Apera spica-venti* (L.) P. Beauv.) were used in the study. Besides, four dicots weed species: catchweed bedstraw (*Galium aparine* L.), scentless mayweed (*Matricaria inodora* L.), gallant soldier (*Galinsoga parviflora* Cav.) and tiny vetch (*Vicia hirsuta* L.) were evaluated. For breaking dormancy, seeds of cleavers and yellow foxtail were stratified by

storage of wet conditions in the cold (+5°C) for 14 days, and seeds of tiny vetch were scarified in concentrated sulfuric acid and rinsed five times with distilled water. Before the experiments, seeds of all plants were sterilized in 70% ethanol for 2 min, 5% sodium hypochlorite for 10 min, and then rinsed one time with 0.01 N HCl and three times with distilled water.

Preparation of 1% aqueous extract of buckwheat

Fourteen-days seedlings of common buckwheat (hypocotyls and cotyledons) were used for preparation of extract. Germination was carried out during four day in darkness at 24 ±1°C by placing buckwheat seeds between two layers of wet filter paper which were then rolled up and placed in a beaker containing tap water. Afterwards, etiolated seedlings were transferred to growth room and were grown for the next 10 days in one fifth-strength Hoagland nutrient solution. Seedlings were grown with a 16/8 h (day/night) photoperiod and temperature was maintained at 24 ±2°C/16 ±2°C (day/night). Light (100–120 μM $m^{-2} \cdot s^{-1}$) was provided by high-pressure sodium lamps (Plantaster, Osram, Germany). After this time, the aboveground parts of buckwheat seedlings (hypocotyl and cotyledons) were freeze dried, ground and used for preparation of extract. To obtain the 1% (w/v) aqueous mixture a 10 g milled freeze-dried buckwheat tissues was suspended in 1000 mL of distilled water. The mixture was left in the dark for 24 h at 4°C, occasionally stirred, centrifuged (3500 rpm), and filtered through a filter paper. The filtrate was brought up to original volume with distilled water.

In vitro tests

The weed seed germination procedure was performed similarly to germination of buckwheat seeds, and then etiolated seedlings were transferred for 3 days to a growth room (growth conditions as above). In 7-days seedlings length of primary roots and shoots was measured. In the *in vitro* experiments the effect of short-term (2 days and 5 days) treatment of weed roots with 1% aqueous extract from buckwheat was examined. The initial lengths was used for calculation of elongation of roots and shoots, i.e. the difference in length after 2 days and 5 days of treatment. Water was used instead of buckwheat extract in control plants. The measurements were carried out in 10 replicates, each contained 5–7 weed seedlings.

Biochemical analyses

In the roots of wild oat, biochemical analyzes determining degree of lipid peroxidation, leakage of electrolytes, total antioxidant capacity and peroxidase activity were carried. Total antioxidant capacity was measured by the reduction in free radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$, Sigma) using the method of Brand-Williams et al. [1995]. The activity of soluble peroxidase was determined according to the method described by Velikova et al. [2000]. The enzyme activity was expressed as a product of guaiacol dehydrogenation. The rate of the lipid peroxidation process was analyzed on the basis of measurements of the malondialdehyde (MDA) content as a result of the reaction with thiobarbituric acid (TBA) according to the method of Hodges et al. [1999]. The degree of the plasma membranes damage, expressed as a electrolytes leakage was de-

termined according to the method Uddin et al. [2014]. Analysis of the total content of phenolic compounds in the extract was carried out before its application, and after 2 and 5 days of experiment using the spectrophotometric method [Singleton et al. 1999]. The analyzes were performed in 5 replicates.

Statistical analysis

The results were analyzed by using STATISTICA 12.0 software (StatSoft, Poland). The results of growth analyses of weed seedlings were subjected to one-way analysis of variance, and chemical analyses to two-way analysis of variance (ANOVA). The comparison of mean results was carried out with post-hoc Tukey's test.

RESULTS AND DISCUSSION

The allelopathic properties of common buckwheat (*Fagopyrum esculentum* Moench) have been studied for many years [Xuan and Tsuzuki 2004, Golisz et al. 2007, Mioduszewska et al. 2013, Falquet et al. 2015]. Buckwheat residues in the soil inhibits the growth of several weed species [Szwed et al. 2019]. In our present study a substantial differences between root elongation of short-term treated and untreated weed seedlings with buckwheat extract (BE) were observed (Fig. 1 A, B, C, D). Root elongation of all species was significantly lower in weed seedlings grown in BE, than in seedlings grown in distilled water, except tiny vetch. Average root elongation after 2 and 5 days of incubation was lowered in monocot weeds by 48%, in comparison to control seedlings. Among investigated monocot species highest suppression of root growth was observed in wild oat, 66% and 69% after 2 and 5 days incubation to BE, respectively (Fig. 1 A, B). Slightly lower suppression of root growth was found in yellow foxtail: 59% after 2 days and 47% after 5 days incubation. The mean inhibitory effect of BE on root growth of monocot weed seedlings reached 52%, both after 2 days and 5 days of use of BE. The mean suppression of root elongation of dicot weed species was 40% after 2 days of incubation with BE and 26% after 5 days of use (Fig. 1 C, D).

Another effect the BE had on the growth of the aerial parts of weed seedlings. Among the species evaluated only in wild oat occurred inhibition of shoot elongation (Fig. 2 A, B, C, D). In the case of the other species, some stimulation of shoot growth was noted. Statistically significant was the phenomenon in yellow foxtail, cleavers, and tiny vetch after 5 days of treatment with BE (Fig. 2 B, D).

It is widely believed that for the allelopathic properties of buckwheat extract are responsible phenolic acids and flavonoids [Golisz et al. 2007, Heidarzade et al. 2012, Gfeller et al. 2018]. According to Wiczkowski et al. [2014] tissues of buckwheat seedlings are rich in C-flavones of luteolin and apigenin, which have high allelopathic potential [de Bertoldi et al. 2009]. Results obtained by Heidarzade et al. [2012] indicate that, cinnamic acid had the highest inhibitory activity in terms of root and shoot elongation of barnyardgrass. The tissues of buckwheat contains a high level of cinnamic acid [Wiczkowski et al. 2016]. Therefore, both C-flavones apigenin and lutein, and cinnamic acid are presumably responsible for the allelopathic properties of BE.

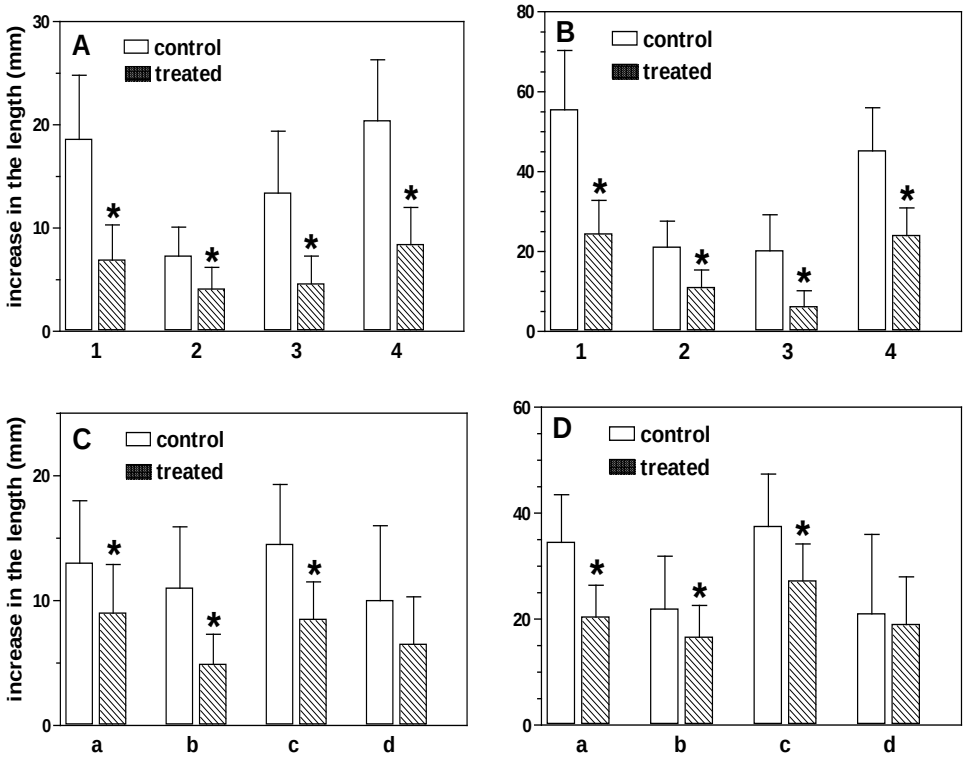

Fig. 1. Elongation of primary root of monocot (A, B) and dicot (C, D) weed species after 2-day (A, C) and 5-day (B, D) incubation with water (control) or 1% aqueous extract of common buckwheat (treated). Monocot weeds: 1 – barnyardgrass (*Echinochloa crus galli* (L.) P. Beauv.), 2 – common windgrass (*Apera spica-venti* (L.) P. Beauv.), 3 – wild oat (*Avena fatua* L.), 4 – yellow foxtail (*Setaria glauca* L.). Dicot weeds: a – scentless mayweed (*Matricaria inodora* L.), b – gallant soldier (*Galinsoga parviflora* Cav.), c – cleavers (*Galium aparine* L.), d – tiny vetch (*Vicia hirsuta* L.). Data are presented as the mean + SD. Mean results marked with (*) were significantly different from the control at $P < 0.05$ as determined by one-way analysis of variance with the Tukey's post hoc test

Growth in the early stages of crop development are considered as important in the allelopathic relationships [Jönsson et al. 1994]. In the study presented here was found that short-term incubation of weed seedlings with 1% buckwheat extract led to inhibition of their root growth. This was observed in the all evaluated weed species, except tiny vetch. Another effect of the buckwheat extract was observed in the case of shoot growth of the weed seedlings. In the seedlings of yellow foxtail, gallant soldier and tiny vetch, stimulation of the growth of shoots under the influence of buckwheat extract was found. The stimulation of plants growth by low concentrations of water extracts from other crops was earlier demonstrated, and the phenomenon of the greater impact of allelochemicals on root growth than shoots is also known [Tawaha and Turk 2003, Mushtaq et al. 2010]. According to suggestion of Esmaeili et al. [2012] and Heidarzade et al. [2012] greater suppression of root elongation than shoot elongation is a result of the direct con-

tact of root with the allelochemicals. Roots are directly affected by phytotoxins what leads to higher accumulation of allelochemicals in their tissues, thus root growth is often stronger inhibited than shoots [Tanveer et al. 2012]. During Kato-Noguchi et al. [2007] study, dicot weeds were more susceptible to the inhibitory effect of buckwheat allelochemicals than monocots. In general, the results of our experiment indicate that the buckwheat aqueous extract more intensely inhibited the growth of seedlings of evaluated monocots than dicots weeds. Similarly, extracts of *Fagopyrum tataricum* suppressed shoot and root growth the grass weeds greater than the broad-leaved species [Uddin et al. 2012]. Probably the impact of buckwheat allelochemicals on young plants is different than in older ones.

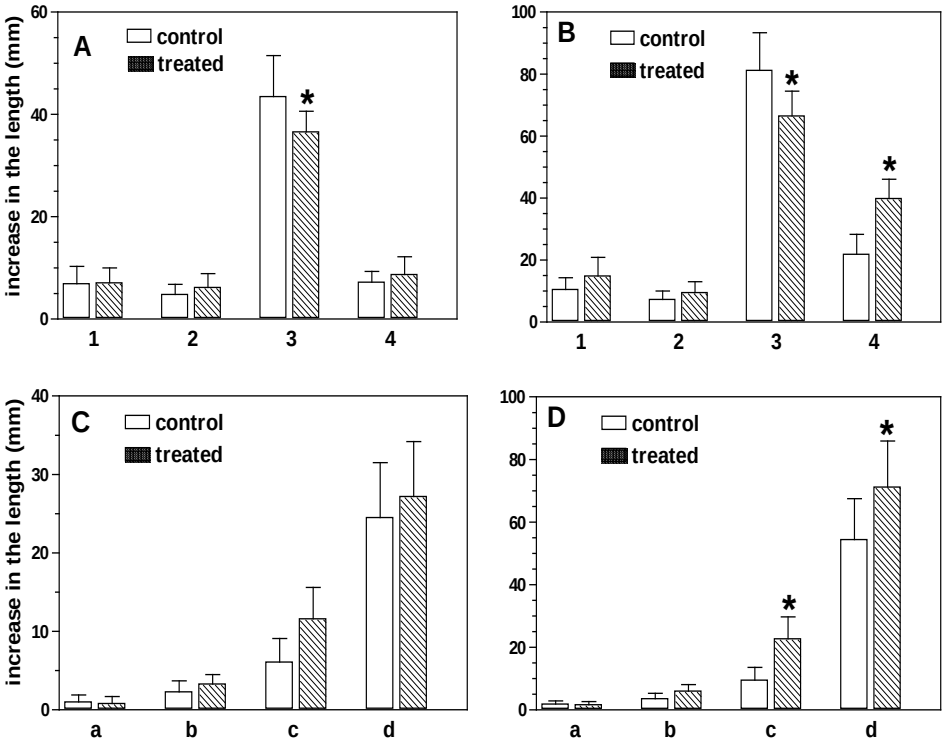

Fig. 2. Elongation of shoot of monocot (A, B) and dicot (C, D) weed species after 2-day (A, C) and 5-day (B, D) incubation with water (control) or 1% aqueous extract of common buckwheat (treated). Monocot weeds: 1 – barnyardgrass (*Echinochloa crus galli* (L.) P. Beauv.), 2 – common windgrass (*Apera spica-venti* (L.) P. Beauv.), 3 – wild oat (*Avena fatua* L.), 4 – yellow foxtail (*Setaria glauca* L.). Dicot weeds: a – scentless mayweed (*Matricaria inodora* L.), b – gallant soldier (*Galinsoga parviflora* Cav.), c – cleavers (*Galium aparine* L.), d – tiny vetch (*Vicia hirsuta* L.). Data are presented as the mean + SD. Mean results marked with (*) were significantly different from the control at $P < 0.05$ as determined by one-way analysis of variance with the Tukey's post hoc test

Table 1. Selected response indicators of wild oat (*Avena fatua* L.) roots incubated for 2 and 5 days with buckwheat extract or water (control). Data are presented as the mean ± SD. Results with the different letter were significantly different at $P < 0.05$ as determined by one-way analysis of variance; post-hoc Tukey's test

Analysed factor	Two-day incubation		Five-day incubation	
	Control (water)	Buckwheat extract	Control (water)	Buckwheat extract
Soluble peroxidase activity (nM · mg protein^{-1} · min^{-1})	77.4 ±6.0^{b}	189.0 ±23.8^{a}	166.7 ±16.4^{a}	89.3 ±6.0^{b}
Rate of electrolytes leakage (%)	6.9 ±2.8^{c}	13.3 ±2.2^{b}	23.5 ±1.5^{a}	22.5 ±1.8^{a}
Antioxidant capacity (% DPPH$^{\cdot}$ reduction)	22.4 ±1.5^{a}	23.8 ±1.0^{a}	17.2 ±2.1^{b}	11.6 ±0.7^{c}
Rate of lipids peroxidation (nM MDA · g^{-1} dry weight)	51.5 ±11.5^{ab}	55.2 ±5.4^{a}	45.3 ±2.9^{ab}	40.4 ±2.9^{b}

Due to the clear inhibitory effect of the BE on the growth of wild oat seedlings, the roots of this species were the object of biochemical analysis (Tab. 1). Root peroxidase (POD) activity increased approximately 2.5-fold after 2 days exposure to BE. However, longer 5-days incubation with the BE led to a marked decrease in POD activity in *A. fatua* roots. Rate of electrolytes leakage after 2 days incubation with BE was almost twice as high as in the roots of control plants. However, after prolonged exposure, there was no difference between the control plants *A. fatua* and those treated by BE.

The antioxidant capacity in the tissues of wild oat roots was higher after 2 days of incubation *in vitro*, than after 5 days. After 2 days of incubation, no differences were found between the control seedlings and those treated by BE. Longer treatment of the roots of *A. fatua* seedlings by BE significantly reduced their antioxidant capacity (Tab. 1). In addition, use of BE did not cause a greater impact on the rate of lipids peroxidation in BE treated and un-treated roots of wild oat seedlings.

The total content of phenolic compounds in BE at the beginning of the experiment was 28.5 mg in 100 mL solution (Tab. 2). Their content was reduced both in the presence of *A. fatua* seedlings, and without them. However, the decline of the phenolics in the presence of oat roots was much faster than in the solution without plants. Rapid absorption of phenolic compounds was also observed in the roots of wheat seedlings [Kobayashi et al. 1996]. Phenolic allelochemicals enter through the plant cells and can change the activity of certain enzymes. In our study a two-day exposure to buckwheat extract resulted in a substantial increase in peroxidase activity (POD) in roots of wild oat. These results confirm the earlier observations regarding the increase of POD activity under the influence of phenolic acids in cucumber tissues [Yu et al. 2003] and maize [Devi and Prasad 1996]. Phenolic acids taken up by roots are also source to the production of free radicals. Their oxidation by POD leads to the production of quinones, which are responsible for the generation of reactive oxygen species [Appel 1993]. Singh et al. [2009] demonstrated that caffeic acid inhibits root growth through generation of reactive

oxygen species in *Phaseolus aureus*. This is one of the reasons for the allelopathic properties of phenolic acids.

Table 2. Changes in the content of total phenolic compounds in buckwheat extracts during the experiment. Data are presented as the mean ± SD. Results with the different letter were significantly different at $P < 0.05$ as determined by two-ways analysis of variance; post-hoc Tukey's test

Duration of the experiment	Extract with wild oat seedlings	Extract without wild oat seedlings
	Content of total phenolic compounds (mg · 100 mL^{-1})	
Before the experiment	28.5 ±0.4[a]	28.3 ±0.1[a]
Two-day incubation	11.3 ±0.1[d]	24.4 ±0.2[b]
Five-day incubation	5.1 ±0.2[e]	14.7 ±0.3[c]

Rapid uptake of phenolic components contained in BE could lead to damage of cell membranes. The rate of electrolytes leakage which was almost twice higher in root tissues for 2-days exposed to BE than in the control roots indicates for such an effect of the extract (Tab. 1). After prolonged 5-day exposure to BE, no significant effect on the rate of leakage was demonstrated. The results are similar to obtained by Baziramakenga et al. [1995], who found rapid increase electrolyte leakage in intact soybean (*Glycine max* L.) seedlings after short term (12-hour) treatment with cinnamic and benzoic acids.

The reaction (POD activity, rate of leakage electrolytes) of wild oat after 5-day of exposure to BE is different than after 2 days. This may indicate the fast adaptation of this species to stress conditions. Adaptability of hexaploid wild oat to many environmental conditions including use of herbicides is known [Beckie et al. 2012, Adamczewski et al. 2013].

CONCLUSIONS

1. The obtained results indicate that the buckwheat extract had lower allelopathic influence on the growth of above-ground parts than on roots of the evaluated weed species.
2. The two-day and five-day presence of buckwheat extract in the growing medium inhibited elongation of primary root in all evaluated species except tiny vetch. In the case of shoot, inhibition of growth by buckwheat extract occurred only in wild oat.
3. In yellow foxtail, scentless mayweed and tiny vetch some stimulation of shoot elongation was demonstrated.
4. These results may indicate that the extracts act on directly exposed tissues. In addition, they may suggest a small uptake and/or transport of allelopathic compounds to other tissues than roots.
5. The metabolic reaction (peroxidase activity, as well as the rate of electrolyte leakage) of wild oat to buckwheat extract after 5 days of exposure is different than after 2 days. This may indicate a quick adaptation of wild oat seedlings to stressful conditions.

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Streszczenie. Oceniano wpływ 1% wyciągu wodnego uzyskanego z 14-dniowych roślin gryki zwyczajnej (*Fagopyrum esculentum* Moench) na siewki wybranych gatunków chwastów. Korzenie siewek owsa głuchego (*Avena fatua* L.), włośnicy sinej (*Setaria glauca* L.), chwastnicy jednostronnej (*Echinochloa crus galli* (L.) P. Beauv.), miotły zbożowej (*Apera spica-venti* (L.) P. Beauv.), przytulii czepnej (*Galium aparine* L.), maruny bezwonnej (*Matricaria inodora* L.), żółtlicy drobnokwiatowej (*Galinsoga parviflora* Cav.) i wyki drobnokwiatowej (*Vicia hirsuta* L.) inkubowano w tym ekstrakcie i porównano z roślinami kontrolnymi rosnącymi w wodzie. Uzyskane wyniki pokazują, że ekstrakt z gryki miał mniejszy wpływ hamujący na wzrost pędów niż korzeni ocenianych gatunków chwastów. Ekstrakt z gryki powodował zahamowanie wzrostu korzeni u wszystkich gatunków, z wyjątkiem wyki drobnokwiatowej. W przypadku pędów zahamowanie wzrostu przez ekstrakt z gryki wystąpiło tylko u owsa głuchego, podczas gdy u włośnicy sinej, maruny bezwonnej i wyki drobnokwiatowej wykazano stymulację wzrostu. Wyniki te mogą wskazywać, że ekstrakty z gryki działają na bezpośrednio narażone tkanki. Odmienna reakcja metaboliczna dzikiego owsa na ekstrakt z gryki po 5 dniach ekspozycji niż po 2 dniach może wskazywać na szybkie dostosowanie siewek owsa głuchego do warunków stresowych.

Słowa kluczowe: gryka zwyczajna, ekstrakt wodny, chwasty, siewki, wzrost

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