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ORIGINAL RESEARCH PAPER

Effect of aqueous extracts of selected medicinal plants on germination of windgrass [*Apera spica-venti* (L.) P. Beauv.] and lambsquarters (*Chenopodium album* L.) seeds

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Abstract
The study aimed to determine the effect of aqueous extracts of medicinal plants (*Matricaria chamomilla*, *Hypericum perforatum*, *Achillea millefolium*, and *Urtica dioica*) containing allelopathic compounds on seed germination in lambsquarters (*Chenopodium album*) and herbicide-resistant windgrass (*Apera spica-venti*). A Petri-dish experiment was carried out, in which the effects of five concentrations of aqueous extracts on the germination of weeds were assessed for 10 consecutive days. It was found that the dynamics of seed germination are closely related to the type and concentration of aqueous extract of medicinal plants. The 8% *U. dioica* aqueous extract posed the strongest inhibitory effect, limiting the germination of both lambsquarters and windgrass. Additionally, weed germination was delayed by 12–72 h in the presence of extracts, compared with the control. Summing up, the aqueous extracts of medicinal plants, especially their higher concentrations, pose a desirable inhibiting effect against the germination of lambsquarters and herbicide-resistant windgrass seeds.

Keywords
allelopathy; dynamics of germination; German chamomile; initiation of germination; St. John's wort; stinging nettle

Introduction

Biological methods of plant protection against weeds create the new direction of crop protection, which incorporates the phenomenon of allelopathy. The definition of allelopathy includes biological interactions involving plants and microorganisms [1]. The new generation of natural herbicides, produced based on allelochemicals, represent an opportunity to create the conditions for sustainable development in agriculture [2]. Therefore, more and more attention is paid to herbal plants, well known for their medicinal properties [3–5]. These plants can be a rich source of allelochemicals effective against weeds [6].

In this study, we tested four herbal species, known for their allelopathic effects: *Hypericum perforatum* [3], *Urtica dioica* [5,7,8], *Achillea millefolium* [9,10], and *Matricaria chamomilla* [4,11]. The *Hypericum perforatum* contains mainly hypericin (0.1%), but also triterpene saponins, tannins (8%), polyphenols, phenolic acids (chlorogenic, coffee, nicotine), phytosterols, essential oils, flavonoids (rutoside and quercitine; 4%) [12]. The flowers of *M. chamomilla* (*Chamomillae anthodium*) contain

essential oil (azulene, α-bisabolol, and spiroeter), carotenoids, valeric acid, salicylic acid, coumarin derivatives, tannins, and flavonoids (apigenin, quercimetrin, luteolin) [13]. The herbal material of *U. dioica* consists of leaves (*Urticae folium*) or stolons. The active compounds of *U. dioica* include tannins, large amounts of vitamins, organic acids (formic acid and acetic acid, glycolic acid, glyceric acid), carotenoids, xanthophyll, flavonoids, phytosterols (such as β-sytosterol), amine compounds (histamine, acetylcholine, serotonin), silica, easily absorbable mineral salts, and a number of other useful substances, e.g., iodine and iron [14]. Shoots of *A. millefolium*, during blooming stage, contain essential oils (cineol, azulene, proazulen, chamazulene – 0.25–0.5%) and many bitter substances, flavonoids (apigenin, luteolin), tannins (3%), furocoumarins, alkaloids, achilleine acid, isovaleric acid, and salicylic acid [15].

Natural products of new structures and new modes of action, friendly to the environment and humans, and obviously effective against weeds, are needed [1]. In the era of rapid development of resistant weed populations [16], the effect of these substances on biotypes resistant to herbicides has a practical dimension, as it can form the basis for new methods of weed management.

The aim of the study was to determine the effect of aqueous extracts of medicinal plants (*Matricaria chamomilla*, *Hypericum perforatum*, *Achillea millefolium*, and *Urtica dioica*) containing allelopathic compounds on seed germination of harmful weeds *Chenopodium album* and *Apera spica-venti*.

Material and methods

The experiment was conducted at the Experimental Station of the University of Agriculture in Krakow. Two Petri-dish experiments were set up for windgrass [*Apera spica-venti* (L.) P. Beauv.] and lambsquarters (*Chenopodium album* L.). Lambsquarters seeds were harvested in the autumn from maize fields near Krakow. Herbicide-resistant windgrass seeds were received from Prof. K. Adamczewski from the Institute of Plant Protection in Poznań. Seeds were cleaned and stored in paper bags at room temperature until the start of the experiments.

Prior to the experiment, the seeds were surface-sterilized for 3 min in a 5% aqueous solution of sodium hypochlorite. Next, the seeds were washed several times with tap water and distilled water.

The following certified, dried medicinal plants, purchased from a drugstore were used as sources of allelochemicals: herb of *H. perforatum* L., leaves of *U. dioica* L. (producer: Flos, PL), herb of *A. millefolium* L. and flower heads of *M. chamomilla* L. (producer: Herbapol in Krakow SA, Poland). Four different aqueous extracts of medicine plants were prepared from each species, at five concentrations: 0.5%, 1%, 2%, 4%, and 8% (w/v). A weighed amount of each herb was quenched with distilled water up to 100 cm^3 in glass flasks, and put aside in a dark place at a room temperature for 24 h. After this time, the extracts were filtered few times through filter paper and used immediately.

Petri dishes were lined with sterilized filter paper discs and 50 seeds of *Apera spica-venti* and *Chenopodium album* were placed into each one. Into each dish, 3 cm^3 of extract of appropriate concentration was pipetted. The control dishes consisted of seeds and distilled water. Each concentration was repeated 5 times. The dishes were placed in a growth chamber (MDF 500, Biogenet) at a temperature of 20°C/10°C with a 12/12 h photoperiod.

For 10 consecutive days, germination measurements were taken every 12 h, starting from the first 12 h following the experimental set up. The seeds were considered germinated when a radicle (2 mm long) was noticed. Germinated seeds were counted and removed from the dish. After 10 days of measurements, a viability test was performed for non-germinated seeds left in the dishes, based on the color of the embryo. To test viability, the seed was pressed by tweezers near the embryo, and if the occurring embryo was white and firm, it was considered a living embryo; yellow, brownish, or milky were recognized as dead. Non-germinated living as well as non-germinated dead seeds were counted and a proportion (in %) of germinated seeds was calculated for the each treatment.

Tab. 1 Descriptive statistics of a percent of total germinated seeds for *Apera spica-venti* and *Chenopodium album*.

Variable	Mean	*SD*	Min.	Median	Max.
Apera spica-venti					
Germinated seeds (%)	55.6	14.7	18.9	56.0	88.0
Non-germinating seeds (%)	28.5	13.8	7.6	26.1	73.6
Dead seeds (%)	15.9	8.5	1.9	13.8	38.3
Chenopodium album					
Germinated seeds (%)	67.4	15.6	8.0	68.0	98.1
Non-germinating seeds (%)	31.8	15.2	0.0	32.0	86.0
Dead seeds (%)	0.8	1.4	0.0	0.0	6.1

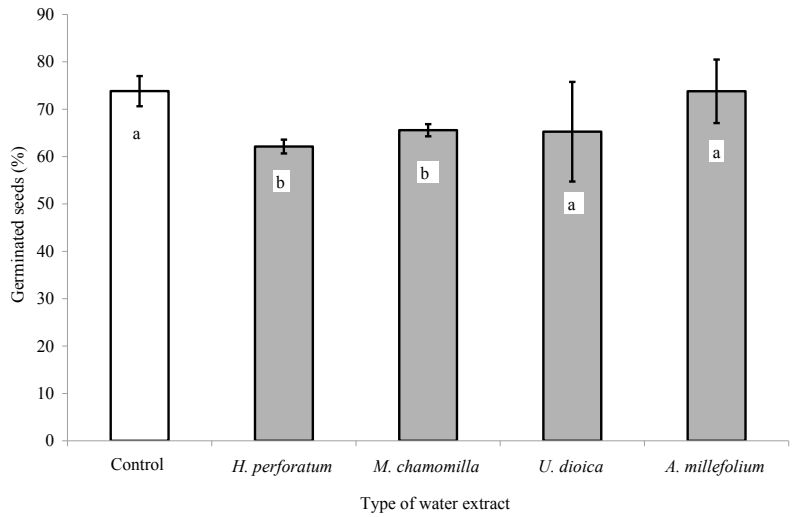

Fig. 1 Percentage of germinated seeds of *Chenopodium album* after 10 days germination process. Values indicated with different letters differ significantly, according to HSD Tukey test. One-way ANOVA *F*-test value = 4479.6; *df* = 20; $p = 0.004$.

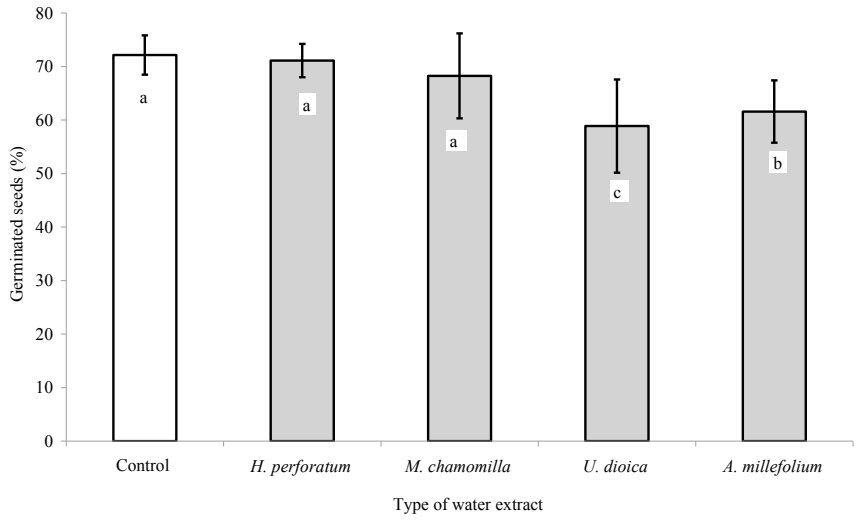

Fig. 2 Percentage of germinated seeds of *Apera spica-venti* L. after 10 days germination process. Values indicated with different letters differ significantly, according to HSD Tukey test. One-way ANOVA *F*-test value = 5279.8; *df* = 20; $p = 0.0004$.

Descriptive statistics (arithmetic mean, standard deviation, median, minimum, and maximum values), testing of the null hypothesis and a one-way analysis of variance was performed using the STATISTICA 10 software for Windows (StatSoft). Homogeneous groups were analyzed by Tukey's test, at $p \leq 0.05$.

Results

In total, slightly more than half of the *Apera spica-venti* seeds and more than 60% of the *Chenopodium album* seeds germinated (Tab. 1). The percentage of non-germinating but living seeds was similar for both species, but in the case of *A. spica-venti*, a high percentage (nearly 32%) of dead seeds was recorded. In the case of *Ch. album*, the percentage of dead seeds was very low (median = 0; Tab. 1).

On average, after 10 days of germination, particular extracts affected the germination of the tested species to a different extent. Germination in *Ch. album* and *A. spica-venti* was significantly reduced by aqueous extracts of *H. perforatum* and *M. chamomilla* (Fig. 1), and *U. dioica* and *A. millefolium* (Fig. 2), respectively.

Overall, aqueous extracts of *H. perforatum* and *M. chamomilla* inhibited the germination of *Ch. album* seeds compared to the control; however, a detailed statistical analysis of different concentrations of these extracts showed no significant effects on *Ch. album* seed germination (Tab. 2). On the other hand, analysis of the particular concentrations of the *U. dioica* extract has shown that the two highest concentrations (4% and 8%) substantially hindered the germination of *Ch. album* seeds. The least uniform results were obtained for the germination of *Ch. album* seeds in the presence of *A. millefolium* extracts:

Tab. 2 Influence of different concentrations of aqueous extracts of medicinal plants on germination of *Chenopodium album* after 10 days.

Concentration	*H. perforatum*	*M. chamomilla*	*U. dioica*	*A. millefolium*
0%	74.0 a	74.0 a	74.0 a	74.0 abc
0.5%	66.9 a	65.5 a	80.9 a	83.0 ab
1%	61.7 a	67.0 a	73.1 a	79.3 ab
2%	62.6 a	60.6 a	90.5 a	90.3 a
4%	61.7 a	67.3 a	47.4 b	61.5 bc
8%	57.7 a	67.4 a	34.5 b	54.9 c
	F-test = 2.37	*F*-test = 2.16	*F*-test = 15.7	*F*-test = 7.05
	df error = 24	*df* error = 24	*df* error = 24	*df* error = 24
	$p = 0.07$	$p = 0.09$	$p = 0.000001$	$p = 0.0004$

none of the concentrations significantly inhibited the germination of *Ch. album* seeds, compared with the control, but a significant difference was noted between lower and higher concentrations of this extract (Tab. 2).

The dynamic of *Ch. album* seed germination in the presence of various concentrations of extracts coincides with an earlier analysis. The *Ch. album* growing in the presence of water (control) and of lower concentrations of extracts started the process of germination in the 36th hour. In the presence of higher concentrations of extracts of *H. perforatum* or *M. chamomilla*, this process began 12 h later. The germination curves for higher concentrations of extracts coincided with the shape of the control curve, but the number of germinated seeds was much lower, particularly from the 84th hour of germination onward (Fig. 3a,b). The shape of the germination curves for *Ch. album* growing in the lower concentrations of *A. millefolium* or *U. dioica* extracts coincided with the control curves. In contrast, *Ch. album* seeds germinating in the presence of, 4% and 8% of each of these extracts, germinated poorly. *Chenopodium album* seeds in the presence of 8% *U. dioica* extract began to germinate at the 84th hour, 48 h later than control seeds. *Chenopodium album* seeds in the presence of 8% *A. millefolium* extract did not begin to germinate until the 72nd hour (Fig. 3d).

The seeds of sulfonylurea herbicide-resistant *A. spica-venti* reacted differently to the various concentrations of the tested extracts (Tab. 3). Compared to the control, germination was inhibited mostly by extracts at the highest concentration (8%), especially those of *U. dioica*, *A. millefolium*, and *M. chamomilla*. Extracts of *H. perforatum* showed divergent reaction in the *A. spica-venti* germination (Tab. 3).

The dynamics of *A. spica-venti* germination coincided with the results obtained for the percentage of germinating seeds. *Apera spica-venti* germinating in the presence of water (control) began to germinate at the 48th hour (Fig. 4). The germination curves for the lower concentrations (0.5–2%) of *M. chamomilla* and *H. perforatum* extracts coincided fairly well with those of the control. The seeds of *A. spica-venti* in the presence of 8% *H. perforatum* extract started germinating at the 72nd hour. These seeds initially germinated poorly compared with the others; however, in the 180th hour, the germination process was more intense (Fig. 4a). *Apera spica-venti* seeds germinating in the presence of the two highest concentrations of *M. chamomilla* extract germinated very poorly compared with other seeds (Fig. 4b). In the initial three days, all of the *A. spica-venti* seeds germinated poorly in the presence of all concentrations of *U. dioica* extract, compared to the control. Later (at 120 hour), however, seeds germinating in the presence of lower concentrations of *U. dioica* extract (0.5–2%), germinated similarly to the controls. The poorest germination of *A. spica-venti* seeds was noted in the presence of *U. dioica* at a concentration of 8%; the germination process started in the 132nd hour and did not exceed 17 germinated seeds at the 228 hour (Fig. 4c). In the 4% and 8% of *A. millefolium* extracts, seeds of *A. spica-venti* began germination 24 and 48 h later, respectively, compared to control (Fig. 4d).

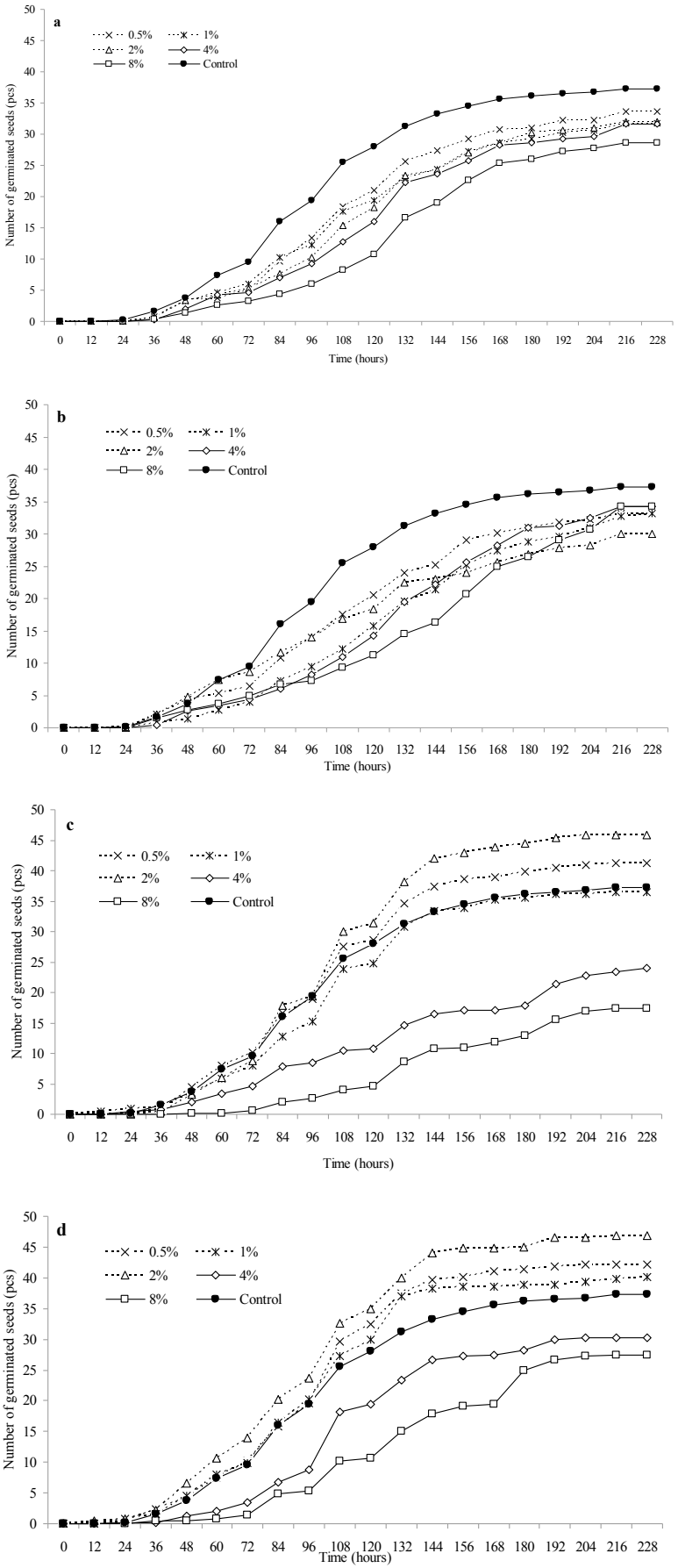

Fig. 3 Dynamics of *Chenopodium album* germination in the presence of different concentrations of aqueous extracts of medicinal plants: *H. perforatum* (**a**), *M. chamomilla* (**b**), *U. dioica* (**c**), *A. millefolium* (**d**).

Tab. 3 Influence of different concentrations of aqueous extracts of medicinal plants on germination of *Apera spica-venti* after 10 days.

Concentration	*H. perforatum*	*M. chamomilla*	*U. dioica*	*A. millefolium*
0%	72.2 ab	72.2 ab	72.2 a	72.2 a
0.5%	70.7 ab	75.7 ab	68.5 ab	65.4 a
1%	77.3 a	87.4 a	71.8 a	72.1 a
2%	61.9 b	73.9 ab	71. 7 a	71.9 a
4%	78.5 a	64.0 b	56.5 b	57.8 a
8%	67.1 ab	40.2 c	25.9 c	40.8 b
	F-test = 3.80	*F*-test = 16.4	*F*-test = 34.8	*F*-test = 12.7
	df error = 24	*df* error = 24	*df* error = 24	*df* error = 24
	$p = 0.01$	$p < 0.001$	$p < 0.001$	$p = 0.000004$

Discussion

In the present study, we demonstrated the impact of aqueous extracts of medicinal plants on the germination of *Ch. album* and herbicide-resistant *A. spica-venti* seeds. For *Ch. album*, the most inhibitory aqueous extracts were those of *U. dioica* and *A. millefolium*, at concentrations of 4% and 8%. In the case of *A. spica-venti*, the greatest inhibition was noted in 8% extracts of *U. dioica* and *M. chamomilla*. At lower concentrations however, the extract either had no effect or was slightly stimulatory. As suggested by Khan et al. [5], the dose of allelochemicals is very important to the allelopathic effect. Our results are also in agreement with others [17,18] who pointed to the stimulatory effect of lower doses of allelochemicals. According to Komorowska et al. [7], the stimulatory effect of allelochemicals may also result from the fact that in the early stages of plant development, some allelochemicals may stimulate plant growth. On the other hand, the absence of an effect of aqueous extracts of medicinal plants on weeds germination may be explained by the lack of allelopathic substances in a cold-water extract as well as a relatively short time of action of such extracts [19]. This would explain the initial inhibition and later (after 6 days) increase in *A. spica-venti* germination in the presence of lower concentrations of *U. dioica* extract.

For both of the tested weeds, the least influence on germination was displayed by the aqueous extract of *H. perforatum*. Alipour et al. [20] showed inhibitory properties of the extracts of *H. perforatum* on the germination of *Ch. album*, but this applied to a much higher concentration of extract (10–20%) than those used in our study.

In addition to the inhibition of germination, the highest concentrations of aqueous extracts of medicinal plants significantly delayed the time of initiation of the germination process in *Ch. album* and *A. spica-venti*. This phenomenon can have a measurable effect, because, under field conditions, competitive crops may be favored over these weed species. As was shown by O'Donovan et al. [21] the later weed emergence in a relation to crop emergence the lower crop-yield losses.

Conclusions

The inhibitory or stimulatory effects of aqueous extracts of *H. perforatum*, *M. chamomilla*, *U. dioica*, and *A. millefolium* on the seed germination of *Ch. album* and herbicide-resistant *A. spica-venti* depended on the concentrations of the extracts. The highest inhibition of *Ch. album* and *A. spica-venti* seeds germination was observed in 8% aqueous extracts of *U. dioica*, *M. chamomilla*, and *A. millefolium*. Low concentrations (0.5–2%) of aqueous extracts had no effect on weed germination. Both weed species, in the presence of aqueous extracts, delayed germination by 12–36 h for *Ch.*

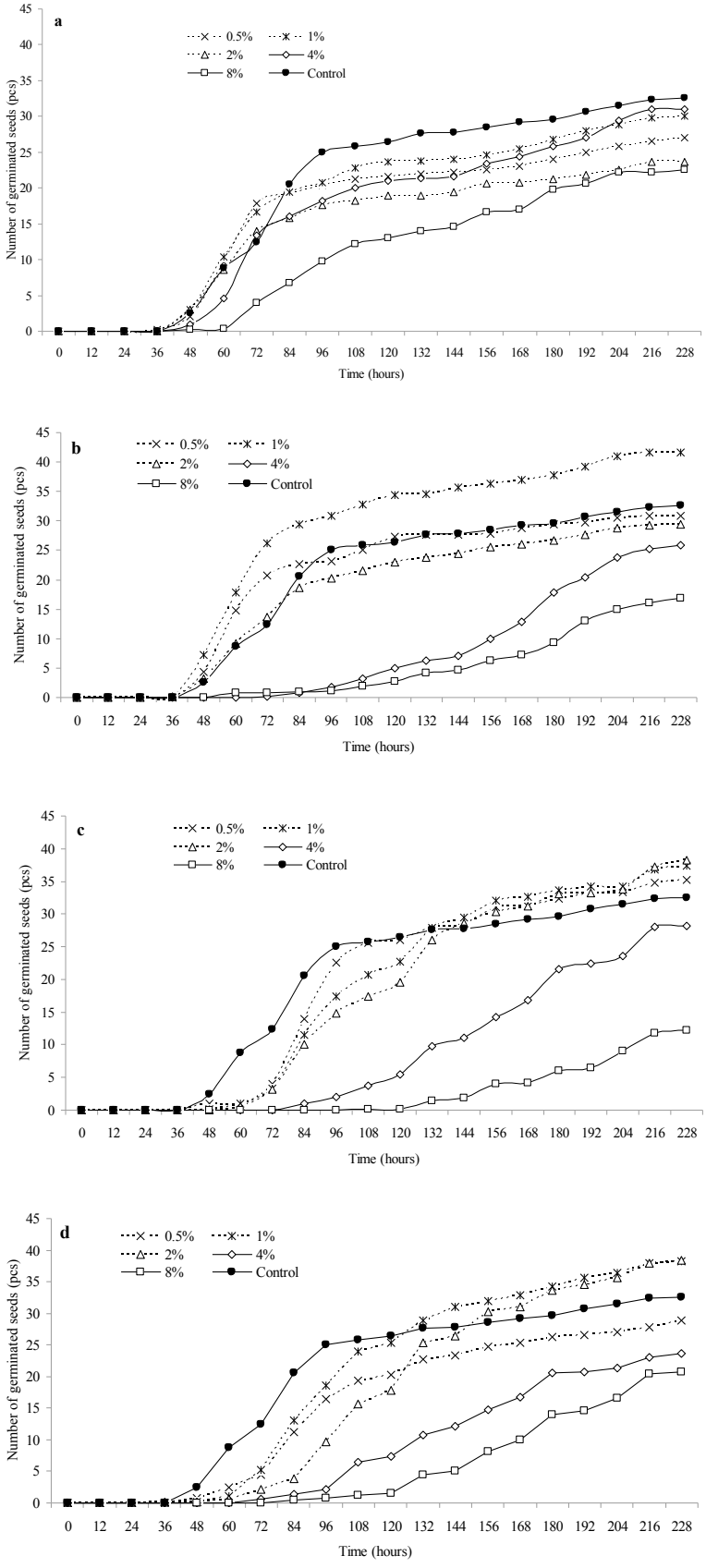

Fig. 4 Dynamics of *Apera spica-venti* germination in the presence of different concentrations of aqueous extracts from medicinal plants: *H. perforatum* (**a**), *M. chamomilla* (**b**), *U. dioica* (**c**), *A. millefolium* (**d**).

album and by 12–72 h for *A. spica-venti*. The initiation of germination was delayed the most by 8% aqueous extract of *U. dioica*. We conclude, that the delayed germination of weeds may favor the competitive abilities of crops, which is of a high importance especially in the case of herbicide-resistant *A. spica-venti*, when herbicides cannot be applied.

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Wpływ wodnych wyciągów z wybranych ziół na kiełkowanie nasion miotły zbożowej [*Apera spica-venti* (L.) P. Beauv.] i komosy białej (*Chenopodium album* L.)

Streszczenie

Celem badań była ocena wpływu wyciągów wodnych z ziół (*Matricaria chamomilla*, *Hypericum perforatum*, *Achillea millefolium* i *Urtica dioica*), zawierających związki allelopatyczne, na kiełkowanie nasion komosy białej i odpornej na herbicydy miotły zbożowej. Przeprowadzono doświadczenie szalkowe, w którym oceniano kiełkowanie nasion roślin testowych przez 10 kolejnych dni. Stwierdzono, że dynamika kiełkowania nasion zależy zarówno od donora związków allelopatycznych, jak i stężenia wyciągu. Najsilniej kiełkowanie nasion obu gatunków hamował 8% wyciąg wodny z ziela pokrzywy. Ponadto w obecności wyciągów stwierdzono opóźnioną o 12–72 h inicjację procesu kiełkowania, w porównaniu do nasion kiełkujących w obecności wody. Konkludując, wyciągi z ziół a szczególnie wyższe ich stężenia, wykazują pożądane właściwości allelopatyczne względem komosy białej jak i odpornego na herbicydy biotypu miotły zbożowej.