

Response of 33 weed species germination to allelocompounds contained in sunflower (*Helianthus annuus* L.)

DOROTA CIARKA, MARTA STANKIEWICZ-KOSYL*, MARIKA SZTYBER, HELENA GAWRONSKA, STANISŁAW W. GAWRONSKI

Laboratory of Basic Research in Horticulture, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences – SGGW

Abstract: *Response of 33 weed species germination to allelocompounds contained in sunflower (Helianthus annuus L.).* The inhibitory effect of sunflower on weeds has been reported in the literature, but most studies deal with a limited number of species. The objective of this work was to study the effect of allelocompounds contained in leaf extracts (at five concentrations: 1.25, 2.5, 5, 7.5 and 10% DM w/v) of the ‘Ogrodowy’ variety of sunflower on the germination of 33 weed species. In response to allelocompounds derived from sunflower, the germination of the weeds, with few exceptions, was delayed and reduced, with complete inhibition in some cases. The response depended on the weed species and on the concentration used. Among weed species, the greatest reduction was observed for *Papaver rhoeas* L., with full inhibition recorded at a concentration of just 5% DM w/v. Complete inhibition was also found for other weed species, but at higher concentration. By contrast, almost no inhibitory effect of sunflower allelocompounds was recorded for *Vicia cracca* L. and *Echinochloa crus-galli* (L.) Beauv.

Key words: germination, *Helianthus annuus* L., Piper Index, phytotoxicity, aqueous extract, weed species

INTRODUCTION

Allelopathic effects on agro-ecosystems and on their exploitation in agriculture have been considered [Bárberi 2002, Bhowmik and Inderjit 2003] with regard

to using crops known for their strong allelopathic potential [Wu et al. 1999] as an alternative strategy for weed management [Weston 1996]. There are already several examples of crops being used for satisfactory weed suppression with minimal or even zero herbicide use [Hoffman et al. 1996a,b, Leather 1983a]. One of the crops often listed among species that exhibit strong allelopathic activity is sunflower (*Helianthus annuus* L.) [Irons et al. 1982, Leather 1983a, b, Leather 1987, Purvis 1990, Purvis and Jones 1990, Narwal 1999, Batish et al. 2002, Azania et al. 2003]. Although the inhibitory effect of sunflower on weeds is quite well documented [Leather 1983a, b, Morris and Parrish 1992, Macias et al. 1999], most authors deal with a relatively limited number of species, while the list of weeds accompanying crops in the field is usually quite long. Moreover, the results obtained so far are sometimes contradictory, which might be due to (i) differing experimental conditions, and/or (ii) the use of different genotypes of both donors and acceptors of allelochemicals. Besides, local populations of the same species can differ in terms of both the allelopathic activity of the donor plants

*e-mail: marta_stankiewicz_kosyl@sggw.pl

and in the responses of acceptors. Therefore, it seems to be important to perform a comparative study with a wide range of weed species under the same conditions using a donor plant with the same genetic background.

The objective of this work was to evaluate the response of 33 weed species to allelochemicals contained in aqueous extracts of leaves of the 'Ogrodowy' variety of sunflower (*Helianthus annuus* L.) under uniform experimental conditions.

MATERIAL AND METHODS

A total of 33 weed species, commonly occurring among cereal crops in Central Europe, were used as acceptors of allelochemicals, and sunflower as their donor. The following weed species were tested: *Achillea millefolium* L. (8), *Aegopodium podagraria* L. (13), *Amaranthus retroflexus* L. (17), *Anthemis arvensis* L. (28), *Apera spica-venti* (L.) Beauv. (21), *Avena fatua* L. (14), *Capsella bursa-pastoris* (L.) Medicus (22), *Centaurea cyanus* L. (30), *Chenopodium album* L. (15), *Cirsium arvense* L. (12), *Consolida regalis* Gray (29), *Convolvulus arvensis* L. (16), *Daucus carota* L. (18), *Echinochloa crus-galli* (L.) Beauv. (33), *Elymus repens* (26), *Conyza canadensis* L. (5), *Erodium cicutarium* (L.) L'Hérit (2), *Galeopsis tetrahit* L. (23), *Galium aparine* L. (3), *Lamium purpureum* L. (6), *Matricaria inodora* L. (25), *Papaver rhoeas* L. (1), *Polygonum convolvulus* L. (9), *Polygonum persicaria* L. (7), *Raphanus raphanistrum* L. (19), *Rumex crispus* L. (4), *Setaria glauca* (L.) Beauv. (20), *Sinapis arvensis* L. (31), *Stellaria media*

(L.) Vill. (24), *Taraxacum officinale* Weber (27), *Thlaspi arvense* L. (10), *Vicia cracca* L. (32), and *Viola arvensis* Murray (11). For the sake of simplicity, in Figure 1, each scientific name has been assigned a number (in parentheses).

The allelopathic activity of sunflower on the tested weed species was evaluated based on the effect of water-extracted allelochemicals in a germination biotest. For the extracts, air-dried (denoted here as DM) leaves of field-grown sunflower of the 'Ogrodowy' variety were used. The extracts were prepared as described by Ciarka et al. [2009]. Weed seeds were placed on Petri dishes layered with filter paper moistened with 5 ml of extract at five concentrations (1.25, 2.5, 5, 7.5 and 10% DM w/v) and with water only (control). The seeds were cultured in a cabinet, in darkness, for 14 days at 20 or 25°C (the tested weed species naturally germinate at different times of the vegetation season, thus in the experiment different temperatures were applied for germination). Germinating seeds were counted daily and removed. Due to the natural uneven germination of the weeds, the data are expressed as percentages of the number of seeds germinating in H₂O (the control).

For each weed species and extract concentration, six replicates were used, with 100 seeds per replication (Petri dish). With regard to the large number of Petri dishes needed for evaluation of the 33 weed species and the five concentrations (more than 1000 dishes in total) the tests were performed in several series. To ensure the comparability of results between series, fresh extracts were prepared for every series, and germination

in H₂O (the control) was conducted in parallel with each series.

The results obtained were analysed statistically using the ANOVA function of Statgraphics Plus 4.1 (Statistical Graphics Corp., USA), and differences between combinations were estimated by Tukey's Honestly Significant Difference (HSD) test. Values of HSD or indices (following values) are given when the differences were significant at $P \leq 0.05$. Data are presented as mean \pm SE, $n = 6$.

RESULTS AND DISCUSSION

The results showed that the germination of all 33 tested weed species was

affected by allelochemicals contained in aqueous extracts of the 'Ogrodowy' variety of sunflower. With few exceptions, germination was delayed and reduced. However, the range of these responses depended on the weed species and the concentration.

In response to leaf extracts, on average for the five concentrations used, 18 out of 33 weed species germinated at a rate of below 25% compared with germination under control conditions (Fig. 1).

A further 13 species germinated at a rate of between 25 and 50%, but two species, *V. cracca* and *E. crus-galli*, were only slightly affected, with 81% germination, comparable to germination in

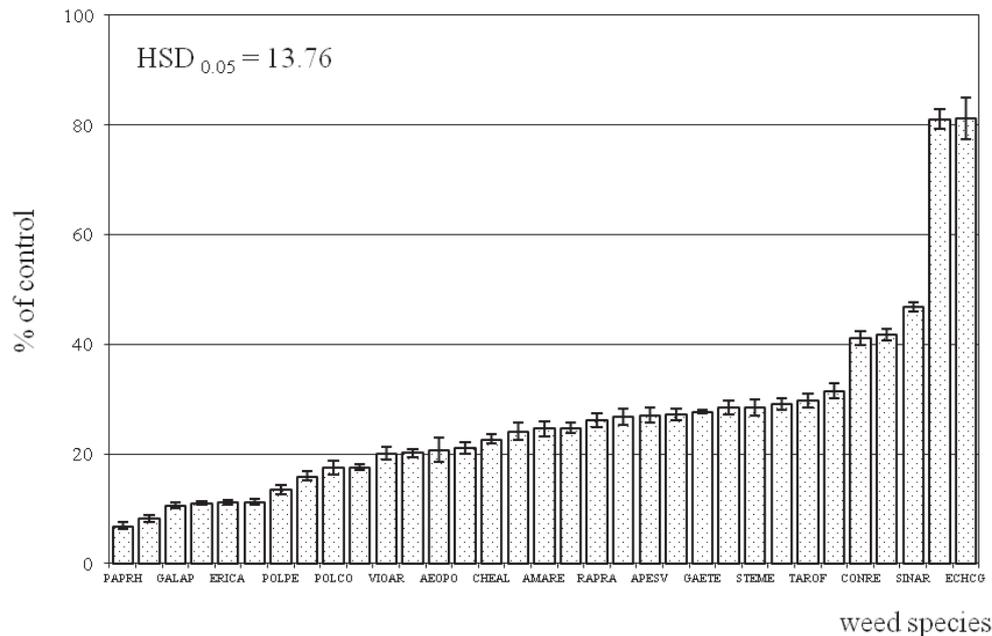


FIGURE 1. Germination of 33 weed species in the presence of allelochemicals contained in aqueous extracts of leaves of the 'Ogrodowy' variety of sunflower (*Helianthus annuus* L.) evaluated during 14 days of culture. Due to natural uneven weed germination, the data are expressed as percentages of the number germinating in the control (H₂O). Significant differences were estimated by Tukey's HSD test at $P \leq 0.05$. Data are given as the average for all five concentrations, \pm SE, $n = 6$ with 100 seeds in each

H₂O (Fig. 1). It is noteworthy that at the highest concentration of extract, the reduction in seed germination was strong for almost all of the tested weed species (Table 1).

The greatest reduction in germination was recorded for *P. rhoeas*, for which the average germination rate for all concentrations was 6.9% (compared with the control), and already at a concentration of 5% germination was fully inhibited. Complete inhibition of germination was also observed for other weed species, but at higher concentrations (13 species at 7.5% and 18 species at 10%) – Table 1.

The effect of allelochemicals contained in stem extracts was evidently weaker for all tested species. On average for all concentrations used, only two weed species – *P. rhoeas* and *D. carota* – germinated at a rate below 25%, and for a further 19 species the rate was between 25 and 50%. Allelochemicals derived from sunflower stems hardly influenced *V. cracca* and *S. arvensis*, as these germinated at a rate of 86%. It is interesting to note that germination of *E. crus-galli*, which was only slightly inhibited by leaf extracts, was in fact stimulated in the presence of stem extract in concentrations of 1.25 and 2.5% DM w/v (35.6 and 7% more seeds germinated than in the control). At the same concentrations a slight stimulation of germination was also recorded for *C. regalis* (data not shown).

These results correspond well to those of Leather [1983a], who similarly showed not only that sunflower stem extract was less effective than leaf extract, but that in some cases weed germination was even stimulated.

On the other hand, the results of the present study, except in the case of *E. crus-galli*, do not support the conclusions that seeds of grass weeds are unaffected by sunflower [Leather 1983b] or that sunflower is less effective against species of the family Poaceae [Azania et al. 2003]. In our study the germination rate (on average for five concentrations) of four species of the family Poaceae – *E. repens*, *A. spica-venti*, *S. glauca* and *A. fatua* – was reduced, by an amount ranging from 71.6 to 78.9% depending on species in the case of leaf extracts (Fig. 1). With the highest extract concentrations, seeds of these four species either did not germinate at all or else germination was very strongly reduced (by 82–96%). The results for the noxious weed *E. crus-galli*, which also belongs to the family Poaceae, are in agreement with results reported by Azania et al. [2003] and Leather [1983b].

It is noteworthy that allelopathic effects depend also on the organs used for extract preparation. In another study performed by us it was clearly shown that the negative impact of allelochemicals derived from stems, petioles and inflorescences, in terms of both reduction and delay of seed germination, was less evident than in the case of leaf extracts (data not presented).

Narwal [1999], in a review of sunflower allelopathy, reported that aqueous extracts from sunflowers inhibited the germination of the following weed species: *A. theophrasti* L., *D. stramonium* L., *Ipomoea* spp., *Brassica kaber* Wheeler, *Trianthema portulacastrum* L., *Amaranthus viridis* L., *Portulaca oleracea* L., *Flaveria australasica* L.

TABLE 1. Germination of 33 weed species in the presence of allelochemicals contained in aqueous extracts of leaves of the 'Ogrodowy' variety of sunflower (*Helianthus annuus* L.), at five extract concentrations, evaluated during 14 days of culture

Weed species	Concentration of air-dried leaves (% DM w/v)				
	1.25	2.5	5.0	7.5	10.0
<i>Papaver rhoeas</i>	17.1 b	17.1 b	0.0 a	0.0 a	0.0 a
<i>Erodium cicutarium</i>	38.6 b	1.4 a	1.1 a	0.0 a	0.0 a
<i>Galium aparine</i>	37.5 c	10.4 b	4.7 ab	0.4 a	0.0 a
<i>Rumex crispus</i>	34.0 d	18.2 c	3.0 b	0.0 a	0.0 a
<i>Erigeron canadensis</i>	26.9 d	19.5 c	9.6 b	0.0 a	0.0 a
<i>Lamium purpureum</i>	26.6 c	18.6 bc	10.8 b	0.0 a	0.0 a
<i>Polygonum persicaria</i>	43.1 c	22.0 b	2.4 a	0.0 a	0.0 a
<i>Achillea millefolium</i>	44.6 c	26.2 b	9.0 a	0.0 a	0.0 a
<i>Polygonum convolvulus</i>	41.9 c	27.5 bc	18.1 b	0.0 a	0.0 a
<i>Thlaspi arvense</i>	56.4 b	45.8 b	36.5 b	0.0 a	0.0 a
<i>Viola arvensis</i>	51.6 b	38.7 b	9.0 a	1.3 a	0.0 a
<i>Cirsium arvense</i>	54.2 c	37.2 b	9.2 a	0.5 a	0.0 a
<i>Aegopodium podagraria</i>	51.8 c	37.9 bc	13.9 a	0.0 a	0.0 a
<i>Avena fatua</i>	54.3 c	37.1 c	13.2 b	0.9 a	0.0 a
<i>Chenopodium album</i>	40.6 c	34.9 c	17.1 b	14.7 b	6.3 a
<i>Convolvulus arvensis</i>	38.5 c	29.6 bc	29.0 bc	17.4 ab	6.5 a
<i>Amaranthus retroflexus</i>	73.9 c	44.3 b	5.1 a	0.0 a	0.0 a
<i>Daucus carota</i>	65.1 b	48.7 b	9.6 a	0.0 a	0.0 a
<i>Raphanus raphanistrum</i>	63.7 c	39.0 b	16.0 a	5.8 a	6.3 a
<i>Setaria glauca</i>	57.7 c	51.2 c	19.7 b	4.3 a	1.0 a
<i>Apera spica-venti</i>	58.6 d	37.4 c	21.2 bc	14.0 ab	4.1 a
<i>Capsella bursa-pastoris</i>	52.7 c	34.7 b	30.1 b	18.4 b	0.0 a
<i>Galeopsis tetrahit</i>	64.4 d	51.8 c	15.9 b	5.8 a	0.5 ac
<i>Stellaria media</i>	71.8 b	55.1 b	15.3 a	0.0 a	0.0 a
<i>Matricaria inodora</i>	63.1 d	42.8 c	13.6 bc	12.7 ab	10.3 a
<i>Elymus repens</i>	49.6 c	34.8 c	32.0 b	20.3 a	8.7 a
<i>Taraxacum officinale</i>	59.8 d	36.0 c	32.7 c	19.6 b	0.6 a
<i>Anthemis arvensis</i>	64.7 d	50.7 c	31.0 b	8.5 c	2.7 c
<i>Consolida regalis</i>	76.8 a	54.1 a	49.3 a	25.7 a	0.0 a
<i>Centaurea cyanus</i>	87.4 a	67.6 a	40.9 b	10.4 c	2.7 c
<i>Sinapis arvensis</i>	80.8 c	68.6 c	48.8 b	25.6 a	10.3 a
<i>Vicia cracca</i>	93.3 c	86.8b c	81.0 abc	77.6 ab	66.8 a
<i>Echinochloa crus-galli</i>	89.4 a	87.3 a	83.1 a	79.0 a	66.9 a

Due to natural uneven weed germination, the data (mean of six replicates with 100 seeds in each) are expressed as percentages of the control (H₂O). Values for a given weed species followed by different letters differ significantly according to Tukey's HSD test at $P \leq 0.05$. Highest and lowest values are bolded.

The allelopathic effect of sunflower extract was demonstrated also by changes in the dynamics of weed germination. A delay in germination in response to allelochemicals derived from sunflower was noted for all tested weed species, with a few exceptions at lower concentrations. According to Piper Index values, the number of days needed for one seed to germinate in H₂O ranged from 2.1 to 8 days depending on weed species, while for those exposed to allelochemicals it ranged from 3.8 to 11 days (leaf extract) – Table 2. It must be taken into consideration that at the higher extract concentrations, some species did not germinate at all during the entire experiment (14 days of culture).

For leaf extract, at just 2.5% DM w/v for *E. cicutarium*, and at 5% DM w/v for *E. cicutarium*, *P. rhoeas* and *P. persicaria*, the PI was not determined because of lack of germination. At the higher extract concentrations (7.5 and 10% DM w/v) no PI was determined for 18 and 21 weed species respectively. At the highest extract concentrations at which germination took place, the greatest delay in germination was recorded for *A. retroflexus* (by 5.6 days), and the smallest for *A. spica-venti* and *E. crus-galli* (by 1 day), when compared with the respective controls (Table 2).

In the case of stem extract, values of PI were not determined only for 1 and 9 weed species at concentrations of 7.5 and 10% respectively. The greatest delay was observed in the case of *A. retroflexus*, for which one seed took as much as 7.2 days longer to germinate than in H₂O. The smallest delay in the presence of stem extract was recorded for *S. arvensis*, where germination was delayed only by 0.1 day (data not shown).

The reduction and delay in weed germination reported here can be attributed to chemical, most often toxic, effects of compounds contained in the extracts, this being the basis of the phenomenon of allelopathy. This assumption is based on results of other studies performed at our laboratory, which attempted to evaluate certain physical properties of the extracts used: osmotic potentials, pH, viscosity and EC. Those results showed that changes in the above parameters, along with increased extract concentration, could not generate such severe responses. Moreover, in accompanying studies, mustard germination in the presence of NaCl and PEG 8000, at osmotic potentials corresponding to the extracts used, was reduced either very slightly (by less than 10%) or not at all. Therefore, we may conclude that the negative impact of aqueous extracts of sunflower, as recorded in this study, has a chemical, toxic mode of action, this being the basis of the phenomenon of allelopathy (data not shown, paper in preparation).

Delays in weed seed germination in response to allelochemicals based on observations have been reported in the literature [Narwal 1999, Xuan and Tsuzuki 2004], but an evaluated and statistically analysed delay expressed in number of days, particularly in the case of sunflower, is according to our best knowledge reported here for the first time.

CONCLUSIONS

1. Allelocompounds derived from sunflower leaf extracts, at the concentrations used, generate allelopathic stress leading to delayed and reduced germination.

TABLE 2. Values of Piper Index for 33 weed species seeds germinated in the presence of allelochemicals contained in aqueous extracts from leaves of the 'Ogrodowy' variety of sunflower (*Helianthus annuus* L.)

Weed species	Control (H ₂ O)	Concentration of air-dried leaves (% DM w/v)				
		1.25	2.5	5.0	7.5	10.0
<i>Achillea millefolium</i>	5.8 a	6.0 a	6.2 a	6.5 a	n.d. l	n.d.
<i>Aegopodium podagraria</i>	7.0 a	7.4 ab	7.7 ab	8.2 b	n.d.	n.d.
<i>Amaranthus retroflexus</i>	2.1 a	4.3 ab	7.5 b	7.7 b	n.d.	n.d.
<i>Anthemis arvensis</i>	5.6 a	6.3 ab	6.6 ab	6.6 ab	7.6 b	7.8 b
<i>Apera spica-venti</i>	7.3 a	7.5 a	7.6 ab	8.0 b	8.3 b	8.3 b
<i>Avena fatua</i>	4.9 a	5.3 ab	5.8 bc	6.6 c	n.d.	n.d.
<i>Capsella bursa-pastoris</i>	8.0 a	8.9 ab	8.9 ab	9.1 b	9.5 b	n.d.
<i>Centaurea cyanus</i>	5.1 a	5.2 a	5.3 a	5.3 a	5.8 a	6.6 a
<i>Chenopodium album</i>	4.9 a	6.7 b	7.1 bc	7.4 bc	7.6 c	7.8 c
<i>Cirsium arvense</i>	5.5 a	5.7 a	5.8 a	6.0 a	n.d.	n.d.
<i>Consolida regalis</i>	7.2 a	7.6 ab	7.8 ab	10.4 b	n.d.	n.d.
<i>Convolvulus arvensis</i>	4.4 a	6.5 ab	6.6 ab	7.2 b	7.9 b	8.1 b
<i>Daucus carota</i>	5.0 a	8.5 b	8.9 b	8.9 b	n.d.	n.d.
<i>Echinochloa crus-galli</i>	3.6 a	3.9 ab	4.2 ab	4.3 ab	4.6 b	4.6 b
<i>Elymus repens</i>	6.6 a	7.0 a	7.0 a	7.2 a	n.d.	n.d.
<i>Erigeron canadensis</i>	5.0 a	5.2 a	6.4 ab	7.3 b	n.d.	n.d.
<i>Erodium cicutarium</i>	4.0 a	4.3 a	n.d.	n.d.	n.d.	n.d.
<i>Galepsis tetrahit</i>	6.8 a	7.4 a	7.5 a	7.9 ab	8.8 b	n.d.
<i>Galium aparine</i>	7.7 a	8.8 a	8.9 a	11.9 b	n.d.	n.d.
<i>Lamium purpureum</i>	4.3 a	7.8 b	8.5 b	8.6 b	n.d.	n.d.
<i>Matricaria indora</i>	7.5 a	8.7 ab	8.7 ab	9.2 b	9.3 b	9.6 b
<i>Papaver rhoeas</i>	4.0 a	5.0 b	5.4 b	n.d.	n.d.	n.d.
<i>Polygonum convolvulus</i>	6.1 a	6.2 a	7.2 ab	7.7 b	n.d.	n.d.
<i>Polygonum persicaria</i>	5.5 a	6.8 ab	7.3 b	n.d.	n.d.	n.d.
<i>Raphanus raphanistrum</i>	4.6 a	5.3 ab	5.4 ab	5.7 ab	6.2 ab	6.7 b
<i>Rumex crispus</i>	5.9 a	6.3 a	7.0 a	7.3 a	n.d.	n.d.
<i>Setaria glauca</i>	7.0 a	7.9 ab	7.8 ab	7.9 ab	8.0 ab	11.0 b
<i>Sinapis arvensis</i>	2.9 a	3.8 ab	3.9 ab	4.1 b	5.7 c	5.8 c
<i>Stellaria media</i>	5.3 a	5.7 a	6.2 ab	8.8 b	8.8 b	8.9 b
<i>Taraxacum officinale</i>	5.8 a	6.0 a	6.6 a	6.6 a	6.7 a	n.d.
<i>Thlaspi arvense</i>	6.6 a	8.3 b	8.5 b	9.4 c	n.d.	n.d.
<i>Vicia cracca</i>	4.6 a	6.3 b	6.5 bc	6.5 bc	7.3 c	7.5 c
<i>Viola arvensis</i>	7.3 a	8.2 a	8.7 a	9.0 a	n.d.	n.d.

n.d. – not determined.

Values for a given weed species followed by different letters differ significantly according to Tukey's HSD test at $P \leq 0.05$. Highest and lowest values are bolded.

2. The 33 evaluated weed species significantly differ in their range of response to sunflower leaf extract allelocompounds, with *Erodium cicutarium* and *Papaver rhoeas* being most affected and *Echinochloa crus-galli* and *Vicia cracca* least affected.
3. The response to water-extracted allelocompounds of all tested weed species showed a dependence on concentration.

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- Streszczenie:** Reakcja 33 gatunków chwastów na allelozwiązki pochodzące ze słonecznika (*Helianthus annuus* L.). Słonecznik jest wymieniany w literaturze jako roślina o silnym potencjale allelopatycznym w stosunku do chwastów, większość doniesień dotyczy jednak ograniczonej liczby ich gatunków. Celem pracy była ocena efektu allelozwiązków zawartych w wyciągach z liści (w pięciu stężeniach: 1,25, 2,5, 5, 7,5 i 10% s.m. m/v) słonecznika odmiany 'Ogrodowy' na kiełkowanie 33 gatunków chwastów. W reakcji na allelozwiązki pochodzące ze słonecznika kiełkowanie chwastów, z wyjątkiem kilku gatunków, było opóźnione i obniżone, a w niektórych przypadkach całkowicie zahamowane. Reakcja zależała od gatunku chwastu i stężenia wyciągu. Przy obu typach wyciągów natężenie efektu inhibicyjnego było proporcjonalne do stężenia. Spośród ocenianych gatunków chwastów najsilniej zareagował *Papaver rhoeas* L., którego kiełkowanie zostało całkowicie zahamowane już przy stężeniu 5%. Efekt taki został osiągnięty również dla innych gatunków chwastów, ale przy wyższych stężeniach. Jednakże dla *Vicia cracca* L. i *Echinochloa crus-galli* (L.) Beauv. jedynie niewielki efekt hamujący wyciągów został zaobserwowany.