

Assessment of allelopathic potential of *Solidago gigantea* Aiton on dry weight of *Echinochloa crus-galli* (L.) Beauv. and *Amaranthus retroflexus* L.

Ocena potencjału allelopatycznego *Solidago gigantea* Aiton w odniesieniu do suchej masy *Echinochloa crus-galli* (L.) Beauv. oraz *Amaranthus retroflexus* L.

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Abstract

Laboratory analyses using the 1st generation bioassay were conducted in the years 2013-2014 to investigate the allelopathic potential of wateralcoholic and aqueous extracts from dry weight of rhizomes and roots as well as stems and leaves of *Solidago gigantea*. Analysed acceptors were two weed species, i.e. monocotyledonous *Echinochloa crus-galli* and dicotyledonous *Amaranthus retroflexus*. When the acceptors (*E. crus-galli* and *A. retroflexus*) reached the 2-leaf stage (BBCH 12) they were sprayed with wateralcoholic and aqueous extracts (at concentrations of 12.5%, 10%, 5% and 2.5%) obtained from the donor, i.e. *S. gigantea*. Results indicate an inhibitory effect of wateralcoholic extracts from aboveground parts (leaves and stems) of *S. gigantea* in relation to dry weight of *E. crus-galli* and *A. retroflexus*. The volume of dry weight reduction in acceptors was dependent on the concentration of extracts produced from the donor plant *S. gigantea*. Dry weight of *E. crus-galli* and *A. retroflexus* was reduced most effectively by two concentrations: 12.5% and 10%. In turn, aqueous extracts from rhizomes and roots of *S. gigantea*, irrespective of the applied concentration, caused an increase in dry weight of *E. crus-galli* and *A. retroflexus*. Only aqueous extracts produced from leaves and stems of *S. gigantea*, irrespective of their concentration, reduced dry weight in only *E. crus-galli*.

Keywords: allelopathic potential, cockspur grass (*E. crus-galli*), common amaranth (*A. retroflexus*), dry weight, giant goldenrod (*S. gigantea*), wateralcoholic and aqueous extracts, weeds

Streszczenie

W badaniach laboratoryjnych przeprowadzonych z użyciem biotestu I generacji w latach 2013-2014, porównywano potencjał allelopatyczny wyciągów wodno-alkoholowych oraz wodnych uzyskanych z suchej masy kłaczy i korzeni oraz łodyg i liści *Solidago gigantea*. Akceptorami były dwa gatunki chwastów: jednoliścienny *Echinochloa crus-galli* oraz dwuliścienny *Amaranthus retroflexus*. W monocyklu uzyskania przez akceptory (*E. crus-galli* i *A. retroflexus*), fazy 2 liści właściwych wykonano oprysk wyciągiem wodno-alkoholowym oraz wodnym, o stężeniu 12,5%, 10%, 5% i 2,5%, otrzymanym z donora, którym było *S. gigantea*. Uzyskane wyniki badań wskazują na efekt inhibicyjnego oddziaływania wyciągów wodno-alkoholowych sporządzonych z nadziemnych części roślin (liści i łodyg) *S. gigantea* w odniesieniu do suchej masy *E. crus-galli* oraz *A. retroflexus*. Wielkość redukcji suchej masy akceptorów uzależniona była od stężenia wyciągów sporządzonych z rośliny donorowej *S. gigantea*. Najefektywniej suchą masę *E. crus-galli* oraz *A. retroflexus* redukowały dwa stężenia: 12,5% oraz 10%. Natomiast wyciągi wodne sporządzone z kłaczy i korzeni *S. gigantea*, niezależnie od zastosowanego stężenia, powodowały zwiększenie suchej masy *E. crus-galli* oraz *A. retroflexus*. Jedynie wyciągi wodne otrzymane z liści i łodyg *S. gigantea*, niezależnie od stężenia, powodowały redukcję suchej masy *E. crus-galli*.

Słowa kluczowe: chwastnica jednostronna (*E. crus-galli*), chwasty, nawłóć olbrzymia (*S. gigantea*), potencjał allelopatyczny, sucha masa, szarłat szorstki (*A. retroflexus*), wyciąg wodno-alkoholowy i wodny

Streszczenie szczegółowe

Eksperyment przeprowadzono w latach 2013-2014 przy użyciu testu I generacji, który obejmował 3 niezależne serie doświadczeń laboratoryjnych po 3 powtórzenia w każdej serii. W badaniach wykorzystano wodno-alkoholowe oraz wodne wyciągi sporządzone z suchej masy łodyg i liści oraz korzeni i kłaczy nawłoci olbrzymiej (*S. gigantea*). Jako gatunki testowe wykorzystano dwa pospolite chwasty występujące najczęściej w łanie kukurydzy: jednoliścienny chwastnicę jednostronną (*Echinochloa crus-galli*) oraz dwuliścienny szarłat szorstki (*Amaranthus retroflexus*). W fazie 2 liści *E. crus-galli* i *A. retroflexus*, wykonano oprysk wcześniej sporządzonym wyciągiem wodno-alkoholowym oraz wodnym, o stężeniu 12,5%, 10%, 5% i 2,5%, uzyskany z łodyg i liści oraz korzeni i kłaczy *S. gigantea*. Obiekt kontrolny stanowiły rośliny *E. crus-galli* i *A. retroflexus* na które nie nanoszono cieczy opryskowej. Eksperyment miał na celu zbadanie wpływu skuteczności działania (inhibicyjnego lub stymulującego) wyciągu wodno-alkoholowego i wodnego, uzyskanych z *S. gigantea* na wielkość suchej masy dwóch chwastów: *E. crus-galli* oraz *A. retroflexus*. Oceną redukcji bądź stymulacji suchej masy testowanych gatunków chwastów wykonano po

28 dniach od momentu oprysku, poprzez ścięcie, wysuszenie w suszarce w temperaturze 105 °C, a następnie zważeniu na wadze analitycznej. Następnie z uzyskanych wyników, obliczano procentowy ubytek bądź przyrost suchej masy roślin *E. crus-galli* oraz *A. retroflexus* w porównaniu do obiektu kontrolnego. Otrzymane wyniki badań posłużyły do sporządzenia wykresów, na których przedstawiono efekt zastosowania różnych stężeń wyciągów wodno-alkoholowych oraz wodnych sporządzonych z suchej masy łodyg i liści oraz korzeni i kłaczy *S. gigantea* w odniesieniu do wielkości suchej masy: *E. crus-galli* oraz *A. retroflexus*. Uzyskane wyniki badań wskazują na efekt inhibicyjnego oddziaływania wyciągów wodno-alkoholowych sporządzonych z nadziemnych części roślin (liści i łodyg) *S. gigantea* w odniesieniu do suchej masy *E. crus-galli* oraz *A. retroflexus*. Wielkość redukcji suchej masy tych chwastów uzależniona była od stężenia wyciągów oraz części roślinnych *S. gigantea* z których sporządzono ciecz roboczą. Najsilniej suchą masę *E. crus-galli* oraz *A. retroflexus* redukowały dwa stężenia: 12,5% oraz 10%. Natomiast w przypadku cieczy roboczej uzyskanej z wyciągu wodnego, sporządzonego z kłaczy i korzeni *S. gigantea*, stwierdzono (niezależnie od zastosowanego stężenia), zwiększenie suchej masy *E. crus-galli* oraz *A. retroflexus*. Najsilniejszy efekt stymulacji suchej masy *E. crus-galli* był obserwowany po zastosowaniu roztworu wodnego 12,5% oraz 2,5% roztworu wodno-alkoholowego i wodnego uzyskanego z kłaczy i korzeni *S. gigantea*. Natomiast najsilniejszy efekt stymulacji suchej masy *A. retroflexus*, obserwowano po aplikacji najmniejszego stężenia (2,5%) roztworu wodnego i wodno-alkoholowego uzyskanego z korzeni i kłaczy *S. gigantea*.

Introduction

Introducing plants other than those naturally occurring into the agroecosystems was and still is motivated mostly by economic benefits. While the intentions behind the introduction of such species are of course good, the final result of this decision is often negative. Alien species intentionally introduced to agroecosystems may become invasive species. It is assumed (according to the “tens rule”) that every tenth introduced species gets out of control, and only one in ten of such species become naturalized. In contrast, only one out of ten naturalized species may become invasive species (Williamson and Fitter, 1996). Many invasive species in their natural habitat are not so extremely invasive, e.g. *Solidago* spp. in North America.

In Poland, three species of *Solidago* spp. were introduced: *S. gigantea*, *S. canadensis* and *S. graminifolia* (Dajdok and Pawlaczyk, 2009; Dajdok and Śliwiński, 2009). Currently, two of them: *S. gigantea* and *S. canadensis* are widely spread all over the country (mostly in the south-western Poland), only in the north-eastern part their population is much lower (Tokarska-Guzik, 2005; Rola and Rola, 2010; Szymura and Szymura, 2013). Why this kind of species becomes highly invasive after introducing into different ecosystem? Theories trying to explain this issue are numerous, but only a few of them could explain this phenomenon. One of the most cited hypothesis indicates an empty ecological niche, but even more popular are other theories, e.g. lack of natural enemies, or the allelopathic effect (Mack et al., 2000; Sakai et al., 2001; Callaway and Ridenour, 2004; Hierro et al., 2005).

It seems, however, that such strong expansion of these species results mainly from their intense growth, large quantities of seeds and allelochemical compounds released into the soil, which have inhibitory impact on soil microflora and plants (Cornelius, 1990; Abilasha et al., 2008; Tang et al., 2009; Zhang et al., 2009).

Allelochemicals released to soil by donor species (including *Solidago* spp.) and having inhibitory impact on seeds of other plant species (acceptors) are discussed in many scientific papers (Bing-Yao et al., 2006; Sekutowski et al., 2012a; Baličević et al., 2015). However, there are few articles on the inhibitory or stimulating effect of water and alcohol extracts prepared from donor leaves or roots and applied on leaves of acceptor plants (Yang et al., 2007; Kieć and Wieczorek, 2009; Sekutowski et al., 2012b).

The aim of this study was to evaluate the effectiveness of the hydroalcoholic and aqueous extracts obtained from *S. gigantea* in relation to the dry weight of two weeds: *E. crus-galli* and *A. retroflexus*.

Materials and methods

The experiment was conducted in 2013-2014 using the 1st generation bioassay (Sekutowski, 2011). The tests included 3 independent series of laboratory experiments with 3 repetitions in each series. The tests were carried out using hydroalcoholic and aqueous extracts from dry weight of rhizomes and roots as well as stems and leaves of *S. gigantea*, obtained from its natural habitat.

Phase I (sowing the weed seeds) – first the substrate was prepared using a specially formulated peat-mineral mixture with pH=6.5 and sand with grain size of 0.6-0.8 mm - mixed in ratio 2:1. Then the seeds of *E. crus-galli* were sown followed by seeds of *A. retroflexus* in the amount of 8 seeds per pot having a diameter of 5 cm. After 14 days of sowing, thinning was performed to leave 5 plants in each plot. After a further 7 days, plants *E. crus-galli* and *A. retroflexus* reached the expected development phase - 12 in BBCH scale (Adamczewski and Matysiak, 2005).

Phase II (preparation of extracts) – both hydroalcoholic and aqueous extracts were prepared from 125 g, 100 g, 50 g and 25 g of crushed dry weight (leaves and stems, rhizomes and roots) of *S. gigantea* in 1,000 cm³ of solvent. The specified amount of crushed dry weight of leaves and stems, rhizomes and roots of *S. gigantea*, was treated with hydroalcoholic solution of 5% MeOH (option I) and distilled water (option II), leaving this extract in the dark for 24 h. After this period, the hydroalcoholic and aqueous extracts were filtered through filter paper obtaining a working fluid of appropriate concentrations (12.5%, 10%, 5% and 2.5%), which was subsequently used for the application (Table 1). The spraying was performed in phase 2 on leaves of *E. crus-galli* and *A. retroflexus* in “Aporo” spray chamber. The reference samples included plots with plants *E. crus-galli* and *A. retroflexus*, which were not sprayed with extracts.

Table 1. Experimental variant of wateralcoholic and aqueous extracts from dry weight of rhizomes and roots as well as stems and leaves of *S. gigantea*Tabela 1. Kombinacje roztworów wodno-alkoholowych i wodnych zastosowanych w doświadczeniu uzyskanych z suszu z łodyg i liści oraz korzeni i kłączy *S. gigantea*

	Treatments Obiekty	Concentration Stężenie
K	Control Kontrola	0%
	<i>S. gigantea</i> (roots and rhizomes) <i>S. gigantea</i> (korzenie i kłączy)	12.5%
		10%
Wwa	Wateralcoholic extract Wyciąg wodno-alkoholowy	5%
		2.5%
		12.5%
	Aqueous extract	10%
Ww	Wyciąg wodny	5%
		2.5%
	<i>S. gigantea</i> (stalks and leaves) <i>S. gigantea</i> (łodygi i liście)	12.5%
		10%
Wwa	Wateralcoholic extract Wyciąg wodno-alkoholowy	5%
		2.5%
		12.5%
	Aqueous extract	10%
Ww	Wyciąg wodny	5%
		2.5%

Phase III (performing and completing the experiment) – in this phase, pots with plants *E. crus-galli* and *A. retroflexus* were placed in a greenhouse, where they were kept for 28 days in controlled conditions ((temperature 25 °C (±5 °C), humidity 70% (±5%)). After 28 days of applying the extracts, the dry weight of the aboveground

parts of plants *E. crus-galli* and *A. retroflexus* was determined by cutting these parts off and drying them in a dryer at 105 °C, this was followed by weighing dry weight on an analytical balance. Then the percentage loss in dry weight was calculated comparing results to the reference samples of *E. crus-galli* and *A. retroflexus*. Gained results were analysed statistically, using ARM8 programme, comparing differences between the means for each variant for the level of significance $P \leq 0.05$.

Results and discussion

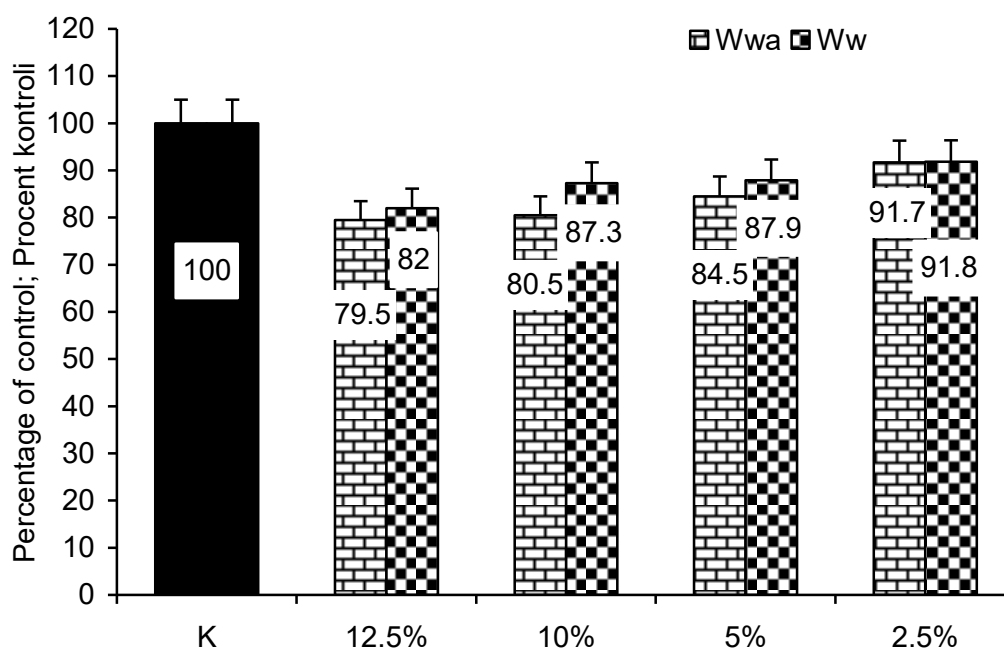
Experiment was conducted in the laboratory conditions using the 1st generation bioassay to evaluate the allelopathic potential of hydroalcoholic and aqueous extracts prepared from dry weight of rhizomes and roots as well as stems and leaves of *S. gigantea* applied on two weed species: *E. crus-galli* and *A. retroflexus*.

The literature on this topic reveals that species of *Solidago* spp. have very strong effect (usually inhibitory) on other species of plants growing in their proximity (Yang et al., 2007; Tang et al., 2009; Zhang et al., 2009). Probably, the strong phytotoxic effect on other species results from secondary metabolites generated by *Solidago* (e.g. polyacetylenes, diterpenoids, saponins, phenols or essential oils), which in favourable environmental conditions may reveal the allelochemical properties (Inose et al., 1991; Lu et al., 1993; Lu et al., 1995; Tori et al., 1999; Choi et al., 2004; Lendl and Reznicek, 2007).

Acceptor – cockspur grass (*E. crus-galli*)

The inhibitory impact of extracts prepared from leaves and stems of *S. gigantea*, on the dry weight of *E. crus-galli* was dependent on the solvent used and the concentration of the extract. A significant reduction of dry weight was obtained after using the hydroalcoholic extract with the concentration of 12.5%, 10% and 5%. Dry weight of *E. crus-galli* for the above three concentrations was reduced respectively by 20.5%, 19.5% and 15.5% compared to reference samples. A similar trend was also observed after applying the aqueous extract, while the only difference was that the reduction in dry weight of *E. crus-galli*, was lower and amounted to 18%, 12.7% and 12.1%, respectively. The concentration of 2.5% resulted in the lowest, but still statistically proven, difference. This concentration caused (regardless of the solvent used) the reduction of dry weight of *E. crus-galli* at the level of 8% compared to reference samples (Figure 1).

The literature indicates that the leaves are considered to have the highest amount of allelochemical compounds that may be released to the environment through evaporation, leaching or decomposition in the soil (Wójcik-Wojtkowiak et al., 1998; Sturz and Christie, 2003; Gniazdowska et al., 2004). Research carried out by Pisula and Meiners (2010), indicates that applying aqueous extract obtained from leaves of *S. graminifolia* resulted in the strongest inhibitory effect on *Lactuca sativa*. When the acceptor plant was changed to *Raphanus sativus*, it turned out that the strongest inhibitory effect was obtained after applying aqueous extracts from the leaves of *S. canadensis* and *S. gigantea*.



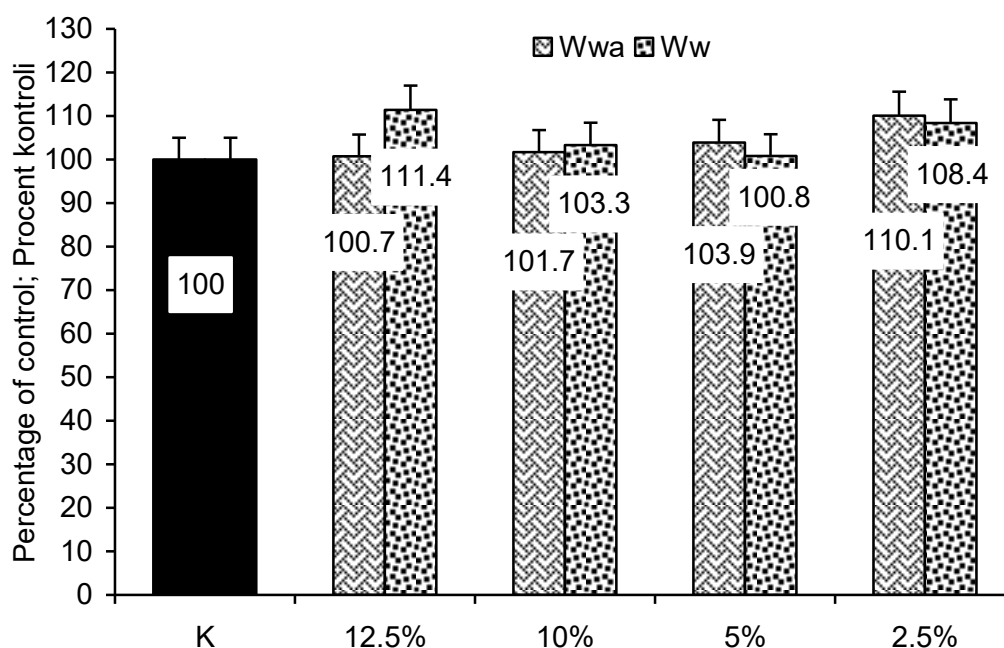
K – control, Wwa – wateralcoholic extract, Ww – aqueous extract
 K – kontrola, Wwa – wyciąg wodno-alkoholowy, Ww – wyciąg wodny

Figure 1. Effect of wateralcoholic and aqueous extracts from leaves and stems of *S. gigantea* on dry weight of *E. crus-galli*

Rysunek 1. Wpływ wodno-alkoholowych oraz wodnych wyciągów z liści i łodyg *S. gigantea* na suchą masę *E. crus-galli*

Stimulating effect on the dry weight of *E. crus-galli* was obtained after applying hydroalcoholic and aqueous extracts prepared from the roots and rhizomes *S. gigantea*. Also, in this case, the stimulating effect was dependent on the concentration of the extract. The largest increase in the dry weight of *E. crus-galli*, was noted after applying the aqueous extract at a concentration of 12.5%. The increase amounted to 11.4% and was confirmed statistically. Similar results with respect to the stimulation of dry weight were also achieved using the lowest concentrations of extract, 2.5%. When compared to the reference samples, the increase of the dry weight for this concentration was 10.1% (hydroalcoholic extract) and 8.4% (aqueous extract). The other two concentrations of 10% and 5% also caused an increase in dry weight of *E. crus-galli*, but differences obtained (vs. reference samples) were not statistically validated (Figure 2).

Research carried out by Baličević et al. (2015) indicates that aqueous extracts obtained from the leaves of *S. gigantea* may stimulate the seedlings of some weed species to faster growth, e.g. *Abutilon theophrasti*.



K – control, Wwa – wateralcoholic extract, Ww – aqueous extract

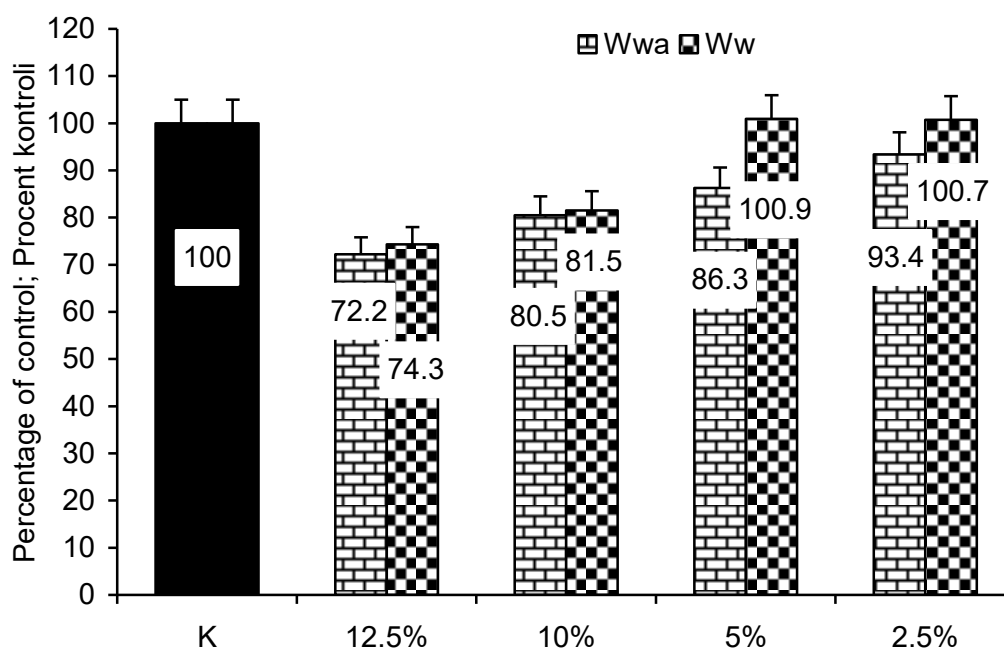
K – kontrola, Wwa – wyciąg wodno-alkoholowy, Ww – wyciąg wodny

Figure 2. Effect of wateralcoholic and aqueous extracts from rhizomes and roots of *S. gigantea* on dry weight of *E. crus-galli*

Rysunek 2. Wpływ wodno-alkoholowych oraz wodnych wyciągów z korzeni i kłaczy *S. gigantea* na suchą masę *E. crus-galli*

Acceptor – common amaranth (*A. retroflexus*)

Extracts made from the leaves and stems of *S. gigantea* showed inhibitory effects to *A. retroflexus*. The reduction of dry weight was dependent on the solvent used and the concentration of the extract. A significant reduction of dry weight was obtained after using the hydroalcoholic extract with the concentration of 12.5%, 10% and 5%. Dry weight of *A. retroflexus* for the above three concentrations was reduced respectively by 27.8%, 19.5% and 13.7% compared to reference samples. A similar trend was observed after applying the aqueous extract, however the only difference was that the reduction in the dry weight of *A. retroflexus* was caused only by two concentrations (12.5%, 10%) and amounted to: 25.7% and 18.5% when compared to reference samples. On the other hand, concentrations of 5% and 2.5% compared to reference samples, did not influence the amount of dry weight of *A. retroflexus* (Figure 3).



K – control, Wwa – wateralcoholic extract, Ww – aqueous extract
 K – kontrola, Wwa – wyciąg wodno-alkoholowy, Ww – wyciąg wodny

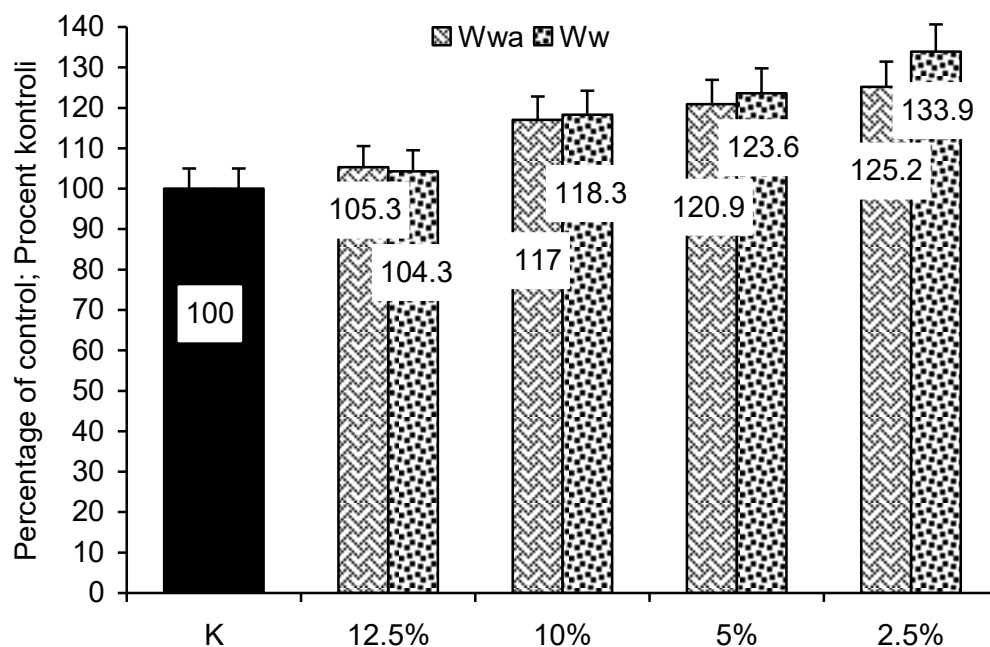
Figure 3. Effect of wateralcoholic and aqueous extracts from leaves and stems of *S. gigantea* on dry weight of *A. retroflexus*

Rysunek 3. Wpływ wodno-alkoholowych oraz wodnych wyciągów z liści i łodyg *S. gigantea* na suchą masę *A. retroflexus*

Research carried out by Kieć and Wieczorek (2009) on the suitability of various extracts and potions for reducing bioweight of *Chenopodium album*, indicates that the most of them have inhibitory properties. In addition, research carried by Baličević et al. (2015) reveal that aqueous extracts from the leaves of *S. gigantea* may inhibit the sprouting of *A. retroflexus*, *Daucus carota*, *Coriandrum sativum* or crop plants i.e. *Hordeum sativum*. Similarly, promising results were obtained by Nimbale et al. (1996), who applied water extracts from the stems and leaves of *Sorghum bicolor* to control weeds. It turned out that the main allelopathin of *S. bicolor* - sorgoleon, inhibited the growth of some weeds. The concentration of 100 μ M inhibited by 80% the growth of *Solanum nigrum*, *A. retroflexus* and *Ambrosia artemisiifolia*, and by 40% the growth of *Cassia obtusifolia*, *Digitaria sanguinalis* and *A. theophrasti*.

Hydroalcoholic and aqueous extracts prepared from roots and rhizomes of *S. gigantea* showed stimulating effects on *A. retroflexus*. The extent of the stimulation was dependent on the concentration of the extract and to a lesser degree on the solvent used. The largest increase in dry weight of *A. retroflexus* compared to the reference sample was obtained after using the concentration of 2.5%. In case of hydroalcoholic extract this increase amounted to 25.2%, while for aqueous extract it was 33.9%. Similar results with respect to the stimulation of dry weight were also achieved using the extracts at the concentration of 5% and 10%. When compared to the reference samples, the increase of the dry weight for these two concentrations

was respectively 20.9% and 17% (hydroalcoholic extract) and 23.6% and 18.3% (aqueous extract). The highest concentration of 12.5% resulted in a highest increase of dry weight of *A. retroflexus*, but the obtained differences (compared to reference samples) were not statistically significant (Figure 4).



K – control, Wwa – wateralcoholic extract, Ww – aqueous extract
K – kontrola, Wwa – wyciąg wodno-alkoholowy, Ww – wyciąg wodny

Figure 4. Effect of wateralcoholic and aqueous extracts from rhizomes and roots of *S. gigantea* on dry weight of *A. retroflexus*

Rysunek 4. Wpływ wodno-alkoholowych oraz wodnych wyciągów z korzeni i kłaczy *S. gigantea* na suchą masę *A. retroflexus*

Similar results were also obtained Bing-Yao et al. (2006), who used in their research aqueous and ethanol extracts prepared from rhizomes of *S. canadensis*. The tests carried out by these researchers show that ethanol extract with the lowest concentration, increased the germination of seeds of *Brasica napus var. napus*. Also, the length of coleoptile was dependent upon the concentration of the extract and the solvent (water, ethanol). The strongest effect of significantly extended coleoptile of *B. napus var. napus* and *Convolvulus arvensis*, was obtained by the researchers also after applying the lowest concentrations of aqueous and ethanol extracts.

Conclusions

The obtained results indicate that the inhibitory or stimulating effect on the dry weight of tested test weeds was dependent on the applied solvent, the extract concentration and parts of the donor plant *S. gigantea*.

The strongest inhibitory effect in relation to dry weight of the two acceptors: *E. crus-galli* and *A. retroflexus*, was obtained after using 12.5% and 10% of hydroalcoholic solution prepared from leaves and stems of *S. gigantea*. In contrast, the stimulating effect on the dry weight of *E. crus-galli* was observed after applying aqueous solution of 12.5% and hydroalcoholic and aqueous solution of 2.5% prepared from rhizomes and roots of *S. gigantea*. However, the strongest stimulating effect on the dry weight of *A. retroflexus*, was observed after applying the lowest concentration (2.5%) of aqueous and hydroalcoholic solution prepared from roots and rhizomes of *S. gigantea*.

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