Original scientific paper
Оригиналан научни рад
UDC 582.998.16:633.16
DOI 10.7251/AGREN2303105M

University of Banjaluka, Faculty of Agriculture
Agro-knowledge Journal

Allelopathic effect of ragweed (*Ambrosia artemisiifolia* L.) on seed germination and early seedling growth of barley (*Hordeum vulgare* L.) and white clover (*Trifolium repens* L.)

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Abstract

Allelopathy is a unique phenomenon in nature that refers to direct or indirect, positive or negative, impact of one plant (including microorganisms, insects, and herbivores) on another plant through chemical secretions (allelochemicals). *Ambrosia artemisiifolia* (ragweed) is a well-known invasive plant possessing allelochemicals that have been found to inhibit the growth and development of plants in their immediate environment. This study aimed to determine the allelopathic impact of the aqueous leaf extract isolated from ragweed leaves (concentrated extract, 1:2, 1:4, and 1:8) on seed germination and early seedling growth of barley (*Hordeum vulgare* L.) and white clover (*Trifolium repens* L.). The examined aqueous extract of ragweed had a higher inhibitory effect on the germination of white clover seeds compared to barley. The germination of barley seeds was the lowest at the concentrated leaf extract (up to 70%), while the same process in barley seeds was not determined at this concentration. In the other treatments, germination was significantly lower in both examined species compared to the control. Shoot and root growth of both researched species was inhibited in all treatments compared to the control. The obtained results indicate that the aqueous ragweed extract significantly inhibits the growth of roots and shoots of both tested species, which increases the need for more intensive research on this topic.

Key words: Ambrosia artemisiifolia, barley, white clover, allelopathy, aqueous leaf extract, germination

Introduction

Allelopathy includes the positive or negative, but also the direct or indirect action of one plant, fungus, or microorganism on another by the release of various chemical compounds called allelochemicals (Rice, 1984). Allelochemicals can affect the composition of the weed flora, the growth and yield of crops, and can be potentially used as a weed control measure (Singh et al., 2001; Bonea et al., 2018; Kincel et al., 2019). Some studies (Bertin et al., 2003; Jabran et al., 2015) have confirmed that allelochemicals can be found in different concentrations in various plant organs (leaf, stem, root) as well as that there are different pathways by which they are released into the environment acting on neighbouring plants or grown crops causing phytotoxic effects (Novak et al., 2018). Allelopathic interactions are also investigated to reduce the negative impact of allelochemicals on crop growth and yield (Šćepanović et al., 2007). Ragweed is an annual herbaceous plant of the family Asteraceae. Native to North America, it was introduced to Europe in the 19th century. It spreads rapidly, grows on abandoned land and meadows, in gardens, along roadsides, and as such is a highly invasive species (Nikolić et al., 2014). Ragweed is known to form a large biomass above ground as well as possess a strong root system that greatly suppresses the surrounding crop (Buzhdygan & Baglei, 2016). The study of the effect of a ragweed aqueous extract on cultivated species was the subject of many scientific studies in the past. Thus, Buzhdygan & Bagley (2016) noted in their research that the germination of barley under the influence of ragweed was stimulatory, while inhibitory to the germination of alfalfa, sunflower, clover, and fleawort. The inhibitory effect of ragweed was also noted by Vidotto et al. (2013) on seed germination and the growth of tomato shoot, green lettuce, black nightshade, and finger grasses. Kincel et al. (2019) in their research confirmed the inhibitory effect of aqueous ragweed extract on the growth and germination of corn and amaranth. However, it has not yet been sufficiently studied how the aqueous extract of ragweed affects seed germination and seedling growth of cultivated species. According to a research by Buzhdygan and Bagley (2016), it was found that ragweed contains chlorogenic acids, glucose esters, and caffeic acids that inhibit the germination of many plants.

Considering the ragweed potential to reduce plant growth and crop yields, as well as its negative impact on human health, the examination of the sensitivity of some cereals to its effects is of great importance. Therefore, this study examined the allelopathic effect of aqueous leaf extract isolated from the leaves of ragweed (*A. artemisiifolia* L.) on seed germination and early seedling growth (root length and shoot height) of barley (*Hordeum vulgare* L.) and white clover (*Trifolium repens* L.).

Material and Methods

The plant material was collected during August in a field located in Banja Luka's Borik suburb. Fresh leaves were first washed under tap water and then distilled water, and, subsequently, dried and pressed. An extract of the plant's dry parts was made in a ratio of 1:10 (100 g of a plant mass in 1000 ml of water) and left at room temperature for 24h, after which the extract was filtered through filter paper by the method given by Elis and Roberts (1981) and Khandakar and Bradbeer (1983) in order to obtain a 10% aqueous ragweed extract. Distilled water was used as the control. Seed surface of barley (*Hordeum vulgare* L., var. nudum) and white clover (*Trifolium repens* L.) was sterilized with 1% NaOCl, and then 30 barley and white clover seeds were placed on filter paper in sterilized Petri dishes (Figure 1). Two mL of aqueous ragweed extract, i.e. distilled water as a control, was added to the filter paper of each Petri dish. The treatment was as follows: control - distilled water (C), undiluted extract - 10% (CE), diluted at a 1:2 ratio (3.3%), diluted at a 1:4 ratio (2%), and diluted at a 1:8 ratio (1.25%). The next step was keeping these dishes in a thermostat (26˚C) in the dark for 7 days. The last part of the experiment was monitoring the effect of different concentrations of aqueous ragweed extract on the final germination percentage (FGP), mean germination time (MGT), seed germination rate index (GRI) and growth parameters (root length and shoot height).

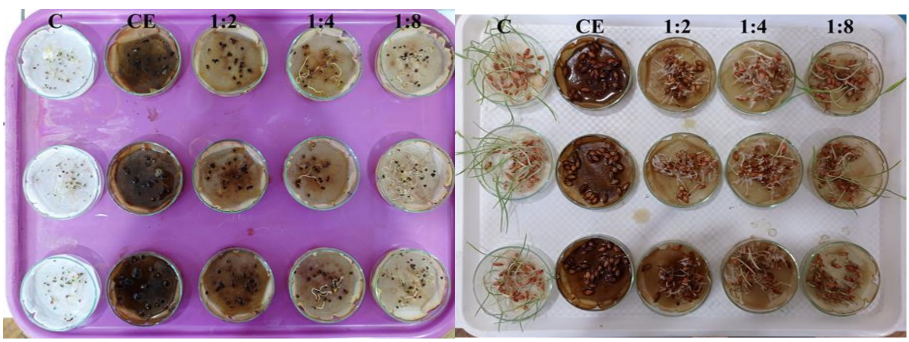

Figure 1. Effect of different concentrations of aqueous ragweed extract on seed germination and seedling growth of barley and white clover (C - Control (distilled water), CE - concentrated extract, at a dilution of 1:2, 1:4, and 1:8).

The percentage of germination was monitored on the third, fifth, and seventh day, and calculated according to the following formula:

Final germination percentage (FGP) = number of germinated seeds / total number of seeds at the beginning of the experiment × 100.

The mean germination time (MGT) was calculated according to the formula (Ellis and Roberts, 1981): MGT = Σ (Dn) / Σ n, where n is the number of seeds that germinated on day D, and D is the number of days from the beginning of germination.

The germination rate index (GRI) was calculated using the following formula (Khandakar and Bradbeer, 1983): GRI = n1 / d1 + n2 / d2 + n3 / d3 + Nn/n x 100, where n is the number of germinated seeds germinating n-days after setting up the experiment, d is the number of days.

All data were statistically processed in the SPSS 20.0 (Statistical Package for the Social Sciences) program. The analyses were performed in three independent replicates and the analyzed parameters were processed by the nonparametric Mann-Whitney U test, at the significance level p<0.05.

Results and Discussion

The results of this study showed that the germination of barley and white clover seeds varied depending on the applied concentration of the aqueous extract of ragweed leaves and the length of the treatment (Figures 2A and 2B). The highest percentage of white clover germination was recorded in the control (up to 80%), while germination at lower concentrations (1:4 and 1:8) was 30-50% lower compared to the control. In the concentrated extract (10%), white clover seed germination was almost completely inhibited, since FGP % at both varieties was approximately 0.5-1% (Figure 2A and 2B).

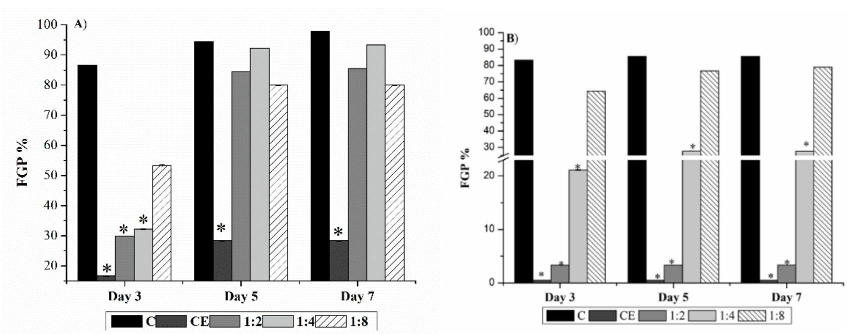

Figure 2. Final germination percentage (FGP %) of barley (A) and white clover (B) treated with different concentrations of the aqueous extract of ragweed leaves. (*) indicates statistically significant difference between control and treatments per day (p <0.05).

It was observed that the barley seeds showed the lowest germination at the concentrated extract and 1:2 on the third day of monitoring, but on the fifth and seventh day, the germination was similar to the control. Extract concentrations of 1:4 and 1:8 did not significantly inhibit the germination of barley seeds (Figure 2B). Compared to white clover (Figure 2A), a higher percentage of germination was recorded in barley during all treatments.

The mean germination time (MGT) of barley seeds in the control treatment was the fastest and amounted to 5.07 days (Figure 3A). Compared to the control, concentrations 1:2 and 1:4 had the highest inhibitory effect, where the mean germination time was 5.56 days. Also, it was observed that the mean germination time was longer for barley in comparison to white clover.

The mean germination time of white clover was approximately the same in the control and the treatment with 1:2 concentration leaf extract and was 5.01 days. The highest inhibitory effect was in the concentrated leaf extract, while concentrations of 1:4 and 1:8 had a similar inhibitory effect, where the mean germination time was 5.2 days (Figure 3A).

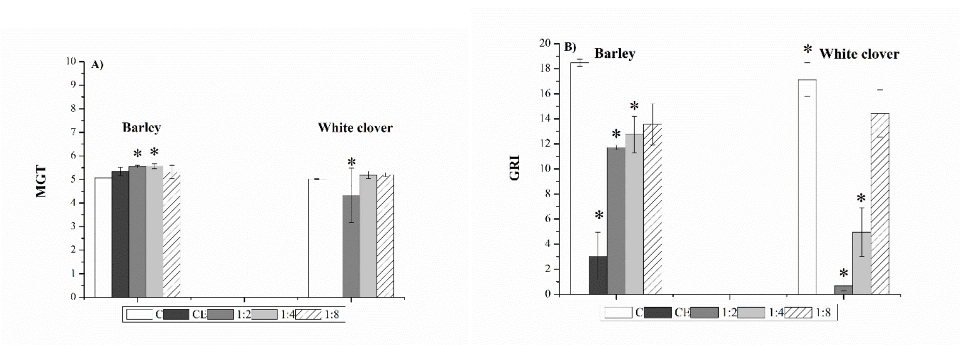

Figure 3. Mean germination time (MGT) (A) and germination index (GRI) (B) of the tested species in control and at different concentrations of the extract. (*) indicates a statistically significant difference between the control and treatments in one examined species ($p<0.05$).

Applied concentrations of the aqueous extract of ragweed leaves had a significant effect on the germination rate index of barley and white clover seeds. The germination rate index (GRI) of barley was significantly higher than that of white clover. The GRI of barley was maximum in the control treatment (18.47), while it was significantly lower in the concentrated extract (3.05). In other treatments, the GRI of barley was lower compared to the control (Figure 3B). The white clover GRI was the highest in the control (17.12), and then decreased with increased extract concentrations. The GRI of white clover was 0.44 at a concentration of 1:2, 4.96 at 1:4 and finally 14.42 at 1:8 concentration treatment. A significant difference in the GRI was determined between the control and all applied concentrations in both examined species except for the 1:8 treatment.

The applied concentrations of the aqueous extract of ragweed leaves (CE, 1:2, 1:4) significantly inhibited the growth of shoot and root of both examined species (Figure 4A and 4B). The extract concentration 1:8 did not significantly affect the growth parameters of the tested species, except for white clover roots, where a reduction of 15% was observed. The impact of the aqueous leaf extract on the root growth of both test plants was significant.

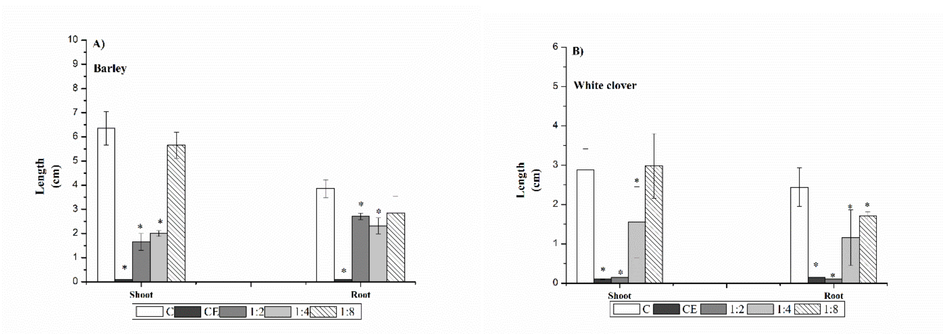

Figure 4. Influence of different concentrations of Ambrosia artemisiifolia extract on root and shoot length of barley (A) and clover. (*) indicates a statistically significant difference in the measured parameter between the control and treatments in one examined species ($p<0.05$).

Some plant species express tolerance to the presence of ragweed (Buzhdygan & Bagley, 2016), while for most of them, an inhibitory effect was found, which is reflected in the reduction of the percentage of germination, growth, development as well as yield of young plants (Beres & Kazinczi, 2000; Cicek & Tilki, 2007; Hodisan et al., 2009; Vidotto et al., 2013; Novak et al., 2018; Kincel et al., 2019). The results obtained in this work showed that the aqueous extract isolated from the leaves of the weed species *A. artemisiifolia* caused a decrease in seed germination, extended the germination time, and caused weaker initial growth of young plants. Most of the inhibitory effects were detected in the initial stages of germination (3rd and 5th day) at the highest extract concentration. The highest inhibitory effect was recorded in white clover with a concentrated extract where germination was not determined, while the other concentrations had no inhibitory effect. The obtained results on the inhibitory effect of ragweed were recorded by Vidotto et al. (2013) on germination and seedling growth of tomato, lettuce, black nightshade, and finger-grasses.

Kincell et al. (2019) determined a significant decrease in the germination of *Amaranthus retroflexus* seeds (germination of 4.83% - 8.33%) under the influence of ragweed extract. They showed that at different concentrations *A. artemisiifolia* inhibits the germination and growth of corn plants, although in some variants (using the 30% extract concentration) some stimulating effects in the growth initiation appeared. The results obtained in this work showed that the extract isolated from the ragweed leaves inhibited germination in all treatments, with the fact that it was lower in clover compared to barley, and also decreased in the following order: concentrated extract <1:2 <1:4 <1:8. On the other hand, Buzhdygan & Bagley (2016) in their research found a stimulating effect of ragweed on barley, which was contrary to the results obtained in this work. Differences in the effect of the ambrosia extract on germination can be attributed not only to different concentrations but also to different parts of the material used for the extract preparation, as well as the sensitivity of the tested species seeds (Novak et al., 2018). Cicek & Tilki (2007) state in their research that the seed size plays a key role, i.e. larger seeds have a higher survival rate (80%), while the smaller ones significantly lower (71%), which is probably one of the reasons for the higher germination of barley compared to the clover in this research.

The applied aqueous leaf extract of ragweed slightly increased the MGT of barley compared to white clover. The average germination time of barley and white clover seeds was the shortest in the control treatment and lasted about 5 days (Figure 2A and B), while it was about 5.56 days for barley and 5.29 days for white clover, which was recorded in the treatment of 1:4. The germination rate index was maximum and minimum in the lowest and highest concentration (concentrated and 1:8) of the aqueous ragweed extract. Also, it can be noted that the GRI of white clover was significantly lower in the concentrated extract (0.58) compared to barley (2.19). Extracts taken from the leaves of the weed Chenopodium murale had an inhibitory effect on the GRI of wheat, corn, and beans (Islam et al., 2014).

The results of this study have confirmed the common fact that the allelopathic effect on the initial sprout's growth is stronger compared to the germination of the test species (Novak et al., 2018). The applied water extracts of ragweed significantly inhibited sprout and root growth compared to the control (by more than 60%), with this effect being more visible on young clover plants compared to barley. Therefore, it is obvious that allelopathy depends on the applied concentration and species. This was confirmed in other studies by Vidotto et al. (2013); Novak et al. (2018); Kinzel et al. (2019) who reported similar results. Experiments conducted by Lehoczky et al. (2011) showed that ragweed sprout extracts significantly reduced the growth of wheat, rye, and oats, while this effect was absent in corn. On the other hand, the inhibitory effect of ragweed interfered with germination more than corn growth. In our research, all applied concentrations inhibited the root growth in both tested species compared to the control. However, when it comes to clover shoot, a slight stimulating effect was observed at the 1:8 concentration. The results are also in agreement with those obtained by Buzhdygan & Bagley (2016) who recorded the inhibition of root length in sunflower, wheat, barley, alfalfa, and clover under the influence of the aqueous ragweed extract. In contrast, Šćepanović et al. (2007) found that date extract inhibited sprout length but stimulated corn root length. The results of our study are similar to previous research done on this topic (Lehoczky et al., 2011; Novak et al. 2018; Maksimović et al. 2021) and they confirmed that allelopathic effects were improved by increasing concentrations of allelochemicals. Certain studies have shown that the allelopathic effects of plants in controlled conditions are not always the same as those in the natural environment (Inderjit, 2003). Therefore, it is necessary to carry out more detailed research in controlled and natural conditions to gain a clearer insight into the impact of the aqueous ragweed extract on seed germination and seedling growth of agricultural crops, like barley and white clover.

Conclusion

The results obtained in this research have shown a more notable allelopathic effect that depends on the applied concentration and the treated species. A significantly lower percentage of seed germination under the influence of the aqueous extract of ragweed leaves has been determined in white clover compared to barley. The aqueous extracts have prolonged the average germination time and reduced the germination rate index of both test plants. Higher sensitivity of the roots compared to the shoots has been proved in both researched species. Taking into account the ragweed potential to reduce the plant growth and crop yield, our research points to the crucial importance associated with the sensitivity of cereals to the presence of ragweed. All of this indicates an increasing need to control the spread of ragweed, with a recommendation to plan the cultivation of less sensitive species before growing more sensitive horticultural ones. In conclusion, the research emphasized the importance of suppressing this invasive weed considering its negative allelopathic influence.

References

Béres, I., & Kazinczi, G. (2000). Allelopathic effects of shoot extracts and residues of weeds on field crops. *Allelopathy Journal*, 7, 93-98.

Bertin, C., Yang, X., & Weston, L. A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil*, 256 (1), 67-83. https://doi.org/10.1023/A:1026290508166

Bonea, D., Elena Bonciu, E., Niculescu, M., & Olaru, A. L. (2018). The allelopathic, cytotoxic and genotoxic effect of *Ambrosia artemisiifolia* on the germination and root meristems of *Zea mays*, *Caryologia*, 71, 24. https://doi.org/10.1080/00087114.2017.1400263

Buzhdygan, O., & Baglei, O. (2016). Developmental traits in grassland and agricultural plants under the influence of ragweed. *Biological systems*, 8 (2), 202-207. http://ibhb.chnu.edu.ua/uploads/files/vb/BS_T8_V2_2016/8_Buzhdygan.pdf

Cicek, E., & Tilki, F. (2007). Seed size effects on germination, survival and seedling growth of *Castanea sativa* Mill. *Journal of Biological Sciences*, 7, 438-441. https://doi.org/10.3923/jbs.2007.438.441

Ellis, R. H., & Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, 9, 373-409.

Hodisan, N., Morar, G., & Neag, C. M. (2009). Research on the allelopathic effect between the invasive species *Ambrosia artemiisifolia* L. ("Floarea Pustei") and some agricultural crops. *Bulletin of the University of Agricultural Sciences &Veterinary*, 66 (1), 554-563. https://doi.org/10.15835/buasvmcn-agr:4265

Inderjit, Callaway, R.M. (2003). Experimental designs for the study of allelopathy. *Plant and Soil*, 256, 1–11. http://www.jstor.org/stable/24123355

Islam, Irum-Us., Ahmed, M., Asrar, M., & Siddiqui, M. F. (2014). Allelopathic effects of *Chenopodium murale* L. on four test species, *Fuuast Journal of Biology*, 4 (1), 39-42. https://fuuastjb.org/index.php/fuuastjb/article/view/214/199

Jabran, K., Mahajan G., Sardana, V., Bhagirath, S. & Chauhan, B. S. (2015): Allelopathy for weed control in agricultural systems. *Crop Protection*, 72, 57-65. https://doi.org/10.1016/j.cropro.2015.03.004

Khandakar, A. L., & Bradbeer, J. W. (1983). Jute seed quality. *Bangladesh Agricultural Research Council*, Dhaka.

Kincel, K., Ramona, S., & Alin, C. (2019). Influence of *Ambrosia artemisiifolia* extract on germination and growth of *Amaranthus retroflexus* and *Zea mays*. *Research Journal of Agricultural Science*, 51 (2),127-135. https://www.rjas.ro/paper_detail/3092

Lehoczky, E., Golya, G., Szabo, R., & Szalai A. (2011): Allelopathic effects of ragweed (*Ambrosia artemisiifolia* L.) on cultivated plants. *Communications in Agricultural and Applied Physiological Sciences*, 6 (3), 545-9.

Maksimović, T., Marković, L., & Hasanagić, D. (2021). Influence of *Ambrosia artemisiifolia* extract on germination and growth of *Pisum sativum* L. and *Phaseolus vulgaris* L. seedlings. Soil and Plant, 70(2), 33-41. https://doi.org/10.5937/ZemBilj2102033M

Nikolić, T., Mitić, B., & Boršić, I. (2014). Flora Hrvatske – invazivne biljke. Alfa d. d., Zagreb.

Novak, N., Novak, M., Bari K., Šepanović, M., & Ivić, D. (2018). Allelopathic potential of segetal and ruderal invasive alien plants. *Journal of Central European Agriculture*, 19 (2), 408-422. https://doi.org/10.5513/JCEA01/19.2.2116

Rice, E. L. (1984). Allelopathy. 2nd Edition, Academic Press London.
Singh, H.P., Batish, D.R., & Kohli, R.K. (2001): Allelopathy in Agroecosystems: An Overview. *Journal of Crop Production*, 4 (2), 1-41. https://doi.org/10.1300/J144v04n02_01
Šćepanović, M., Novak, N., Barić, K., Ostojić, Z., Galzina N., & Goršić, M. (2007). Alelopatski utjecaj korovnih vrsta *Abutilon theophrasti* Med. i *Datura stramonium* L. na početni razvoj kukuruza. *Agronomski glasnik*, 6, 459-472. http://www.agronomski-glasnik.agronomsko.hr/Arhiva/2007/06/03_M_Scepanovic_i_sur_Alelopatski_utjecaj_korovnih.pdf
Vidotto, F., Tesio F., & Ferrero A. (2013). Allelopathic effects of *Ambrosia artemisiifolia* L. in the invasive process. *Crop Protection*, 54, 161-167. https://doi.org/10.1016/j.cropro.2013.08.009

Алелопатски утицај пеленасте амброзије (*Ambrosia artemisiifolia* L.) на клијавост сјемена и почетни раст клијанаца јечма (*Hordeum vulgare* L.) и бијеле дјетелине (*Trifolium repens* L.)

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Сажетак

Алелопатија је јединствен феномен у природи који се односи на директан или индиректан, позитиван или негативан утицај једне биљке (укључујући микроорганизме, инсекте и биљоједе) на другу биљку путем хемијских излучевина (алелохемикалија). *Ambrosia artemisiifolia* (пеленаста амброзија) је позната инвазивна биљка која посједује алелохемикалије за које је утврђено да инхибирају раст и развој биљака у њеној непосредној околини. Сврха овог истраживања била је да се утврди алелопатски утицај воденог екстракта изолованог из листова амброзије (концентровани екстракт, 1:2, 1:4 и 1:8) на проценат клијавости, средњу вриједност клијања, брзину клијања, раст поника и коријена јечма (*Hordeum vulgare* L.) и дјетелине (*Trifolium repens* L.). Испитивани водени екстракти амброзије имали су већи инхибиторни утицај на клијавост сјемена дјетелине у односу на јечам. Клијавост сјемена јечама је била најнижа при концентрованом екстракту (за 70% нижа у односу на контролу) док клијавост сјемена дјеталине није утврђена при овој концентрацији. При осталим третманима клијавост је била значајно нижа код обе истраживане врсте у односу на контролу. Раст поника и коријена обе истраживане врсте је била инхибирана при свим третманима воденог екстракта амброзије у односу на контролу,што указује на већу осјетљивост поника у поређењу с клијавошћу сјемена. Добијени подаци указују да водени екстракт амброзије значајно инхибира раст коријена и поника обе испитиване врсте, што повећава потребу за интензивна истраживања на ову тему.

Кључне ријечи: Ambrosia artemisiifolia, јечам, бијела дјетелина, алелопатија, водени екстракт листа, клијавост

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Received: February 03, 2023
Accepted: August 31, 2023