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Original scientific paper

Antioxidant activity, phytotoxicity and allelopathic potential of green walnut (*Juglans regia* L.) fruit extract

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SUMMARY

The potential allelopathic effect of a green walnut fruit extract on seed germination and early growth of three weed species (*Chenopodium album*, *Amaranthus retroflexus*, *Daucus carota*) was tested, as well as its phytotoxic effect on seed germination and early growth of maize (*Zea mays*). Another objective was to analyze the plant extract and assess its antioxidative activity.

Antioxidative activity of the plant extract was evaluated based on its ability to neutralize DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and capacity for iron reduction using the FRAP method. Inhibition of the evaluated parameters (total germination and seedling length) decreased proportionally with decreasing concentrations of extract solution of green walnut fruit. The parameters of seedling growth were also found to show greater susceptibility than total seed germination of all three of the tested weed species. Although the seed bioassay results indicated a high inhibitory effect on germination and early growth of the tested weeds, they also revealed phytotoxic activity during early growth of the cultivated *Z. mays*.

Keywords: alleopathy, walnut, plant extract, weeds, maize

INTRODUCTION

Detrimental effects of herbicides on the environment and human health, increase in the number of weed species developing resistance to herbicides, slower development of novel herbicides, retraction of a significant number of herbicides from use, and increasing growth of organic agriculture have been the main factors over the past several decades for promoting environment-friendly approaches to weed control (Hatcher & Melander, 2003; Vilà et al., 2010; Villa et al., 2017; Hossard et al., 2017; Kanissery et al., 2019). Allelopathy is one such approach that refers to any direct or indirect, beneficial or detrimental impact of plants on the growth and development of other plants (Narwal, 2010). Plants may exude their products of metabolism by roots,

by evaporation from above-ground plant parts or by decomposition of plant matter in the soil (Wu et al., 2000; An et al., 2003; Gatti et al., 2010), which may cause inhibition of seed germination or its delay, inhibition of root system growth or radicle burst, lack of root hair, elongation of coleoptile, etc. (Gella et al., 2013). Natural products are sources of a variety of compounds that can be used as leading structures in the synthesis of new bioherbicides intended either for independent use or combinations with synthetic herbicides within the framework of integrated control strategies (Duke et al., 2000). Bioherbicides are made from naturally synthesized components having allelopathic properties that are readily degradable, which makes their application safer than synthetic herbicides (Belz, 2007).

Walnut is one of the plant species most abounding in antioxidants, and rich in lipids and oils (up to 70%), proteins, carbohydrates, vitamins (C and E), essential fatty acids, phenolic acids (p-coumarin, chlorogenic, vanillic, syringic), flavonoids (quercetin, catechin, myricetin) and chinones (juglandin and juglone), as well as saponins, tannins, alkaloids, glucosides, etc. (Cosmulescu et al., 2014; Rusu et al., 2020; Zurek et al., 2022). Walnut is a significant source of phytochemicals that may have allelopathic properties. The purpose of the present study was therefore to test the allelopathic potential of an unripe walnut fruit extract, more specifically to: (1) analyze the extract and estimate its antioxidative activity; (2) estimate *in vitro* the allelopathic effect of the extract of green (unripe) walnuts on seed germination and early growth of three weed species, namely *Amaranthus retroflexus*, *Chenopodium album,* and *Daucus carota*, as well as its phytotoxic effect on seed germination and early growth of maize (*Zea mays*).

MATERIAL AND METHODS

Plant material

Seed bioassays included: *A. retroflexus* (seed collected from Surčin), *C. album* (seed collected from New Belgrade), and *D. carota* (seed collected from New Belgrade). The collected seeds were cleansed and kept in paper bags in the laboratory at a room temperature of 20-22°C. Commercial maize seed (hybrid NS3022), a product of the Institute of Field and Vegetable Crops of Novi Sad, were used in the bioassay. Immature fruits of walnut (*J. regia*) were picked in fields around Krčedin on 5th May 2022.

Seed bioassay

Testing of allelopathic potential of the extract of *J. regia* green fruit on seed germination and early seedling growth of *A. retroflexus*, *C. album,* and *D. carota*, and its phytotoxic effect on seed germination and early seedling growth of *Z. mays,* was carried out in a controlled environment. Before the bioassay, seeds of all tested species were immersed in thiourea solution for 24 h and then sterilized with 3% hydrogen peroxide solution for 5 minutes before they were well rinsed with distilled water. The disinfected seeds of three weed species were transferred into sterile Petri dishes (25 seeds/Petri dish of 9 cm) and 5 ml of prepared green walnut extract, diluted in distilled water in the initial concentration of 1% and a series of dilutions of 0.75%, 0.50%, 0.25%, 0.10%, and 0.05%, was added, while 25 maize seeds were transferred to each Petri dish (14 cm Ø) and 10 ml of extract was added in corresponding concentrations. Petri dishes were sealed with parafilm and kept in an incubator (Velp Scientifica, Europe) at 27°C temperature in the dark. Distilled water was used for the control treatment. All treatments were done in four replicates and the bioassay was repeated twice. Germinated seeds were counted and seedling length was measured 7 days after treatment (DAT). Ungerminated seeds were subjected to a standard tetrazolium test (TTC) to distinguish dormant from dead seeds and determine seed viability for all test plants.

Sequential ultrasonic extraction from plant material

The extract was obtained from unripe walnuts by sequential ultrasonic extraction using solvents with different polarities to achieve a satisfactory yield of phenolic compounds, as well as other plant secondary metabolites of different polarities. The ratio of ground plant material and solvent was 1:1 (w/v). Extraction included homogenization in Erlenmeyer flasks in an ultrasonic bath (lasting 15 minutes at 30°C temperature at 37 kHz frequency and ultrasonic bath power of 168 W) recurring in seven successive extraction cycles with hexane, ethyl-acetate, acetone, acetonitrile, ethanol, methanol, and distilled water. Extracted aliquots from each extraction cycle were filtered through quantitative filter paper into evaporator flasks and evaporated on a rotary vacuum evaporator at 40°C to dryness, while plant remains were used in the next extraction step with respective solvents. All dry matter was washed with distilled water into one container. The extract was

then frozen at -80°C in a lyophilization container and dried by lyophilization. The resulting dry sample was kept in vials at -20°C until analyses.

Phytochemical analyses of extract

Total phenolic content

Total phenolic content in the extract was determined by a modified Folin-Ciocalteu method. The principle of the assay is the reduction of Folin-Ciocalteu reagent (mixture of phosphomolybdate and phosphotungstate) in the presence of phenolics, resulting in the production of molybdenum-tungsten blue that is measured spectrophotometrically at 760 nm, and the intensity increases linearly with concentrations of phenolics in the reaction medium. For the analysis, 0.25 g of dry green walnut extract was dissolved in distilled water (25 ml). A volume of 100 µl of extract solution was mixed with 0.5 ml of Folin-Ciocalteu reagent and 6 ml of distilled water in test tubes and vortexed well. A volume of 2 ml of 15% Na_2CO_3 was then added to the solution, and supplemented further with distilled water up to 10 ml, and the solution was then incubated for 60 min at room temperature. Absorbance was measured at 760 nm wavelength by spectrophotometer. A solution containing only the reagents, beside extract, was considered a blank. Gallic acid (GAE) was used as an equivalent for calibration curve preparation, and the results were expressed as mg of gallic acid per g of dry extract (mg GAE g^{-1} d.e.).

Total flavonoid content

Total flavonoid content in green walnut extract was determined using a modified aluminum chloride colorimetric assay. The principle involved in the aluminum chloride colorimetric method is that $AlCl_3$ forms stable acid complexes with C-4 keto groups and either C-3 or C-5 hydroxyl group of flavones and flavonols, resulting in the production of pink color, which is measured spectrophotometrically at 510 nm. For analysis, dry green walnut extract (0.125 g) was dissolved in methanol (25 ml). A volume of 375 µl of extract solution was mixed with 1.5 ml of 5% $NaNO_2$ solution in test tubes and incubated at room temperature for 6 min. A volume of 150 µl of 10% $AlCl_3$ solution was added, vortexed well, and left for another 5 min. A volume of 750 µl of 1M NaOH solution was added and further incubated for 15 min. Absorbance was measured at 510 nm wavelength by spectrophotometer. A solution containing only the reagents, besides extract, was considered a blank. Quercetin (QE) was used as an equivalent for calibration curve preparation, and the results were expressed as mg quercetin per g of dry extract (mg QE g^{-1} d.e.).

Evaluation of antioxidant activity – determination of DPPH scavenging activity

Antioxidant activity of the obtained extract was tested by determining its DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. In a DPPH assay, the violet-colored DPPH solution is reduced to the yellow-colored product diphenylpicryl hydrazine by antioxidants present in the sample. For the analysis, 0.25 g of dry green walnut extract was dissolved in distilled water (25 ml), and a series of dilutions were prepared from the stock solution to obtain concentrations ranging from 0.01 to 0.2 mg/ml. A volume of 0.5 ml of each dilution was mixed with 1 ml of DPPH solution (0.2 mM in methanol) and 3 ml of methanol in test tubes and vortexed well. The volume was then augmented to 5 ml by adding methanol, and the solution was incubated for 30 minutes at 25°C in the dark. Absorbance was measured by a spectrophotometer at 517 nm. The solution containing only reagents, besides extract, was considered as a control, and antioxidant activity was calculated using the formula: % inhibition = $[(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated. Ascorbic acid was used as a positive control.

Evaluation of antioxidant activity - determination of ferric ion reducing antioxidant power (FRAP assay)

Antioxidant activity of the obtained extract was tested also by measuring its ferric ion-reducing potential (FRAP). FRAP assay is based on rapid reduction in ferric-tripyridyltriazine (FeIII-TPTZ) by antioxidants present in the test sample, forming ferrous-tripyridyltriazine (FeII-TPTZ), a blue-colored product. Acetate buffer (pH 3.6) (300 mM), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3 \times 6H_2O$ solution (20 mM) were mixed (10:1:1 v/v) for the analysis into a FRAP solution. Dry green walnut extract (150 µl, concentration 0.5 mg/ml in methanol) and 150 µl of distilled water were mixed with 3 ml of FRAP solution and incubated in the dark at 37°C for 30 minutes. FRAP solution was used as a blank. Absorbance was measured at 593 nm by spectrophotometer. Ferrous sulphate heptahydrate

($FeSO_4 \times 7H_2O$) was used as the equivalent for calibration curve preparation, and the results were expressed as μmol of ferric ion (Fe^{2+}) per g of dry extract. Butylated hydroxytoluene (BHT) was used as a positive control.

Statistical analyses

The results are shown as means ± standard deviation. The data were analyzed by single factorial analysis of variance (ANOVA) using the STATISTICA 8.0. software. When F values were statistically significant ($p < 0.05$) treatments were arranged according to LSD test. Effective concentrations (EC_{50}) of the plant extract were calculated using the BIOASSAY 97 software.

RESULTS AND DISCUSSION

Antioxidant activity of unripe walnut extract

The results of chemical analyses of the extract of unripe walnuts showed that total phenol content (TPC) was 173.7±7.9 mg GAE/g dry extract, while total flavonoid (TF) content was 870.9±16.3 mg QE/g dry extract. The antioxidative activity data, expressed in inhibition percentage, i.e. as neutralized free DPPH radicals against the control, are shown in Figure 1 by the dependence curve of plant extract and percentage of inhibition of DPPH radical. The IC_{50}, which is the concentration of green walnut extract needed to inhibit 50% of the initial amount of DPPH radicals, was 0.047 mg/ml. Comparing that value with the IC_{50} for ascorbic acid, which is used as a commercial antioxidant and rates 0.016 mg/ml, the obtained extract of green walnuts showed a significant antioxidative activity. The value received by analyzing ferric ion-reducing antioxidant power (FRAP) was 631.0 μmol Fe^{2+}/g dry extract. A comparison with the obtained FRAP value for BHT (6908 μmol Fe^{2+}/g) reveals that the reducing potential of green walnut extract was significantly weaker than the reducing potential of synthetic antioxidant but, having in mind that the extract contained only antioxidants of natural origin, such antioxidative activity is by no means insignificant.

Many studies have shown that the contents of total phenols and flavonoids, as well as antioxidative potential, change with ongoing maturity of plants and their parts (Jahanban-Esfahlan et al., 2019; Rusu et al., 2019, 2020). Besides, clear differences emerged between the values obtained from fresh, as opposed to dry materials, as well as different solvents, which ultimately resulted in varying results of these parameters reported from different studies (Zarghami Moghaddam et al., 2017).

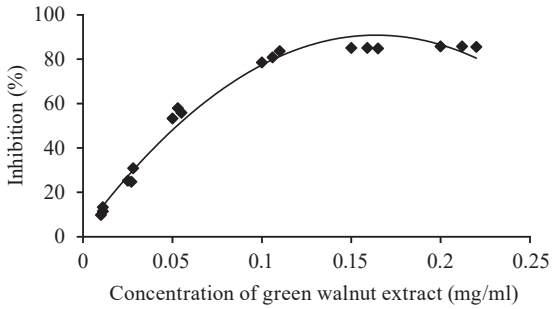

Figure 1. Dependence curve of green walnut extract concentrations (mg/ml) and DPPH radical inhibition (%)

Allelopathic effect of green walnut extract on seed germination and seedling growth of *A. retroflexus*, *C. album* and *D. carota*

The results of seed bioassays testing the allelopathic effects of green walnut extract on weed seeds are shown in Figures 2-4 and Table 2. The percentage of viable seeds after the TTC test for all weed species was over 80% for all concentrations, and 100% for maize seed (Table 1).

Table 1. Percentage of viable seeds of tested plants at different concentrations of extract

Test plant	Concentration of extract						
	Control	0.05%	0.1%	0.25%	0.5%	0.75%	1%
Amaranthus retroflexus	99.0	96.0	91.0	92.0	86.0	88.0	84.0
Chenopodium album	98.8	92.5	92.5	88.8	82.5	87.5	87.5
Daucus carota	98.8	96.3	100.0	85.0	85.0	83.8	87.5
Zea mays	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Seed germination of *A. retroflexus* ranged between 5% and 97%, and the highest germination was found in the control (97%) and treatment with the lowest concentration (0.05%) of extract (90%). Treatments with higher extract concentrations (1%, 0.75%, 0.50%, 0.25%, 0.10%) caused germination inhibition of 95%, 88%, 87%, 49% and 11%, respectively (Figure 2A), and statistical difference was significant between all extract concentrations and the control. The parameter of radicle length showed the highest susceptibility, compared to hypocotyls and overall seedling lengths, as all applied concentrations caused inhibition of 13-94% (Figure 2B). Statistically significant differences ($p<0.05$), noted between the control and all applied concentrations, confirm the finding. On the other hand, the extract of green walnuts had a stimulating effect (7-26%) on shoot length and total seedling length at the two lowest concentrations (0.05% and 0.1%) (Figure 2C and 2D). Higher extract concentrations (1%, 0.75% and 0.50%) resulted in shoot length inhibition of 70% to 100%, while total seedling length was inhibited by 8% to 97%. Statistically significant differences were missing only between the control and the two lowest concentrations (0.05% and 0.10%) in both parameters, while significant differences ($p<0.05$) were detected between 0.25%, 0.50%, 0.75% and 1% concentrations and the control (Figure 2C and 2D).

The bioassay results revealed that *C. album* was more susceptible than the other two weed species as inhibition was noted for all parameters even under the lowest concentration. On the other hand, the lowest concentration (0.05%) had a stimulating effect

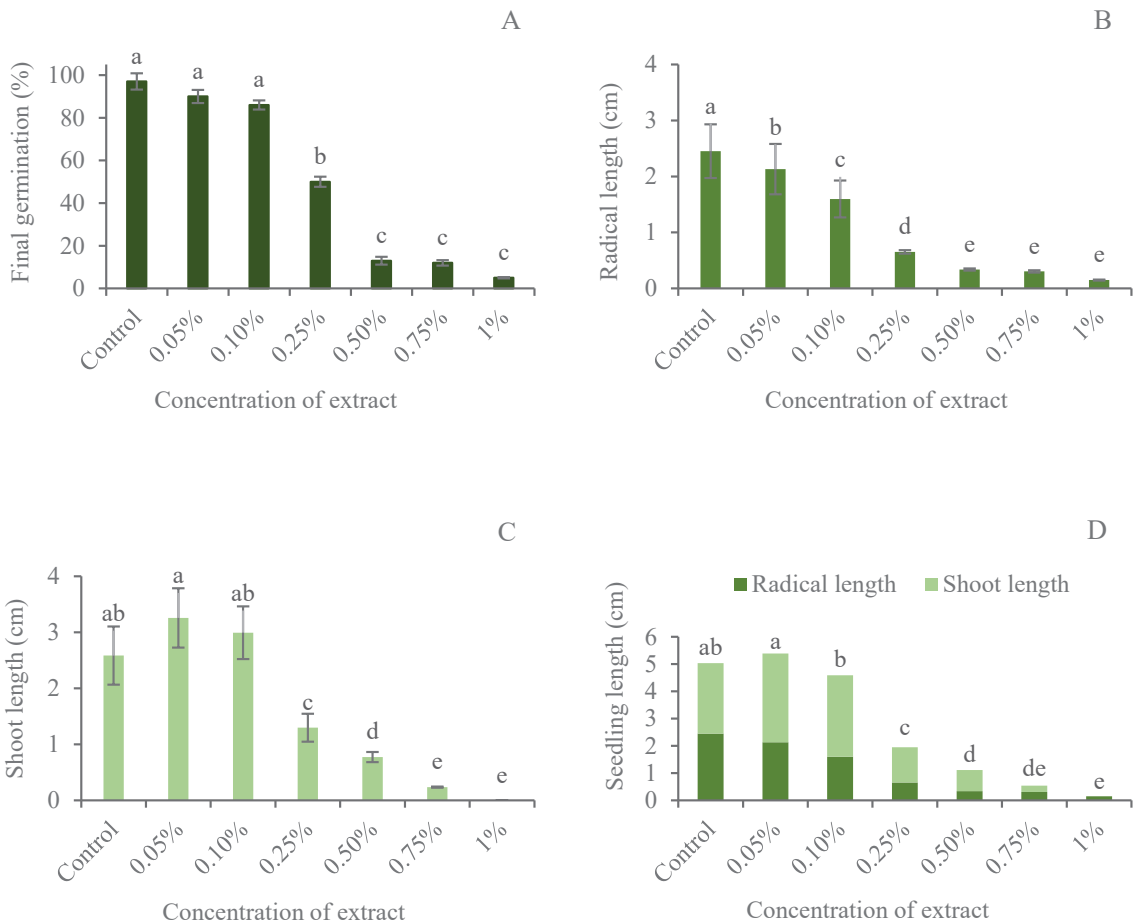

Figure 2. Effect of green walnut extract on seed germination (A), and seedling growth (B, C, D) of *A. retroflexus*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d, e) differ significantly ($p<0.05$) for all parameters at different concentrations

on *A. retroflexus* and *D. carota*. The inhibition of *C. album* germination under the lowest concentration was 33%, while higher concentrations caused 38% to 78% inhibition. Statistically significant differences ($p < 0.05$) were noted between the control and all extract concentrations (Figure 3A). Regarding the shoot length of *C. album* seed, no significant difference was detected between the control and the lowest extract concentration (0.05%), while inhibition of three lower concentrations did not exceed 33%. Conversely, higher concentrations caused significantly higher inhibition (92-94%) and a significant difference ($p < 0.05$) was confirmed between those concentra tions and the control (Figure 3C). Although inhibition of radicle length by the lowest extract concentration was 31%, it was lower than inhibition of shoot length, which ranged from 60% to 85%. The sensitivity of this parameter was confirmed by statistically significant differences ($p < 0.05$) found between the control and all tested concentrations (Figure 3B).

Inhibitory activity of the unripe (green) walnut extract was evidenced by all measured parameters of *D. carota*. Inhibition grew proportionally to rising extract concentrations, reaching 20-98%, 8-96%, 16-100%, and 12-98% based on seed germination, radical length, shoot length and total seedling length results, respectively (Figures 4A, B, C and D). However, a stimulative activity of the lowest extract concentration (0.05%) was also found for all measured parameters, ranging from 1% to 6%. Statistically significant differences ($p < 0.05$) were found between the control and concentrations of 0.25%, 0.50%, 0.75%, and 1% for all parameters, while the lowest concentration showed no significant difference from the control (Figure 4).

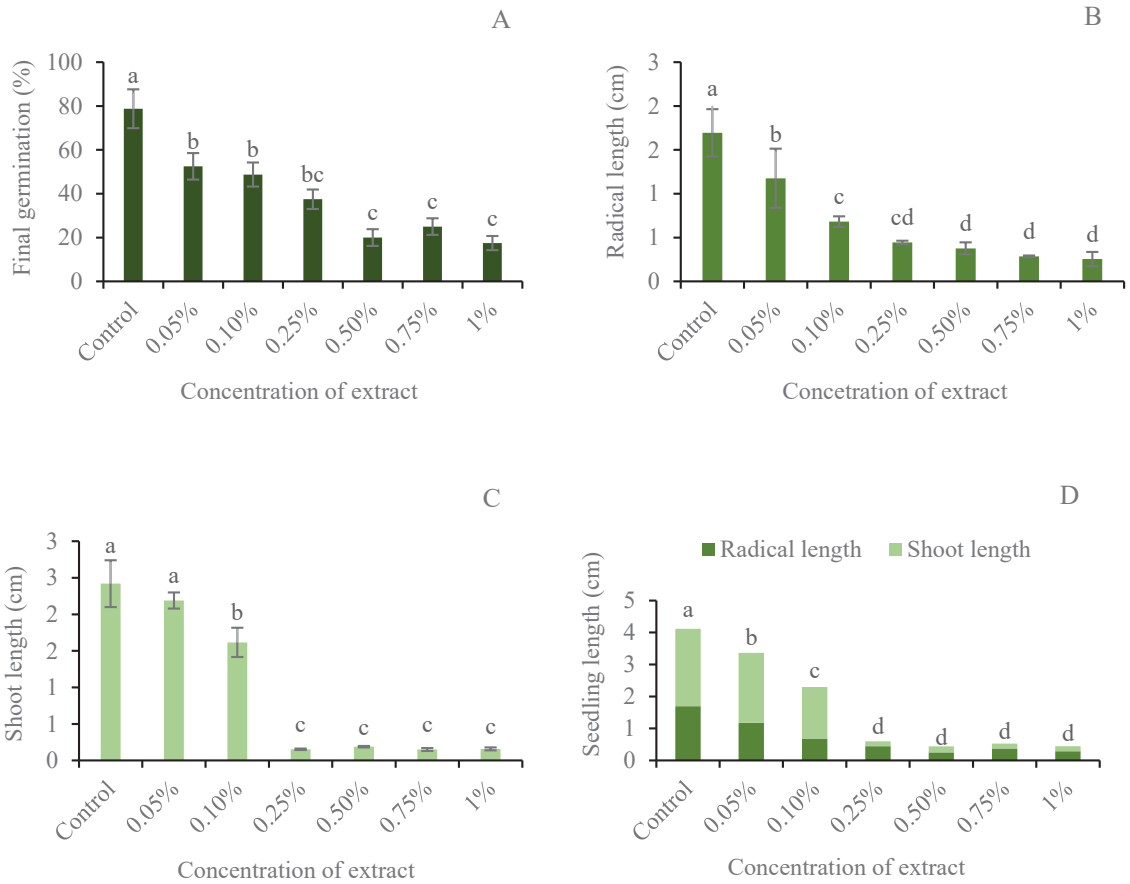

Figure 3. Effect of green walnut extract on seed germination (A) and seedling growth (B, C, D) of *C. album*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d) differ significantly ($p<0.05$) for all parameters at different concentrations

The described inhibition levels clearly showed that the parameters of seedling growth (radical and hypocotyl length, and total seedling length) were more sensitive in all three test weed species than total seed germination. Greater sensitivity of the growth parameters was further confirmed by lower EC_{50} values for those parameters in all tested plant species, *C. album* showing the lowest (Table 2).

Most literature data indicate that juglone has a predominantly toxic effect on plants of many species,

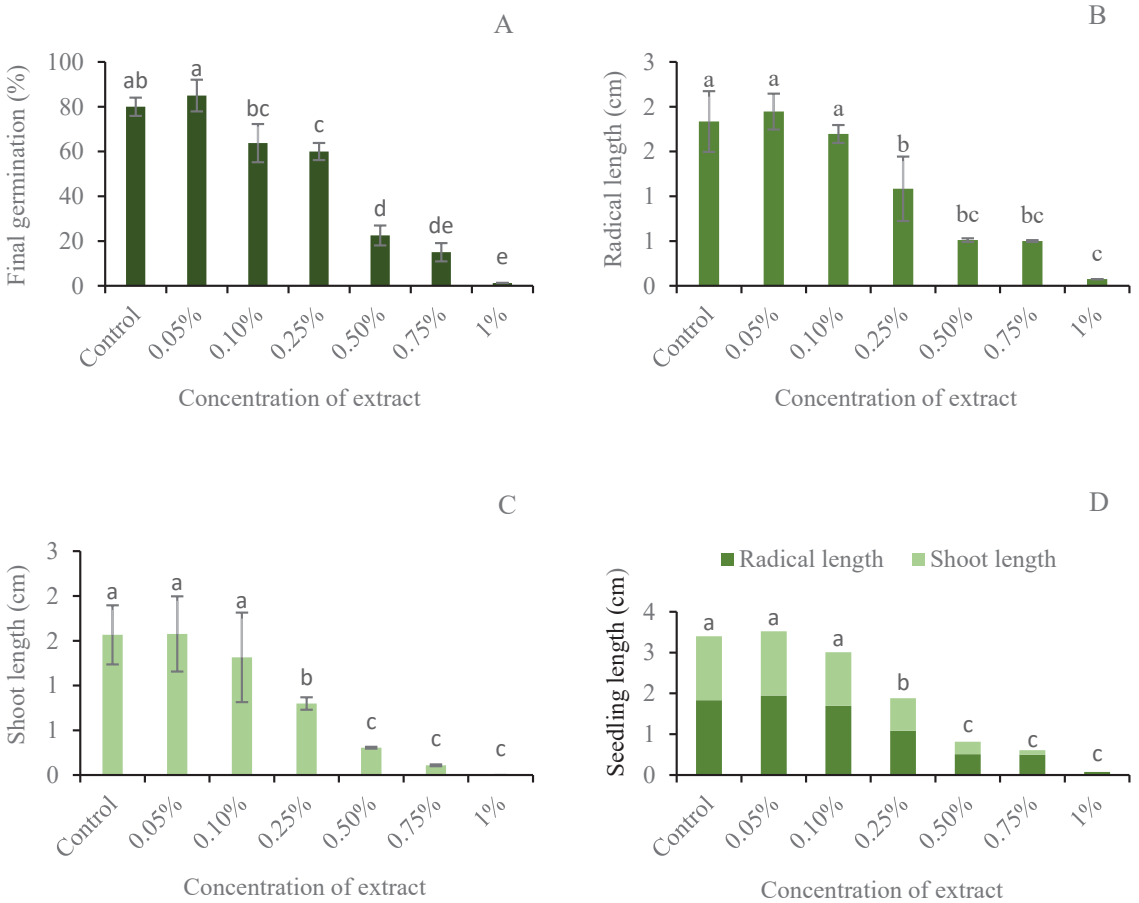

Figure 4. Effects of green walnut extract on seed germination (A) and seedling growth (B, C, D) of *D. carota*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d, e) differ significantly ($p<0.05$) for all parameters at different concentrations

Table 2. EC_{50} for seed germination and seedling growth of tested plants

Test plant	EC_{50}±SE			
	Final germination	Radical length	Shoot length	Seedling length
Amaranthus retroflexus	0.24 ± 0.02	0.13 ± 0.01	0.23 ± 0.05	0.21 ± 0.06
Chenopodium album	0.27 ± 0.09	0.06 ± 0.01	0.11 ± 0.01	0.14 ± 0.03
Daucus carota	0.59 ± 0.17	0.30 ± 0.09	0.28 ± 0.02	0.30 ± 0.09
Zea mays	–	0.70 ± 0.13	0.29 ± 0.17	0.70 ± 0.13

inhibiting their seed germination and early growth of young plants (Kocaçalikan & Terzi, 2001; Terzi, 2008; Maksimović & Hasanagić, 2020). Maksimović and Hasanagić (2020) confirmed the allelopathic activity of juglone on seed germination of tomato and green salad. Germination of tomato seed and early seedling growth were most intensively inhibited by concentrated juglone, while its lower concentrations resulted in a weak stimulative effect. On the other hand, compared to tomato, all applied juglone concentrations had a significant inhibitory effect on germination and early seedling growth of green salad. Some other studies (Kocaçalikan & Terzi, 2001; Terzi, 2008) also confirmed the inhibitory activity of juglone on seed germination of specific plant species, such as cabbage, beetroot, green salad, cucumber, alfalfa, etc. Inhibition of seed germination was shown to weaken proportionally to decreasing extract concentrations, which indicates that the allelopathic effect of a specific extract depends significantly on its concentration.

Phytotoxic effect of green walnut extract on seed germination and seedling growth of *Zea mays*

The results of bioassay testing aimed at clarifying the phytotoxic effects on seed germination and early seedling growth of a cultivated species (*Z. mays*) are shown in Figure 5. Consistent with the results of weed species seed bioassays, where a small inhibitory effect on seed germination was found, compared to the parameters of early seedling growth (radical and hypocotyls length and total seedling length), similar trends were revealed for *Z. mays*. Phytotoxic effect on maize seed germination was

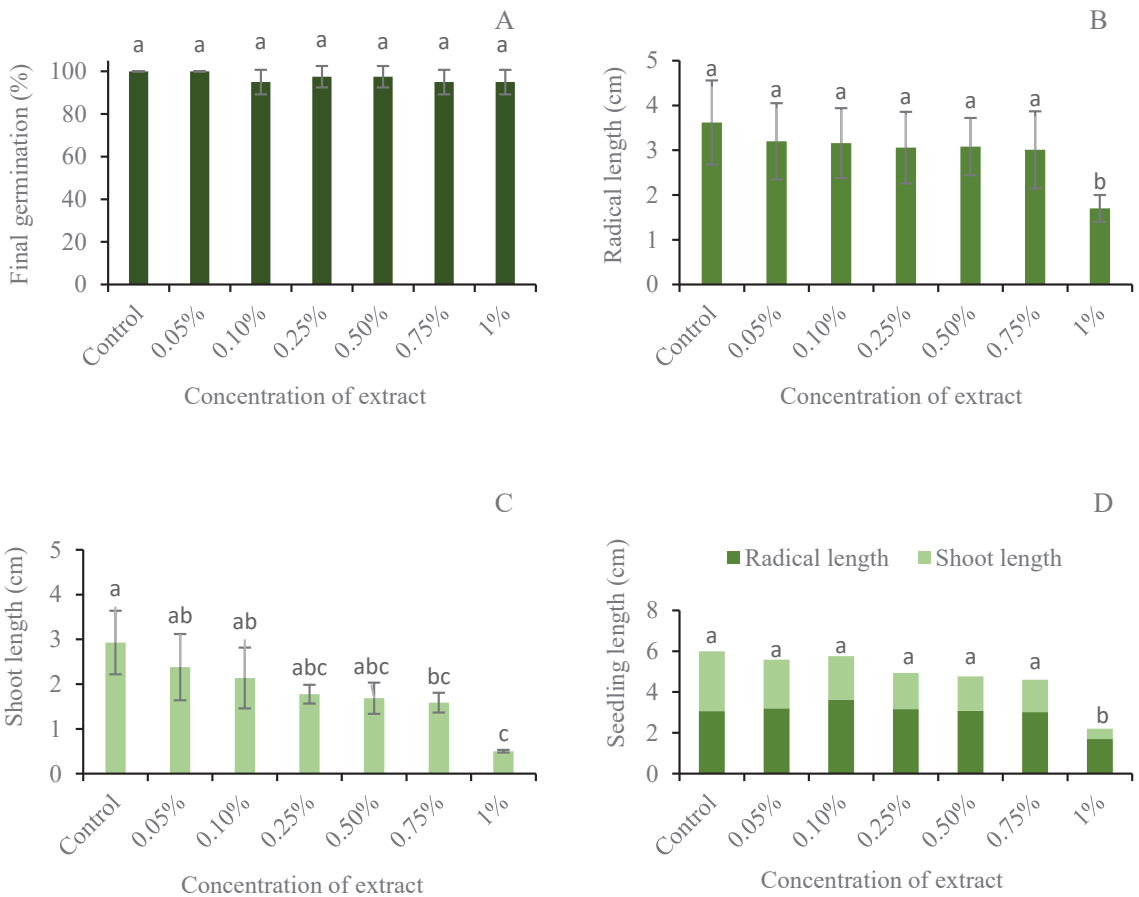

Figure 5. Effect of green walnut extract on seed germination (A), and seedling growth (B, C, D) of *Z. mays*. Differences were evaluated by one-way analysis of variance (ANOVA) completed with Fisher's least significant difference (LSD) test, $p<0.05$. Means marked by different letters (a, b, c, d, e) differ significantly ($p<0.05$) for all parameters at different concentrations

evident from growing inhibition caused by increasing extract concentrations, which ranged from 2.5 to 5% (Figure 5A). Inhibition of radical length and total seedling length was similar, ranging from 12 to 66% (Figures 5B and 5D), while greater inhibition percentages were found for hypocotyl length (19-83%) (Figure 5C).

Statistical data analysis revealed no significant difference ($p=0.485$) in maize seed germination, while seedling growth parameters showed significant differences ($p < 0.05$) between the control and the highest concentration (1%). Significant difference was also found for shoot length between control and concentrations of 0.75% and 1% (Figure 5).

CONCLUSION

Promising results of the initial screening of content (total phenols and flavonoids) and antioxidative properties of the obtained extract of green walnuts inspire further research steps which should include a complete HPLC analysis of that plant extract. The seed bioassay results indicate an inhibitory effect of different concentrations of green walnut extract on seed germination of the weed species *A. retroflexus*, *C. album,* and *D. carota*. Inhibitory activity of a series of extract concentrations on early seedling growth parameters (radical length, shoot length, and total seedling length) was revealed, which showed their greater sensitivity than seed germination. Although the seed bioassay results show a high inhibitory effect on germination and early growth of all three weed species, they also reveal phytotoxic effects on early seed growth of the cultivated species *Z. mays*. Future research needs to include pot trials with different plants, as well as field micro-trials, to determine bioherbicidal effectiveness on weeds, and phytotoxic effects on cultivated species.

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REFERENCES

An, M., Liu, D.L., Johnson, I.R., & Lovett, J.V. (2003). Mathematical modeling of allelopathy: II. The dynamics of allelochemicals from living plants in the environment. *Ecological Modelling, 161*, 53-66. Doi: https://doi.org/10.1016/S0304-3800(02)00289-2

Belz, R.G. (2007). Allelopathy in crop/weed interactions – An update. *Pest Management Science*, *63*, 308-326. Doi: 10.1002/ps.1320

Cosmulescu, S., Trandafir, I., Nour, V., Ionica, M., & Tutulescu, F. (2014). Phenolics content, antioxidant activity and color of green walnut extracts for preparing walnut liquor, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*, 551-555. Doi: https://doi.org/10.15835/nbha4229649

Duke, S.O., Romagni, J.G., & Dayan, F.E. (2000). Natural products as sources for new mechanisms of herbicidal action. *Crop Protection*, *19*, 583-589.

Gatti, A. B., Ferreirall, A. G., Arduin, M., & Andrade Perez, S. C. G. (2010). Allelopathic effects of aqueous extracts of *Artistolochia esperanzae* O. Kuntze on development of *Sesamum indicum* L. seedlings. *Acta Botanica Brasilica*, *24*, 454-461.

Gella, D., Ashagre, H., & Negewo, T. (2013). Allelopathic effect of aqueous extracts of major weed species plant parts on germination and growth of wheat. *Journal of Agricultural and Crop Research*, *1*(3), 30-35.

Hatcher, P.E., & Melander, B. (2003). Combining physical, cultural, and biological methods: Prospects for integrated non-chemical weed management strategies. *Weed Research*, *43*, 303-322. Doi: 10.1046/j.1365-3180.2003.00352.x

Hossard, L., Guichard, L., Pelosi, C., & Makowski, D. (2017). Lack of evidence for a decrease in synthetic pesticide use on the main arable crops in France. *Science of the Total Environment*, *575*, 152-161.

Jahanban-Esfahlan A., Ostadrahimi A., Tabibiazar M., & Amarowicz R. (2019). A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (*Juglans regia* L.) fruit and tree. *Molecules*, *24*, 2133. Doi: 10.3390/molecules24112133

Kanissery, R., Gairhe, B., Kadyampakeni, D., Batuman, O., & Alferez, F. (2019). Glyphosate: Its environmental persistence and impact on crop health and nutrition. *Plants*, *8*, 499. doi: 10.3390/plants8110499

Kocaçalikan, I., & Terzi, I. (2001). Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. *Journal of Horticultura Science & Biotechnology*, *76 (4)*, 436-440.

Maksimović, T., & Hasanagić, D. (2020). Allelopathic influence of *Juglans regia* L. aqueous extract on the germination and growth of lettuce and tomato. *Journal of Agricultural, Food and Environmental Sciences*, *74(2)*, 5-12.

Narwal, S.S. (2010). Allelopathy in ecological sustainable organic agriculture. *Allelopathy Journal*, *25*, 51-72.

Rusu, M.E., Fizesan, I., Pop, A., Mocan, A., Gheldiu, A., Babota M. ... Popa, D. S. (2020). Walnut (*Juglans regia* L.) septum: assessment of bioactive molecules and *in vitro* biological effects. *Molecules, 25,* 2187.

Rusu, M.E., Mocan, A., Ferreira, I.C.F.R., & Popa, D.S. (2019). Health benefits of nut consumption in middle-aged and elderly population. *Antioxidants, 8*(8), 302.

Terzi, I. (2008). Allelopathic effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. *African Journal of Biotechnology*, *7(12),* 1870-1874.

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S. ... Hulme, P. E. (2010). How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment, 8*(3), 135-144.

Villa, F., Cappitelli, F., Cortesi, P., & Kunova, A. (2017). Fungal biofilms: Targets for the development of novel strategies in plant disease management. *Frontiers in Microbiology, 8,* 654.

Wu, H., Haig, T., Pratley, J., Lemerle, D., & An, M. (2000). Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in root tissues. *Journal of Agricultural and Food Chemistry, 48,* 5321-5325.

Zarghami Moghaddam, P., Mohammadi, A., Feyzi, P., & Alesheikh, P. (2017). *In vitro* antioxidant and antibacterial activity of various extracts from exocarps and endocarps of walnut. *Pakistan Journal of Pharmaceutical Sciences, 30,* 1725-1731.

Zurek, N., Pawłowska, A., Pycia, K., Grabek-Lejko, D., & Kapusta, T. I. (2022). Phenolic profile and antioxidant, antibacterial, and antiproliferative activity of *Juglans regia* L. male flowers. *Molecules, 27,* 2762. Doi: 10.3390/molecules27092762

Antioksidativno delovanje, fitotoksičnost i alelopatski potencijal ekstrakta zelenog ploda oraha (*Juglans regia* L.)

REZIME

Cilj ovog rada je bio da se ispita alelopatski potencijal ekstrakta zelenih nezrelih plodova oraha na klijanje i rani porast semena tri korovske vrste (*Chenopodium album*, *Amaranthus retroflexus*, *Daucus carota*), kao i fitotoksični efekat na klijanje i rani porast semena kukuruza *Zea mays*. Pored toga, cilj je bio i da se uradi analiza biljnog ekstrakta i procena njegove antioksidativne aktivnosti.

Antioksidativna aktivnost biljnog ekstrakta određena je kroz sposobnost neutralizacije DPPH (2,2-difenil-1-pikrilhidrazil) radikala i ispitivanjem sposobnosti redukcije gvožđa FRAP metodom. Inhibicija merenih parametara (ukupna klijavost i dužina klijanaca) se proporcionalno smanjivala sa smanjenjem koncentracije rastvora ekstrakta zelenih nezrelih plodova oraha. Takođe, parametri rasta klijanaca su ispoljili veću osetljivost u odnosu na parametar ukupne klijavosti semena kod sve tri korovske vrste. Iako dobijeni rezultati iz biotesta sa semenima pokazuju značajan inhibitorni efekat na klijanje i rani porast testiranih korovskih vrsta, ujedno ukazuju i na fitotoksične efekte kod ranog porasta semena gajene vrste *Z. mays*.

Ključne reči: alelopatija, orah, biljni ekstrakt, korovi, kukuruz